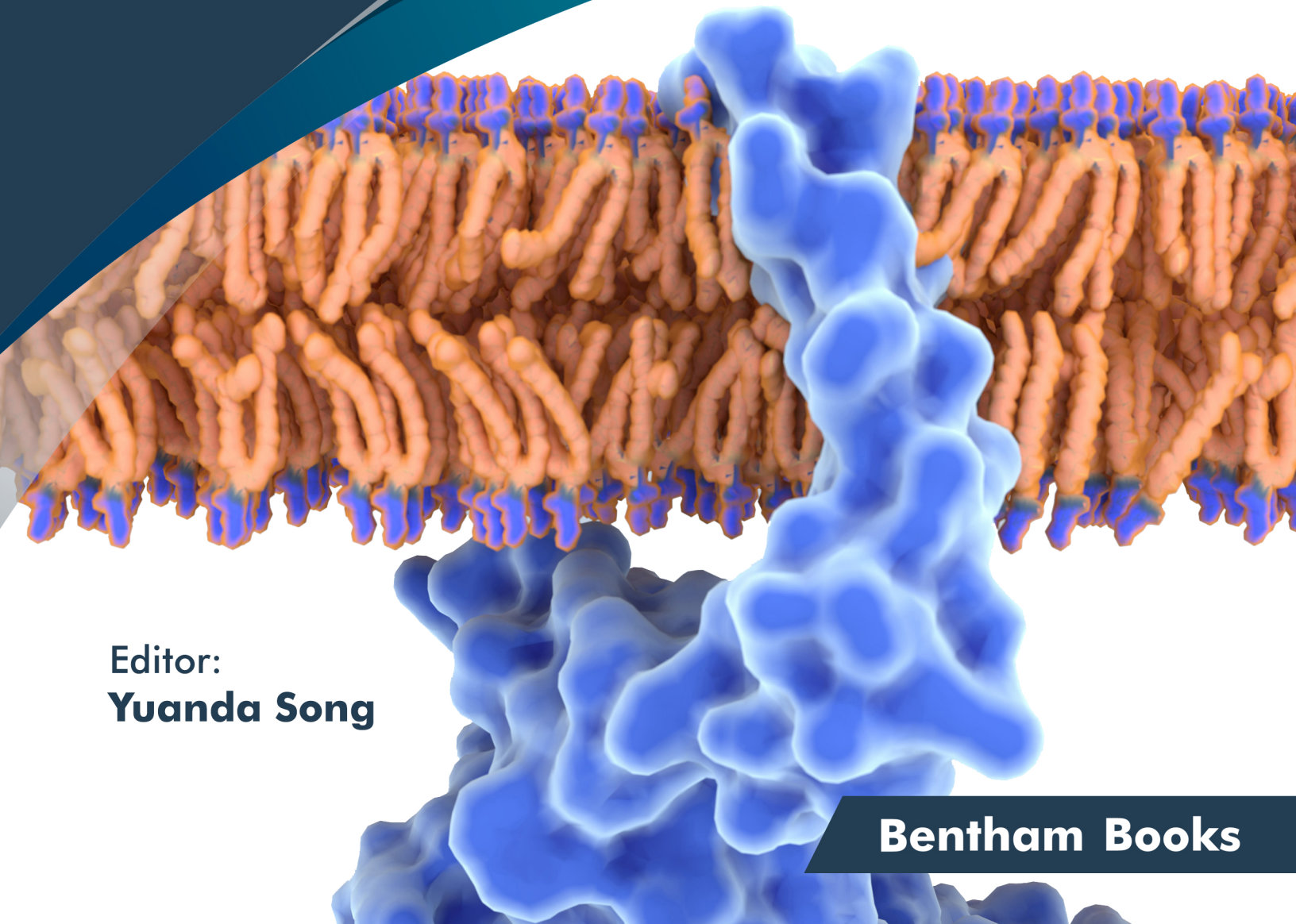
FUNGAL LIPID BIOCHEMISTRY



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Fungal Lipid Biochemistry

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CONTENTS

FOREWORD

This book fills a huge gap in our knowledge of microbial lipids, otherwise known as oils and fats but encompasses a much more bewildering array of molecules than just those containing fatty acids. The coverage of lipids of fungi is a vast subject, not least because those microorganisms that make up the kingdom of fungi are amazingly diverse in their own right. Fungi vary in physiological complexity from the fairly simple examples of yeasts and unicellular 'lower fungi' to the so-called 'higher fungi, ' many of which are able to differentiate and have complex life cycles. Altogether, the number of known fungal species is about 150,000, but some experts have suggested that the total number could be over 3 million, a truly huge array of life forms.

Professor Song, who many years ago was an exceptionally diligent and hard-working Ph.D. student of mine at the University of Hull in the UK, is, therefore, to be congratulated on producing this long-awaited book that then describes in detail the various lipids that can be found in many fungi. Lipids themselves can be relatively simple molecules, such as hydrocarbons and fatty acids – though these can be very diverse materials, to the more complex steroids, sphingolipids and glycosphingolipids. To bring together such an array of knowledge is a major accomplishment; to present this knowledge in a clear and easily understandable single volume is an achievement that could only have come about by someone who has dedicated much of his professional career to the study of these fascinating molecules.

But, for all our knowledge of the lipids that can be found in fungi, there are many things that we still do not understand. For example, while it has been known for nearly a century that fungi can produce a large number of different polyunsaturated fatty acids, no one knows with any certainty what role they play: why do fungi devote so much of their available energy-producing molecules that seem to fulfill no useful purpose. But purpose they must have. If they did not participate in some key role within the producing cells, cells would have quickly evolved so as not to produce them. So what could be their role? Signaling molecules or highly specific receptor roles? Similarly, we can ask what is the role of complex molecules, such as cerebrosides and glycosphingolipids. And, perhaps ultimately, ask why some fungi, particularly the basidiomycetes, produce vitamin D? An intriguing question that remains completely unanswered. The role of vitamin D in fungi cannot be involved in calcium metabolism and bone formation as it is in animals, but the role of vitamin D in animals now seems to be much more complex than just having a role in bone metabolism. Some think it has multiple roles in animals, including ourselves, and should be considered more of a hormone than a vitamin. If we could work out the role of vitamin D in fungi, and surely this would be a simpler proposition than trying to work out its role in animals, then we could find something that transcends microbiology and leads us to discover something vital concerning ourselves. Thus, by studying fungi, we could be helping to study ourselves. A study of fungal lipid metabolism, therefore, might be a way to unravel some of the questions surrounding our own biochemistry.

Also, we still do not completely understand why some fungi can accumulate considerable amounts of lipid – mainly in the form of triacylglycerols – but what controls the actual amount that an individual fungus can produce? Why do some fungi accumulate up to 50 to 70% of their biomass as storage lipids, but other fungi, placed in the same growth environment, will only produce half this amount? If these stored lipids are valuable reserves for a fungal cell to build – as indeed they are, then why do not all fungi not produce the highest amount of lipid possible? Why stop half-way? But, if we could learn more about lipid accumulation in microorganisms, might it not have repercussions for studying obesity in

animals, including ourselves? If we could find out the mechanism of high lipid accumulation in fungi, could this lead us to control the extent to which humans accumulate fat? Are there corresponding signal molecules in fungi that will turn out to be related to the hormones in humans that seem to be involved with obesity? These remain intriguing questions, and some of the answers may be hinted at in the chapters of this book. But, we should always remember that a study of lipid biochemistry in fungi could easily reveal what goes on in higher life forms. A study of fungi, and particularly fungal lipids, could therefore be an easier way to unravel the complexities of metabolism in ourselves. Study the simpler forms of life, and this will reveal what goes on in higher life forms.

This is a book that will be needed by all researchers, teachers and their students who work with, or have an interest in, microbial oils and fats or whose work brings them into occasional contact with lipids within the fungal group of microorganisms. The book will also be of key importance to those working in the industry who are engaged in developing some of the key lipids into commercial biotechnological products. Already a number of fungal lipids are in commercial production, but many others may be found that have applications that will require careful development to achieve large-scale manufacture. The diligent reader may well find key material herein that strikes the vital chord and provokes the response, "Now, that is interesting!" Interesting and potentially valuable?

This book sets out to give clear information and guidance about an amazing array of lipid molecules; it should become the first source of information for all those who wish to know about this topic. Professor Song has assembled a first-class group of contributors who, between them, have produced this comprehensive coverage. The book is a distinguished contribution to lipid chemistry, biochemistry, genetics, and microbiology itself- a veritable store-house of knowledge and information. Well done, Yuanda.

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PREFACE

Lipids are sources of energy (ATP), carbon and water (hence the hump on the camel). As nutrients, lipids provide fatty acids, many colorants, and flavors that are of value to us. With fungi, some produce long-chain polyunsaturated fatty acids, such as gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid, which are beneficial to human health. Fungal lipids had been investigated for well over 100 years, and some of this early research work had been comprehensively summarized in the monograph "Fungal Lipid Biochemistry, Distribution and Metabolism" written by John D. Weete in 1974, and later updated in 1980 as well as comprehensively summarized in the monograph Lipid Biochemistry of Fungi and Other Organisms passed with the emerging of cutting edge analytical techniques and instrumentation. Massive progress has been made in the biochemical research of fungal lipids, pioneered by Colin Ratledge and later on by many outstanding scientists around the world. During this period, there has been an increasing demand concerning the production, distribution and metabolism of microbial lipids as alternatives to animal and plant oils, and as possible alternative energy sources to reduce greenhouse gas emissions and strive towards a sustainable economy. Lipid biosynthesis is largely conserved among all microorganisms, however, the accumulation of large amounts of lipids is largely confined to yeasts and moulds, and each species has its own unique genetic background.

With these considerations in mind, this book focuses on the recent advances in the distribution, classification and biochemistry of fungal lipids and, in many instances, their accompanying metabolism. The classes of lipids are presented in a comparative context. This book aims to satisfy the need of being the main comprehensive reference to which academics, scientific researchers and industrial scientists will be able to use for information on lipid composition, biochemistry, biotechnology, food science and nutrition, and genetics/molecular biology in fungi.

The book is divided into four sections, starting with an introduction to fungal lipids, which includes definition, classification, nomenclature and some historical aspects of fungal lipid research, followed by an overview of fungal lipids and environmental and nutritional cultural conditions affecting lipid production. In the second section (Chapters 3-6), the metabolism of fatty acids, and their biosynthetic pathways together, with their storage mainly in the form of triacylglycerols, have been discussed. The latter includes a key description of the recently discovered lipid droplet acting as a highly specific cellular compartment for the storage of neutral lipids. The third section (Chapters 7-11) is concerned with the relatively recent interpretation of other major lipid classes, which include glycerophospholipids, sphingolipids, aliphatic hydrocarbons, sterols, carotenoids, and polyprenols and their occurrence and biosynthesis. The final chapter is focused on lipid metabolism during fungal development and sporulation.

While this book has not included all potential publications on fungal lipids, the data presented was compiled and inspired by many recently published articles. Selection of the key papers being cited has been made critically, leading to each chapter displaying an up-to-date and informative coverage of the subject content.

I would like to express my appreciation to Professor Colin Ratledge, University of Hull, UK, as the pioneering scientist for his suggestions for the preparation of this book. I would also like to thank Dr. Hassan Mohamed and Dr. Aabid Manzoor Shah for their own chapter contribution, typing assistance and final preparation of this book. I would acknowledge

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CHAPTER 1

Introduction to Lipids

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Abstract: Fats and oils, which are present in a wide variety of foods, are classified as lipids, a group of biomolecules. In addition to storing energy, lipids serve a diversity of biological purposes. Lipids are not characterized by the presence of specific functional groups, such as carbohydrates, but by their physical property and solubility. Multiple compounds obtained from body tissues are categorized as lipids if they are more soluble in nature in organic solvents. Thus, the lipid classification includes not only oils and fats, which are esters of the trihydroxy alcohol glycerol and fatty acids, but also compounds that merged functional groups derived from carbohydrates, phosphoric acid, or amino alcohols, in addition to steroid compounds such as cholesterol. In this chapter, we discussed the various kinds of lipids by considering classification and pointing out structural similarities, history, and nomenclature.

