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Intriguing Insights into Mycosporine-Like Amino Acids (MAAs), Chemical Complexity, Distinctive Identification and its Biomedical Applications

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Abstract

Mycosporine-like amino acids (MAAs), renowned for their low molecular weight and water-soluble nature, demonstrate the capability to absorb ultraviolet (UV) radiation across the wavelength spectrum spanning from 310 to 365 nm. These compounds are notably accumulated by a diverse array of organisms, including marine macroalgae, corals, and various marine life forms, as well as prokaryotic cyanobacteria and eukaryotic microorganisms such as yeasts, fungi, and microalgae. It is widely acknowledged that MAAs serve as protective agents, functioning effectively as natural sunscreens to shield against the deleterious effects of high levels of UV radiation. A growing body of evidence suggests that Mycosporine-like amino acids (MAAs) may assume diverse roles. For example, they might potentially serve as antioxidant molecules, efficiently scavenging harmful oxygen radicals. Furthermore, MAAs have been observed to amass as compatible osmolytes in response to salt stress, desiccation, or thermal stress, and in some organisms, these conditions can incite the formation of MAAs. Additionally, these compounds may function as a reservoir for cellular nitrogen. This article will delve into intriguing discoveries regarding Mycosporine-Like Amino Acids (MAAs), exploring their biomedical significance, intricate chemistry, and distinctive identification features.

Keywords: *MAAs, Biological Significance, Intricate Chemistry, and Distinctive Identification Features.*

Introduction

Mycosporine-like amino acids (MAAs) are diminutive UV-absorbing compounds characterized by a molecular weight spanning from 188 to 1050 Da. These compounds exhibit a colourless appearance and dissolve readily in water. Their distinctive chromophore, featuring an absorption peak ranging from 268 to 362 nm, comprises either a cyclohexenone or cyclohexenimine moiety linked to the nitrogen group of an amino acid or its aminoalcohol [1]. Initially dubbed as mycosporine, due to their role in fungal sporulation under light exposure, MAAs has garnered remarkable recent acclaim for their exceptional ability to absorb UV radiation.

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Beyond their established role as protective agents against UV radiation, MAAs have recently unveiled a multitude of biological functions, that encompass safeguarding life forms and their embryonic stages from the harmful effects of UV radiation, serving as antioxidants while effectively scavenging reactive oxygen species (ROS), participating in osmotic regulation, countering desiccation, and providing defence mechanisms against oxidative and thermal stresses [2] [3]. The global community of researchers, hailing from various nations, reflects the international interest in MAA research, which continues to burgeon. The multifaceted secondary metabolites also offer diverse possibilities in biomedical and commercial applications. Due to their remarkable UV-absorbing capacity, MAAs can find valuable roles in the realms of toiletries and cosmetics as both UV filters and cell division stimulators [3]. Within human skin, a limited subset of MAAs has demonstrated the remarkable capability to shield fibroblast cells from the detrimental effects of UV-induced cell death and ageing. Recent reports indicate that MAAs play a pivotal role in maintaining the skin's antioxidant defence system and fostering the expression of Hsp70. In the marketplace, tetrahydropyridine-based sunscreen products, akin to MAAs, have gained traction. Furthermore, two commercially available products, Helioguard® and Helionori®, feature MAAs extracted from the red alga *Porphyra umbilicalis*. Notably, MAAs have found application as photostabilizing additives in paints, varnishes, and polymers, serving as UV blockers [4]. This review delves into a comprehensive exploration of their biomedical relevance, intricate chemical complexity, and distinctive identification features, and gives valuable insights into these multifaceted compounds.

Historical Origins: Tracing the Roots of MAA Recognition

In the realm of terrestrial fungi, mycosporine, a type of water-soluble nitrogenous metabolites, has emerged as a key player within the realm of chemicals associated with light-induced sporulation. Casting back to 1965, an experiment involving near-UV irradiation that triggered fungal sporulation brought forth a fascinating revelation [5]. This revelation unveiled the existence of P-310, a compound with UV-absorbing properties λ_{max} at 310 nm, residing within these organisms. Leading the charge in scientific identification, the inaugural component of P-310 to be unveiled was mycosporine-serinol. This intricate molecule showcases a composite structure comprising two-methoxy-3-bis (hydroxymethyl) methylmethylamino-5-hydroxy-5-hydroxymethyl-2-cyclohexene-1-one [6]. At its core lies serinol, a counterpart of the amino acid serine intricately bound to a cyclohexenone ring within the molecular configuration of mycosporine-serinol. Mycosporine, a term denoting a class of fungal metabolites, characteristically absorbs UV light while incorporating amino acid residues instead of conventional components. Notably, within six *zooxanthellate scleractinian* corals sourced from the Great Barrier Reef, aqueous extracts yielded UV-absorbing compounds. This intriguing collection comprised five distinct *Acropora* species and one *Pocillopora* species. The UV absorption spectrum of the S-320 substance exhibited a broad peak centred around 320 nm. As time progressed, S-320 gained recognition as a subset of mycosporine derivatives, eventually being categorized as MAAs (mycosporine-like amino acids) [2]. The defining characteristic of MAAs is that the center around their core chromophore features a cyclohexenone or cyclohexenimine ring embellished with a methoxy group positioned at C2. Within the class of MAAs,

