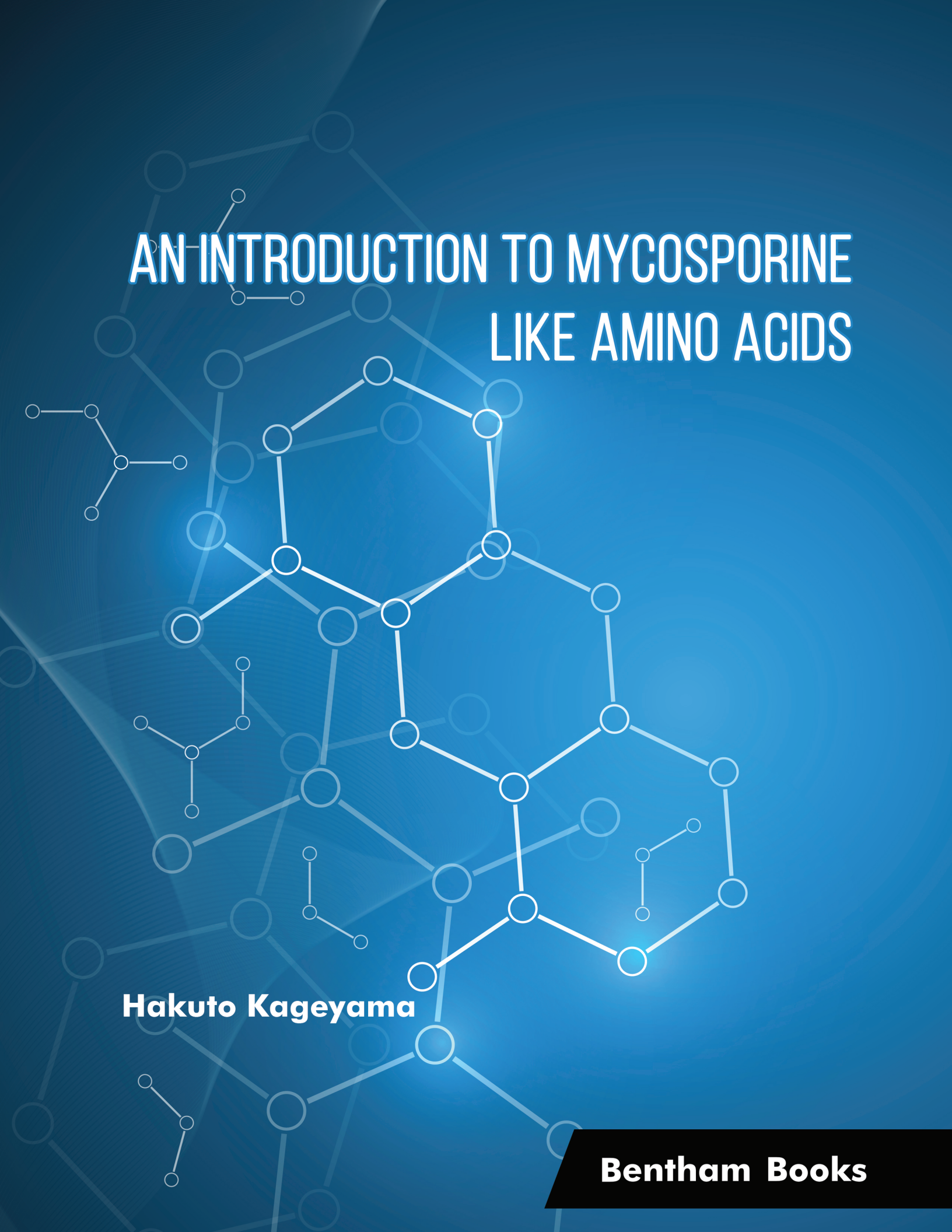
AN INTRODUCTION TO MYCOSPORINE LIKE AMINO ACIDS



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An Introduction to Mycosporine-Like Amino Acids

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FOREWORD

This book is a good introduction to those interested in Mycosporine-like amino acids (MAAs), and will be an asset for students, university researchers, and corporate researchers.

MAAs are natural compounds synthesized by microorganisms to protect themselves from UV irradiation. Their usefulness is expected in various biological and industrial fields. This book describes MAAs in detail, from the basics to the applied perspectives. This book is composed of 11 sections. First, starting with the molecular structure of MAAs (Chapter 1), their distributions are outlined, focusing on cyanobacteria (Chapter 2). Chapter 3 has a detailed description of the MAAs biosynthetic pathway. These chapters will help readers gain a basic understanding of MAAs. Chapter 4 introduces the knowledge accumulated so far regarding the method of analysis and preparation of MAAs. The characteristics of MAAs are described in chapter 5 to 11. Each chapter is compactly organized from the point of view application so that readers will find the usefulness of MAAs. It should be noted that this book has abundant appendices. Information on more than 60 types of MAAs reported so far is summarized. Correlation diagrams of the molecular structures of these many MAAs are also available. This book thus can serve as a key reference work to all those working on MAAs.

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PREFACE

Mycosporine-like amino acids (MAAs), which have the property of absorbing ultraviolet rays, are natural compounds that are applied in the cosmetics field as the active ingredient in sunscreens. In recent years, it has become clear that MAAs have various useful functions such as antioxidant activity and anti-inflammatory action as well as ultraviolet absorption ability. Therefore, applications may be considered and developed not only in the cosmetics field but also in various fields such as pharmaceuticals and foods. Patents have already been filed by companies around the world, and cosmetic ingredients and skin care products containing MAAs have been launched on the market.

This book details the molecular structures, activities, and application examples of MAAs and is intended to be used as an introductory book for undergraduate and graduate students in science or as a handbook for researchers. I aim to make the descriptions as clear as possible. In the text, references to academic research are cited as appropriate. I hope that this book will help to deepen the knowledge of MAAs.

This book is based on an English translation of my book Mycosporine-like Amino Acids Nyumon, published by Sankeisha in Japan in 2021. I was able to take the opportunity to review and remake the full text of the book and to incorporate the latest information. I would like to thank all the people involved in the production of this book. I would also like to take this opportunity to thank Dr. Rungaroon Waditee-Sirisattha of Chulalongkorn University, a collaborator who has been conducting research on cyanobacteria and MAAs for many years.

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CHAPTER 1

Mycosporine-like Amino Acids and their Biomolecular Properties

Abstract: Mycosporine-like amino acids (MAAs) are natural ultraviolet (UV)-absorbing compounds that are attracting attention in the industrial field including cosmetics and pharmaceuticals. This book provides a wide range of descriptions of MAAs, from fundamentals to applications. In order to discuss the properties of MAAs, an understanding of their chemical structures would be required. The purpose of this chapter is to understand the basic molecular structure of MAAs. In general, MAAs have structures in which amino acids are bound to the core structures of cyclohexenone or cyclohexenimine. In addition to the basic structure, the resonance hybrid structures of MAAs are also described here. Delocalization of electrons is considered to affect the stability and the absorption maximum wavelength of MAA molecules. We will also discuss the environmental factors that can affect the structure of MAAs. Finally, databases of molecular structure information of MAAs will be described.

Keywords: Cyclohexenimine, Cyclohexenone, Environmental factors, Maximum absorption wavelength, Molar absorption coefficient, Molecular structure, Mycosporine-like amino acid, Resonance hybrid structure, Ultraviolet.

INTRODUCTION

Mycosporine-like amino acids (MAAs) are water-soluble small organic compounds containing nitrogen in their molecular structure and are known as natural sunscreens. “Mycosporine” is a secondary metabolite that originally existed in fungi. It has a specific molecular structure in which amino acids bound to its basic structure are called MAAs [1]. To explain in a little more detail, it is as follows. It has long been known that fungi have substances with maximum absorption in the UV region (310 nm). Since this substance was thought to be involved in sporulation, it was called mycosporine (myco- + spore + -ine) together with the prefix myco-, which means fungi. In 1976, one of the chemical structures of mycosporine was reported (currently this substance is called mycosporine-serinol) [2]. After that, it became clear that compounds with similar structures also exist in various organisms other than fungi. Since amino acids were contained in the molecular structure, these compounds came to be called mycosp-

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orine-like amino acids (hereinafter referred to as MAA). To date, more than 60 kinds of MAA compounds have been reported.

MAAs are well-known UV-absorbing compounds. The maximum absorption wavelengths of MAAs are in the range of 310 to 362 nm. In addition, the value of the molar absorption coefficient is as large as ε = 20,000 to 50,000 M^{-1} cm^{-1}. As an example, the absorption spectrum of mycosporine-2-glycine, which is a type of MAA purified from salt-tolerant cyanobacteria, is shown in (Fig. **1**). Given that UV rays are classified into UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm) according to wavelength, MAAs are substances that efficiently absorb UV-A and UV-B. They are also considered to be compounds with the strongest UV-A absorption capacity in nature [3]. MAAs can release the absorbed UV energy to the surroundings as heat without producing harmful substances such as reactive oxygen species (ROS) [4].

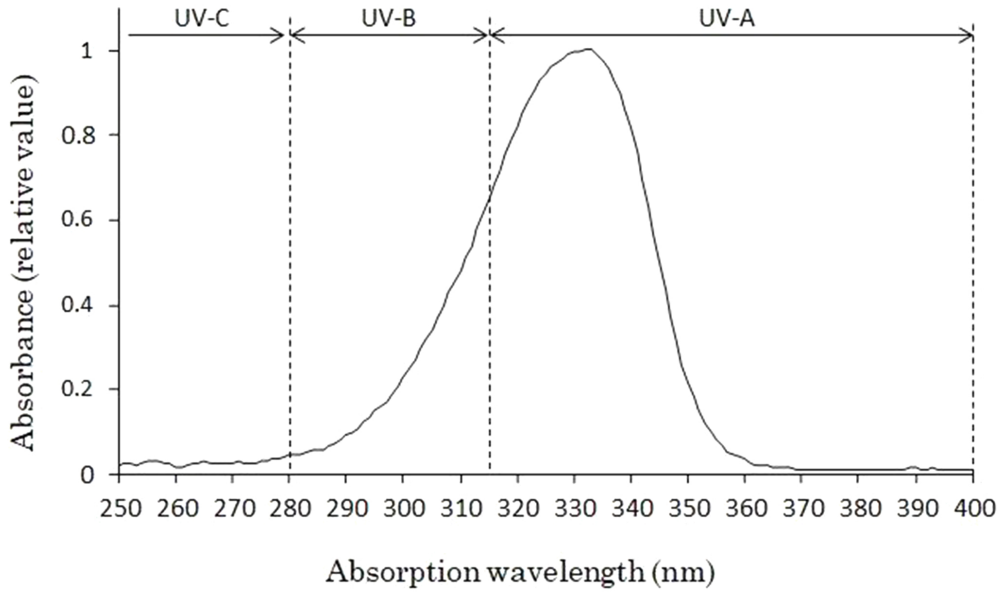

Fig. (1). Absorption spectrum of an MAA (mycosporine-2-glycine).

Many species that biosynthesize MAAs have been reported. So far, MAAs are found in various marine, freshwater, and terrestrial species, including micro and macroalgae, cyanobacteria, and animals [5 - 9]. It is thought that MAAs accumulated in the organism contribute to the reduction of damage to nucleic acids and proteins caused by UV rays. In addition, as will be described later, various physiological activities other than UV absorption have been reported.

MOLECULAR STRUCTURES OF MAAS

Basic Chemical Structure

The core part of the molecular structure of MAAs is a cyclohexenone structure or cyclohexenimine structure (Fig. **2**) [10, 11]. Basically, the substituted amino acids are bound as moieties R_1 and R_2. MAAs with a cyclohexenone structure contain one amino acid, and when R_1 is replaced with glycine, it produces mycosporine-glycine (Fig. **3**). However, in the cyclohexenimine structure, two substituents are substituted. For example, when R_1 and R_2 are substituted with glycine and serine, respectively, they produce shinorine (Fig. **3**). In disubstituted MAAs, the amino acid corresponding to the R_1 moiety is often glycine. This is because glycine first binds to the basic structure to produce mycosporine-glycine, and then the second amino acid binds to mycosporine-glycine to form disubstituted MAAs in the MAA biosynthetic pathway. (Details of MAA biosynthetic pathways will be described in Chapter 3.) Table **1** shows the molecular structure, substituents, and absorption maxima of representative MAAs.

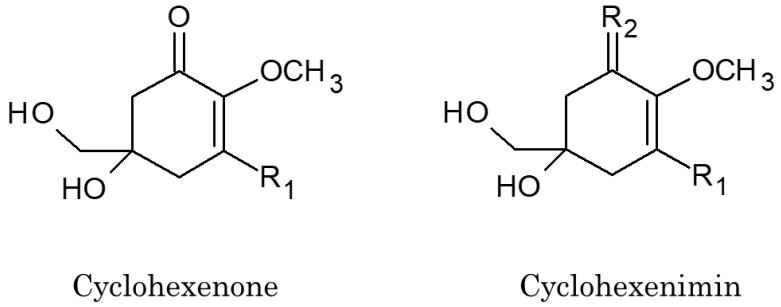

Fig. (2). Core structures of MAAs.

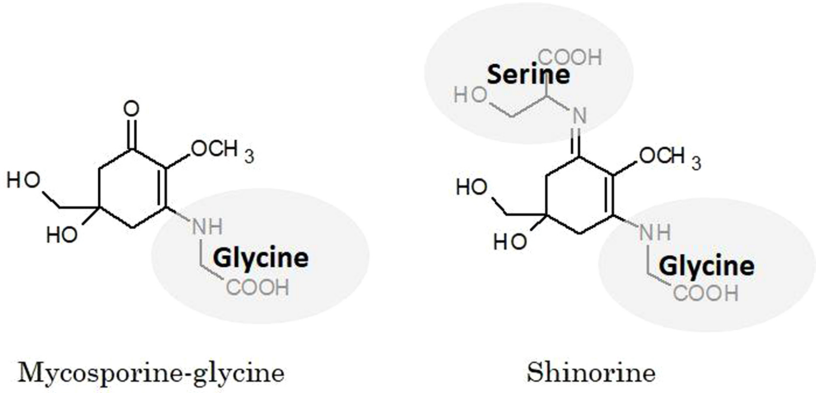

Fig. (3). Amino acid substitutions of core structures.