Keywords: Fat and oils, Fatty acid nomenclature, Fermentation, Filamentous fungi, Historical aspect, Lipid systematic, Lipid definition and classification, Polyunsaturated fatty acids.

DEFINITION AND CLASSIFICATION

Lipids have been largely established as carbon-based molecules that are typically hydrophobic naturally and in numerous instances, might soluble in organic solutions as solvents [1]. Lipid includes carbon, hydrogen and oxygen, with various configuration having fewer oxygen atoms than carbohydrate. They occur naturally in most plants, animals and microorganisms, and are used as cell membrane composition, energy storage molecules, insulation, and hormones. The chemical characteristics of lipids cover a wide range of biological substances, including fatty acids, sterols, sphingolipids, terpenes, phospholipids, and other related compounds [2]. They are categorized based on their physical properties at

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ambient temperature (solid or liquid, respectively, oils and fats), on polarity, or on their value for humans, but the preferable classification is due to their structure.

More precisely, lipid definition is applicable when they are well thought out from a bio-synthetic perspective and its structure, and based on various classification approaches that have been employed last multiple years. Nevertheless, for the main goal of lipid identification and further classification, here we defined lipids as amphipathic molecules or hydrophobic that may become completely or in part of thioesters by carbanion-based condensations like (polyketides, fatty acids, *etc.*) in addition, isoprene units (prenols, sterols, *etc.*). Moreover, lipids are subdivided into two groups, simple and complex, with simple lipids being those yielding at most two different kinds of products based on their hydrolysis (*e.g.*, acylglycerols, fatty acids, and sterols) and complex lipids (*e.g.*, glycosphingolipids and glycerophospholipids) yielding more than three products also on hydrolysis.

The schematic categorization given here arranges lipids into well-defined classes that include prokaryotic and eukaryotic biological sources. Due to the chemical and functional backbone, lipids are classified as polyketides, prenols, acylglycerols, sphingolipids, or saccharolipids (Table **1**). By using the advantage of historical and bioinformatics studies, fatty acyls should be separated from the glycerolipids, glycerophospholipids, polyketides, and sterol lipids from other prenols that resulted in eight different essential groups. In order to handle the existing and emerging arrays of lipid structures, this scheme leads to the subdivision of the major classifications into classes and subclasses that is important for the study of the lipid research field and the improvements of structured approaches to data processing. The lipid classification shown here is based on chemical, hydrophobicity molecules that represent the lipid. Bio-synthetically such compounds which not subjected to lipids because of their water solubility are enclosed for completeness in this systematic [3].

Table 1. Main classes of lipids

Fatty acyls [FA]
Octadecanoids
Fatty acids and conjugates
Docosanoids
Eicosanoids
Fatty alcohols
Fatty aldehydes [FA]
Fatty (esters, amides, nitriles, and ethers)
Hydrocarbons

(Table 1) cont.....

Fatty acyls [FA]
Oxygenated hydrocarbons and others
Glycerolipids [GL]
Mono-, Di-, and Triradylglycerols,
Glycerophospholipids
Glycerophosphoethanolamines
Glycerophosphocholines
Glycerophosphoserines
Glycerophosphoglycerophosphates
Glycerophosphoglycerols
Glycerophosphoinositol monophosphates
Glycerophosphoinositols
Glycerophosphoinositol trisphosphates
Glycerophosphoinositol bisphosphates
Glycerophosphates
CDP-glycerols
Glycerophosphoglycerophosphoglycerols (cardiolipins)
Glyceropyrophosphates
Glycerophosphonocholines
Glycerophosphoglucose lipids
Glycerophosphoinositolglycans
Di-glycerol tetraether phospholipids (caldarchaeols)
Glycerophosphonoethanolamines
Glycerol-nonitol tetraether phospholipids
Oxidized glycerophospholipids and others
Sphingolipids [SP]
Sphingoid bases and Ceramides
Phosphosphingolipids
Phosphonosphingolipids
Neutral, Acidic, and Basic glycosphingolipids
Amphoteric glycosphingolipids
Arsenosphingolipids and others
Sterol lipids [ST]
Sterols
Cholesterol and derivatives

CHAPTER 2

Fungal Lipids

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Abstract: Lipids are considered a heterogeneous group of organic compounds which contain fats and their derivatives. This chapter achieved the data available on the nature and composition of lipids in filamentous fungi, and their distribution within the cell. The chapter describes some aspects of lipid metabolism, including fatty acid biosynthesis, lipid accumulation mechanisms, and different fermentation strategies. The lipid content of vegetative hyphae varies between 1% and more than 50%, of spores between 1% and 35%, and of yeast cells between 7% and approximately 15% of the tissue dry weights. The amount of lipids produced by a given species of fungus depends on the developmental stage of the growth and on the culture conditions. Culture parameters that influence the growth and the lipid contents of fungi have been found to be temperature, carbon and nitrogen sources, pH, inorganic salts, and others. The qualitative and quantitative nature of the extracellular lipids is influenced by the different growth parameters. The extracellular lipids known in a large number of oleaginous strains include polyol fatty acid esters, glycolipids, hydroxy fatty acids, sugar alcohols, acetylated sphingosines, and acetylated fatty acids. The main purpose of this chapter was to explain the biochemistry behind fungal lipid accumulation in oleaginous filamentous fungi, their distribution and functions, and the current applications of fungal fermentation strategies.

Keywords: Environmental and nutritional factors, Fungal lipid contents, Fatty acids synthesis, Filamentous fungi, Fungal growth, Fermentation strategies, Lipid functions and distribution, Lipid composition.

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INTRODUCTION

In the last decades, there has been an increasing demand for the improvement of alternative energy sources and a significant rise in the number of research publications in the global literature with a focus on the production of lipids by microbial sources (the "single-cell oils; SCOs" that are produced by "oleaginous" microorganisms). For many years, there has been significant concern over the nutritional difficulties accompanying the rapid growth of the world population. With the high need for lipids and fats in the energy sector, agricultural production, and other industrial applications, microbial lipids accumulated from microorganisms, especially oleaginous filamentous fungi and yeasts, have been an important scope of several recent research studies. Oleaginous (oil-bearing) fungi are well known for their ability to accumulate inside their cells or mycelia large quantities of lipid more than 20% *w/w* in their dry cell weight (DCW). Several studies have been reported since, but they have added little more to our understanding of the relationships between environmental and nutritional factors and lipid production. Recent advances have been in the development of different fermentation cultures, substrates bio-modifications, metabolic engineering, lipid accumulation mechanisms, techniques, and methods to produce high-value-added lipids, and the application of these techniques in studying lipid production and fungal metabolism is limited.

This chapter summarizes the current status of knowledge and technology about lipid production by oleaginous fungi, reproductive structures, and subcellular components, and a brief summary of the composition and functions of major lipids. This chapter describes the relationships between environmental and nutritional conditions and lipid production

Total Lipid Content

The lipid content of various fungal species has been reported and is highly variable depending upon the species and their growth conditions. Indeed, lipid production can be manipulated by varying culture conditions; and studies to determine the potential of fungi as commercial sources of fat have led to an understanding of the relationships between fungal growth and lipid production as they relate to environmental conditions and nutritional requirements. As we shall see, the optimum growth conditions are not necessarily the best for maximum lipid production. Other studies, on the other hand, have been concerned simply with fungal lipid content under suitable, but not necessarily optimum growth conditions rather than conditions for maximum lipid production. While some species appear to have greater potential for lipid production than others, there is no apparent taxonomic value of fungal lipid content. However, this cannot be

fairly evaluated from the available data since it would require a comparison of lipid content under optimum growth conditions for each fungal species, and relatively not many species have been studied with taxonomic objectives in mind. Lipid accumulation in a microbial cell begins only when nitrogen is exhausted from the medium. The medium, therefore, must be formulated with a high C/N ratio to confirm that nitrogen is exhausted while other chemical nutrients, including carbon, remain in excess. In fact, this is about 40–50:1 (C/N), although the optimum ratio needs to be determined for each individual organism. Lipid content relies on the individual organism-lipid accumulation may vary between 20% and 70% of the dry biomass [1, 2]

In fungi, lipids occur not only as major components of cell membrane systems, but also as cell wall constituents, as storage material in high quantity and readily observed lipid bodies, and, in some cases, as extracellular products. The greater cell size and density of fungi are accompanied by a corresponding variety of lipid components. The quantities and types of lipids at individual fungal sites differ not only from one microorganism to another but also with other factors, including age, stages of development, nutrition, and environmental conditions [3].

The lipid fractions from a variety of fungi showed a variety of values for the contents of both polar and neutral lipids. Triacylglycerols are considered the main component of lipids used for energy and carbon skeletons during cell growth and development. Other major lipid constituents of oleaginous fungi are several sterols, squalene, and other hydrocarbons. There is a confirmation that sterols exhibit a condensing or liquefying effect on acyl lipids depending upon their physical state. They may regulate permeability by affecting the internal viscosity and molecular motion of lipids in the membrane systems.

The majority of fungal species contain, in order of quantities, oleic acid (C18:1), palmitic acid (C16:0), and linoleic acid (C18:2) as the major acids, with stearic acid (C18:0), linolenic acid (C18:3) and palmitoleic acid (C16:1) as the minor ones, in addition, high levels of polyunsaturated fatty acids (PUFAs), C18:2 and C18:3. On the other hand, Mucorales uniquely contain γ-linolenic acid (C18:3, n-6) rather than α-linolenic acid. Additionally, arachidonic acid (AA, C20:4), eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA C22:5) occur in the same species [4, 5]. The total lipid content in some oleaginous fungi and yeasts is listed in Table **1**.

CHAPTER 3

Fatty Acid Metabolism in Fungi

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Abstract: Fatty acids, in terms of their chemical structure, are aliphatic monocarboxylic acid that represents the most abundant class of lipids in nature. The major roles of fatty acids in the biological system are they constitute the building blocks of cell membranes, serve as reservoirs of energy, and their derivatives act as signaling molecules with various effects and functions. The type and composition of fatty acids vary from organism to organism. Recent research has revealed that cellular fatty acid profiles can be utilized to distinguish and identify yeast and yeast-like organism genera, species, and strains. Fatty acids commonly range from C:14 to C:20, and are predominant and most common in all organisms. Palmitic acid is the predominant saturated fatty acid of most organisms, and oleic, linoleic, and linolenic acids are the major unsaturated acids. Furthermore, several fungi have been suggested as potential sources for biodiesel production. As such, this chapter will focus on fatty acid metabolism in fungi and its characteristics that will broaden the fatty acid metabolism in fungi biology.

Keywords: Fatty acids, Fungi, Fatty acid biosynthesis, Fatty acid degradation, Regulation of fatty acid synthesis, Unusual fatty acids.

INTRODUCTION

Fatty acids, in terms of chemical structure, are aliphatic monocarboxylic acids. Chevreul gave the term fatty acid (acide gras) in 1813 by Chevreul [1 - 4]. All living organisms produce fatty acids. The majority of naturally occurring fatty acids lack branching in the aliphatic chain and have an even number of carbon atoms, ranging from C-10 to C-36 in chain length. Moreover, fatty acids C:14 to C:20 are commonly predominant and common in all organisms. Palmitic acid is the main saturated fatty acid in most organisms, whereas oleic, linoleic and lino-

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Yuanda Song (Ed.)