the conventional oxo or imino moiety found at the C1 location on the ring structure is often substituted, commonly replaced by an amino compound at the C3 position. Within the domain of cyanobacteria, shinorine, a significant MAA, exhibits serine and glycine attachments at the 1st and 3rd position Carbon, respectively, of the ring structure of the central chromophore [7]. To date, there are about more than 30 discerned MAAs available, encompassing a diverse array of over 30 distinct variations.

Geographical Presence of MAAs

In the natural world, MAAs exhibit widespread distribution. Since their initial identification in fungi, reports of MAAs have emerged across various microorganisms and macroorganisms, encompassing dinoflagellates, sea stars, cyanobacteria, corals, and red algae. Notably, the absence of MAA discovery in bacteria remains notable. While animals do harbour MAAs, it's understood that they don't directly generate these substances. Instead, mounting evidence suggests animals obtain MAAs either through the food chain or via symbiotic microbes. Intriguingly, the possibility of de novo MAA synthesis in coral and sea anemones stems from the homologous gene clusters that bear semblance to the synthesis genes of MAAs of cyanobacteria. Furthermore, within this group of organisms, the synthesis of gadusol, a compound akin to MAAs, was also observed. The different kinds of MAAs produced are porphyra-334, mycosporine-glycine, with shinorine being produced in most abundance, especially in the cyanobacteria [8]. The shinorine MAA, gets produced across the cyanobacterial family encompassing *Microcystis aeruginosa*, *Nodularia baltica*, *Chlorogloeopsis* PCC 6912, *N. spumigena*, *N. commune*, *Anabaena variabilis*, *Scytonema sp*., *Nodularia harveyana*, and *Anabaena doliolum*. Alongside shinorine, these organisms express other MAAs as well except in *A. variabilis* as well as *Scytonema sp*. where Shinorine is the only MAA produced. Also interestingly, when these organisms undergo UV exposure, we often can record a change in the expression level of different MAA amongst them. Following exposure to UVB radiation, *Chlorogloeopsis* accumulated both mycosporine-glycine and shinorine. In three different *Nodularia* strains, *Porphyra*-334 exhibited higher prevalence compared to shinorine, both before and after UVB irradiation. Under UV conditions, *A. doliolum* exclusively contained shinorine and porphyra-334, while mycosporine-glycine was naturally produced [9].

In the moisture-absorbing cyanobacterium, *N. commune* glycosylated MAAs are detected, featuring a mass of 1050 Da. These MAAs comprised two distinct chromophores: 3-aminocyclohexen-1-one and 1,3-diaminocyclohexen. The structures include entities like pentose-bound shinorine, poryphyra-334 and 7-O-(β-arabinopyranosyl)-porphyra-334, alongside hexose-bound derivatives of palythine and threonine. It was found that the variation in genetics among the studied *N. commune* led to this distinct MAA accumulation pattern and is expected to accumulate in its extracellular matrix. Another microorganism *M. aeruginosa* also exhibited dominant Shinorine accumulation, comprising over 95% of its total MAAs. Notably, recent findings unveiled shinorine presence in *M. aeruginosa* strain PCC 7806 [10], and no presence of porphyra-334. Other than Shinorine, MAAs that have been documented over time are palythene, asterina 330 and palythinol. A separate discovery highlighted that the halophilic cyanobacterium *Euhalothece sp*., found in the upper layer of gypsum crust within a hypersaline pond, is a producer of mycosporine-2-glycine. Moreover, *Aphanothece halophytica*, a halotolerant cyanobacterium, exhibits the production of only mycosporine-2-glycine among all the MAAs. Within *Synechocystis* sp. PCC 6803,

three distinctive MAAs have been unveiled: M-343, dehydroxylusujirene, and mycosporine-taurine. Mycosporine-tau synthesis in this cyanobacterium responded to both UVA and UVB radiation, whereas dehydroxylusujirene and M-343 production were exclusively induced by UVA exposure. Nevertheless [11] research leaves the precise nature of these unique *Synechocystis*sp. PCC 6803 MAAs somewhat uncertain. Additionally, other cyanobacterial MAAs, including those awaiting characterization, have been documented alongside these compounds.