CHAPTER 2

Distribution of MAAs

Abstract: Accumulation of mycosporine-like amino acids (MAAs) has been reported in a wide range of species in nature, including microalgae, macroalgae, cyanobacteria, phytoplankton, fungi, and some animals. This chapter describes the distribution of MAAs with a focus on macroalgae and cyanobacteria. MAAs biosynthesized by macroalgae have already been applied in cosmetic products, such as Helioguard 365 and Helinori. Macroalgae tend to accumulate multiple types of MAAs, and the types and accumulation levels are affected by changes in environmental factors. Regarding cyanobacteria, we focus on UV, salt, and osmotic stresses, temperature changes, and drought stress as environmental factors, and describe the species in which the accumulations of MAAs are induced by these stresses. UV-B irradiation is a common environmental factor that can induce the accumulation of MAAs in cyanobacteria, but induction by other abiotic stresses has been reported. These findings suggest that MAAs act as a multifunctional molecule that responds to a variety of environmental factors, not just as a UV absorber.

Keywords: Asterina-330, Cyanobacteria, Drought, Induction, Macroalgae, Mycosporine-2-glycine, Mycosporine-glycine, Osmotic stress, Porphyra-334, Pterin, Palythine, Red algae, Shinorine, Scytonemin, Temperature, UV-B.

INTRODUCTION

MAAs are widely distributed in nature. A wide variety of organisms, including microalgae, macroalgae, cyanobacteria, phytoplankton, and fungi, are known to biosynthesize MAAs. There have been no reports of intracellular accumulation of MAAs in bacteria (except cyanobacteria) or archaea, but a species of Actinomycetales, a Gram-positive bacterium, has been reported to contain trace amounts of shinorine depending on culture conditions [1]. MAAs have not been detected in higher plants, in which flavonoids act as UV-absorbing compounds. In animals, MAAs are sometimes detected, but this is thought to be due to uptake from other organisms through the food chain or symbiosis with microorganisms capable of biosynthesizing MAAs. However, it should be noted that the existence of homologs of cyanobacterial MAA synthetic genes has been reported in corals and sea anemones, and the possibility that these animals biosynthesize MAA can-

not be ruled out [2]. In this chapter, the distribution of MAAs in marine macroalgae and cyanobacteria will be described.

MACROALGAE

Among macroalgae, red algae (rhodophytes) are known to accumulate MAAs. MAAs have also been detected in some green algae (chlorophytes) and brown algae (phaeophytes). According to a survey by Sun *et al.*, 572 species of macroalgae accumulating MAAs were reported in the 30 years from 1990 to 2019, of which 486 were red algae (Fig. **1**) [3]. In particular, MAAs were abundant in strains belonging to the orders Bangiales, Ceramiales, and Gracilariales [4].

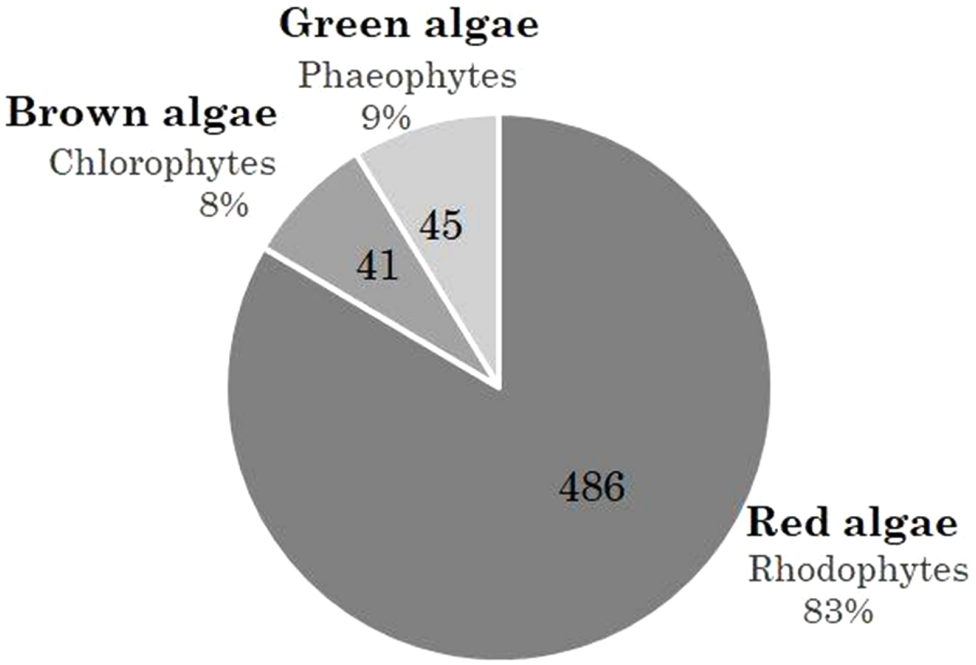

Fig. (1). Distribution of macroalgae that accumulate MAAs (Created based on the data in Sun Y *et al.* (2020) *Mar. Drugs*).

Seven types of MAAs are detected in red algae (mycosporine-glycine, porphyra-334, shinorine, palythine, palythene, palythinol, asterina-330), and the absorption wavelength range covered by these MAAs is wide (310–360 nm). Most red algae accumulate 4 to 5 types of MAA, which means that they can absorb a wide range of UV-A and UV-B [5]. In addition to these MAAs, usujirene has been reported to accumulate in species such as *Palmaria palmate*, *Gracilaria tenuifrons*, and *Porphyra yezoensis* [3]. In addition, in 2019, bostrychine A, B, C, D, E, and F

were identified as new MAAs in *Bostrychia scorpioides* [6]. Red algae can adapt to the amount and types of MAAs accumulated and the regulation of biosynthesis of MAAs in response to fluctuating environmental factors, such as the degree of UV irradiation in the habitat, the concentration of nitrogen sources, salinity, and temperature. Helioguard 365 and Helinori, which are commercially available as cosmetic ingredients, contain MAAs extracted from the red alga *Porphyra umbilicalis*. Helioguard 365 is a formulation containing liposomal porphyra-334 and shinorine, and Helinori is a formulation containing porphyra-334, shinorine, and palythine. However, other MAAs extracted from red algae do not appear to be used in new product development so far. The main reason is that the content of MAAs in red algae collected from the sea is not sufficient. A maximum of 12 mg of MAAs content per gram of dry weight of red algae has been reported, but in most cases, less than half of that amount has been detected [5]. Exploration of algal strains with higher MAA contents or optimization of culture conditions that cause efficient biosynthesis of MAAs may lead to a solution. In addition, a method for efficient extraction and isolation of MAAs from red algae is required. From the viewpoint of industrial production, it is also considered effective to produce MAAs using microorganisms that are easy to culture and genetically transform, such as cyanobacteria.

For the identification of genes involved in MAA biosynthetic pathways and the characterization of the enzymatic reactions, analysis using cyanobacteria has preceded other organisms. However, in 2017, the cyanobacterial type MAA biosynthetic gene cluster was also reported in the genomic sequence of red algae including *Porphyra umbilicalis* and *Chondrus crispus* [7]. So far, there have been no further reports on the molecular regulatory mechanisms of these MAAs biosynthetic gene clusters in red algae, and future investigations are expected.

CYANOBACTERIA

In addition to algae, cyanobacteria are commonly used in the research of MAAs. Cyanobacteria are Gram-negative bacteria and are thought to have contributed greatly to the supply of oxygen and organic substances to the Earth by oxygen-evolving photosynthesis. Cyanobacteria are distributed throughout the waters and lands of the Earth. Also, being the primary producer of photosynthetic products in marine ecosystems, nitrogen-fixing cyanobacteria play a role as a nitrogen source in terrestrial ecosystems. Cyanobacteria also inhabit extreme environments such as deserts, hot springs, salt lakes, and polar regions [8]. In order to adapt to these extreme environments, cyanobacteria are thought to have acquired unique environmental adaptation strategies during their evolution. In particular, cyanobacteria are exposed to UV irradiation in sunlight when absorbing the solar energy required for photosynthesis. Therefore, it is considered that UV protection

CHAPTER 3

Biosynthetic Pathways of MAAs and their Regulatory Mechanisms

Abstract: The biosynthetic mechanism of mycosporine-like amino acids (MAAs) has been roughly elucidated. In 2010, the genes responsible for MAA biosynthesis were identified in cyanobacteria. In this chapter, first, we will describe the reaction mechanisms responsible for the biosynthetic pathways of MAAs, mainly based on results from cyanobacteria. Next, as a regulatory mechanism for MAA biosynthesis, the response patterns of MAA accumulation in response to abiotic stresses, such as UV irradiation, salt, and osmotic pressure, will be explained. There are many points to be clarified regarding the detailed regulatory mechanisms, and further analyses are awaited in the future. Because MAAs have useful activities in addition to UV absorption, they are substances that are expected to be used in cosmetics and pharmaceuticals. This chapter also includes discussions from the perspective of future industrial production.

Keywords: ATP-grasp enzyme, Biosynthetic pathways of MAAs, D-Ala-D-Ala ligase, Gene resources, Nonribosomal peptide synthetase (NRPS), Localization, Osmotic stress, Pentose phosphate pathway, Shikimate pathway, Regulatory mechanism, Substrate specificity, Salt stress, Temperature, UV irradiation, 4-deoxygadusol, 3-dehydroquinate synthase (DHQS), *O*–methyltransferase (*O*–MT), 2-*epi*-5-*epi*-valiolone synthase (EVS).

INTRODCUTION

Biosynthetic Pathways of MAAs

The biosynthetic pathways of MAAs have been intensively analyzed using cyanobacteria. It is known that the precursor compounds of MAAs are produced from metabolic intermediates of primary metabolic pathways. Mono- or di-substituted MAAs are produced based on the precursor compound through several enzymatic reactions.

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Biosynthetic Pathway of 4-deoxygadusol, A Precursor Compound of MAAs

In cyanobacteria, the precursor compound of MAAs is 4-deoxygadusol (4-DG) (Fig. **1**). 4-DG is thought to be produced from metabolic intermediates of theprimary metabolic pathways, the shikimate pathway, or the pentose phosphate pathway.

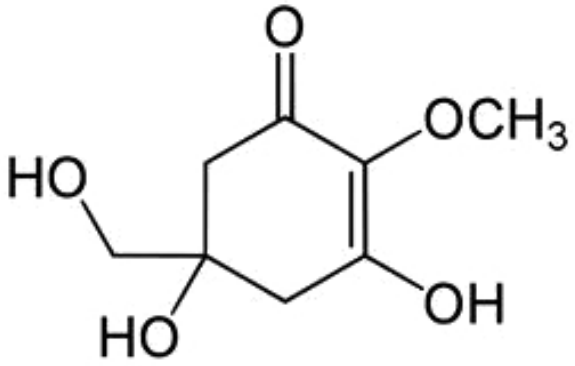

Fig. (1). Molecular structure of 4-deoxygadusol (4-DG).

The shikimate pathway (Fig. **2**) is a biosynthetic reaction pathway for the aromatic amino acids tyrosine, phenylalanine, and tryptophan, which has not been found in animals but is present in most microorganisms and plants. The pentose phosphate pathway (Fig. **3**) is a pathway involved in the production of various pentoses. In this pathway, glucose-6-phosphate (G6P), which is an intermediate of glycolysis, is converted into glyceraldehyde-3-phosphate (G3P), which is also an intermediate of glycolysis. In the pentose phosphate pathway, one molecule of G6P produces one molecule of CO_2 and two molecules of NADPH. Therefore, it is a source of NADPH*.

* NADPH is a reduced form of nicotinamide adenine dinucleotide phosphate (NADP), which is used as an electron carrier in photosynthetic pathways and glycolysis.

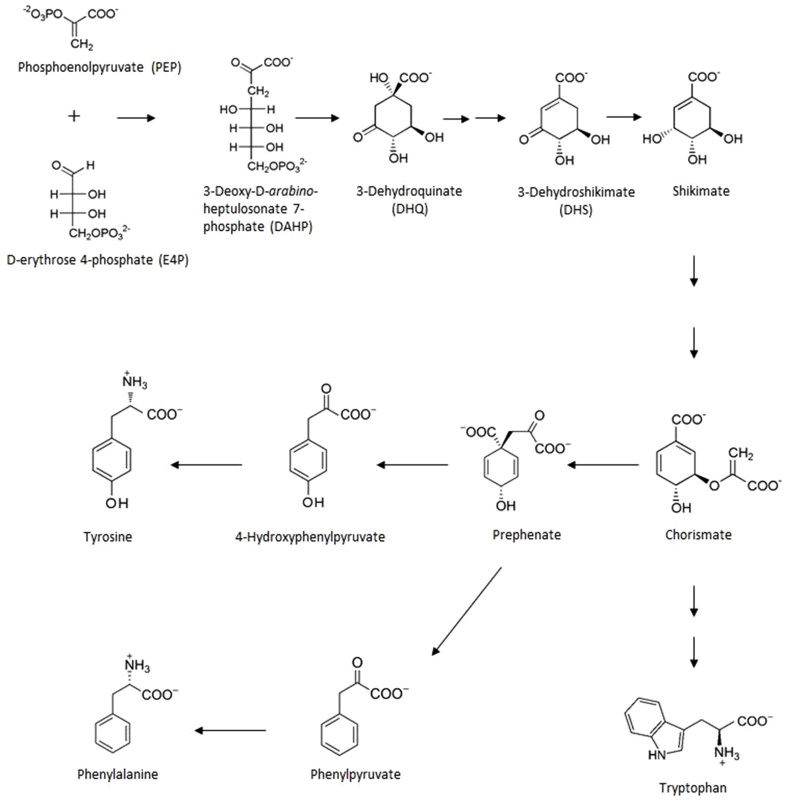

Fig. (2). The shikimate pathway.