-lenic acids are the major unsaturated acids. The aliphatic chain of fatty acid is either saturated (no double bond) or unsaturated (double bonds). They can also be substituted with keto, epoxy, methyl, or hydroxyl functional groups. Cyclic (cyclopropyl- and cyclopentyl-) and odd-chain fatty acids also occur in some organisms. The carboxyl group of the fatty acids is responsible for the hydrophilic characteristics of fats or oils, while the long nonpolar tails of the fatty acids are hydrophobic in nature. When fatty acids are put in an aqueous solution, they form spherical clusters or micelles due to their hydrophilic and hydrophobic properties. The polar carboxyl groups of the micelles face outward, contacting the water, while the nonpolar straight chain extends toward the center of the formation. Fatty acids are found as esters in natural fats and oils and are not found free in nature. When a fatty acid is part of an ester, it is referred to as an acyl group. In biological systems, fatty acids can be found as glycerol esters, sterols, sugars, sphingolipid bases, and hydroxy fatty acids. The major roles of fatty acids in the biological system are they constitute the building blocks of cell membranes, serve as reservoirs of energy and their derivatives act as signaling molecules with various effects and functions. The type, concentration, and composition of fatty acids vary from organism to organism. The analysis of cellular fatty acid composition is also frequently used to classify, distinguish, and identify bacteria genera, species, and strains [5 - 10]. The fatty acids generated and their respective quantities distinguish taxa. FAs and their derivatives are also significant signaling molecules. They act as important constituents in the establishment of plant-fungal interactions. Fungi generally store considerable amounts of lipids in hyphae and spores, and these lipids serve as carbon and energy sources during starvation and spore germination. In mutualistic interactions like mycorrhizas, the fungal partner is reliant on the host for carbon. Plant-derived carbohydrates are converted to lipids by arbuscular mycorrhizal fungi, resulting in lipid bodies that can be transferred from intraradical to extraradical hyphae; lipids are partially eaten during their journey to the hyphal tips, although re-shuttling also occurs. The composition of FAs varies dramatically during the establishment of ectomycorrhizas, most likely due to lipid transfer. FAs have also been utilized as markers to define microbial communities and soil food webs [11 - 14]. Fungi produce fewer different fatty acids than bacteria, yet some particular characteristics are specific to various fungi. Recent research has revealed that cellular fatty acid profiles can be utilized to distinguish and identify yeast and yeast-like organism genera, species, and strains [15, 16]. Furthermore, several fungi have been suggested as potential sources for biodiesel production. The fatty acid content of diverse fungus species has been reported since the discovery of gas chromatography in the early 1950s.

Nomenclature

The majority of naturally occurring fatty acids consist of an unbranched carbon atom chain with a carboxyl group (–COOH) at one end and a methyl group (–CH_3) at the other. The systematic naming of fatty acids is based on a hydrocarbon chain containing the carboxyl group. The final “e” in the name of the corresponding alkane is replaced by “oic acid” for instance, a fatty acid that contains 10 carbons is known as decanoic acid. The numbering of the carbon

atoms recommended by the IUPAC in a fatty acid's backbone is normally indicated by counting from 1 at the -COOH end and increasing in number sequentially toward the terminal carbon. Carbon number x is often abbreviated or C-x (or sometimes Cx), with x=1, 2, 3, *etc.* Another practice is to utilize the Greek letters in alphabetical order, beginning with the first carbon and following the carboxyl. Thus, carbon α (alpha) is C:2, carbon β (beta) is C:3, and so forth. Even though fatty acids can be of various lengths, the final carbon in the chain is always labeled as ω (omega) in this second convention. A third numbering convention counts the carbons from that "ω" end, using the labels "ω −1", and " ω −2". Alternatively, the label "ω−x" is written as "n−x", where the "n" is meant to represent the number of carbons in the chain. The presence and number of double bonds in the hydrocarbon chain are indicated by inserting ene, diene, *etc.*, into the fatty acid name. The position of the double bond is designated by the number of the carbon atom closest to the carboxyl end. Thus, in an 18-carbon fatty acid, a double bond between C:12 (or ω−6) and C:13 (or ω−5) is said to be "at" position C-12 or ω−6. The IUPAC naming of the acid, such as "octadec-12-enoic acid" (or"12-octadecanoic acid"), is always based on the "C" numbering. The notation Δx,y, is traditionally used to specify a fatty acid with double bonds at positions x,y, (The capital Greek letter "Δ" (delta) corresponds to the Roman "D", for Double bond). Thus, for example, the 20-carbon arachidonic acid is Δ5,8,11,14, meaning that it possesses double bonds between carbons 5 and 6, 8 and 9, 11 and 12, and 14 and 15. Fatty acids are frequently represented using the shorthand system. For instance, C18:2, octadecadienoic acid, uses the letter C followed by a subscript figure to show the number of carbons in the hydrocarbon chain, followed by a colon and a number to denote the number of double bonds. The position of the double bond is denoted by adding Δ and then the double bond's carbon number; for example, Δ9,12 Cl8:2. The cis and trans configurations of double bonds are denoted by the letters c and t, respectively, as in Δ 9c,12c Cl8:2. Fatty acids can also be classified as unusual acids if their structures differ from other structures. Saturated fatty acids with chain lengths of 10 to 28 carbons have melting values ranging from 31.6 to 90.9 C. The inclusion of double bonds in a molecule lowers the melting point, for example, oleic acid is 10.5 C; linoleic acid is -5.0 C; a-linolenic acid is -11 C, and arachidonic acid is -49.5 C. Majority of fatty acids which occur in nature have cis double bonds; but, trans fatty acids can be found in few organisms. Cis fatty acids are less stable than their trans-isomer counterparts. The introduction of a cis double bond leads the molecule to bend, and the number of methylene-interrupted double bonds increases, bringing the methyl end of the molecule closer to the carboxyl end. Increased polarity is accompanied by increased unsaturation of fatty acids. Substituents also change the physical properties of fatty acids, for example, methyl branching reduces the boiling point of a straight-chain acid.

CHAPTER 4

Multienzyme Complex in Fatty Acid Biosynthesis

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Abstract: Fatty acid biosynthesis is a fundamental process that occurs in all living organisms and involves multiple reaction steps. Thus, a systematic transfer of the intermediates between the different catalytic sites is highly required for the efficient regulation of a pathway as well as for sustaining growth. Multienzyme complex, a protein complex that comprises a group of interacting enzymes in a specific metabolic pathway, has been identified to catalyze numerous metabolic pathways, including fatty acid synthesis. The existence of a lipogenic multienzyme complex that involves protein interaction between numerous enzymes that took part in fatty acid biosynthesis plays a key fundamental role in channelling the intermediate substrates. Herein, the growing evidence for the formation of multienzyme complexes in fatty acid synthesis and the properties of the complex will be elucidated in this chapter.

Keywords: Protein-protein interaction, Fatty acid biosynthesis, Fatty acid synthase, Lipogenic multienzyme complex.

INTRODUCTION

Metabolism is a highly branched and connected network of fundamental physiological processes, well-defined as the sum of the biochemical processes occurring in all living organisms that either yield or consume energy [1, 2]. This process involves metabolic pathways that are formed by a series of enzymes responsible for interconverting a substrate into a product *via* a sequence of intermediate steps [2]. To ease this process, complexes or known as metabolons, have been discovered to catalyse various crucial reactions in the central metabolic processes, including photosynthesis, respiration and amino acid synthesis [3]. The origin of the 'multienzyme complex' concept was dated back to 1947 when Green

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et al., 1948 [4] presented the first study documenting the properties of a multienzyme complex, known as cyclophorase, which catalyses the complete oxidation of pyruvic acid in the citric acid cycle. The enzyme complex was associated with the mitochondria and visualised as 'an organised mosaic of enzymes in which each of a large number of component enzymes is uniquely located to permit efficient implementation of consecutive reaction sequences' [5].

The term 'metabolon' was first proposed by Srere, 1985 [6] as a simpler term to refer supramolecular complex of sequential metabolic enzymes and cellular structural elements that could be defined as multienzyme protein complexes which comprise numerous enzymes that catalyse a sequential reaction in a metabolic pathway [7]. The formation of metabolon generally involves specific interaction between some 'soluble' enzymes that are anchored to a membrane either by membrane-bound structural protein. This might act as a 'nucleation' site for the metabolon formation or by membrane-bound proteins [8]. The metabolons are temporary complexes that are non-covalently bounded, easing the regulation of a metabolic pathway flux by dynamic association and/or dissociation [9]. The transient interaction formed between the enzymes could be either weak interaction *via* protein phosphorylation and dephosphorylation or strong interaction *via* changes in protein quaternary structure [10]. Most of the cellular multi-cascaded reactions are catalysed by multienzyme complexes and not by free-floating isolated enzymes [11]. Several observations suggested enzymes are not always homogeneously distributed in cells but will come together in time and space as a result of protein-protein interaction [12]. The component of a metabolon could be specific to one metabolon or dynamically shared with other metabolons as an adaptation strategy towards alteration in environment conditions and cellular requirements [13].

The multienzyme complex could be composed of different monofunctional proteins connected by non-covalent interaction or multifunctional protein where a single covalent unit contains more than one enzyme activity; in some cases, it could be a combination of both [14]. In multifunctional enzyme proteins, multiple enzymatic activities are allied with a single polypeptide chain which results from evolutionary gene-fusion events [15]. Among the well-characterised multi-functional enzymes are tryptophan synthesis pathway, phosphoribosylanthranilate and isomerase-indoleglycerol synthetase, whereas for the multi enzymatic complex are protein CAD and pyruvate dehydrogenase [16].

PROTEIN ORGANISATION IN CELLS

The cell interior is a highly organised medium with a dense cytoskeleton [17]. The cytoplasm is comprised of macromolecules with low (*e.g.*, ATP and NADH)

and high (*e.g.*, protein, lipid, and polysaccharides) molecular weight, together with macromolecule arrays [18] occupying 20-30% of cellular interiors [19]. Even though the cell interior could be regarded as a highly crowded environment with a protein cellular concentration of 200-300 mg/mL, it displays a high degree of dynamic organisation [18]. The intracellular organisation is achieved *via* quinary interaction, which is a type of soft interaction that stabilise or destabilise the globular proteins. This interaction establishes the fifth level of protein organisation known as the quinary structure, which majorly functions in the spatial organisation of the intracellular environment and formation of membrane-less organelles [20]. In 1982, the term 'quinary' was proposed by McConkey [21] as it constitutes the fifth level of protein organisation to define the set of evolutionarily selected weak interactions that is crucial for the maintenance of a fragile functional organisation of biological macromolecules [21]. This interaction has been presumed to drive the formation of reversible multienzyme complexes such as the tricarboxylic acids (TCA) cycle that involves interactions with structural elements of the cells [6].

Moreover, compartmentalisation in the form of membrane-bound organelles is common in eukaryotes [22]. One of the approaches for compartmentalisation in cells is the formation of a multienzyme complex which enables substrate channelling and allows the transfer of intermediates directly to the active site of subsequence reaction in a pathway [22]. This phenomenon has been observed in *Arabidopsis thaliana,* where enzymes of glycolysis are functionally associated with the mitochondria [23].