Inherent Qualities: Delving into the Intrinsic Traits of MAAs

The MAAs, low molecular weight, secondary metabolites, are considered as "UV-screening molecule". It is widely believed that MAAs stand as the most effective natural compounds for absorbing UV-A radiation. The UV-A radiation is marked by their molar extinction coefficients (ε) spanning from 28,100 to 50,000 mol^{-1} cm^{-1}, and their absorption peaks ranging from 310 to 362 nm, distinguishing them from other radiations. To illustrate, Porphyra-334 and shinorine exhibit specific molar extinction coefficients of around 40,000 mol^{-1} cm^{-1}, respectively, at a wavelength of 334 nanometers. Upon absorbing highly potent ultraviolet radiation (UVR), MAAs demonstrate the ability to dissipate additional energy as heat, to avoid the release of reactive oxygen species (ROS) [2]. The list of organisms capable of synthesizing MAAs encompasses a range of life forms, including microalgae and macroalgae, fungi, eukaryotic phytoplankton, and cyanobacteria. Accumulation of MAAs within these organisms is believed to counteract the adverse impacts of UVR. Unlike substances like scytonemin and cyanobacterial carotenoids, MAAs also exhibit increased water solubility due to their zwitter ionic characteristics originating from amino acid substitution. This results in the gathering of MAAs as solutes within the cytosolic realm [12]. It has also been demonstrated that the cells of the worldwide terrestrial cyanobacterium *Nostoc commune* release mycosporine derivatives bound to amino acids and oligosaccharides. These components unite with the extracellular glycan sheath, forming remarkably substantial complexes through noncovalent associations. Notably, recent findings unveiled that the cyanobacterium *M. aeruginosa* strain PCC 7806, known for causing algal blooms, accumulates shinorine within its extracellular matrix.

MAA Molecular Configuration

With discoveries surrounding MAA, the structural decoding of it became very important. At the core of MAAs' structural framework are cyclohexenone or cyclohexenimine rings. One notable MAA, mycosporine-glycine, showcases an absorption peak at 310 nm and features a cyclohexenone ring where glycine, an amino acid, attaches at the C3 position. Diverse species encompass oxocarbonyl-MAAs, such as mycosporine-glycine, exemplifying monosubstituted MAAs. Recent revelations in cyanobacteria show that it produces oxocarbonyl MAAs like mycosporine-taurine and mycosporine GABA (mycosporine-γ-aminobutyric acid). In the cyanobacterial context, mycosporine-glycine plays a pivotal role as an intermediary in the biosynthesis of disubstituted MAAs like shinorine, porphyra-334, and mycosporine-2-glycine [13]. Notably, with a few exceptions, the C1 position of mycosporine-glycine tends to conjugate with other groups like amino alcohol, or a second amino acid or anenaminone system when disubstituted MAAs get synthesised. Disubstituted MAAs exhibit absorption peaks ranging from 320 to 362 nm, influenced by nitrogen substitutions [2]. Additionally, amino acid moiety of certain specific MAAs undergoes processes like dehydration, sulfonation, condensation, decarboxylation, reduction, oxidation, and

glycosylation. Also, the recent spectrum of compounds that have been decoded in *N. commune* cyanobacteria, as discussed above, hold potential, not only within the realms of cosmetics and hygiene, but also in contributing resilience against stress. Similarly, another research group uncovered MAAs, in a variety of strains of *Scytonema crispum* that are found to be glycosylated. These glycosylated derivatives were connected to hexose-bound shinorine and palythine-serine.

Environmental Impacts on the Molecular Configuration of MAAs

The absorption spectra of MAAs are known to be influenced by pH variations. Notably, porphyra-334's absorption peak experienced alterations in highly acidic solutions. At pH levels of 1-2 and 3, the absorption maximum was observed at 330nm and 332nm, respectively. These extremely acidic conditions led to a notable decrease in overall absorbance. Within such circumstances, with a pH below 3.0, the porphyra molecule undergoes protonation of the nitrogen atom, at its unbound electron pair, leading to disruption in resonance delocalization. Conversely, alkaline solutions did not impact the maximum absorption or the extinction coefficient. Recent findings have unveiled shared pH dependence in both shinorine and mycosporine-glycine [2]. The zwitter ionic characteristics of these compounds prompted protonation within their respective chromophoric amino cyclohexenone and amino cyclohexenimine components. It was affirmed that the shift in their absorption peaks at pH 1-2 occurred due to the protonation of the amino acid residue at the carboxylate anion. Moreover, there is impact of solvent properties on UV-vis spectral features is widely acknowledged. Furthermore, temperature plays a significant role in the stability of porphyra-334. Notably, as the temperature increased, there was an observable rapid decline in porphyra-334's absorbance.

MAA Regulatory Standards

The impact of various environmental variables, particularly cyanobacteria, on the intracellular MAA levels in species that accumulate them is well documented. We have investigated these effects of MAA production, especially of stress caused due to UV Radiation and other such abiotic stress, within cyanobacteria. However, the different signalling pathways that control the biosynthetic processes of the MAAs are not yet fully understood and leave room for further research.

UVR Impact on MAA Regulatory Guidelines

MAA biosynthesis is commonly stimulated by exposure to UV radiation, like the UV-A, UV-B and photosynthetically active radiation (PAR), which all are involved in boosting MAA synthesis within cyanobacteria. Across most species of cyanobacteria, the most robust induction occurred under UVB radiation. In the case of *A. variabilis* PCC 7937, when exposed to the combined radiation of PAR + UVA + UVB, the shinorine production was notably higher in cells as compared to those exposed to only one kind of those radiations. As in the case of *A. doliolum*, UVB radiation led to enhancement of the the production of shinorine, mycosporine-glycine and porphyra-334 [13].