CHAPTER 4

Analytical and Preparative Methods for MAAs

Abstract: This chapter describes the basics of analytical and preparative methods for mycosporine-like amino acids (MAAs). For samples whose molecular structures are known, high-performance liquid chromatography is widely used as a simple quantitative or qualitative analytical method for MAAs. However, if the molecular structures are unknown, they are often identified by combining several analytical methods, such as liquid chromatography-mass spectrometry and nuclear magnetic resonance analysis. In MAA preparation, the first key factor is how efficiently MAAs can be obtained in the extraction process from biological samples. The second key factor is how efficiently high-purity MAAs can be obtained from the separation process. This chapter also discusses the production of MAAs from an industrial perspective.

Keywords: Absorption spectrum, Amino acid analysis, Extraction, High-performance liquid chromatography, Industrial production, Liquid chromatography-mass spectrometry, Mass spectrometry, Nuclear magnetic resonance, Octadecyl silyl, Preparation, Purification, Reversed-phase chromatography.

INTRODUCTION

Analysis of Maas and their Molecular Structures

This section outlines various methods for the analysis of MAAs and methods for the identification of MAA molecules whose molecular structures are unknown.

HPLC Analysis of MAAs

When analyzing MAAs accumulated in organisms such as algae and cyanobacteria, samples extracted using a protonic solvent such as methanol or methanol are usually separated by HPLC instrument. MAAs are detected using wavelengths close to their absorption maximum. A technique called reversed-phase chromatography (RPC) is commonly used to analyze MAAs [1 - 3]. One of the column packing materials used in RPC is a silica gel to which alkyl groups are chemically bonded. The most widely used column is an ODS column (C18 column) filled with silica gel to which octa decyl silyl (ODS, $C_{18}H_{37}Si$) groups are

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bonded. Because octadecylsilyl groups show a small polarity, a substance with a smaller polarity and a higher hydrophobicity interacts more strongly with the ODS column and takes longer to elute from the column. By utilizing this property, the target substance contained in the mixed solution can be separated. For example, as shown in (Fig. **1**), in shinorine, mycosporine-2-glycine, and porphyra-334, the amino acid residues substituted at the C1 position of the cyclohexeneimine ring, which is the core structure of an MAA, are serine, glycine, and threonine, respectively.

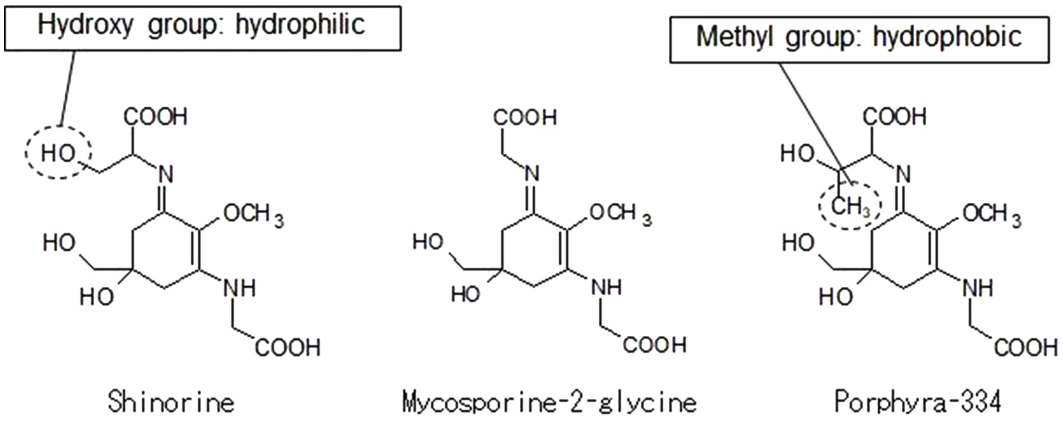

Fig. (1). Molecular structure of shinorine, mycosporine-2-glycine, and porphyra-334.

Fig. (**2**) shows the results of analyzing a mixed solution of these three MAAs using an ODS column. In this example, after injection of the sample into the column, shinorine is eluted first, followed by mycosporine-2-glycine, and finally porphyra-334. This result can be explained as follows. By comparing the molecular structures of shinorine and mycosporine-2-glycine, it can be seen that hydrophilic hydroxyl groups are present in the serine residue of shinorine (Fig. **1**). It is considered that the retention of shinorine in the column is weakened due to the influence of the presence of highly polar hydroxyl groups. However, the threonine residue of porphyra-334 also has a hydroxyl group like shinorine, but it is considered that the methyl group existing near it has a greater hydrophobic influence (Fig. **1**). Because the methyl group is hydrophobic, it is considered that its presence enhances the interaction between porphyra-334 and the column packing materials, resulting in a longer retention time. In the analysis of Fig. (**2**), 1% acetic acid aqueous solution was used as the mobile phase, but the separation pattern will differ depending on the solvent used as the mobile phase even if the same column is used. Although several methods have been proposed as general purpose methods for analyzing MAAs, it is important to find the optimum separation conditions according to the molecular structures of the target MAAs.

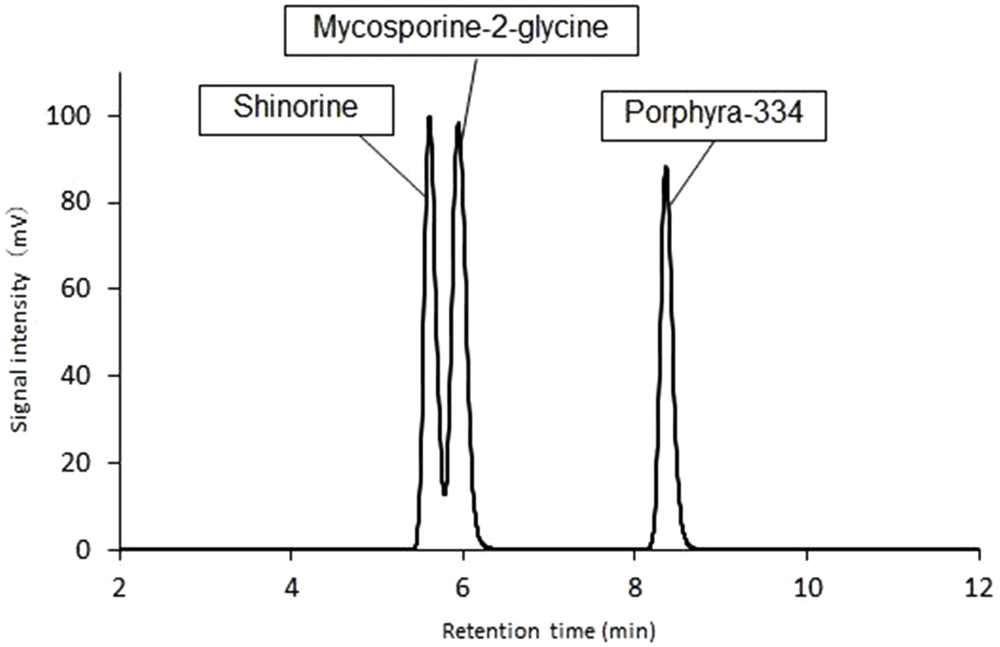

Fig. (2). HPLC chromatograph of a mixture that contained shinorine, mycosporine-2-glycine, and porphyra-334.

Methods to Determine the Molecular Structures of MAAs

When determining the types of MAAs by HPLC analysis, it is essential to confirm that the retention time of the analyte is consistent with that of the reference sample (authentic standard sample). In addition to matching the retention times, it is also necessary to confirm that the shape of the overlapping peak does not change when a mixture of the analyte and the reference sample is analyzed. This process is called spiking. When spiking is performed, if the analyte and the reference sample are different substances, changes in peak shape such as widening of the peak may be observed. After confirming these properties, if the retention time of the analyte and the reference sample match even if the analytical conditions, such as the composition of the mobile phase are changed, it is considered that these are the same substances. If the above analyses are performed and the retention times do not match, the same analyses should be repeated using other reference materials. Alternatively, the molecular structures of the analyte MAAs can be determined using analytical techniques other than HPLC. These analytical methods include measurement of absorption maximum wavelength, determination of molecular weight by mass spectrometry (MS), identification of amino acid substituents contained in the analyte MAA by amino acid analysis, and nuclear magnetic resonance (NMR) analysis.

CHAPTER 5

Biological Activities of MAAs and their Applications 1: UV-protective Activity of MAAs and their Application as Sunscreens

Abstract: In this chapter, we focus on the UV absorption characteristics of MAAs and describe the application examples. UV rays that pass through the ozone layer and the atmosphere and reach the surface of the Earth consist of UV-A and UV-B. Because these rays are harmful to biomolecules, MAAs, which can efficiently absorb these wavelength regions and detoxify their by-products, are promising natural organic compounds such as sunscreens. Products containing MAAs extracted from red algae are already on the market. With a focus on Helioguard 365 and HELINORI, the biological effects of MAAs against UV irradiation will be described.

Keywords: Collagen, DNA damage, Elastin, Helioguard 365, HELINORI, Melanin, Melanocyte, Photoaging, *Porphyra umbilicalis*, Red alga, UV-A, UV-B, UV-C.

INTRODUCTION

The skin is the largest human organ and is constantly exposed to the surrounding environment. Among various environmental stresses, UV rays are known to have a negative effect on the skin. Exposure to UV light damages the skin, and its reaction mechanism depends on the wavelength of light.

UV rays are divided into three wavelength regions: UV-A, UV-B, and UV-C [1]. Of the UV rays contained in the Sun's rays, UV-A (315–400 nm) and UV-B (280–315 nm) reach the surface of the Earth without being absorbed by the ozone layer and the atmosphere (Fig. **1**). Among them, UV-A accounts for 95% of the total UV radiation. Although the proportion of UV-B is small, it is high energy and is considered to be more harmful to the skin and eyes than UV-A [2]. In recent decades, the amount of UV-B rays reaching the surface of the Earth has increased due to the depletion of the ozone layer [3, 4]. Both UV-A and UV-B are known to be genotoxic and cause photochemical damage to biopolymer compounds such as intracellular DNA and proteins [5, 6]. As a result, these irradiations accelerate skin aging and can lead to skin cancer. In contrast, although

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UV-C (100–280 nm) is high energy, it does not affect living organisms because it is absorbed by the ozone layer and the atmosphere and does not reach the surface of the Earth (Fig. **1**) [2, 7].

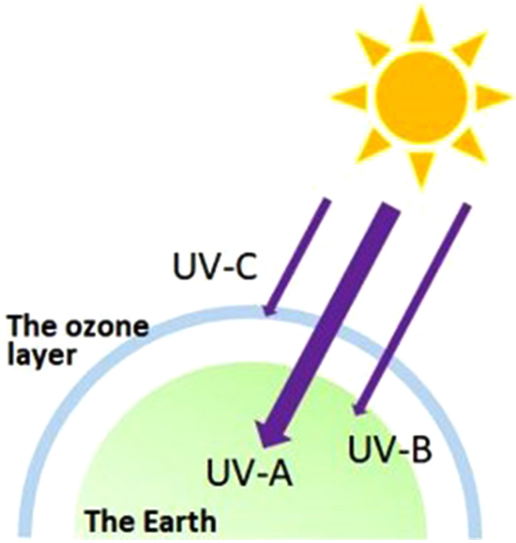

Fig. (1). UV irradiation that reaches the surface of the earth. 95% of the UV irradiation that reaches the surface of the Earth is UV-A. Some UV-B also reach the surface of the Earth. UV-C is completely absorbed by the ozone layer and the atmosphere.

UV-A

UV-A has a weaker energy than UV-B, but it has a large effect on the skin because the amount of radiation that reaches the surface of the Earth is large. UV-A irradiated to the skin reaches the dermis and interferes with the functions of collagen and elastin, which give the skin its firmness and elasticity (Fig. **2**). It also damages fibroblasts that produce collagen and elastin. As a result, the skin becomes less firm and elastic, causing wrinkles and sagging. In addition, by activation of melanocytes, it promotes the biosynthesis of melanin pigments and creates age spots. These actions are called photoaging.

Characteristics of UV-A and its Effect on the Skin

• UV-A is 95% of the UV rays that reach the surface of the Earth.

• Although its energy is weak, long-term exposure causes chronic damage.

• The wavelength is long and reaches the dermis.

• Causes wrinkles, sagging, and age spots.

UV-B

UV-B is only about 5% of the UV rays that reach the surface of the Earth, but it has higher energy. Because the depth of the reach of UV-B is shallow, it reaches only the epidermis (Fig. **2**). Because it damages the DNA in epidermal cells, the risk of developing skin cancer increases with long-term exposure*. Even short exposures can cause an inflammation reaction, resulting in redness of the skin. This is called sunburn. UV-B also causes suntan, which causes the skin to brown due to the deposition of melanin pigments several days after exposure. It can also cause age spots and freckles.