RATIONALE BEHIND THE FORMATION OF MULTIENZYME COMPLEX

The formation of the metabolon can be rationalised with many key reasons linked to metabolic channeling. The catalytic efficiency could be greatly improved as the active site of the enzymes involved is brought close to each other, hence decreasing the transit time for the intermediates [8, 24]. This could enable the preservation of high local concentrations of the metabolites, which is vital for unstable compounds [24]. At the same time, high enzyme concentration could be achieved, which could increase the probability of the substrate binding to the active sites, thus contributing to an increase in flux [25]. This is also particularly beneficial for securing rapid conversion of labile and/or toxic intermediates into more stable and less toxic constituents by sequestration and preventing compounds that might exert an inhibitory effect on the enzyme from reaching the active site [8]. Typically, the channel could be defined as a compartment with the size of a nanometre that could highly correlate its structure, size, and physiochemical nature with the nature of the intermediates that are being shuttled

CHAPTER 5

Acylglycerols in Fungi

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Abstract: Over the past decades, fungi have been increasingly recognized as the potential source of lipids that can be applied in various sectors, including nutraceutical and biofuel. Thus, many studies have been conducted to understand the structural and functional roles of lipid molecules, particularly in the potential oleaginous strains. Lipids produced by oleaginous fungi comprise different classes, and acylglycerol, which are esters formed from different fatty acids and alcohols such as glycerol, represent the major components of the lipid. The biosynthesis of acylglycerol in fungi involved a series of reactions involving the central carbon and glycerol-3-phosphate (G-3-P), while its catabolism *in vivo* generally involved the degradation of triacylglycerol (TAG) by intracellular lipase resulting in the release of fatty acids and glycerol. The resulting glycerol will be phosphorylated, oxygenated, and enter glycolysis, whereas the fatty acids will undergo β-oxidation into acetyl-CoA and be used for various physiological functions. On the other hand, several fungi species, particularly from the *Mucoralles* sp, have been documented to be able to utilize the oil and fat as the alternative substrate for growth and reproduction due to its capability to produce extracellular lipases which hydrolyze the ester bond of the TAG. This chapter will comprehensively discuss the functional role of acylglycerol in fungi, its biosynthesis, as well as *in vivo* and *ex vivo* degradation in fungi, which will be a bridge toward the development of the industrial application.

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Keywords: Acylglycerol, Biosynthesis of Triacylglycerol, β-oxidation, Fungi, Glycosylglycerides, Glycolipids, Lipases, Metabolism of Acylglycerol, Nomenclature.

INTRODUCTION TO ACYLGLYCEROL AND ITS OCCURRENCE IN FUNGI

Lipids are widely distributed in both prokaryotes and eukaryotes; they comprise different classes, and acylglycerol represents the main component in most cells [1]. Acylglycerols, also known as glycerides, are esters that are formed from different fatty acids and alcohols, such as glycerol. Glycerol, a three-carbon molecule, commonly acts as the backbone for a range of glycerides, and it may be fully or partially acylated to form triglycerides (TAG), monoglycerides (MAG), or diglycerides (DAG). The mono- or diglyceride could also be replaced at the third carbon with phosphate or phosphorylated molecules like phosphorylcholine, phosphorylserine, *etc*., through a phosphodiester bond or with carbohydrates to form phosphoglyceride, phospholipid or glycosylglycerol, respectively. Furthermore, several glycerides may include hydroxylated acyl groups, which will successively be esterified to long-chain fatty acids to form estolides (tetra-, Penta-, and hexaglycerides). Aliphatic chains can also be linked to a DAG *via* an ether (alkyl) or ether (alkenyl) linkage. TAG serves mainly as a storage material, an important reservoir of power or energy in the majority of the cells, which could yield over twice or more calories/gram of TAG upon oxidation compared to carbohydrates or proteins. TAG is also the main component of natural fats or oils in the cells, including fungi [2].

In fungi, TAG is the major constituent of lipids, and it is commonly stored in the mycelium, spore, or cytoplasmic space. Furthermore, lipids constituted the majority of the membranes of the cells as well as the cell walls of fungi. The composition of TAG, however can vary depending on the species, stage of development, and growth conditions, which may reach up to 90% of the total lipid content in various fungi, including the mycelia of *Mucor* sp, *Cunninghamella*sp, *Mortierella* sp, *Phycomycesblakesleeanus*, *Lipomyceslipoferus*, *Glomerel-lacingulata*, and *Coprinuscomatus* [2]. Analysis of the TAG by thin-layer chromatography (TLC) divides *G. cingulata* TAG into five classes based on the degree of unsaturation. One of these classes includes saturated and monoenoic fatty acids, another contains saturated, mono-, and dienoic acids, and two others contain different amounts of these acids plus a triene. TAG is also found to be the major class of lipids, but the conidia of *Neurosporacrassa* contain less than 0.6% triglyceride [3, 4].

STRUCTURE AND NOMENCLATURE OF ACYLGLYCEROL

To date, there are several nomenclature systems for naming the acylglycerols, such as the L-α, D/L, and R/S systems, but none of them has been universally accepted. The concerns that arise in naming the substituted glycerols are the reaction of the two primary hydroxyl groups with the enzymes is not identical. For example, upon the formation of an ester bond, or phosphorylation at one of the primary hydroxyl groups of glycerol, the C:2 meso carbon becomes asymmetric, and the molecule is optically active. Asymmetry and optical activity are maintained with substitution at the second primary hydroxyl group, provided the substituent is not identical to that at the first primary hydroxyl. A system of nomenclature capable of distinguishing between stereoisomers or between the C:l and C:3 carbinols is required [2, 4, 5]. Several systems have been developed, including designating each of the carbons with Arabic numerals or Greek letters, of which, primary carbons C:1 and C:3 are named α and γ, while C:2 is named β (Fig. **1a**). On the other hand, the naming of the acylglycerol was based on the number of alcohol groups esterified by FAs, where acylglycerols are described as monoacylglycerols, diacylglycerols, or triacylglycerols (Fig. **1b**).

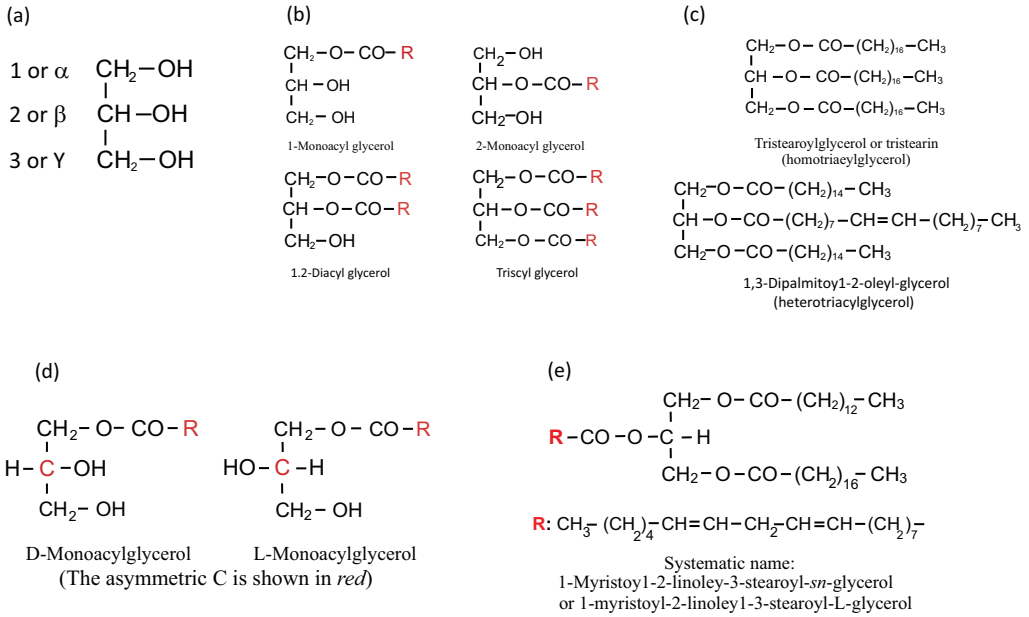

Fig. 1(a-e). Structure and nomenclature of acylglycerols

However, according to Blanco [5], the use of monoglycerides, diglycerides, and triglycerides, which have been broadly used, is incorrect and should be deserted.

CHAPTER 6

An Overview of Lipid Droplets in Oleaginous Fungi

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Abstract: Triacylglycerols and steryl esters are the most common nonpolar lipids found in Fungi. When cells are provided with an abundance of carbon-rich nutrients, these storage lipids accumulate. Nonpolar lipids are sequestered from the cytosolic environment in lipid droplets (LDs) because they cannot be incorporated into biomembranes in large quantities. Triacylglycerol lipases and steryl ester hydrolases mobilize lipids from this compartment upon demand. The degradation products act as energy sources or building blocks for membrane formation. This chapter covers the mechanisms of triacylglycerol and steryl ester synthesis, storage of these lipids in lipid droplets, and subsequent mobilization in oleaginous fungi. The information on fungi's LD biology, like size and distribution, their composition, mechanism and dynamics of formation, and their role in cell physiology, is important from a physiological and biotechnological point of view that will facilitate these organisms as model systems and also promote biofuel development.

Keywords: Lipid droplet, Oleaginous fungi, Triacylglycerols, Steryl esters, Biogenesis, Biofuel.

INTRODUCTION

Lipid droplets (LDs) are ubiquitous cellular organelles composed of a hydrophobic core enriched in neutral lipids, mostly triacylglycerols and sterol esters, surrounded by a polar lipid monolayer. LDs are primary lipid-storage compartments that stock and supply lipids in all eukaryotic and some prokaryotic cells for energy metabolism. Lipid droplets are known by various names in

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different organisms, including lipid particles, lipid bodies, oil bodies, oil globules, oleosomes, adiposomes and cytoplasmic inclusions. The structure of lipid droplets are relatively well-known in animals and plant; although LDs or their related organelles have also been observed in fungi and yeasts, little is known about the biogenesis and molecular organization. Lipid accumulation in fungi is a biological process that has several roles. Lipid storage contributes to energy storage for survival, lipid transport, membrane biogenesis, lipotoxicity relief, cell division, cell growth and stress response, and in some cases, plays a role in pathogenicity. Some microorganisms that have the potential to accumulate more lipids in their dry cell weights are known as oleaginous microorganisms that have great economic and technological importance [1 - 3]. A significant number of more than 64 fungal species have been characterized, accumulating more than 25% of lipids in their biomass [4). Fungi are considered favorable and potential producers of polyunsaturated fatty acids that have crucial dietary and pharmaceutical importance. The most common genera of oleaginous fungi are *Mucor, Yarrowia, Rhodosporidium, Candida, Rhodotorula, Trichosporon, Cryptococcus and Lipomyces* [2, 5]. In oleaginous fungi, LDs accumulate in response to high carbon and low nitrogen in the culture media, indicating that nutritional conditions might be the key to the increase in lipid production [6]. The high carbon stimulates lipid production due to the divergence of the available carbon atoms from glucose to fatty acid synthesis, generating the TAGs accumulated in the LDs [7 - 10]. Lipid droplets are the primary source of oil for human consumption produced by oleaginous microbes. Thus, a better knowledge of LD biology could have far-reaching implications for food security and alternative biofuels [11]. The study of LDs in oleaginous fungi was prompted by the fact that microbes have a lot of potential for converting waste materials into fatty acid-derived fuel molecules and other chemicals. The information of fungi's LD biology, like size and distribution, their composition, mechanism and dynamics of formation, and their role in cell physiology, is important from a physiological and biotechnological point of view that will facilitate these organisms as model systems and also promote biofuel development using these organisms.

Lipid Droplet Structure

Nonpolar lipids like triacylglycerol's (TAG) and sterol esters (SE) in extra amounts cannot be integrated into biomembranes; instead, these are stored in special compartments known as lipid droplets. These lipid droplets are composed of a hydrophobic core surrounded by a phospholipid monolayer and various proteins embedded within the monolayer (Fig. **1**).