UVB exposure led to increased shinorine synthesis in *Anabaena* sp., *N. commune*, and *Scytonema sp.*, while PAR and UVA did not show significant effects. Essential for shinorine and porphyra-334 induction, UVB radiation was observed in three *Nodularia* strains. Notably, UVB light notably enhanced mycosporine-glycine biosynthesis in *Arthrospira sp.* CU2556. Apart from cyanobacteria, other species such as macroalgae, yeast, and marine microalgae, including diatoms, dinoflagellates, and prymnesiophytes demonstrated increased MAAs production in response to UVR. The

cyanobacterium *Chlorogleopsis* PCC 6912 provided compelling documents for the existence as well as attributes of a photoreceptor-specific UVB [14]. In this study, the concept of a pterin acting as a potential photoreceptor was proposed. This idea gained credibility from the targeted reduction of MAA levels in UV-stressed cells upon the addition of N-acetylserotonin (NAS), an inhibitor of pterin production. However, the specific molecular mechanisms underlying signal transduction and the interplay between MAA production and gene expression remain uncertain. Moreover, the study unveiled that UVR exposure triggered the production of the WspA, a water stress protein, expressed in *N. commune*. It is hypothesized that WspA plays a crucial role in the overall stress response of the microorganism by altering the 3D structure of the extracellular matrix. This, in turn, could impact the transportation, distribution, and molecular arrangement of pigment complexes involved in UV absorption as well as the MAAs.

Impacts of Salt and Thermal Stressors

Beyond radiation, additional abiotic stressors have been identified for their impact on MAAs production. Notably, salt stress has been observed to trigger MAA accumulation in various cyanobacterial species. Intriguingly, both in the presence and absence of UVB exposure, NaCl has been found to stimulate the synthesis of mycosporine-glycine and shinorine in *Chlorogloeopsis*. Interestingly, the highest MAA content was attained at a concentration of 513mM NaCl. Moreover, other salts such as Na_2SO_4, KCl, and $MgCl_2$ have shown similar effects to NaCl in inducing MAAs. Noteworthy is the synergistic effect observed between the NaCl stress treatment and UV exposure in inducing shinorine in *A. variabilis* PCC 7937 [15]. Furthermore, NH4Cl has proven to have a major effect on shinorine induction.

The production of mycosporine-2-glycine was significantly increased in the halotolerant cyanobacterium *A. halophytica* when exposed to a stress condition generated by 2.0 M NaCl. Furthermore, in *A. halophytica*cells, the application of salt stress significantly increased the expression levels of all four MAA synthetic genes *(Ap3858-Ap3855)*. Recent findings have also shown that salt stress goes beyond cyanobacteria, enhancing the total concentration of MAAs in the marine dinoflagellate *Gymnodinium catenatum*. Additionally, thermal stress led to a substantial increase in MAA accumulation levels in corals like *Lobophytum compactum* and *Sinularia flexibilis*. Interestingly, during periods of elevated temperature, the cyanobacteria *Chlorogloeopsis* PCC 6912 [14] and *A.variabilis* PCC 7937 did not experience a rise in MAA accumulation.

Influences of Nutrient Accessibility

The synthesis of MAAs in cyanobacteria is influenced by the concentration of nutrients. The MAAs presence was altered in cyanobacterium *A. variabilis* PCC 7973 due to deficiency in sulphur [15]. Normally, only shinorine was found in this cyanobacterium under regular growth conditions; however, a shortage of sulfur prompted the production of the secondary MAA palythine-serine. The addition of methionine helped reduce the accumulation of palythine-serine in cultures with low sulfur levels. The production of methionine also had an impact on the conversion of mycosporine-glycine into shinorine. Moreover, the availability of nitrogen, in addition to sulfur, plays an important role in the synthesis of MAAs. The nitrogenous nature of MAAs has led to suggestions that they could serve as intracellular nitrogen storage molecules [13]. When ammonium salts were introduced, elevation in the shinorine level was observed in *A. variabilis* PCC 7973. Intriguingly, increased production of shinorine and

porphyra-344 was observed in the marine red algae *P. columbina* when exposed to a combination of ammonium and UVR. Additionally, the availability of nitrate influenced MAA production in cyanobacterium *A. halophytica*as it responded to changes in salinity. Notably, this cyanobacterium exhibited increased intracellular levels of mycosporine-2-glycine under high salinity conditions, coinciding with a rise in nitrate content.