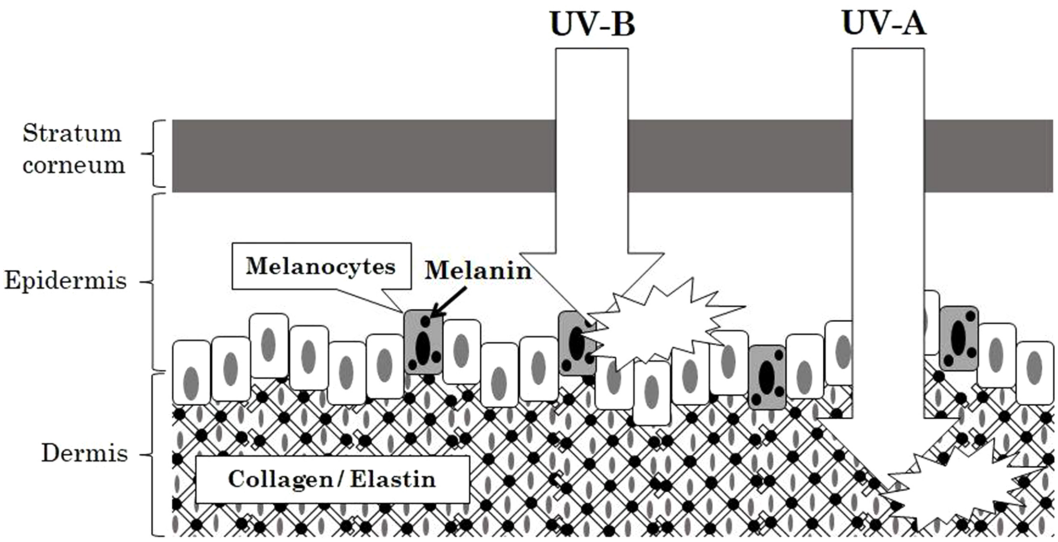

Fig. (2). Effects of UV-A and UV-B on the skin.

* UV-A is also known to indirectly damage DNA. UV-A irradiation produces reactive oxygen species (ROS) in the body, which causes oxidative damage to biopolymer compounds such as DNA [8].

Characteristics of UV-B and its Effect on the Skin

• It has higher energy than UV-A and causes DNA damage.

• The wavelength is short, and the reach of depth is shallow.

• Short-term exposure causes acute sunburn and suntan.

MAAs have an absorption maximum of 310 ~ 362 nm, which is in the range including UV-A and UV-B, and have large molar extinction coefficients (ε =

CHAPTER 6

Biological Activities of MAAs and their Applications 2: Antioxidative Properties

Abstract: It is known that the generation of reactive oxygen species (ROS) caused by UV irradiation and oxidative reactions accelerate skin aging. Substances that suppress or eliminate the generation of ROS are called antioxidants. So far, various mycosporine-like amino acids (MAAs) have been reported to have antioxidative activities. To prevent damage to the skin caused by ROS and maintain the homeostasis of the epidermis, skin cells have an endogenous antioxidant system consisting of enzymatic reactions. Although many points are unclear about the regulatory mechanisms, it has been suggested that MAAs are involved in the regulation of genes encoding enzymes that are involved in this system. This chapter provides a comprehensive overview of the antioxidant activities of MAAs.

Keywords: Antioxidant, Catalase, Glutathione peroxidase, Glutathione reductase, Peroxiredoxin, Oxidation, Reactive oxygen species, Superoxide dismutase, Thioredoxin reductase.

INTRODUCTION

Reactive oxygen species (ROS) that trigger oxidative stress include the hydroxyl radical (•OH), superoxide anion radical ($•O_2^-$), hydrogen peroxide (H_2O_2), and singlet state molecular oxygen ($^1O_2$). Within the skin, exposure to UV irradiation is associated with the production of ROS. The ROS generation reaction by UV irradiation is diverse and depends on its wavelength. For example, the production of $^1O_2$ and $• O_2^-$ was promoted in the skin of UV-A irradiated mice [1]. There are also reports that • OH, $• O_2^-$, and H_2O_2 were generated from advanced glycation end products (AGE) during UV-A irradiation [2]. Although the mechanism of formation is unknown, it has been reported that UV-B irradiation also induced • OH, $• O_2^-$, and H_2O_2 [3].

Oxidation is an essential reaction in energy production and metabolic pathways, and these reaction processes are responsible for the production of ROS. The generated ROS function as signal transduction molecules that cause cell division, inflammation, immune function, stress response, *etc* [4]. In addition, in plants and

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photosynthetic microorganisms such as cyanobacteria, excess light energy is absorbed by the photosynthetic reaction system, and if this energy is not safely dispersed, ROS such as $^1O_2$ and H_2O_2 are generated.

Skin cells have an endogenous antioxidant system to prevent damage to the skin by ROS caused by UV irradiation and oxidative reactions and to regulate epidermal homeostasis. The system consists of six enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), thioredoxin reductase (TRXR), and peroxiredoxin (PRDX). SOD and CAT erase O_2^- and H_2O_2, respectively, and convert them into H_2O (Figs. **1** & **2**). In contrast, GPX, GR, TRXR, and PRDX eliminate H_2O_2 by regulating the redox state of glutathione and thioredoxin (Fig. **3**). In addition to this enzymatic system, non-enzymatic molecules such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), and glutathione play important roles as antioxidants in the skin tissue [5]. These small molecule compounds eliminate free radicals by acting as electron donors (Fig. **4**).

Fig. (1). The reaction catalyzed by SOD.

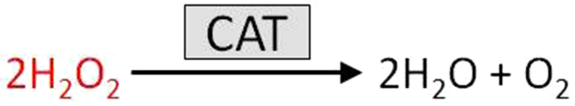

Fig. (2). The reaction catalyzed by CAT.

ANTIOXIDATIVE ACTIVITIES OF MAAS

To date, many MAAs have been reported to exhibit antioxidative activites (Table **1**). Exposure to UV radiation causes the production of ROS, one of the factors that promote skin aging. Therefore, antioxidant molecules with ROS-scavenging activity are commonly used as cosmetic ingredients to prevent aging. Mycosporine-glycine is considered to be one of the molecular structures of MAAs with the strongest antioxidant activity. It has been reported that the antioxidant activity of mycosporine-glycine isolated from the lichen *Lichina pygmaea* is higher than that of shinorine and porphyra-334 under the condition of pH 8.5 by the ABTS assay. The half-maximal (50%) inhibitory concentration (IC_{50})* of mycosporine-glycine was 3 μM, which was significantly lower than the well-known antioxidant ascorbic acid (26 μM) [6]. Mycosporine-2-glycine has also

been reported to have a higher antioxidant activity than shinorine or porphyra-334 [7]. However, the following points should be noted when discussing the antioxidative capacity of each MAA molecule. As can be seen from Table **1**, the evaluation of the antioxidant activity of MAAs differs depending on the measurement method and the research group. For example, three studies investigated the DPPH-free radical scavenging activity of porphyra-334. Of these reports, the activity of porphyra-334 was detected in two cases, but the IC_{50} values differed significantly between them [8 - 10]. One of the reasons for these discrepancies is considered to be the purity of the MAAs used in the experiments. As mentioned in Chapter 4, it is generally not easy to purify MAAs with high purity, so it is possible that impurities may have affected the measurements. There may also have been subtle differences in the measuring methods and the type and condition of the measuring instruments.

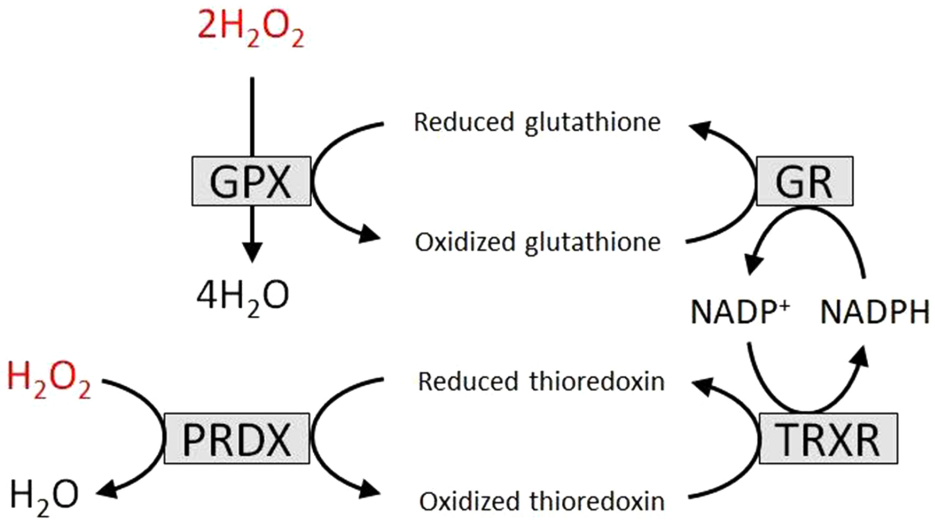

Fig. (3). The reactions catalyzed by GPX, GR, TRXR, and PRDX.

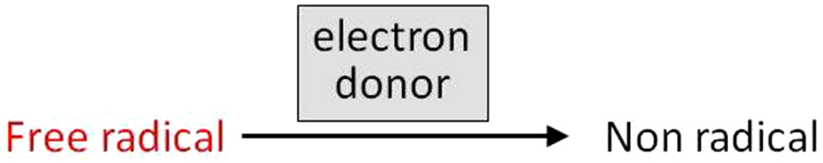

Fig. (4). A nonenzymatic reaction to neutralize free radicals by donating electrons from electron donors.

CHAPTER 7

Biological Activities of MAAs and their Applications 3: Anti-inflammatory Effects

Abstract: Inflammation is the defensive reaction system that occurs when the body receives a harmful stimulus and tries to remove it. In general, the area where the reaction occurs has a fever, swelling, redness, and pain. The stimuli that cause inflammation are diverse but include UV irradiation and reactive oxygen species. This chapter briefly describes the inflammatory response pathways caused by these stimuli. After that, it outlines the effects of mycosporine-like amino acids (MAAs) on the accumulation, activity, and regulation of factors contained in the inflammatory pathway. Although research findings are accumulating, the molecular mechanisms are still unknown. Details of the relationship between the molecular structures of MAAs and their functions in the inflammatory pathway await further study.

Keywords: Antioxidant, Cyclooxygenase-2, Interleukin-1, Interleukin-6, Inducible NO synthase, Inflammation, Inhibitor protein of NF-κB, Lipopolysaccharides, Mycosporine-like amino acid, Nuclear factor-kappa B, Nitrogen monoxide, Prostaglandin E_2, Reactive oxygen species, Tumor necrosis factor α, Ultraviolet.

INTRODUCTION

Inflammation is a physiological defense mechanism against molecular and cellular damage caused by various stresses, including irradiation, oxidative stress, infection, and exposure to endotoxins, such as lipopolysaccharides (LPS) [1]. UV irradiation is known to induce inflammation. Distinct patterns of inflammation caused by UV irradiation are caused by exposure to rays of a specific wavelength. The three groups, UV-A, UV-B, and UV-C, are categorized based on these different inflammatory patterns [2]. In particular, the erythema caused by UV-B irradiation is called sunburn. It is well known that the inflammatory response induced by UV-B irradiation is associated with a variety of factors. These factors include nitrogen monoxide (NO), inducible NO synthase (iNOS), prostaglandin E2 (PGE2), cyclooxygenase-2 (COX-2), tumor necrosis factor (TNF-α), and cytokines such as interleukin-1 (IL-1) and interleukin-6 (IL-6) (Fig. **1**). These molecules are produced mainly in keratinocytes, the major cell type of the epider-

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mis, and are regulated by nuclear factor-kappa B (NF-κB) [3]. NF-κB regulates the expression of genes involved in inflammation, oxidative stress response, differentiation, proliferation, apoptosis, and cell adhesion [4, 5]. In the cytoplasm, the NF-κB protein interacts with the inhibitor protein of NF-κB (IκB) and is inactivated. When IκB is degraded in response to stress stimuli, activated NF-κB translocates to the nucleus [4, 6]. It is known that iNOS and COX-2 are involved in the molecular response to inflammatory stimuli. *iNOS* gene expression is induced as inflammation progresses, followed by overproduction of the pro-inflammatory factor NO. It has also been reported that the expression of COX-2, which is involved in the production of PGE2, was induced by UV-B irradiation in the human skin tissue and cultured human keratinocytes [7]. PGE2 is a bioactive lipid associated with the induction of inflammation and cancer. In addition, reactive oxygen species (ROS) are associated with the inflammatory response. In fact, it has been found that COX-2 expression is induced by ROS in various cells [8].

Anti-inflammatory molecules have been widely studied not only for skin care applications, but also for the treatment of chronic inflammatory diseases including rheumatoid arthritis, psoriasis, chronic obstructive pulmonary disease, multiple sclerosis, and inflammatory bowel disease. Anti-inflammatory compounds are also useful in the treatment of cardiovascular diseases such as atherosclerosis and neurodegenerative diseases such as Parkinson's disease.

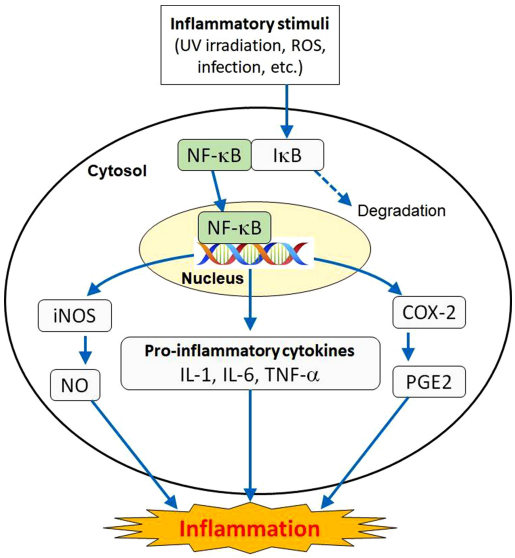

Fig. (1). Inflammatory response induced by the stimuli, including UV-irradiation. The NF-κB protein activated *via* inflammatory stimuli can translocate into the nucleus and regulate genes involved in the inflammatory response. See the text for further explanation.