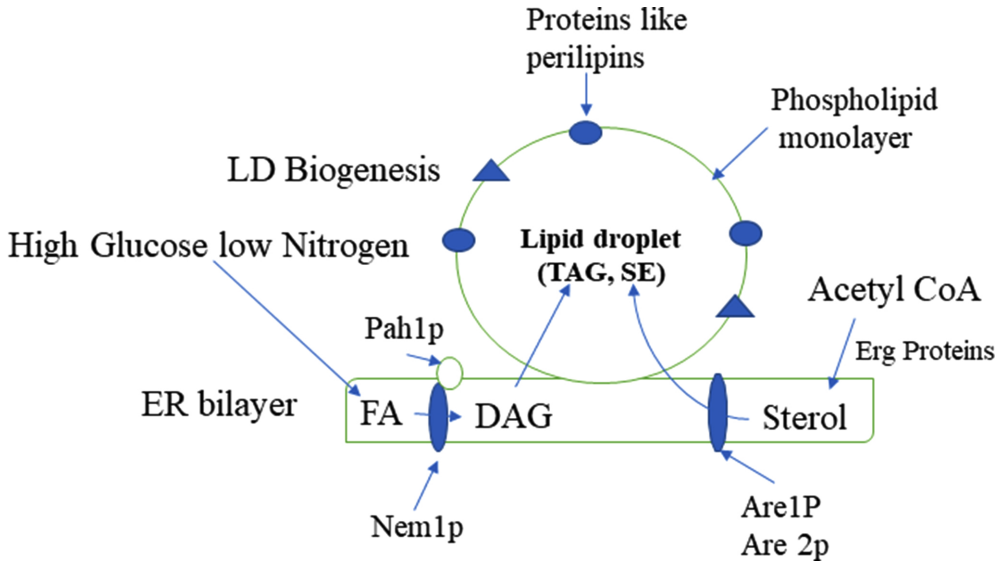

Fig. (1). .Graphical representation of lipid droplet biosynthesis in oleaginous fungi.

Polar lipids, primarily phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, combine to form a micellar structure that stabilizes the hydrophobic center. In *Saccharomyces cerevisiae,* the structural investigations of the hydrophobic core of lipid droplets revealed that TAG and SE are well organized, but their distribution is random [12]. There are several layers of SE beneath the phospholipid monolayer, further covering the inner core of TAG. The composition of the hydrophobic core depends on the ratio of TAG to SE that differs between the different kingdoms as well as between different species. The ratio of TAG to SE in the hydrophobic core, number and size of lipid droplets vary depending on the source and environment of a living cell. In the wild-type of *S. cerevisiae* average diameter of lipid droplets ranges between 0.3 and 0.4 μm, however, some larger droplets have also been observed with a diameter of 1.2–1.6 μm [13]. Nearly equal amounts of TAG and SE have been observed in the hydrophobic core of the budding yeast *S. cerevisiae* [13, 14]. In oleaginous yeast like *Y. lipolytica*, *P. pastoris* TAG is the main component of lipid droplets [15]. From the comparative study of lipid droplet size, it was observed that oleaginous yeast *Y. lipolytica* has a twofold bigger size of the droplets than the budding yeast grown under the same conditions. In an oleic acid-containing medium, the lipid droplets size of *Y. lipolytica* increases from 0.65 to 2.5 μm [mean diameter) and also with some changes in the nonpolar lipid composition and protein content [16]. Several studies have confirmed that environmental conditions significantly influence the size and composition of lipid droplets in the budding yeast S. cerevisiae [14]. Yet, it was shown that the size and lipid composition of lipid

CHAPTER 7

Glycerophospholipids

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Abstract: Glycerophospholipids are defined as phosphatidyl esters attached to the terminal carbon of glycerol in the triglyceride structure. Glycerophospholipids are the most abundant phospholipids, which form essential structural components of cellular or vesicle membranes. This chapter defines the mechanisms and regulations of glycerophospholipid synthesis, such as phosphatidic acids, phosphatidylcholines, phosphatidylethanolamines, phosphatidylserine, and phosphatidylinositol, and the roles of glycerophospholipids in fungi and yeast lipid metabolism. Some essential enzymes that catalyze the degradation of phospholipids were also discussed, such as phospholipase C, phospholipase D and phospholipase B.

Keywords: Fungi, Glycerophospholipids, Phospholipids synthesis, Phospholipase, Yeast.

INTRODUCTION

Phospholipids constitute the major components of biological membranes. Phospholipids can be natural or synthetic, which can be used as a model for biochemical and membrane research. Numerous studies have been made about the synthesis and characterization of lipid bilayers, natural and synthetic membranes where phospholipids make the highlight. The synthesis of phospholipids, including phosphatidic acids, phosphatidylcholines, phosphatidylethanolamines, phosphatidylserine, and phosphatidylinositol, has been well-developed. These synthetic routes are described that lead to unusual phospholipids, such as compounds containing the polar group at position 2 of the glycerol moiety, glycerophospholipids containing alkanolamines of different chain lengths, and glycol-phospholipids. The synthesis of these classical phospholipids is now possi-

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ble without the use of protecting groups during the phosphorylation step. Enzymes that catalyze the hydrolysis of phospholipids are called phospholipases and are included in several reviews on phospholipid metabolism. These enzymes are ubiquitous and have been found in plants, animals, bacteria, and fungi. The preparation and possible use of liposomes from phospholipids has gained substantial attention in the recent past since they have been discussed as the delivery system.

NOMENCLATURE AND STRUCTURE

The structure of different kinds of phospholipids shows great variation from each other. However, almost all the natural phospholipids are made of glycerol which contains a fatty acid group at positions 1 and 2 that is linked *via* ether or ester linkage. In addition, the glycerol molecule also contains a phosphate group at position 3. The precise and accurate positioning of these substituents on 1, 2, and 3 carbon of glycerol results in chirality. Besides the hydrogen atoms of the glycerol molecule may be substituted by different groups like choline, serine (Figs. **1** and **2**), *etc.*

The IUPAC-IUB Biochemical Nomenclature Committee has recommended naming glycerophospholipids using the stereospecific numbering (*sn*) system that has already been defined in Chapter 5 for the nomenclature of neutral acyl derivatives of glycerol [1 - 4].

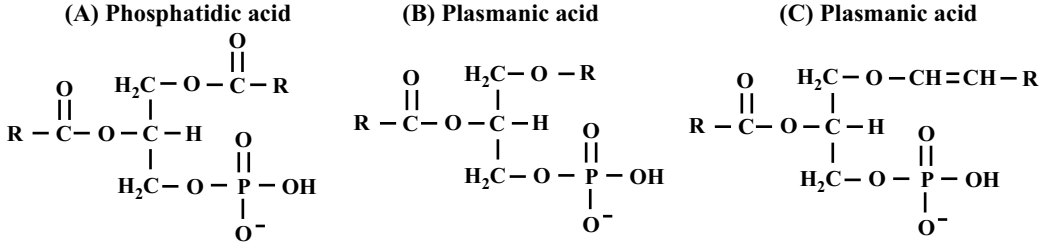

Fig. (1). (A) O-(1-acyl), (B) O-(1-alkyl), and (C) O-(1-ackenyl) glycerophospholipids [1-4].

According to this nomenclature, C-1 is named as the carbon atom that is positioned at the top of the Fischer projection and the corresponding carbon atom is attached to the hydroxyl molecule. Consequently, the *sn* name of the simplest phospholipid (common name: phosphatidic acid) is 1,2-diacyl-sn-glyce-o-3-phosphate (Fig. **2**). The phosphatidic acids may be categorized into different species depending on the position of acyl groups at positions 1 and 2 of the glycerol molecule and the products obtained by the incomplete hydrolysis of these phosphatidic acids are designated by using “lyso” as the prefix. For instance, 2-lysophosphatidic acid specifies that the hydrolysis has taken place at C-2, which

results in the addition of a hydroxyl molecule at the same position. The glycerophospholipids where the hydrogen molecule at the position x of the phosphatidic acid is replaced by other substituent groups are named accordingly (Fig. **2**); for instance, the 1,2-diacyl-sn-glycero-3-phosphocholine represents choline derivative of phosphatidic acid.

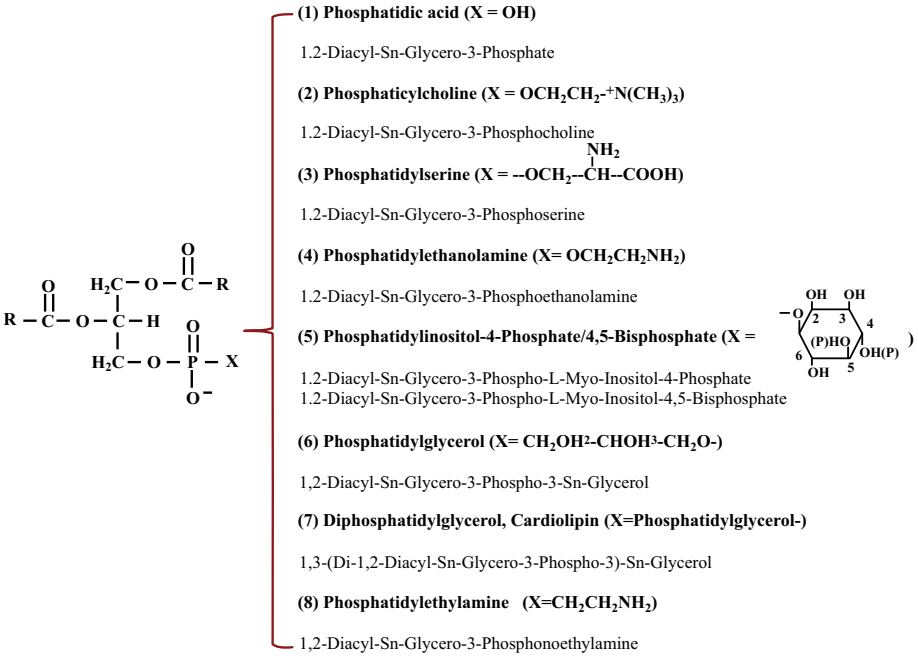

Fig. (2). Common glycerophospholipids [1-4].

The term plasmalogen is used for glycerophospholipids that contain O-(l-alkenyl) molecule and an acyl molecule at position 2. The *sn* name for such plasmalogen is 2-acyl-alkenyl-sn-glycero-3-phosphate. The name 'plasmenic acid' is used as a simple name for lipids having a vinyl ether and an acyl group, *i.e.,* plasmenylcholine, for example, 2-Lysoplasmenylcholine is a plasmonic acid containing a hydroxyl group at position 2. The Glycerophospholipids that are substituted by an alkyl (ether) group and an acyl group at position 1 and 2 respectively are named 2-acyl-l-alkyl-sn-glycero-3-phosphate. Plasmonic acid is a recommended shorthand name for these described above for lipids containing vinyl ether linkage, *i.e.,* plasmanylcholine and lysoplasmanylcholine. Glycerophospholipids also occur as esters of phosphonic acid besides phosphodiesters, *i.e.,* 1,2-diacyl-sn-glycero-3-phosphono-ethylamine or phosphatidylethylamine (Fig. **2**).