MAA Production Pathways

The original cyanobacterial production of the genes necessary for MAA synthesis might have been an early adaptation for countering cellular stress induced by exposure to UVR. This concept is supported by various lines of evidence, and it's suggested that the shikimate pathway, known for generating aromatic amino acids, underwent conversion. A precursor in this process is 3-dehydroquinate (3-DHQ), which forms the basis of the 6 membered carbon ring present in every MAAs. This transformation leads to the creation of 4-DG (4-deoxy gadusol and gadusol from three-DHQ. Interestingly, differing research posits that sedoheptulose-7-phosphate (SH-7P), an intermediate in the pentose phosphate cycle, could be the origin of 4-DG [16]. Figure 1 demonstrates the biosynthesis pathway of MAA.

Even with experimental support for the pentose phosphate pathway, the application of inhibitors of the shikimate pathway like tyrosine and glyphosate has shown the ability to suppress the production of MAA in corals and cyanobacteria. Intriguingly, even when the gene for the enzyme cyclase-2-*epi*-5-*epi*-valiolone synthase (EVS) was removed from the cyanobacterium [17]. *Anabaena variabilis* ATCC 29413, the synthesis of shinorine persisted. These findings suggest that the shikimate pathway remains the major route for generating enough MAAs for photoprotection, while MAAs produced via the pentose phosphate pathway likely fulfill biological roles. Nonetheless, it's evident that the pentose phosphate as well as shikimate pathways are interconnected [18].

The fundamental core structure of MAAs is 4-DG, shared by both pathways [19]. Mycosporine-glycine is produced when glycine is added. A single amino acid residue, such as serine or threonine, is introduced to create di-substituted (aminocyclohexenimine-type) MAAs. This simple mono-substituted cyclohexenone-type MAA typically serves as a transitional step in the process. This process results in the development of well-known MAAs as shinorine and porphyra-334.

Encoded within this step is a protein reminiscent of D-alanyl-D-alanine ligaseprotein or NRPS (nonribosomal peptide synthase) that could play a role in MAA biosynthesis. The creation of various MAAs occurs through the modification of nitrogen substituents and associated side groups (including amidation, dehydration, esterification, decarboxylation, glycosylationhydroxylation and sulfonation). Variations in the absorption spectra of MAAs are a result of discrepancies in amino acid sidechains.

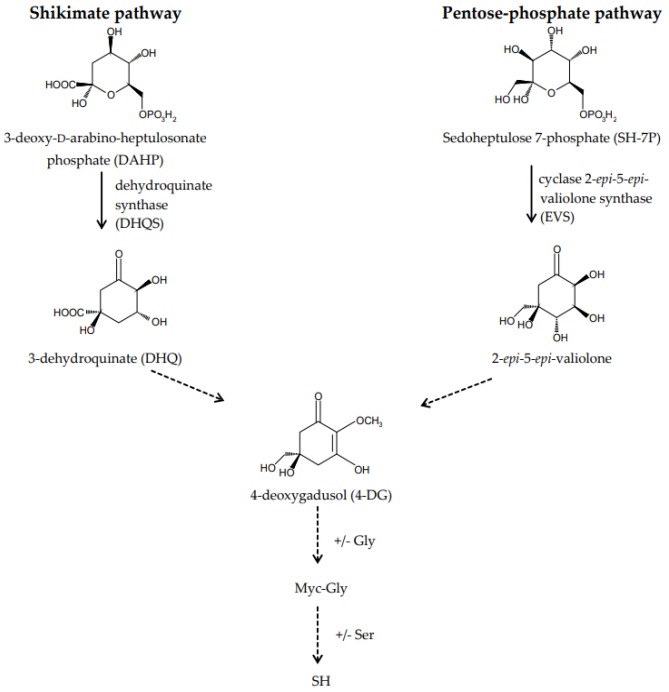

Figure 1. Biosynthesis Pathways of MAAs

Synthetic Chemistry and MAA Analogues

The motivation for addressing the challenges of the modest extraction yields from the organisms producing MAA and the extensive production necessity has fueled the pursuit of chemically synthesizing these MAAs.This endeavor has resulted in the development of a diverse array of artificially produced compounds showcasing captivating photoprotective and antioxidant attributes. Among these, tetrahydropyridine, an artificial equivalent of mycosporine-glycine, has garnered interest due to its perceived resilience against hydrolysis and oxidation, positioning it as a promising contender for incorporation into sun-protection formulations [20].

Utilizing a combination of microwave and ultrasonic processes, MAA analogues are synthesized with efficacy and ecological mindfulness. These analogues have demonstrated substantial antioxidant prowess, coupled with minimal invitro cytotoxicity and robust absorption capacities for both UVA and UVB wavelengths. Recent strides in the development of promising UV sunscreens have harnessed straightforward synthetic techniques, providing a viable reservoir for integration into commercially viable products.