EFFECTS OF MAAS ON UV-B-INDUCED INFLAMMATORY PATHWAY

So far, the results of studies on the anti-inflammatory effect of mycosporine-like amino acids (MAAs), including shinorine, porphyra-334, mycosporine-glycine, and mycosporine-2-glycine, have been reported (Table **1**). Suh *et al.* showed that the upregulation of *COX-2* gene expression in immortalized human keratinocyte HaCaT cells by UV irradiation was suppressed by shinorine or mycosporine-glycine but not porphyra-334 [9]. However, Becker *et al.* reported that porphyra-334 suppressed LPS-induced NF-κB activity in human myelomonocyte THP--Blue cells. In contrast, shinorine promoted LPS-induced NF-κB activity [10]. Ying *et al.* showed that the expression levels of TNF-α, IL-1β, and IL-6 were reduced in the skin tissue of mice treated with a mixture of shinorine and porphyra-334. Treatment of MAAs at a concentration of 20 mg/ml reduced mRNA levels of NF-κB, IL-1β, and IL-6 by 21.05%, 25.10%, and 42.13%, respectively [11]. In addition, Tarasuntisuk *et al.* revealed that mycosporine-glycine significantly inhibited NO production in LPS-stimulated RAW264.7 macrophage cells. Although mycosporine-glycine, palythine, shinorine, and porphyra-334 showed a maximum inhibitory effect of about 20%, mycosporine-glycine showed an inhibitory effect of about 50% at 10 μM. The relationship between the molecular structures of MAAs and their inhibitory effects is still unclear, but it is an interesting research subject. Besides, it was reported that mycosporine-2-glycine strongly suppressed the expression of *iNOS* and *COX-2* genes [12]. Cheewinthamrongrod *et al.* reported that oxidative stress-induced NF-κB accumulation in human skin fibroblasts was suppressed by the addition of mycosporine-2-glycine [13]. Given that antioxidant molecules such as vitamin E is shown to inhibit the NF-κB signaling pathway [14], it is possible that the strong antioxidative capacity of mycosporine-2-glycine is related to this phenomenon.

Table 1. Effects of MAAs on UV-B-induced inflammatory pathways.

MAAs (Treatment)	Target Cells or Tissue	The Effects of MAAs
Shinorine, Mycosporine-glycine (Addition to the medium) [9]	HaCaT cells	The upregulation of *COX-2* gene expression caused by UV irradiation stress was suppressed.
Porphyra-334 (Addition to the medium) [10]	THP-1-Blue cells	The induction of NF-κB activity caused by LPS was suppressed.
Shinorine (Addition to the medium) [10]	THP-1-Blue cells	The induction of NF-κB activity caused by LPS was promoted.
Mixture of shinorine and porphyra-334 (Application of an aqueous solution containing MAAs) [11]	Mouse back skin	The induction of NF-κB activity caused by UV irradiation was suppressed.

CHAPTER 8

Biological Activities of MAAs and their Applications 4: Anti-glycative Properties

Abstract: Advanced glycation end products (AGEs) are formed by a series of chemical reactions initiated by non-enzymatic glycation reactions. In this process, the reducing sugar binds to the free amino group of the protein. The formation of AGEs that accompany the aging process is thought to be associated with various diseases such as diabetes and Alzheimer's disease. A number of inhibitors derived from synthetic compounds and natural products have been developed and evaluated to prevent the formation of AGEs. Compared to synthetic compounds, natural products are considered to be relatively safe for human consumption, so there is an increasing demand for compounds derived from natural products. From this perspective, this chapter focuses on mycosporine-like amino acids as naturally occurring inhibitors against AGEs formation.

Keywords: Advanced glycation end products, Amino guanidine, Glycation, Maillard reaction, Natural inhibitors, Reactive oxygen species, Synthetic inhibitors.

INTRODUCTION

Glycation is a series of chemical reactions involving the non-enzymatic binding of sugar molecules to free amino groups and hydroxy groups of biocompounds such as proteins and lipids, which is also called the Maillard reaction [1]. The Maillard reaction was discovered in 1912 by the French chemist Louis-Camille Maillard as a reaction associated with browning during the cooking and storage of food [2]. The final product of the glycation reaction between the free amino group of a protein and a reducing sugar such as glucose is called an advanced glycation end product (AGE) [3]. Accumulation of AGEs impairs the structure and function of tissue proteins in the body and is a complication of blood vessels and kidneys in diabetic patients, atherosclerosis, and Alzheimer's disease [1, 4 - 6]. For example, high levels of AGEs have been detected in the blood and tissues of diabetics, which leads to complications such as nephropathy and neuropathy [7, 8]. It is thought that a decrease in enzyme activity due to changes in the charge state and the formation of crosslinks by the glycation of proteins is related to the adverse

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effects of these AGEs. Changes in protein properties can affect a variety of biological phenomena, including tissue homeostasis and regulation of gene expression. In addition, because there are many structures of AGEs, it is highly possible that many proteins are modified even if the amount of each AGE structure accumulated is small.

AGEs are also associated with the aging process, and the amount of AGEs accumulated in the body increases with normal aging. Thus, endogenously formed AGEs are a common product of metabolism, but it is also known that AGE formation is enhanced by oxidative stress [9]. A number of studies have shown that elevated levels of reactive oxygen species (ROS) caused by oxidative stress are involved in the development of diabetes and its complications [10].

AGEs are generated through a complicated multi-step reaction that can be roughly divided into two stages (Fig. **1**). In the reaction process of the early stage, the electrophilic carbonyl group (R_1−C(=O)−R_2) in a reduced sugar reacts with the free amino group (−NH_2) in the N-terminal of the protein and the side chains of the basic amino acid residues lysine, arginine, and histidine [11]. Subsequently, an unstable Schiff base (R_1R_2C=N−R_3) is formed [12]. The rearrangement of this compound results in the formation of a stable Amadori product. Then, in the late reaction process, the Amadori product irreversibly binds to an amino acid residue of the protein to form a cross-linked product [12, 13]. Further oxidation, dehydration, polymerization, and oxidative decomposition of Amadori products produce a variety of AGEs [14].

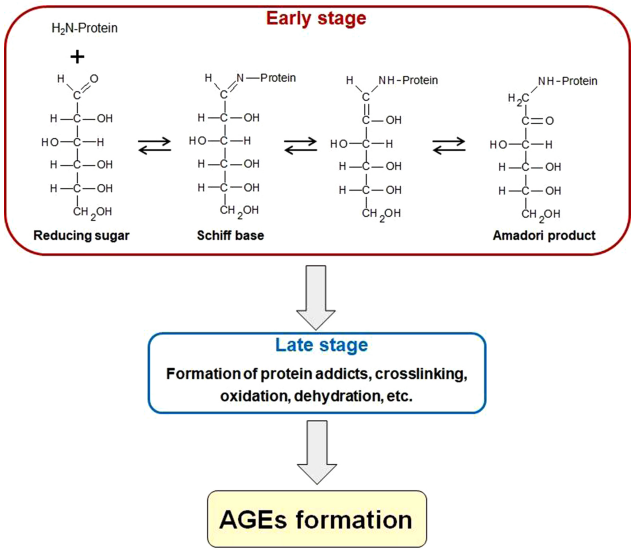

Fig. (1). The formation of AGEs by the glycation reaction.

At present, more than 40 types of structures of AGEs have been reported [15], [16]. Some of them are crosslinked structures, such as pentosidine, glucosepane, crossline, glyoxal-lysine dimer (GOLD), and methylglyoxal-lysine dimer (MOLD). Pentosidine and glucosepane are AGEs that crosslink lysine and arginine. In contrast, crossline, GOLD, and MOLD crosslink two lysine residues. Others are non-crosslinked AGE adducts to the protein, such as N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)lysine (CEL), and pyrraline (Fig. **2**).

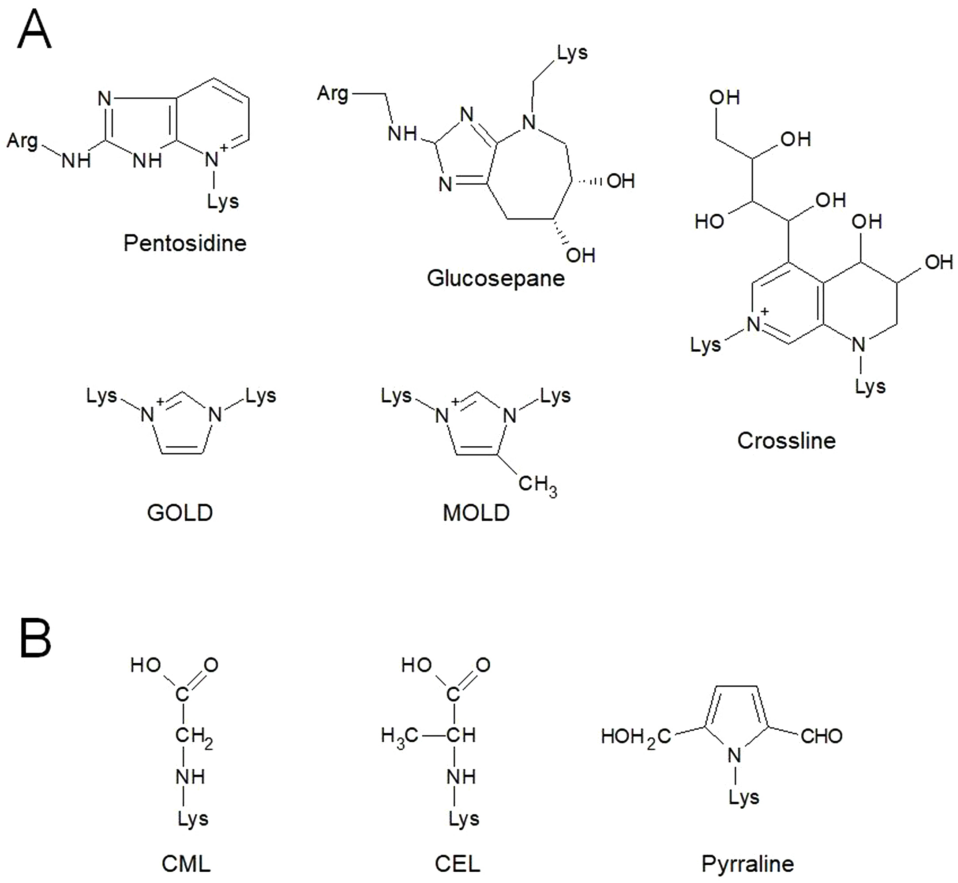

Fig. (2). Schematic representation of structures of cross-linked type AGEs (A) and non-crosslinked AGE adducts (B).

Inhibitors of various glycation reactions have been derived from synthetic compounds to suppress the formation of AGEs. An example of a well-known synthetic inhibitor is aminoguanidine, which has a nucleophilic hydrazine [17]

CHAPTER 9

Biological Activities of MAAs and their Applications 5: Inhibition of Collagenase Activity

Abstract: Enzymes involved in the degradation of the extracellular matrix (ECM) are deeply involved in skin aging. Compounds that suppress the degradation of collagen and elastin, constituents of the ECM, are of significant value to the cosmetics field. So far, more than 10 types of MAAs have been reported to inhibit the activity of collagenase, which belong to the family of matrix metalloproteinases. It has been suggested that the metal-chelating activity of MAAs is involved in these mechanisms of action. However, MAAs have not been reported to have an inhibitory activity on elastase. This chapter briefly summarizes these observations.

Keywords: Collagenase, Elastase, Extracellular matrix, Matrix metalloproteinase, Metal chelating activity.

INTRODUCTION

Collagen is a fibrous protein that constitutes various tissues including skin, bone, and blood vessels in vertebrates (Fig. **1**). Collagen accounts for 30% of proteins that make up humans. Collagen is produced by fibroblasts within the dermis layer. It has high strength and is involved in the maintenance of structure in the skin tissue, which greatly affects the elasticity and firmness of the skin. Because collagen depletion and denaturation cause skin aging, prevention of collagen depletion leads to skin anti-aging. Therefore, compounds with an inhibitory effect on collagenase, which is a collagen-degrading enzyme, are useful in the cosmetics field.

Collagenase, which is an endopeptidase, belongs to the family of matrix metalloproteinases (MMPs). By degrading collagen and elastin, MMPs play an important role in a variety of biological processes, including tissue homeostasis and post-wound repair. However, MMPs are activated with aging and change the composition of collagen and elastin in the extracellular matrix (ECM), resulting in wrinkles and sagging of the skin [1]. Several types of MMPs are expressed in mammalian skin [2, 3]. Mammalian collagenases are secreted by keratinocytes and skin fibroblasts in response to UV irradiation, oxidative stress, and cytokine

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stimulation. Repeated induction of these collagen-degrading enzymes over a long accelerates skin aging. Bacterial collagenase is known to be one of the factors of bacterial virulence. It destroys the extracellular structure by attacking the collagen helix and is involved in the pathogenic process of some bacteria, such as *Clostridium* [3, 4].

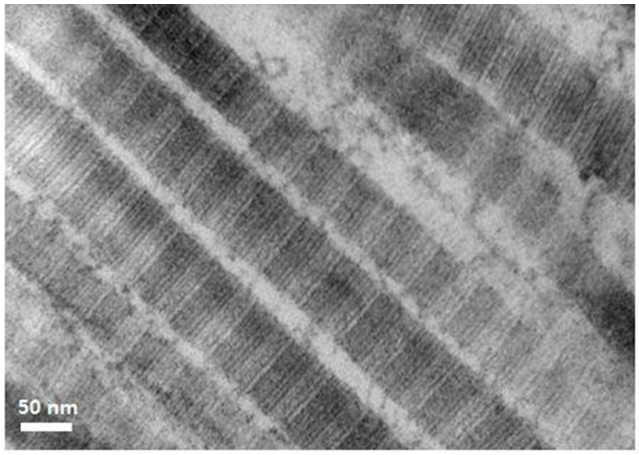

Fig. (1). Transmission electron microscope image of fibrous collagen (type I collagen).