CHAPTER 8

Sphingolipid

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Abstract: Sphingolipids are a class of lipids containing the backbone of long-chain amino-alcohol bases in their structure, which are synthesized in the endoplasmic reticulum. Modification of this base gives rise to a variety of such lipids ranging from simple to complex sphingolipids that play a significant structural and functional role in membrane biology as well as regulate various cellular processes. Sphingosine, dihydrosphingosine and phytosphingosine are nature's most frequently occurring bases. Ceramides are the simplest sphingolipids after the backbone. These fatty acids are amide-linked derivatives of sphingoid bases and central intermediates of sphingolipid metabolism. Ceramides perform various biological functions and constitute the hydrophobic backbone of all complex sphingolipids. The best-characterized sphingolipids in fungi and yeast are glycosphingolipids (GSLs), which could be categorized into two groups, neutral GSLs (glucosyl and galactosylceramide) and acidic GSLs, (glycosylinositol-phosphorylceramides). Due to the several important functions of sphingolipids in cell biology, it is crucial to understand the regulation and metabolism of sphingolipids. Despite the diversity of structure and function of sphingolipids, their synthesis and degradation are governed by common synthetic and catabolic pathways. In recent years, significant progress in the field of sphingolipids has been made. Recent developments in sphingolipid biology, including the construction of analytical and genetic tools and the development of computer visualization techniques for sphingolipids analysis, have highlighted the role of sphingolipids in developing anticancer and antifungal therapeutics. Recent advances in sphingolipid biology continue to provoke and inspire vigorous investigations in sphingolipidology.

Keywords: Anticancer, Antifungal, Biological functions, Biosynthesis, Ceramide, Dihydrosphingosine, Degradation, Glycosphingolipids, Long-chain bases, Metabolism, Nomenclature, Occurrence, Phytosphingosine, Sphingolipid structure, Sphingolipids, Sphingosine, SphingoViz.

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INTRODUCTION

Sphingolipids constitute a group of lipids characterized by their long chain amino-alcohol backbones which are synthesized in the endoplasmic reticulum (ER) from nonsphingolipid precursors. Modifying this basic structure gives rise to the variety of sphingolipids that play important roles in membrane biology and regulate cell function by providing many bioactive metabolites [1]. Sphingolipids are widely distributed in nature and found in all animals, plants, fungi, and some prokaryotes and viruses. They are one of the major structural constituents of cellular membranes [2]. Earlier, their biological functions were not well understood, but recently, they have emerged as important bioactive molecules mediating diverse and essential cellular processes such as cell growth, angiogenesis, migration, inflammation, apoptosis, differentiation, cell signaling, and senescence [3]. Sphingolipids present large structural diversity and any changes in their composition can strongly impact various biophysical properties of membranes like fluidity, thickness, hydration, passive permeability, membrane curvature, and lateral diffusion of components [4 - 6]. These changes are responsible for the mechanisms by which sphingolipids fulfill their different biological functions. Certain groups of these lipids exhibit immunological activity as well [7]. High amounts of sphingolipids are associated with nerve tissue. Considerable interest in these lipids developed because of their association with certain diseases called sphingolipidoses [8 - 10]. These diseases result from inborn errors in sphingolipid metabolism; the absence or low activity of certain degradative enzymes resulting in the accumulation of specific sphingolipids in certain tissues, such as cerebroside (Gaucher's disease and Krabbe's globoid cell leukodystrophy), sphingomyelin (Niemann-Pick disease), and ganglioside, an acidic GSLPs, found in the brain of babies with Tay-Sachs disease in the massive amount [11, 12]. Recent studies have shown the involvement of sphingolipids in many common diseases like diabetes, various cancers, microbial infections, Alzheimer's disease, cardiovascular and respiratory disorders and many others [13].

Various biological roles played by sphingolipids can be seen in Fig. (**1**). However, we anticipate many additions to this list of these functions in the near future. Due to the diverse roles mediated by sphingolipids in cellular biology, it is important to explore them in detail and understand the mechanisms by which they get synthesized and catabolized to elucidate their signaling targets. There are different kinds of sphingolipids. So far, more than 300 structures have been reported, and the list is still growing [14, 15]. They vary according to the alkyl chain lengths ranging from 14 to 22 carbon atoms, the degree of saturation, the position of double bonds and hydroxyl groups, and branching positions [16, 17]. It is necessary to develop a nomenclature system for the sphingolipids so that

individual species and sphingolipids can be referred to logically.

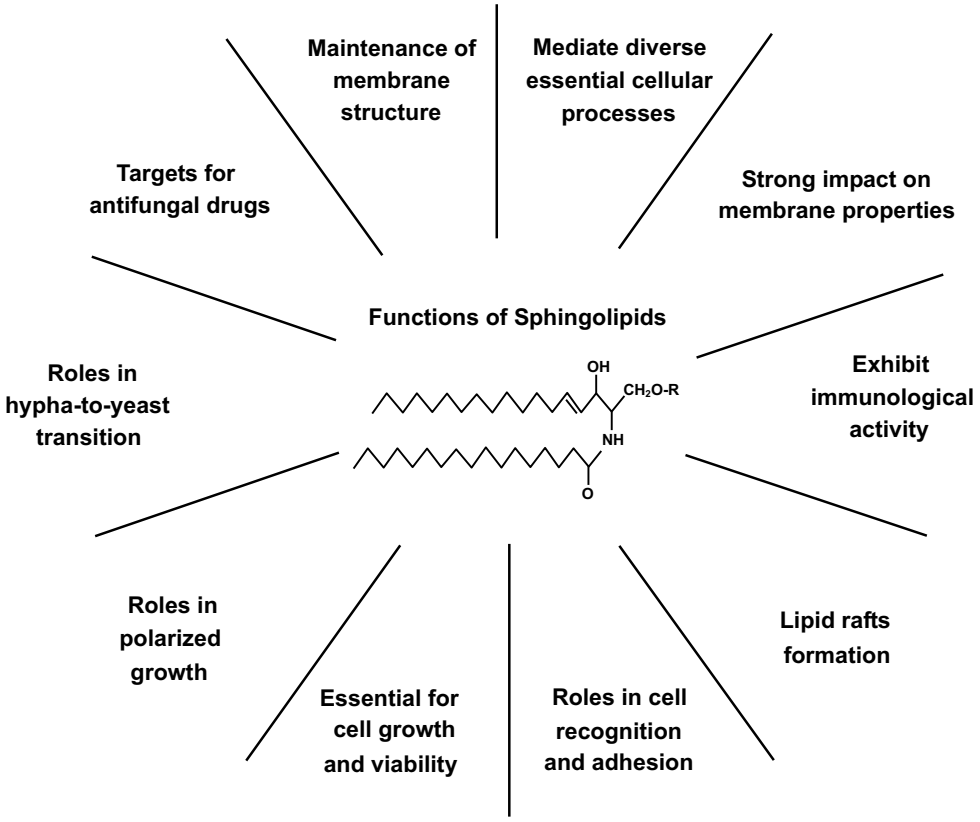

Fig. (1). Biological functions of sphingolipids in the living organisms

STRUCTURE AND NOMENCLATURE

Sphingolipids are a large and complex group of lipids characterized by long-chain 2-amino alcohols (base) as components of their structure. Long chain bases (LCBs) are unique building blocks of sphingolipids, which are synthesized in the ER from nonsphingolipid precursors [18, 19]. Modification of this base gives rise to the vast family of sphingolipids that play significant roles in membrane biology and regulate various cellular processes [20]. Sphingolipids were first discovered and characterized by J.L.W. Thudichum in 1874 while studying the chemical constituents of the brain [21 - 23]. More than 60 natural sphingolipid LCBs have been identified and proposed so far, but four base structures occur most frequently in biological materials and serve as the backbones for the synthesis of complex sphingolipid derivatives [24]. Trivial names of these bases are sphingosine, dihydrosphingosine (DHS) and phytosphingosine (PHS), and dehydrophytos-

CHAPTER 9

Aliphatic Hydrocarbons

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Abstract: Aliphatic compounds are carbon and hydrogen-containing hydrocarbon complexes and are present in almost every plant, animal and microorganism. In 1929, aliphatic hydrocarbons were first discovered by crude chemical methods. These observations were later confirmed and expanded by more sophisticated instruments, such as gas-liquid chromatography and GLC-mass spectrometry. Aliphatic hydrocarbons were detected in the wax of most studied organisms and mainly contained n-alkanes, but may also include n-alkenes, saturated and unsaturated, cyclic alkanes, and isoprenoid hydrocarbons. Similarly, surfaces of higher plants contain a complex waxy coating that consists of primary and secondary fatty alcohols, long chains of fatty acids, ketones, aldehyde, terpenes, diols, waxy esters, glycerides, *etc*. The chemical composition of aliphatic hydrocarbons, chain length predominance, branching, the unsaturation of surface wax and their variation among various organisms, such as plants, algae, bacteria, animals and particularly in fungi have been described in the current chapter. The hydrocarbon distribution in animals is reported to be slightly similar to that of higher plants, while in bacteria, a complex mixture of normal, single- double branched, saturated, or unsaturated structural isomers are reported. A brief description of the biotechnological production of various aliphatic compounds using genetic engineering has also been presented in this chapter. The biosynthesis of aliphatic hydrocarbons by two common routes, "elongation decarboxylation" and "head-to-head condensation," has been studied well in plants and bacteria and are discussed here in detail. Pathways involved in the degradation of hydrocarbons by aerobic and anaerobic microbes and the enzymes involved are also described in this chapter. Aliphatic compounds with different chain lengths have been of biotechnological interest for the past few decades as they perform various biological functions in living organisms apart from their role as the chief component of diesel and jet fuels. The current chapter also highlighted the biological importance of these aliphatic hydrocarbons.

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Keywords: Aliphatic hydrocarbons, Alkanes, Biosynthesis, Biotechnological productions, Carbon chains, Cuticular wax, Degradation, Cesiccation, Elongation decarboxylation, Fungal spores, Head-to-head condensation, Monooxygenases, Odd/even ratios, Oxidation, Paraffin, Predominant.

INTRODUCTION

Hydrocarbons are organic chemical compounds that are entirely composed of carbon and hydrogen atoms. They are the key elements of petroleum and natural gas. Hydrocarbons are present widely in nature. About 98% of raw natural rubber is a polymer of hydrocarbon. The structure and chemistry of individual hydrocarbons largely depend on the chemical bonds with which atoms of constituents' molecules are bound together. Hydrocarbons are classified as aliphatic or aromatic hydrocarbon-based on their properties and sources by 19^{th}-century chemists. An aliphatic compound is a carbon, and hydrogen-containing hydrocarbon complex joined in straight lines, branched chains, or non-aromatic rings. Aliphatic hydrocarbons are obtained by the chemical degradation of oil or fats, while aromatic hydrocarbons are derived from a group of related substances by the chemical degradation of specific plant extracts with pleasant smells [1]. Aliphatic hydrocarbons are classified into three major groups based on the types of bonding they contain, such as alkanes, alkenes, and alkynes. Alkanes have only a single bond; alkene contains double carbon bonds, while alkynes have triple carbon bonds. Alkanes are described as saturated hydrocarbons, while alkenes, alkynes, and aromatic hydrocarbons are defined as unsaturated.

Aliphatic hydrocarbons have been detected in almost every plant, animal, and microorganism examined. They are present in plants and trees in the form of pigments known as carotenoids. Hydrocarbons may comprise up to 90% of the surface as wax, depending on the source. They are present in the wax of most studied organisms and mainly consist of n-alkanes, but may also include n-alkenes, saturated and unsaturated, cyclic alkanes, and isoprenoid hydrocarbons. Alkanes from biological sources were reported in 1929 for the first time when nonacosane (C_{29}) and hentriacontane (C_{31}) were recognized as components of plants [2]. Due to the complex nature of biologically sourced wax, progress in the study of the chemistry of components was slow until the discovery of sophisticated analytical techniques, such as thin-layer chromatography, gas-liquid chromatography, mass spectrometry, and nuclear magnetic resonance. The composition and biosynthesis of hydrocarbons in various plants, animals, and microbial organisms have been reviewed in a book edited by [3], and their occurrence is summarized in Table **1**.