Exogenous Expression of MAAs

The limited utilization of MAA in economically viable industries can be attributed to the incomplete understanding of the intricate biosynthetic pathways governing specific MAA production. A deeper understanding of these biochemical mechanisms may open the door to more efficient large-scale synthesis, particularly in the context of a heterologous bacterial host. For instance, the synthesis of shinorine within *Escherichia coli* was achieved through heterologous expression. This method led to the successful generation of 4-DG, mycosporine-lysine, mycosporine-ornithine, and mycosporine-glycine within *E. coli*. The synthesis of shinorine and mycosporine-glycine-alanine has been accomplished through heterologous expression in *Actinomycetales* through chemical means. Present-day endeavours predominantly focus on generating MAAs using genetically engineered microorganisms as a viable alternative to natural MAA production. The importance of heterologous MAA expression

is magnified by the fact that MAAs serve as the core element in next-generation sunscreens, underscoring their substantial significance within the biotechnological landscape [5].

Extraction, Characterization, and Quantification of MAAs

MAA Extraction

Harnessing the potential of MAAs as sunscreens within cosmetic products requires a strategic refinement of the extraction methodology, tailored to their distinctive physicochemical attributes and light-absorbing traits. This endeavour calls for the selection of cost-effective and environmentally friendly solvents. Conventionally, the extraction procedure involves solid/liquid interactions, encompassing a range of temperatures, conducted on either freshly collected or freeze-dried specimens. Polar solvents, such as aqueous ethanol or methanol, are favoured due to their heightened solubility in aqueous solutions. Notably, there were inconspicuous disparities observed between the extraction outcomes derived from distilled water and a 20% aqueous methanol solution.

The outcomes of their investigation revealed a reduction in the overall levels of MAAs due to the desiccation of the pellets followed by re-dissolution. They showcased a swift and efficient extraction strategy that eliminates the need for preliminary concentration steps [21]. This method exclusively employs water and volatile additives as the extracting agents, promptly channelling the extracts into High-Performance Liquid Chromatography (HPLC) for analysis. Remarkably, this extraction procedure harnesses solvents known for their benign nature, while also being straightforward to handle. Sun et al. [22] successfully extracted and identified several MAAs, including shinorine, palythine, porphyra-334, and palythenic acid, from four red macroalgae species, with first-time identification in *Bangia fusco-purpurea* and *Gracilaria sp.,* and also prepared a polythene monomer from *Gracilaria sp.,* using silica gel column chromatography and HPLC-ESI-MS spectra.

MAA Characterization

The predilect technique for the segregation and delineation of MAAs orbits around HPLC, strategically harnessing UV spectra and retention chronicles. By harnessing the formidable attenuation coefficients **(ε)** inherent to MAAs, this approach manifests heightened acumen in UV detection. However, a shortcoming resides in its susceptibility to potential interference from biosynthetic congeners, lending intricacy to the unequivocal identification of MAAs. Additionally, the scarcity of standard compounds in the commercial milieu, coupled with the paucity of global reference materials, erects impediments to confirming the structural integrity of MAAs. In the face of these challenges, LC-MS emerges as an exquisite alternative, proffering both heightened sensitivity and unparalleled discernment for a comprehensive analysis of MAAs.

A paradigm of MAAs identification has emerged, employing the harmonious integration of ultrahigh-performance liquid chromatography, adorned with diode array detection and the marvel of quadrupole time-of-flight mass spectrometry (UHPLC-DAD-QTOFMS). This symphony is orchestrated by the electrifying prowess of an electrospray ionization source (ESI), manifesting an expedited, dependable, and potent tool that can be used for the screening and identification of MAAs [5]. This virtuoso technique unfurls its canvas across a diverse spectrum of organisms - cyanobacteria, dinoflagellates, macroalgae, and microalgae - weaving a tapestry of insight. Amid the grand symphony, HPLC retains its regal stature, a trusted avenue for the finesse of MAA purification. An opus of precision is

conducted with a 0.2% (v/v) formic acid solution, entwined in the orchestration as buffer A. The crescendo unfolds upon the stage of semi-preparative HPLC-DAD, adorned by the Luna C18 (2) column, a ballet of refinement and elegance [23].

MAA Quantification

In the realm of MAA quantification, the venerable HPLC methodology has been a steadfast companion, leveraging the bedrock of molar attenuation coefficients. However, as the sun of scientific progress climbs higher, a new luminary has ascended - the harmonious union of liquid chromatography and mass spectrometry (LC-MS). This contemporary synergy not only brings heightened sensitivity but also wields the sword of selectivity, carving intricate pathways through the landscape of MAA analysis. This evolution introduces quantitative artistry, allowing the measurement of MAAs to unfurl through the prism of their molecular symphonies, the enigmatic choreography of retention times, and the melodious harmonies of UV absorption maxima. With visionary zeal, a hydrophilic interaction chromatography (HILIC)--based LC-MS technique has been crafted, showcasing the spirit of innovation dancing in step with analytical precision.

A paradigm shift in MAA analysis materialized through the creation of a rapid and quantitative LC-MS/MS method. This technique performed a precise symphony, quantifying MAAs according to their unique retention periods, molecular weights, and particular mass transitions, all skilfully performed using multiple reaction monitoring (MRM) tests. Departing from the conventional full scan technique, this approach yielded graceful linear correlation coefficients, though it left a few essential validation parameters untouched, notably the recovery and matrix effects.