INHIBITORY EFFECTS OF MAAS ON COLLAGENASE ACTIVITIES

So far, 14 types of MAAs have been reported to have an inhibitory effect on collagenase activity in *in vitro* experiments (Table **1**). Hartmann *et al.* reported the collagenase-inhibiting activity of shinorine, porphyra-334, and palythine isolated from red algae [5]. Another research group showed the collagenase-inhibiting activity of mycosporine-2-glycine isolated from halotolerant cyanobacterium *Halothece* sp. PCC7418 [6]. Interestingly, in that report, no collagenase inhibitory activity was detected in the mixture of shinorine and porphyra-334 [6]. Orfanoudaki *et al.* also reported the collagenase-inhibiting activity of 13 MAAs, including shinorine and porphyra-334 [7]. Another report found that the amount of MMPs accumulated after applying UV irradiation to mouse skin tissue was suppressed by the application of MAAs [8]. The mechanism of collagenase inhibition by MAAs is not fully understood. As outlined in Chapter 10, MAAs may be a chelating agent for metal ions [9], which may lead to the inhibition of metal-requiring collagenase.

In addition to collagenase, elastase, a member of the chymotrypsin-type serine protease family, is an enzyme involved in ECM degradation [10]. Degradation of elastin leads to a decrease in skin elasticity. So far, no results have reported that MAAs showed inhibition of elastase activity. In a preliminary study using elastase from porcine pancreas by a research group in Japan, the purified MAAs (M2G,

P334, and SHI) tested showed no inhibitory activity [3]. Unlike collagenase, it should be noted that elastase does not require metal ions to exhibit protease activity.

Table 1. MAAs reported to have an inhibitory effect on collagenase activity.

MAA	Collagenase (Substrate)	Activity (IC_{50})
Shinorine [5]	Collagenase type V from *Clostridium histolyticum* (MMP-2 substrate SCP0192)	104 μM
Porphyra-334 [5]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	106 μM
Palythine [5]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	159 μM
Mycosporine-2-glycine [6]	Collagenase type IV from *C. histolyticum* (4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg-OH)	470 μM
Mycosporine-methylamine-threonine [7]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	251 μM
Mycosporine-alanine-glycine [7]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	158 μM
Aplysiapalythine A [7]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	81 μM
Asterina-330 [7]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	71 μM
Bostrychine B [7]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	105 μM
Bostrychine C [7]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	58 μM
Bostrychine D [7]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	118 μM
Bostrychine E [7]	Collagenase type V from *C. histolyticum* (MMP-2 substrate SCP0192)	163 μM

CHAPTER 10

Biological Activities of MAAs and their Applications 6: Metal Chelating Abilities

Abstract: As mentioned in Chapters 8 and 9, the useful functions of MAAs, such as the anti-glycative property and collagenase inhibitory activity, might be associated with their metal chelating activity. Although there are few reports on the metal-chelating activity of MAAs, a chelating model of MAAs and metal ions has recently been proposed. This chapter briefly summarizes these observations.

Keywords: Chelating, Euhalothece-362, Mycosporine-2-glycine.

INTRODUCTION

A compound in which a ligand (*i.e.*, a molecule or ion) is bonded to a metal ion is called a complex. If the ligand has multiple atoms that coordinate with the metal ion, a ring structure containing the metal ion is formed. This compound is called a chelate compound. The etymology of chelate is 'chēlē', which means "crab scissors" in Greek. A chelating agent binds to a metal ion in solution and reduces the activity of the metal ion. Chelating agents have various applications. For example, they are added to shampoos and laundry detergents to prevent the salt formation of anionic surfactants and maintain detergency. In agriculture, chelate compounds are used as water-soluble metal salt fertilizers due to their high solubility in water.

Fig. (**1A**) shows the structure of a chelate compound of a divalent nickel ion and ethylenediaminetetraacetic acid (EDTA), which is a major chelating agent. The arrows in the figure indicate coordinate bonds, indicating that lone electron pairs are provided by oxygen and nitrogen atoms*. Amino acids with an amino group ($-NH_2$) and a carboxyl group ($-COOH$) are also known to act as chelating agents. Fig. (**1B**) shows the structure of a chelate compound of a divalent calcium ion and glycine. Because MAAs contain amino acids in their molecular structure, they have the potential to function as chelating agents.

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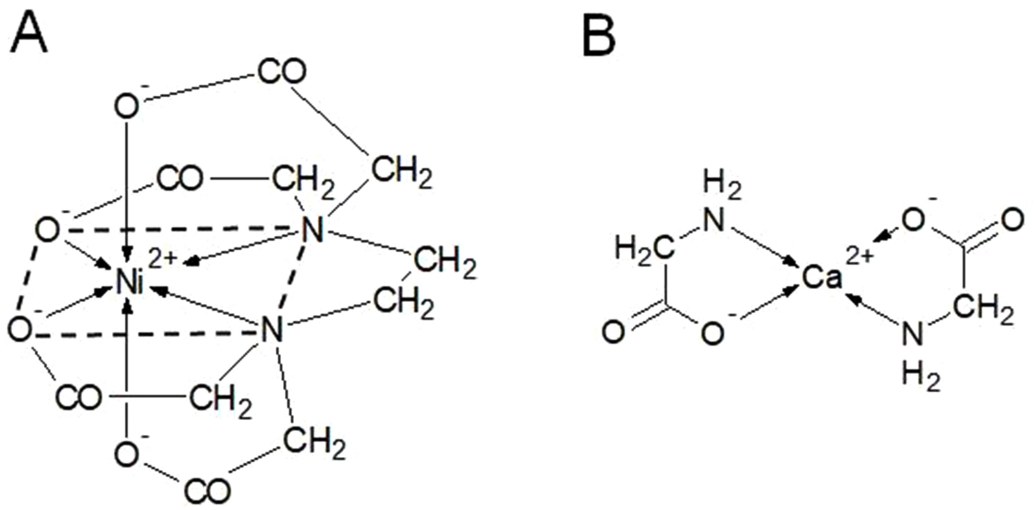

Fig. (1). Representative chelate compounds **A.** Chelate compound of Ni2+ and EDTA (4-) ion. **B.** Chelate compound of Ca^{2+} and glycine.

POTENTIAL METAL CHELATING ACTIVITIES OF MAAS

In 2006, Volkmann *et al.* suggested that euhalothece-362 (Fig. **2**), an MAA derived from the halophilic cyanobacterium *Euhalothece* sp. strain LK-1 may act as a chelating agent [1]. At that time, it was presumed that the four hydroxy groups (−OH) present in the structure of enhalothece-362 and the carboxy group of the alanine residue substituted at the C3 position were involved in chelation.

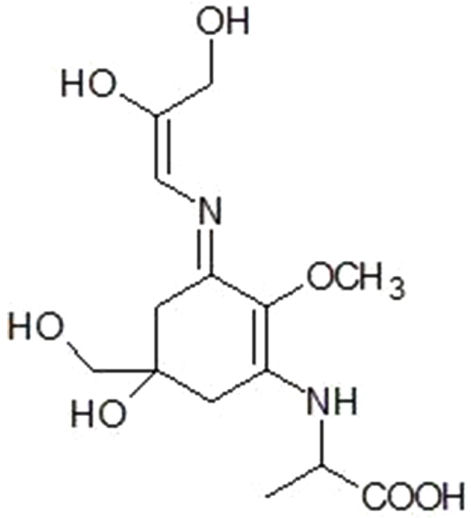

Fig. 2. Molecular structure of euhalothece-362.

Later, Varnali *et al.* proposed a model structure for chelated compounds of metal ions and MAAs [2]. Fig. (**3**) shows two examples of the chelation of mycosporine-glycine and calcium ions. In the first model, the oxygen atom of the methoxy group ($-OCH_3$) at the C2 position and the nitrogen atom and oxygen atom of the carboxy group of the glycine residue substituted at the C3 position of mycosporine-glycine are bound to the calcium ion (Fig. **3A**). In the second model, the two oxygen atoms of the carboxy group of glycine and the oxygen atoms of the hydroxy group and hydroxymethyl group ($-CH_2$-OH) at the C5 position bind to the calcium ion (Fig. **3B**).

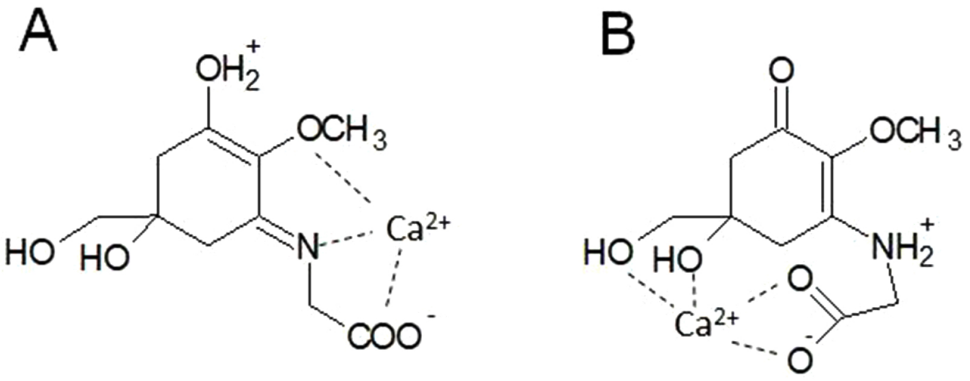

Fig. (3). Two chelation models of mycosporine-glycine and Ca^{2+}.

In addition, in the disubstituted MAs shinorine, a model has been proposed in which the hydroxy group of the serine residues substituted at the C1 position is bound to the metal ion (Fig. **4**).

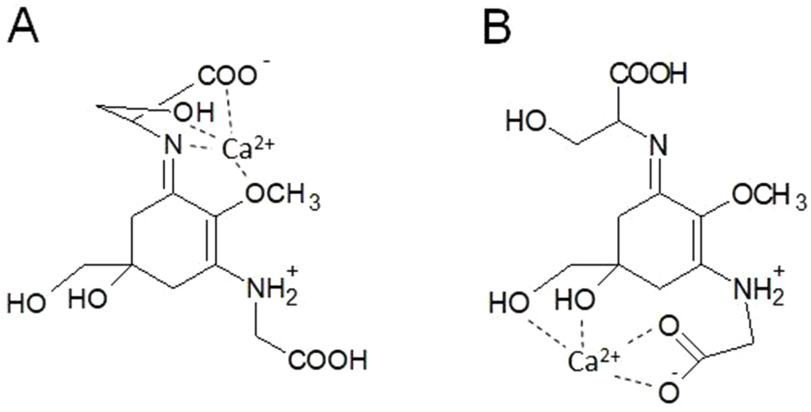

Fig. (4). Two chelation models of shinorine and Ca^{2+}.

CHAPTER 11

Biological Activities of MAAs and their Applications 7: DNA Protective Property, Wound Healing Effects, Anti-Cancer Effects, And Applications in Horticulture and as a Film Material

Abstract: This chapter describes the research findings on the DNA protection, wound healing, and anti-cancer effects of MAAs and their use in horticulture and as a raw material for film-type UV-blocking materials. These applications are additional to the use of MAAs as sunscreen agents and pharmaceuticals applied to humans.

Keywords: Chelating, DNA protection, Euhalothece-362, Mycosporine-2-glycine, Wound healing.

INTRODUCTION

DNA Protective Properties of MAAS

It is known that reactive oxygen species (ROS) and oxidative stress generated by UV irradiation cause oxidative DNA damage. It has been reported that MAAs can reduce the DNA damage caused by these external stresses. It was mentioned in Chapter 5 that Helioguard 365 relieved DNA damage from UV irradiation in human fibroblasts. In addition, DNA damage caused by oxidative stress treatment with hydrogen peroxide in human malignant melanoma A375 cells was suppressed by the addition of mycosporine-2-glycine to the medium [1]. It is thought that MAAs, which have an antioxidant capacity, protect DNA by scavenging ROS.

UV is absorbed by the double bonds in the pyrimidine ring of thymine and cytosine, which are the constituent bases of DNA. As a result, the double bond is cleaved and can react with an adjacent base. A polymer of adjacent thymines and cytosines is called a pyrimidine dimer. Pyrimidine dimers inhibit DNA replication

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and transcription, causing various disorders such as cell death and mutation. It has been reported that red algae extracts containing palythine, shinorine, and porphyra-334 suppressed the production of pyrimidine dimers [2].

WOUND HEALING EFFECTS OF MAAS

MAAs might have a wound healing effect. Orfanoudaki *et al.* showed that shinorine, porphyra-334, mycosporine-alanine-glycine, and bostrychine-B showed the effect of closing scratches made on monolayer cultures of human keratinocyte cells by scratch assay* [3]. These MAAs are thought to have the effect of promoting the proliferation and migration of human keratinocyte cells, but the mechanism of action is still unknown.

* A method that scratches a monolayer culture on a plate and investigates the process in which the area is closed by cell proliferation or migration.