Table 1. Hydrocarbons of Plants, Animals, and Microbes

Hydrocarbons	Occurrence
i. n-alkanes	Distributed almost universally: C_{16}-$C_{36,}$ present in shorter or longer chain lengths but found in certain groups
ii. branched alkanes A. Single branched 1. 2-methyl (iso) 2. 3-Methyl *(anteiso)* 3. Internal	Present in plants, animals, and microorganisms, but the occurrence is generally limited to certain species or genera: odd chain predominance; in insects C_{29}-C_{31}; in plants (tobacco). C_{29}-C_{33} Present in plants, animals, and microorganisms, but the occurrence is generally limited to certain species or genera: even chains predominance C_{24}, C_{26}, C_{28}, C_{30}, C_{32} in insects; C_{30} and C_{32} in plants (tobacco). In Blue-green algae present as 4-, 6-, 7-,8-methyl-heptadecane. In insects, methyl branching, and odd carbon chains predominance.
B. Multiple Branched 1. Dimethyl	In bacteria, C_{23}-C_{30} monounsaturated *(cis)* with *iso-iso, iso-anteiso, anteiso-anteiso* branching. In insects, C_{25}-C_{55} with methyl branching has isoprenoid spacing on odd-carbon atoms. Odd chains predominance
2. Trimethyl Alkanes a. Branches near the end of the chain: 3,7, 11 and 4, 8, 12 b. Internal	Limited occurrence, present only in insect genus *Atta.* Isoprenoid spacing of methyl groups (C_{34} to C_{39}) Limited occurrence, only two insect species. Isoprenoid spacing of methyl groups (C_{22}-C_{44}).
3. Isoprenoids	Two pathways are involved (MVA) and (MEP). Universal occurrence of squalene (C_{30}) but low abundance except under low O_2 tension. The major lipid of shark liver oils (30%). Dihydro squalene in bacteria. Pristane (C_{19}) and phytane (C_{20}) in algae, bacteria, copepods, and zooplankton.
III. Alkenes	Limited occurrence in plants; essential oils of trees and other plants, flower petals, C_{19} to C_{33}*cis*- 5-alkenes (rose petal wax), odd chain predominance; 1- alkenes with even chain predominance in sugar cane wax; some conjugated dienes. Insects, C_{23} to $C_{43,}$ with *cis* double bonds, odd chain predominance. Present in bacteria as monounsaturated, normal and branched alkenes. Not detected in fungi except hexadecatriene A in *Candida utilis.* Predominant olefin in marine phytoplankton is C21:6.
IV. Alkynes	In higher plants, ethylene biosynthesis proceeds *via* "ACC pathway". Rarely present in a few slime mold *Dictyostelium mucoroides* and the fungus *Penicillium citrinum.* KMBA pathway is described in various microorganisms, such as *E. coli*, *C. albidus*, *B. cinerea* and in a few fungi isolated from soil rhizosphere. Act as a plant growth regulator and promote fruit ripening.

The cuticle of a plant is a hydrophobic extracellular sheet that covers the aerial epidermis of all terrestrial plants, protecting them against adverse external stresses and desiccation. In most terrestrial organisms, the cuticular surface is protected with a complex combination of lipophilic non-glyceride elements collectively described as wax [4]. The exact composition of the wax layer not only varies according to species but changes were also observed with the seasonal and

CHAPTER 10

Sterols, Carotenoid and Polyprenols

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Abstract: Isoprenoid compounds are a family of compounds constructed with isoprene as the basic unit but with very different structures, including monoterpenes, diterpenes and polyterpenes. Isoprenoid compounds mainly include ergosterol, steroids, carotene, carotenoids, polyisoprene, and their structures range from relatively simple linear hydrocarbon chains to highly complex cyclic structures, in which the cyclic structure is cyclized by terpenoids. Enzymes, also known as terpene synthase catalyzed by them, are also called cyclic terpenes. Isoprenoids are widely distributed in archaea, bacteria, and eukaryotes, and a variety of isoprenoids are essential components of the biological mechanism of the organism. For example, in mammals, β-carotene (β-carotene) is the precursor substance of Vitamin A. β-carotene has the function of preventing oxidation reactions, and can inhibit and eliminate oxygen free radicals in the body, and has various effects such as slowing down aging and improving resistance. At the same time, carotenoids can be combined with protein. Astaxanthin and protein combine to form astaxanthin, which makes aquatic animals appear body color and has a certain protective effect. Therefore, ergosterol, steroids, carotene, carotenoids and polyisoprene, which are relatively large in terpenoids, are all important products with commercial value and have been widely used in food, medicine, and daily chemical products.

Keywords: Carotenoid, Dolichol, Polyprenols, Pigment, Sterols, Terpenoid.

STEROLS

Introduction to Sterols

Sterol is a general term for terpenoid lipids. There are many kinds of natural sterols, which are widely found in nature. According to their different sources, sterols are divided into three categories: animal sterols, plant sterols and fungal

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sterols [1]. Cholesterol is the main animal sterol. Its physiological function is to form cell membranes, cholic acid, and synthesize hormones [2]. Phytosterols are one of the constituents of cell membranes in plants, which are mainly found in the roots, stems, leaves, fruits and seeds of plants [3]. They are also the precursors for the synthesis of vitamin D, various hormones and steroids [4]. Phytosterols mainly include stigmasterol, sitosterol and campesterol, as well as spinach sterol and oatsterol. The main representative of fungal sterol is ergosterol, which is the precursor of the chemical synthesis of vitamin D2. As early as the late twentieth century, as scientists discovered plant sterols such as sitosterol in vegetable oils, scholars at home and abroad began to conduct extensive and in-depth discussions and research on sterols. In recent years, with the rapid development of lipid engineering technology, the physiological functions and applications of sterols have also been studied in depth, and their applications in food, medicine, cosmetics, chemicals and feed have attracted widespread attention, especially the excellent performance of plant sterols in reducing human cholesterol [5].

High blood cholesterol level is a prominent risk factor for cardiovascular disease and is relatively easy to measure. Therefore, strategies to reduce blood cholesterol are the forefront of preventive measures to reduce cardiovascular disease risk. The use of statins can lower blood cholesterol levels, and this can also be done by increasing dietary phytosterol intake.

In addition to cholesterol-lowering effects, phytosterols have also been reported to have other health benefits, such as anti-atherogenic, anti-inflammatory, anti-cancer neuro modulatory and neuroprotective activities. Of the myriad of phytosterols biosynthesized by plants, four have attracted commercial interest in the pharmaceutical, nutraceutical and functional food industries: campesterol (24-α-methyl cholesterol), β-sitosterol (24-α-ethyl cholesterol), stigmasterol (Δ22, 24-α-ethyl cholesterol) and brassicasterol (24-methyl-cholest-5, 22-dien-3β-ol). All of these can be produced by micro-algae.

The Chemical Structure of Sterols

Sterols are a class of steroids widely found in nature. They are high-carbon cyclic monohydric secondary alcohols with 4 rings as the skeleton of cyclopentane perhydrophenanthrene [1]. The 4 rings of the alcohol are composed of A, B, C, D ring nucleus condensation. The general structure of the sterol is shown in Fig. (**1**). All sterols have a bonded secondary group at the C23 atom on the A ring. Most natural sterols have A/B ring, B/C ring, and C/D .The rings are all trans-condensed. A hydroxyl group is attached to the C-3 position, and a side chain composed of 8-10 carbon atoms is attached to the C-17 position. The C-5 position

of most sterols is a double bond. The structure of the side chain determines the type of sterol. The molecular structure is shown in Fig. (**1**).

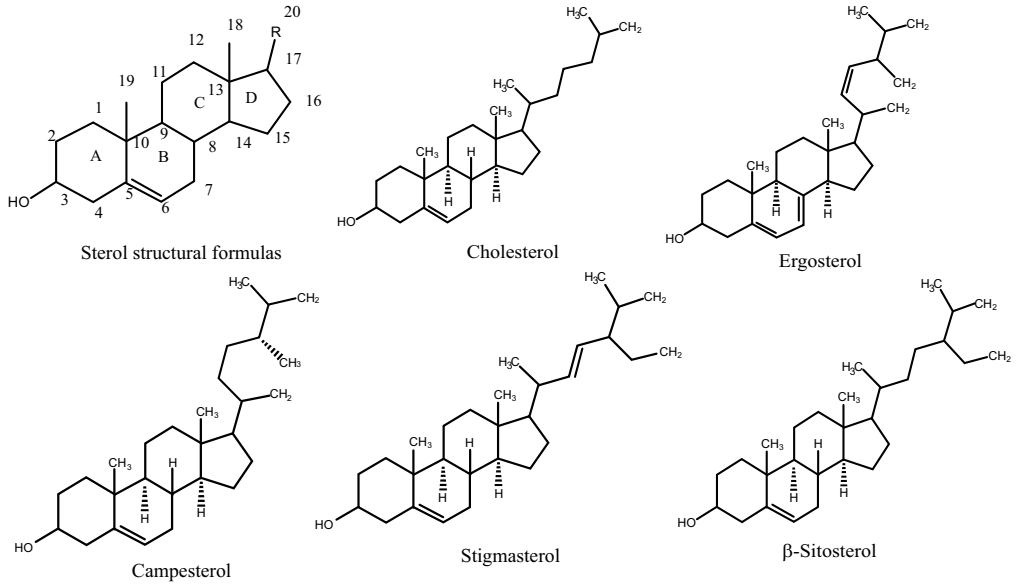

Fig. (1). Sterol structural formulas

A distinguishing feature of plant sterols that distinguishes them from animal sterols is that they are a hydrocarbon group attached to the C-24 atom [4]. Therefore, phytosterol molecules often contain 28 or 29 carbon atoms. The difference between phytosterols often lies in the number and position of double bonds in the molecule.

Due to the differences in the number and position of the double bonds on the sterol nucleus and side chains, whether there are branches at the C-24 position of the side chains and the length of the branches, the structure of sterols varies greatly and the types are extremely diverse. According to the number of methyl groups attached to the C-4 position on the A ring of the sterol nucleus, sterols are divided into: 4-non-methyl sterol (also known as non-methyl sterol or sterol) and 4, 4' bis-methyl sterol according to the chemical structure (Also known as dimethyl sterol or trimethylenol).

The spatial configuration of the ring nucleus is inverse at the junction of the ring zone C, and almost always at the junction of the ring C and D. The configuration at the junction of rings A and B can be either cis (5β) or trans (5α), and C5 is called cholestane and Coprostane based on the C27 hydrocarbon skeleton,

CHAPTER 11

Biosynthesis of Sterols, Carotenoids, and Polyprenols

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Abstract: Sterols are essential lipid components for the cell membrane. In addition to their structural roles, they are critical signaling molecules that regulate metabolism, development, and homeostasis. Due to the functions of sterols being concentration-dependent, the biosynthesis of sterols is tightly controlled. Here, we reviewed the biosynthesis processes of sterols (squalene, lanosterol, ergosterol, carotenoid, and polyprenols) and analyzed the key and limited enzymes in these processes. Although various sterols are identified in nature, their basic synthesis pathways appear to be conserved. Squalene is the key intermediate in the biosynthesis of sterols, and the cyclization of squalene into lanosterol (animals and fungi) or cycloartenol (plants), producing various types of terpenoids. In addition to the synthesis processes of sterols, how to enhance sterols production was also discussed, which provides the strategy for the industrial production of sterol products.

Keywords: Carotenoid, Ergosterol, Lanosterol, Polyprenols, Sterols biosynthesis, Squalene.