To address these lingering questions, a meticulous validation journey unfolded, following the celestial navigation of ICH and EURACHEM guidelines. This voyage embarked on a thorough exploration of the validation landscape, embracing the critical checkpoints with unwavering determination: the realm of specificity, the enchantment of linearity [13], the rhythm of precision (composed of both the echoes of repeatability and the harmonies of reproducibility within the laboratory's sanctum), the melody of accuracy, the discovery of extraction recovery, the subtlety of matrix effects, and the timeless stability that grounds the endeavour. With this meticulous validation as its mantle, the method emerged as an embodiment of credibility, ready to embark on a voyage into the uncharted waters of MAA analysis with the serene assurance of its authenticity. Chaves-Peña et al. [24] demonstrated that distilled water is an effective solvent for extracting MAAs from red macroalgae, with re-dissolution in pure methanol after dryness enhancing qualitative HPLC analysis, particularly with a C8 column, identifying several MAAs, thus highlighting red macroalgae as promising, eco-friendly sources for natural sunscreens.

Moreover, a meticulous evaluation encompassed the thresholds of detection and quantification, intertwined with the expansive expanse of the operational range. Through the embodiment of this methodology, an alchemical metamorphosis unfolded, bestowing upon the method an enhanced discerning faculty and heightened perceptibility. This illustrious transformation bestowed upon the methodology an unparalleled prowess to discriminate the nuanced differentials that set apart isomeric molecules, thus elevating the echelons of analytical finesse to an unprecedented zenith.

Prospects of Mycosporine-Like Amino Acids as Photoprotective Agents

In the celestial realm of UV rays (UV-A and UV-B), MAAs serve as an exquisite

cohort of intrinsic photoprotective sentinels, unveiling their zenith in terms of absorption prowess. The noble mantle of safeguarding light's assault, donned by these MAAs, finds resolute support in a mosaic of biochemical signatures that they unfurl. Noteworthy among them, their UV-absorption crescendos paint the canvas within the 310 to 362 nm spectrum, adorned with elevated molar extinction coefficients (ε) ranging from =28,100 to 50,000 M^{-1} cm^{-1}. A testament to their mettle, they uphold their fortitude in the crucible of nature's sanctums, be it untamed waters or aquatic realms, steadfast even when in the company of photosensitizing allies [25]. With a masterstroke, MAAs deploy an artful defence, orchestrating absorbed energies into thermal reverberations, evading the siren call of reactive oxygen species (ROS). Furthermore, their tenure guards against the inception of cyclobutane pyrimidine dimers and 6-4 photoproducts (6-4PPs). The grand ballet of MAAs' protective symphony hinges on their strategic residence within the cellular domain. Amid the sunlit canvas, the symphony of photons unfolds, and in this majestic choreography, a triumvirate of every ten is intercepted by the watchful grace of MAAs, a cadre primarily domiciled within the hallowed halls of cyanobacterial domains. As the narrative takes a graceful turn, the stage shifts to the ethereal realms of albino hairless mice, their skin a canvas for empirical revelations [26]. Figure 2 showcases the photoprotective nature of MAA against skin cancer. Here, the saga unfurls with a promising prelude - the application of MAA formulations, akin to a protective elixir, yields a scene of subdued thymine dimer choreography, a ballet of defence against UV-B's relentless overtures. A palpable divergence emerges, juxtaposing the untreated with the MAA-enveloped and the UV-B-bathed counterparts. In the unfolding epic of UV-B photoreception, pterin ascends to prominence, a protagonist bedecked in biochemical intrigue. The revelation gleams in the aftermath of pterin inhibitors - 2,4-diamino-6-hydroxypyrimidine and *N*-acetylserotonin - casting shadows and veils, validating its stewardship in the orchestration of MAA's lyrical creation. The narrative's harmony lingers as a poetic interlude, encapsulating the delicate liaison between UV-B's radiant ardour and the burgeoning treasury of MAAs within their cyanobacterial sanctuaries [27]. A symphony of evidence takes centre stage, unravelling a melodic partnership where light and pigment interlace in nature's timeless minute. Babele et al. [28] demonstrated that MAAs, including shinorine, asterina-330, porphyra-334, and mycosporine-glycine, extensively contribute to photoprotection in the cyanobacterium *Microcystis aeruginosa*.

MAAs, with their notable resistance to abiotic stresses such as UVR, pH, temperatures, and various solvents, demonstrate remarkable stability. The impact of UV-B on the initiation and biosynthesis of MAAs, as well as the need for a specific photoreceptor to induce MAA synthesis, suggests that MAAs might function as effective UV sunscreens. These compounds not only protect their producers and consumers from UV radiation but also play a vital role within the food chain.