ANTI-CANCER EFFECTS OF MAAS

It has been reported that MAAs have an inhibitory effect on the growth of cancer cells. For example, Yuan *et al.* showed that an MAA-containing extract prepared from the red alga *Palmaria palmata* inhibited the growth of B16-F1 mouse skin melanoma cell line [4]. The extract prepared from red algae exposed to the high UV irradiation environment (grade II) had a higher growth inhibitory effect than that of the low UV irradiation environment (grade I). Because grade II contained usujirene in addition to palythine, palythinol, shinorine, asterina-330, and porphyra-334 contained in grade I, it is possible that usujirene contributed to growth inhibition. In another report, extracts prepared from wild- and cultivated-red algae showed a growth inhibitory effect on human HeLa adenocarcinoma cervical cell line and U-937 histiocytic lymphoma cell line *in vitro* [5]. These antiproliferative activities were thought to be triggered by the induction of apoptosis. Other reports also suggested that MAAs induce apoptosis. Kim *et al.* investigated the effects of an extract of the red alga *Porphyra yezoensis* containing porphyra-334 and shinorine on human keratinized HaCaT cells treated with UV-B. As a result, UV-B-irradiated cells were protected by promoting cell proliferation through the activation of the JNK and ERK signaling pathways and inducing the apoptosis of damaged cells [6].

APPLICATION OF MAAS TO HORTICULTURE

MAAs may be used to combat sunscald in horticultural crops. Although it is common to physically block direct sunlight to prevent sunscald, it may be possible to chemically protect crops by applying an MAA-containing emulsion to crops. From this perspective, Pedrosa *et al.* prepared a stable emulsion containing

carnauba wax in an ammonium aqueous solution to which Helioguard 365 was added to enhance absorption in the UV-B region (280–300 nm) [7]. However, the actual application to crops has not been examined, so future development is expected.

APPLICATION OF MAAS AS FILM MATERIALS

MAAs are useful as a raw material for film-type UV-blocking materials. For example, Fernandes *et al.* prepared and evaluated MAA-containing (mycosporine-glycine, shinorine, or porphyra-334) films using chitosan as a medium [8]. These films were prepared by forming an amide bond between the carboxy group of MAAs and the amino group of chitosan. As a result, the film effectively absorbed the UV-A and UV-B regions and showed light and heat resistance. The UV absorption pattern was different depending on the MAA species used. In addition, the biocompatibility of these films was confirmed using L-929 murine fibroblasts. Because substances other than chitosan can be used as the medium, it is possible that various functional films can be developed using MAAs.

CONCLUDING REMARKS

This chapter outlined the research findings on DNA protection, wound healing, and anti-cancer effects as potential useful functions of MAAs. Regarding these effects, it is necessary to perform more detailed studies in the future. In addition, application examples of MAAs as a film material and an anti-burning agent were described. By utilizing the properties of MAAs such as UV absorption and antioxidant activity, it is possible that MAAs can be applied as various materials in the future.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

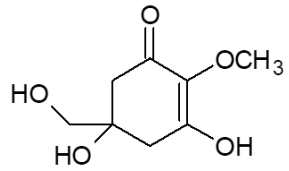
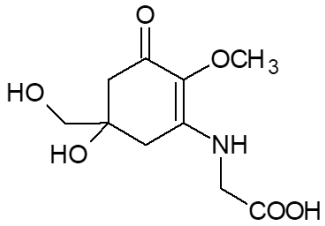
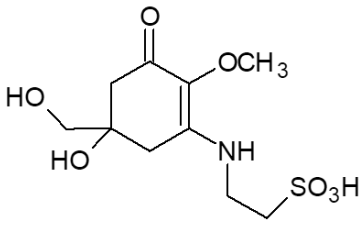
Declared none.

REFERENCES

[1] Cheewinthamrongrod, V.; Kageyama, H.; Palaga, T.; Takabe, T.; Waditee-Sirisattha, R. DNA damage protecting and free radical scavenging properties of mycosporine-2-glycine from the Dead Sea

Appendix A

Molecular structures, molecular weights, absorption maxima, Etinction coefficients of MAAs

MAA	Molecular weight (MW) Apsorption maxima (λ_{max}) Etinction coefficients (ε)
Precursor compound of MAAs	
4-Deoxygadusol [1] 	MW: 188 λ_{max} = 268 nm (pH = 2), 294 nm (pH =7) ε = ND
Monosubstituted-MAAs	
Mycosporine-glycine [2] 	MW: 245 λ_{max} = 310 nm ε = ND
Mycosporine-taurine[3] 	MW: 295 λ_{max} = 309 nm ε = ND

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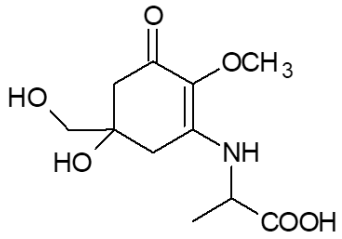
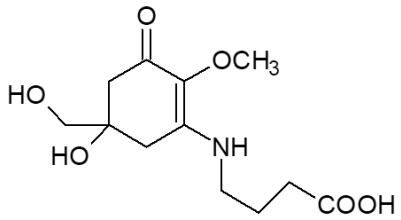
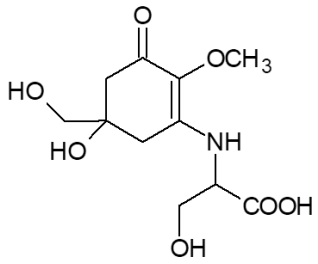
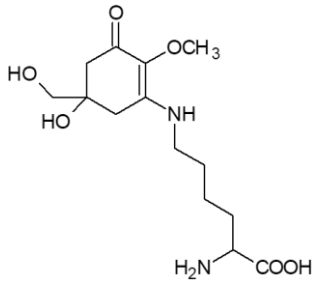
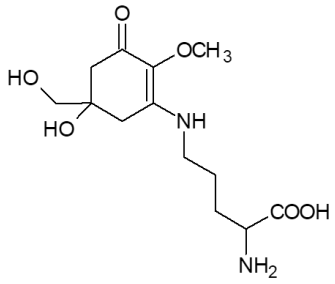
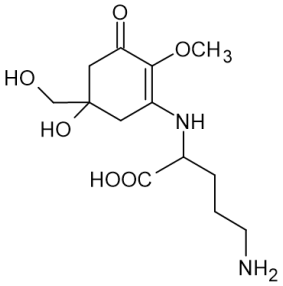
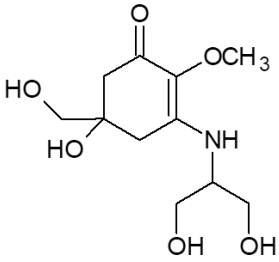
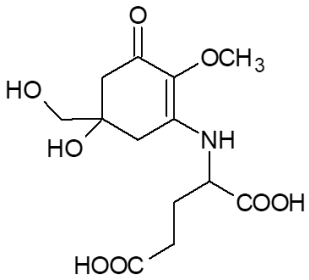
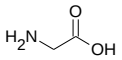
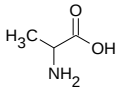
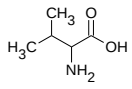
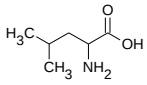
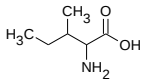
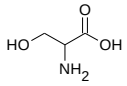
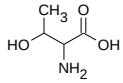
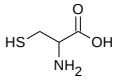
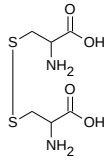
Mycosporine-alanine [4, 5] 	MW: 259 λ_{max} = 310 nm[4], 317 nm[5] ε = 640 M^{-1} cm^{-1}
Mycosporine-GABA[6] **(Mycosporine-γ-aminobutyric acid)** 	MW: 273 λ_{max} = 310 nm ε = 28,900 M^{-1} cm^{-1}
Mycosporine-serine[7] 	MW: 275 λ_{max} = 310 nm ε = ND
Mycosporine-lysine [8] 	MW: 316 λ_{max} = 310 nm ε = ND

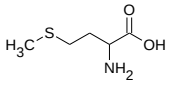
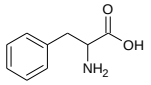
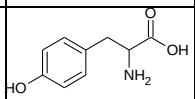
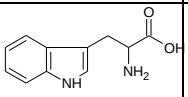
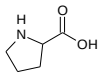
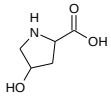
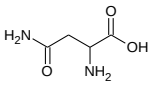
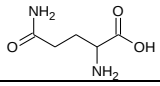
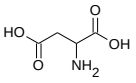
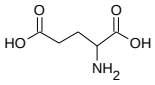
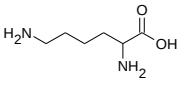
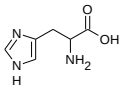
Mycosporine-ornithine [8] 	MW: 302 λ_{max} = 310 nm ε = ND
Mycosporine-ornithine (isomer) [9] 	MW: 302 λ_{max} = ND ε = ND
Mycosporine-serinol [10] 	MW: 261 λmax = 310 nm ε = 27,270 M^{-1} cm^{-1}
Mycosporine-glutamic acid [11] 	MW: 317 λ_{max} = 311 nm ε = 20,900 M^{-1} cm^{-1}

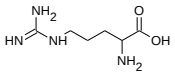
Appendix B

Classification and Molecular Structures of Amino Acids

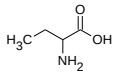
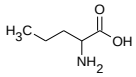
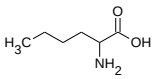
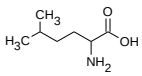
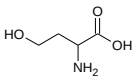
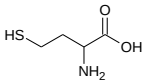
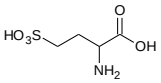
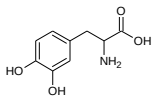
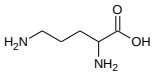
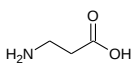
1. Proteinogenic Amino Acids

Classification		Amino acid	Abbr. 3-ltrs	Abbr. 1-ltr	Structure	MW (MF)
Neutral amino acids	Aliphatic amino acids	Glycine	Gly	G		75 ($C_2H_5NO_2$)
		Alanine	Ala	A		89 ($C_3H_7NO_2$)
		Valine	Val	V		117 ($C_5H_{11}NO_2$)
		Leucine	Leu	L		131 ($C_6H_{13}NO_2$)
		Isoleucine	Ile	I		131 ($C_2H_5NO_2$)
	Oxyamino acids	Serine	Ser	S		105 ($C_3H_7NO_3$)
		Threonine	Thr	T		119 ($C_4H_9NO_3$)
	Amino acids containing sulfur	Cysteine	Cys	C		121 ($C_3H_7NO_2S$)
		Cystine	(Cys)$_2$	–		240 ($C_6H_{12}N_2O_4S_2$)

		Methionine	Met	M		149 ($C_5H_{11}NO_2S$)
	Aromatic amino acids	Phenylalanine	Phe	F		165 ($C_9H_{11}NO_2$)
		Tyrosine	Tyr	Y		181 ($C_9H_{11}NO_3$)
		Tryptophan	Trp	W		204 ($C_{11}H_{12}N_2O_2$)
	Imino acid	Proline	Pro	P		115 ($C_5H_9NO_2$)
		Hydroxyproline	Hyp	–		131 ($C_5H_9NO_3$)
	Acid amide	Asparagine	Asn	N		132 ($C_4H_8N_2O_3$)
		Glutamine	Gln	Q		146 ($C_5H_{10}N_2O_3$)
Acidic amino acids		Aspartic acid	Asp	D		133 ($C_4H_7NO_4$)
		Glutamic Acid	Glu	E		147 ($C_5H_9NO_4$)
Basic amino acids		Lysine	Lys	K		146 ($C_6H_{14}N_2O_2$)
		Histidine	His	H		155 ($C_6H_9N_3O_2$)

	Arginine	Arg	R		174 ($C_6H_{14}N_4O_2$)

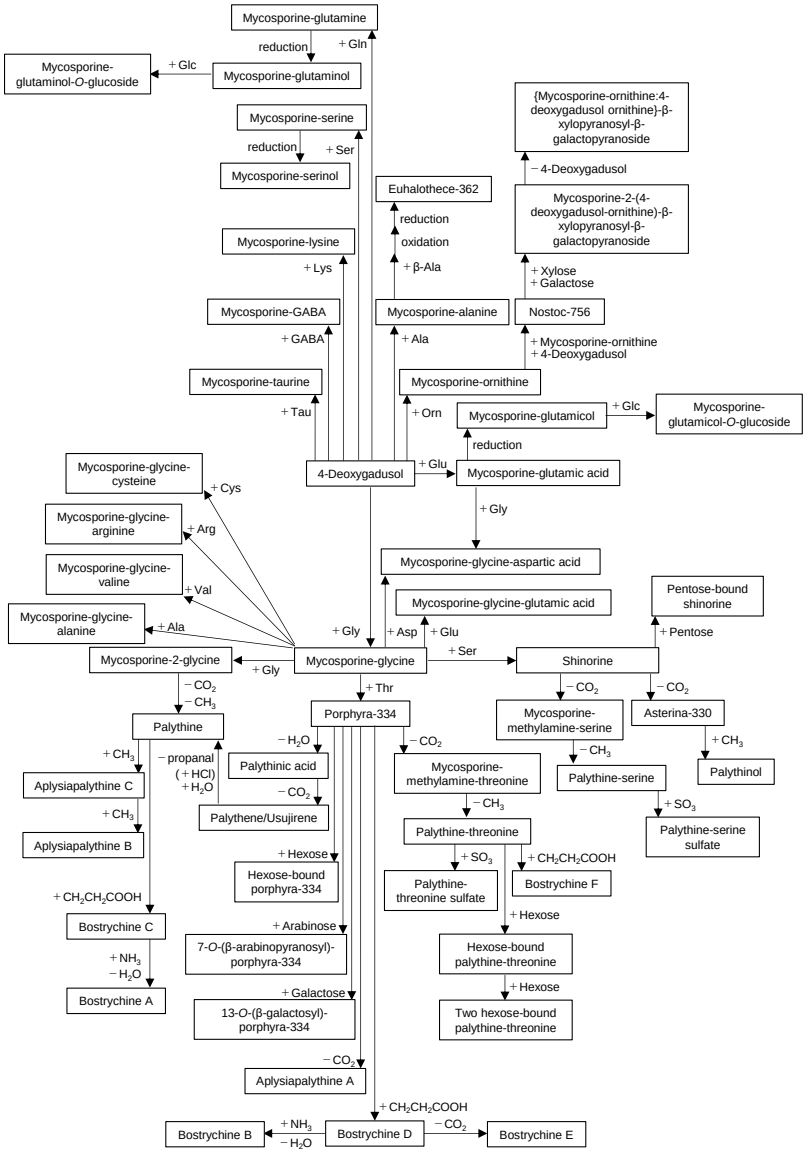
2. Representitive Amino Acids other than Protein Constituent