INTRODUCTION TO STEROL BIOSYNTHESIS

Sterols are an important class of lipids that are essential lipid components of eukaryotic membranes. A large number of studies on sterol biosynthesis have been published and reviewed in various aspects [1 - 4]. One of the most important functions of sterols is to ensure the fluidity of the plasma membrane. Furthermore, sterols have been implicated in many functions in eukaryotic cell membranes, such as membrane ion permeability, vesicle formation, endocytosis and protein sorting [3]. The biosynthesis of sterols is tightly controlled because their functions

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are concentration-dependent. Sterols are synthesized and mature in the endoplasmic reticulum, thus the lowest concentration of the free sterol is found in the endoplasmic reticulum and then appears to increase along the membranes of the secretory pathway to reach a maximum at the plasma membrane [3]. In addition to the membrane-embedded sterol, steryl esters exist and are stored in lipid droplets, which can also be liberated by the action of specific lipases [5]. The balance between the synthesis and hydrolysis of steryl esters in lipid droplets is tightly regulated to maintain sterol homeostasis [6]. In addition, sterols transport plays an important role in maintaining sterol homeostasis. The functions of sterols are concentration-dependent, thus the free sterol has to be efficiently transported to the plasma membrane to prevent toxic accumulation at the endoplasmic reticulum. Although this transport pathway is still incompletely understood, it involves both vesicular and non-vesicular components [7]. Nicolas Jacquier and Roger Schneiter have summarized how sterols are transported and regulated between different intracellular membranes and how these membranes may establish their characteristic sterol concentration [3]. Therefore, we do not repeat this information here.

Early studies concerning the biosynthesis of sterols mainly focused on mammalian (*e.g.*, rat) and fungal (such as *Neurospora* or yeast) systems. Recently, more works have been studied in higher plants and algae. The sterol biosynthetic pathway is the key pathway leading to the production of cholesterol in animals and various C24-alkylsterols in fungi, plants and microalgae in eukaryotes [1, 8]. The basic functions of sterols appear to be conserved, though these mature sterols have slightly different structures. Generally, sterol biosynthesis in eukaryotes consists of three stages: 1) isopentenyl pyrophosphate (IPP) is biosynthesized by using acetyl-CoA or leucine or other alternative carbon sources [9]; 2) IPP is condensed with dimethyl allyl pyrophosphate (DPP) to form squalene; and 3) squalene is cyclized into lanosterol (animals and fungi) or cycloartenol (plants), and then produce the various types of terpenoids (Fig. **1**). Squalene is a key intermediate in the formation of sterols that are cyclized and then converted into final products such as cholesterol, ergosterol, and phytosterol [8]. Eukaryote, especially yeast, have played an important role in the study of sterol biosynthesis.

FORMATION OF SQUALENE

Squalene is the key intermediate in the biosynthesis of sterols. It is a naturally occurring linear triterpene with a formula of $C_{30}H_{50}$. As Dr. Tsujimoto first identified squalene in the shark liver extract in 1903, it received its name. Recently, squalene has gained a large amount of attraction due to its crucial role in steroid (especially dietary cholesterol) synthesis in humans. Meantime, it has

been characterized for its beneficial biological activities, including antitumor, antioxidant and cytoprotective effects [10]. At present, due to its multiple functions, it is used widely in the cosmetics, food and pharmaceutical industries [11].

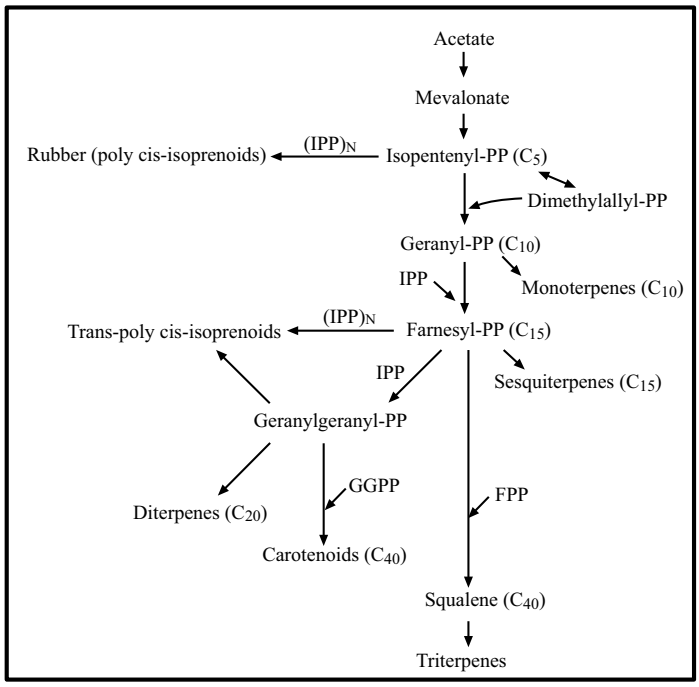

Fig. (1). Schematic map of isoprenoid pathway.

Shark liver oil is rich in squalene, which is relatively higher than some vegetable oils [10]. Currently, squalene has been verified to take part in metabolism as a precursor for the synthesis of steroids and play a crucial role in steroid synthesis in humans. Meantime, squalene carries out the effect of natural skin protection against environmental factors such as ultraviolet light radiation because it is one of the major components of human skin [12]. Squalene exists readily in the human body and can be obtained from natural sources,thus, it has a bright future in the nutraceutical and pharmaceutical industries.

In recent years, many works have attempted to produce squalene by microbial fermentation [13] or microbial strain development by metabolic engineering strategy [14]. As shown in Fig. (**1**), the process of squalene biosynthesis can be divided into two modules: the synthesis pathway of isoprenoid and the fusion pathway [11]. In the synthesis pathway of isoprenoids, all isoprenoids are

CHAPTER 12

Lipid Metabolism in Fungal Growth and Development

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Abstract: Fungal biotechnology has enormously contributed to the growing bio- and circular economy. With the capabilities of individual fungal strains in diverse applications, the fundamentals of their growth development and metabolic traits significantly impact the process development of industrial production. Lipids are cellular biomolecules that play dynamic functions during vegetative growth and development, and environmental adaptation. Regarding the structural and functional roles of lipid molecules, intensive studies have been given to understanding the physiological and molecular regulations in the lipid metabolism of filamentous fungi, particularly in the potential oleaginous strains. Hence, a link between fungal growth, morphological development and lipid phenotypes, is presented. Vegetative growth phases of fungi are distinguishable based on their lipid content and profile. Cell morphology can be controlled by physical and genetic manipulations. Through multi-dimensional technologies and emerging tools, more biological insights into a systematic regulation underlying lipid metabolisms, precursors, and other related metabolites are described. In the end, a correlation of phenotypic and genotype characteristics in growth and lipid dynamics on various substrate and culture conditions is elaborated. The informative data bridging towards industrial biotechnology for the establishment of fungal bio-manufacturing platforms are discussed not only for diversified lipid production but also for developing the eco-friendly and economically feasible production process.

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Keywords: Abiotic factor, Ascomycota, Bioprocess, Cell morphology, Desaturase, Developmental stage, Fatty acid biosynthesis, Fermentation, Filamentous fungi, Lipid degradation, Lipid particle, Membrane lipids, Neutral lipids, Nitrogen source, Oleaginous strain, Polyunsaturated fatty acids, Sporulation, Spore germination, Vegetative growth, Zygomycota.

INTRODUCTION

Filamentous fungi are attractive workhorses for industrial biotechnology. Through biotransformation processes, they can utilize inorganic and organic materials for cell growth and metabolite production. Thus, attention has been focused on the industrial fungal strains empowering the production of high-value products with a wide range of applications, such as enzymes, organic acids, long-chain polyunsaturated fatty acids (LC-PUFAs), and active pharmaceutical ingredients. With advances in fungal biotechnology and emerging tools, it is promising to produce novel compounds of industrial relevance through the synthetic biology approach. Although the fungal platforms offer advantages to industrial production, economic success would require knowledge of how the cells leverage their growth and development and the production of targeted metabolites to achieve a feasible option. A set of biomolecules are essential constituents in the fungal cell structures (*e.g.*, cell wall and cell membrane). Some play pivotal roles in cellular functions, such as adaptation for survival under surrounding conditions. Lipids are dynamic molecules that significantly contribute to the growth and development of filamentous fungi. Phospholipids (PL) are the structural backbone of the bilayer cell membrane. The physical and biological properties of PL are also governed by phosphate linkage group and fatty acyl chain diversity. Neutral lipids (NL) are of considerable interest due to their significant roles in metabolic energy and lipid storage in the so-called lipid particle (LP) or lipid body (LB). Not only for biosynthesis, but the lipid mobilization relevant to subcellular compartmentation is a major subject in the context of cell function and homeostasis. Moreover, cellular fatty acid profiles have been exploited for the chemotaxonomic differentiation of fungal groups [1].

Basically, the growth development in filamentous fungi is distinguished into three main stages, spore germination, vegetative growth, and reproduction (sporulation). Cell behavior can be monitored in response to growth and developmental stages, nutritional conditions, and physical stimuli for a given fungal species. Certain fungal groups, dimorphic fungi, have the ability to yeast-like and filament growths. Morphological switching of the dimorphic fungi can occur in particular culture environments and some chemical induction. Furthermore, the fungal growth in the form of mycelial pellets can be developed under specific fermentation conditions that are strain-dependent. The alterations

in growth characteristics and cell morphology of filamentous fungi are usually linked to their metabolic traits [2]. Therefore, the interplays of lipid metabolisms involved in the multi-level regulation of fungal growth and development are discussed. Prospectively, the metabolism of fungal lipids has implications for the systematic development of both fungal strain and bioprocessing, which offers a solution for the industrial production of functional lipids and lipid-derived products.

Role of Lipids in Spore Germination Process

In filamentous fungi, the dormant spores are formed as a developmental stage, commonly found whenever the mycelial cells are exposed to adverse factors, mainly in nutrient depletion or starvation conditions. The fungi belonging to Ascomycota (*e.g.*, *Neurospora, Penicillium*, and some species of *Aspergillus*), Zygomycota (*e.g.*, *Rhizopus* and *Mucor*), and Basidiomycota fundamentally generate sexual spores, called ascospores, zygospores, basidiospores, respectively. Some of them also produce asexual spores called conidia or sporangiospores. Deuteromycota is a particular division of fungi in which a sexual cycle has not been reported, and thus its member generates only conidia, *e.g.*, *Aspergillus fumigatus*. Typically, asexual spores contained lipids at low amounts, in which NL, PL, and glycolipids were major constituents. For example, 6%, 2−4%, and 11% (w/w) of lipid contents in dry cell weight were detected in *Aspergillus niger*, *Cunninghamella japonica*, and *Blastocladiella emersonii* spores, respectively. The sporangiospores of *Mucor rouxii* contained 3.4−7.6% of total fatty acid (TFA) in dry cell weight, and their fatty acid compositions varied depending on solid substrates [3]. Several roles of intracellular lipids in fungal spores have been documented. Not only for the barrier function, but they are also involved in energy generation, signal transduction, lipid transport, and DNA replication for further vegetative growth [4, 5].

In principle, hyphal growth is initiated by germ tube emergence of spores, the so-called germination process, which is a vital stage in the life cycle of fungi. Germination of fungal spores is a responsive mechanism of a resting cell to the appropriate conditions, which requires some nutrients, such as carbon and nitrogen sources and inorganic salts [6]. The simple computer simulation of fungal morphogenesis has been elaborated by Bartnicki-Garcia *et al.* [7]. The two-stage sequence of fungal morphogenesis commonly occurs during spore germination. Initially, the swelling of the spore occurs as an observation in the increase of either spore diameter or biomass titer. Then, the germ tube emerges from the enlarged spore, known as the polarized growth, followed by hyphal elongation and branching. The duration of each step depends on fungal species, spore age, and environmental conditions, such as temperature, water activity, and

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

R

S

T

U

V

Y

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