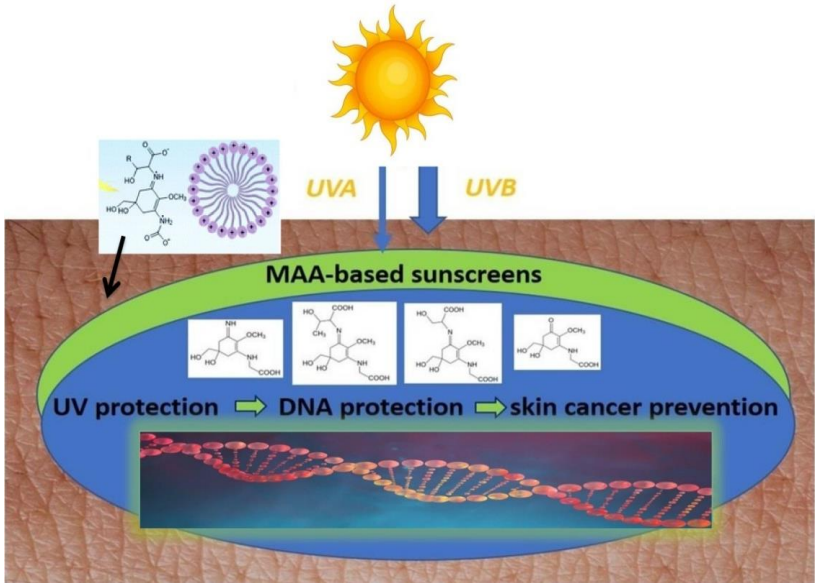

Figure 2. Mycosporine-Like Amino Acids as Photoprotective Agents Against Skin Cancer

Biomedical Applications

MAAs encompass a broad range within the UV spectrum and have recently garnered attention as a significant natural source of sunscreen and an alternative to synthetic products due to their inherent anti-ageing properties [29]. This newfound understanding could pave the way for the development of photo-protective materials, such as sunglasses and clothing, through the incorporation of these compounds into textiles and optics in the future [30]. MAAs derived from the Antarctic diatom Phaeodactylum tricornutum ICE-H, including 4-DG, shinorine, and porphyra-334, have demonstrated notable attributes. These MAA bioactive compounds displayed significant efficacy in scavenging free radicals, mitigating acute skin damage induced by UVB exposure, and reducing collagen degradation in damaged skin. The protective mechanism of MAAs appears to involve the attenuation of UVB-induced ROS generation and oxidative stress, consequently inhibiting the activation of NF-κB and MAPK signalling pathways. Hence, the MAA constituents sourced from *Phaeodactylum tricornutum* ICE-H hold significant applications in photodamage treatment and cosmetic formulations [31-34].

Future Research Priorities and Prospects

MAAs production needs to undergo meticulous refinement to make these photoprotective compounds suitable for the cosmetics industry. This necessitates the strategic allocation of resources to elevate MAA concentrations within organisms, potentially through GMOs, and the optimization of intricate MAA synthesis procedures. Diversifying production methods requires us to simultaneously prioritise investigating alternate approaches like heterologous gene expression or the chemical synthesis of MAA analogues. Furthermore, ongoing research endeavours should be intensively directed towards advancing the sophistication of isolation and purification techniques for MAAs.

To gain widespread acceptance, these approaches should prioritize straightforwardness and long-term effectiveness. It is essential to carry out comprehensive studies on novel photoprotective agents' commercial viability and potential effects on the ecosystem and human health to determine whether to use them on an industrial scale and to guarantee their safety. To diversify our production

methods, we must also prioritise researching alternative strategies such as the synthesis of MAA counterparts chemically or by heterologous gene expression.

Conclusions

Mycosporine-like amino acids (MAAs) belong to the extensive group of naturally occurring UV-absorbing compounds. These remarkable substances have been identified in over 70 different molecules, originating from diverse species across various biological phyla. In nature, cyanobacterial organisms synthesize MAAs as a defence mechanism against the harmful effects of ultraviolet radiation (UVR). MAAs possess unique attributes that make them particularly attractive for potential commercial applications, particularly in the cosmetics industry. Notably, MAAs have the exceptional ability to absorb UV radiation and dissipate it as heat without generating reactive oxygen species (ROS). Additionally, they exhibit the remarkable capacity to prevent the formation of both pyrimidine dimers and 6-4 photoproducts (6-4PPs). The versatility of MAAs extends well beyond cosmetics, offering promising opportunities in various biotechnological and industrial sectors. Their multifunctional nature allows for potential applications in medicine, dietary supplements, functional organic components, toiletries, and numerous other areas, making them a valuable resource for innovation and product development. One of the primary hurdles in the commercial or pharmaceutical development of natural sunscreens with antioxidative properties lies in ensuring the stability of these products in various commercial formulations. A breakthrough in this regard has been the revelation that to create stable sunscreens, it is essential to substitute the amino acid or amino alcohol groups found in MAAs with alkylamino groups. This study delves into the biological significance, chemical intricacy, and unique identifying characteristics of Mycosporine-Like Amino Acids (MAAs).

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Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

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