Classification		Amino acid	Abbr.	Structure	MW (MF)
α-Amino acids	Aliphatic amino acids	α-Aminobutyric acid	Abu		103 ($C_4H_9NO_2$)
		Norvaline	Nva		117 ($C_5H_{11}NO_2$)
		Norleucine	Nle		131 ($C_6H_{13}NO_2$)
		Homoleucine	Hle		145 ($C_7H_{15}NO_2$)
	Oxyamino acids	Homoserine	Hse		119 ($C_4H_9NO_3$)
	Amino acids containing sulfur	Homocysteine	Hcy		135 ($C_4H_9NO_2S$)
		Cysteic acid	—		169 ($C_3H_7NO_5S$)
	Aromatic amino acids	3,4-Dihydroxyphenylalanine	DOPA		197 ($C_9H_{11}NO_4$)
	Basic amino acids	Ornithine	Orn		132 ($C_5H_{11}N_2O_2$)
β-Amino acid		β-Alanine	β-Ala		89 ($C_3H_7NO_2$)

Appendix C

Correlation Diagram of the Molecular Structure of MAAs

1. Overall view



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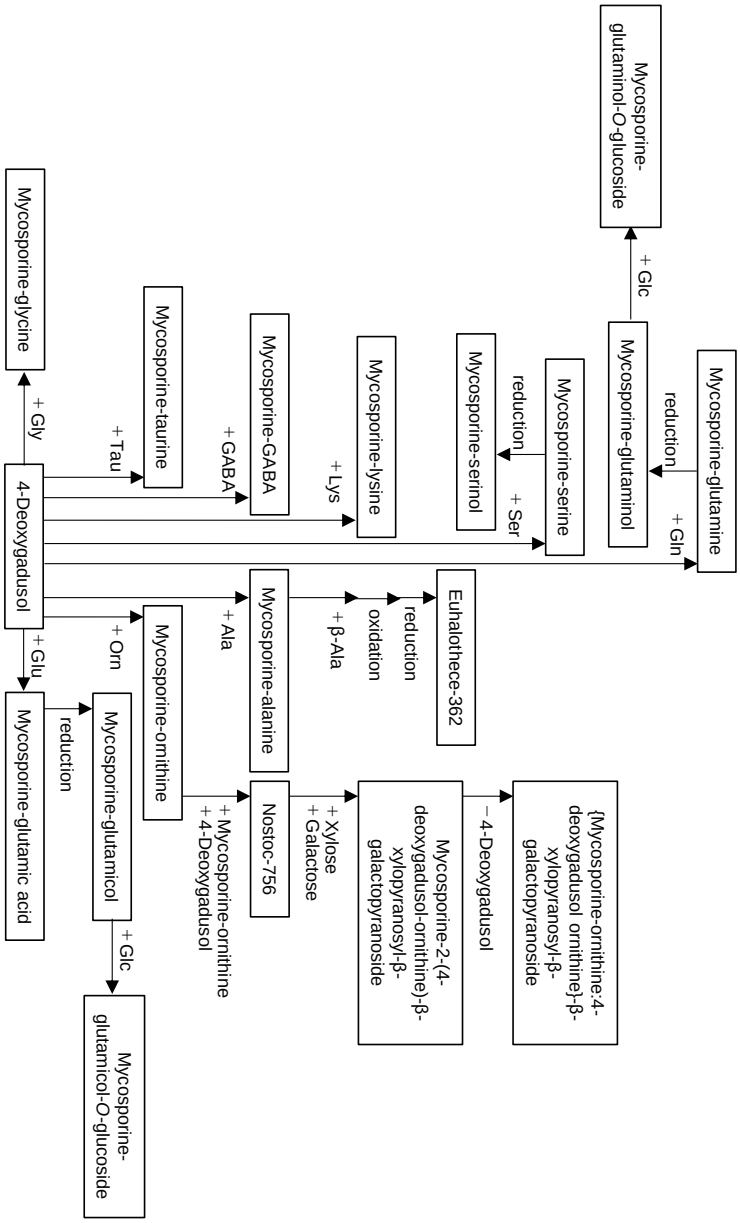
2. Correlation diagram centered on 4-deoxygadusol (bottom of the overall diagram)

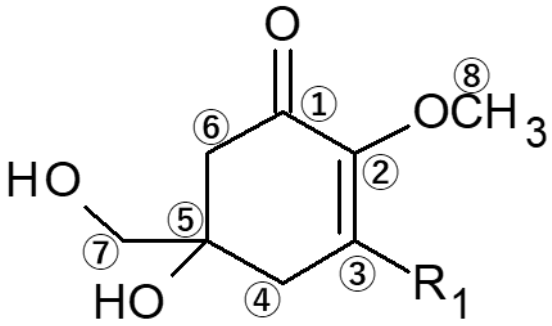
3. Correlation diagram centered on mycosporine-glycine (upper part of the overall diagram)



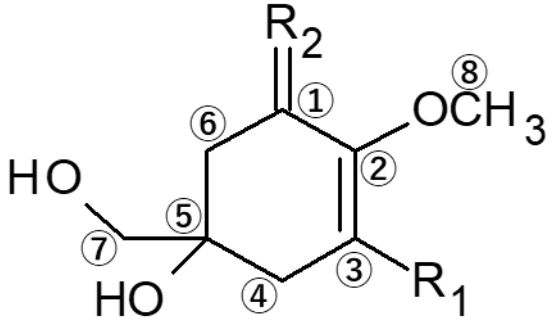
Appendix D

Position Numbering of Carbon Atoms in the Molecular Structures of MAAs

1. Cyclohexenone structure



2. Cyclohexenimine structure



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Appendix E

Outline of the Scytonemin Biosynthetic Pathway

The genes involved in scytonemin biosynthesis were first reported in 2007 [1]. A cluster of 18 genes (*NpR1276–NpR1259*) involved in the scytonemin biosynthetic pathway was identified by random transposon mutagenesis in the cyanobacterium *Nostoc punctiforme* ATCC 29133. This gene cluster was well conserved among various cyanobacterial strains [2]. Scytonemin is thought to be synthesized from derivatives of the aromatic amino acids tryptophan and tyrosine. In fact, the gene cluster identified by *N. punctiforme* included 8 genes associated with the aromatic amino acid biosynthesis pathway (the tyrosine biosynthesis gene *NpR1269* as *tyrA*; tryptophan biosynthesis genes *NpR1266*, *NpR1265*, *NpR1264*, *NpR1262*, and *NpR1261* as *trpE*, *trpC*, *trpA*, *trpB*, and *trpD*, respectively; and shikimate pathway-related genes *NpR1267* and *NpR1260* as *aroB* and *aroG*, respectively).

Fig. (1). Genes involved in scytonemin biosynthetic pathway.

In the scytonemin synthesis gene cluster of *N. punctiforme*, the six genes *NpR1276–NpR1271* are thought to catalyze the core reactions of scytonemin biosynthesis. They were named *scyA–scyF*, respectively. ScyB, which is similar to NADH-dependent oxidoreductase, was identified as an enzyme responsible for the early reaction stages of scytonemin biosynthesis. It is thought to promote oxidative deamination of tryptophan and to synthesize indole-3-pyruvic acid (I3P). I3P is one of the precursors required for the monomeric polycyclic alkaloids of scytonemin. The second precursor compound, *p*-hydroxyphenylpyruvic acid (HPP), is believed to be converted from prephenic acid by TyrA encoded by *NpR1269*. ScyA, which is similar to acetolactic acid synthase, promotes the condensation reaction between I3P and HPP, as revealed by *in vitro* experiments [3]. The product of the condensation reaction is cyclized and decarboxylated by ScyC [4]. The monomer compound thus obtained is thought to be dimerized by ScyD, ScyE, and ScyF,

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but the detailed reaction mechanism of these proteins is still unknown. Given that signal domains are present in the amino acid sequences of ScyD, ScyE, and ScyF, these proteins are thought to be present in the periplasmic space. Therefore, scytonemin biosynthesis is considered to be compartmentalized in cyanobacterial cells. That is, the synthesis of the monomer precursor compound in the early stage and the dimerization reaction in the late stage may occur in the cytoplasmic space and the periplasmic space, respectively. In addition, genes involved in the two-component signal transduction pathway (*NpR1277*/*NpR1278*) located directly upstream of the scytonemin biosynthesis gene cluster were identified, and NpR1278 was essential for scytonemin biosynthesis in *N. punctiforme* [5].

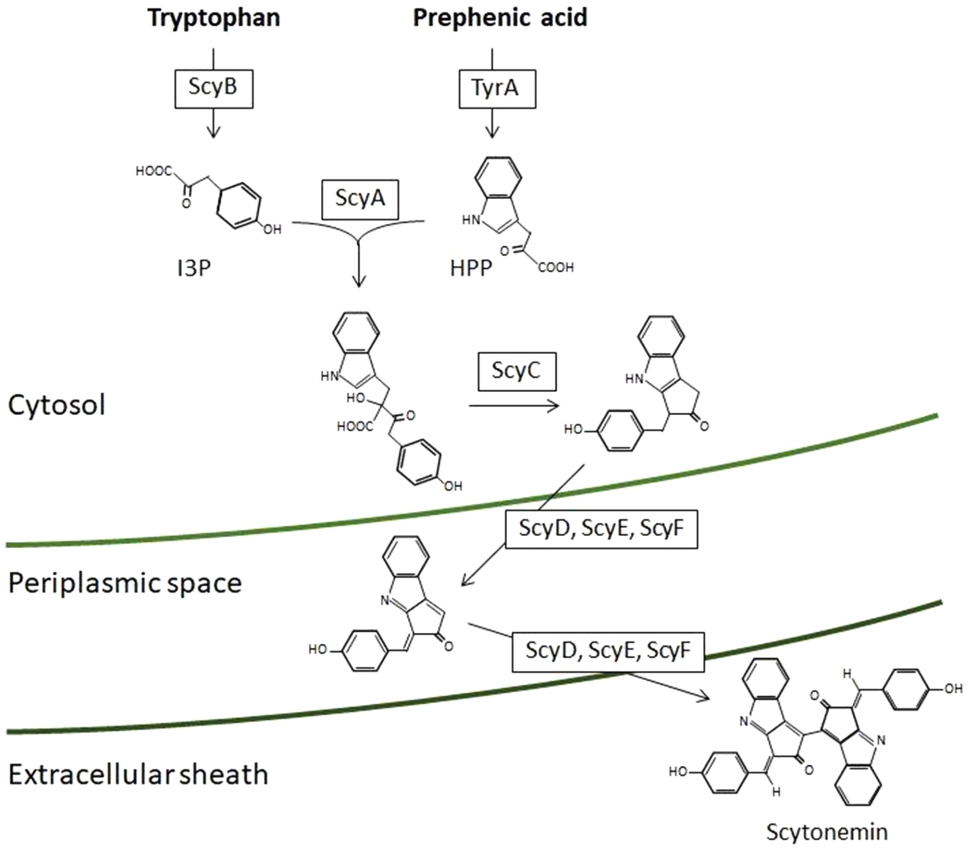

Fig. (2). Scytonemin biosynthetic pathway. See the text for an explanation.

REFERENCES

[1] Soule, T.; Stout, V.; Swingley, W. D.; Meeks, J. C.; Garcia-Pichel, F. Molecular genetics and genomic analysis of scytonemin biosynthesis in Nostoc punctiforme ATCC 29133. *J Bacteriol* **2007**, *189* (12), 4465-4472. DOI: 10.1128/JB.01816-06.

[2] Soule, T.; Palmer, K.; Gao, Q.; Potrafka, R. M.; Stout, V.; Garcia-Pichel, F. A comparative genomics approach to understanding the biosynthesis of the sunscreen scytonemin in cyanobacteria. *BMC Genomics* **2009**, *10*, 336. DOI: 10.1186/1471-2164-10-336.

[3] Balskus, E. P.; Walsh, C. T. Investigating the initial steps in the biosynthesis of cyanobacterial sunscreen scytonemin. *J Am Chem Soc* **2008**, *130* (46), 15260-15261. DOI: 10.1021/ja807192u.

[4] Balskus, E. P.; Walsh, C. T. An enzymatic cyclopentyl[b]indole formation involved in scytonemin biosynthesis. *J Am Chem Soc* **2009**, *131* (41), 14648-14649. DOI: 10.1021/ja906752u.

[5] Naurin, S.; Bennett, J.; Videau, P.; Philmus, B.; Soule, T. The response regulator Npun_F1278 is essential for scytonemin biosynthesis in the cyanobacterium Nostoc punctiforme ATCC 29133. *J Phycol* **2016**, *52* (4), 564-571. DOI: 10.1111/jpy.12414.

[44] Soule, T.; Stout, V.; Swingley, W. D.; Meeks, J. C.; Garcia-Pichel, F. Molecular genetics and genomic analysis of scytonemin biosynthesis in Nostoc punctiforme ATCC 29133. J Bacteriol 2007, 189 (12), 4465-4472. DOI: 10.1128/JB.01816-06.

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