Mycosporine-Like Amino Acids for Skin Photoprotection

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Abstract: Background: Excessive human exposure to solar ultraviolet radiation (UVR) continues to be a major public health concern, with skin cancer rates increasing year on year. The major protective measure is the use of synthetic UVR filters formulated into sunscreens, but there is a growing concern that some of these chemicals cause damage to delicate marine ecosystems. One alternative is the use of biocompatible mycosporine-like amino acids (MAA), which occur naturally in a wide range of marine species. Their role within nature is mainly thought to be photoprotective. However, their potential for human photoprotection is largely understudied.

Objective: To review the role of MAA in nature and assess their potential as natural sunscreens for human skin photoprotection.

Method: A literature review of all relevant papers was conducted.

Conclusion: MAA are natural photostable compounds that are thought to offer photoprotection to marine species. Initially thought of as protective based on their absorption properties in the solar UVR spectrum, it is clear that MAA are multifunctional photoprotective compounds acting as chemical and biological anti-oxidants. This suggests that MAA may offer a novel eco-friendly approach to human skin photoprotection. Most studies have been carried out \textit{in vitro} and current data strongly suggest that MAA have potential for development as natural biocompatible sunscreens that protect against a diverse range of solar UVR induced adverse effects on human health.

Keywords: Photoprotection, mycosporine-like amino acids, skin, natural products, mechanisms, solar radiation.

1. INTRODUCTION

Exposure to terrestrial solar ultraviolet (\( \sim 295-400\text{nm} \)) and visible (\( 400-700\text{nm} \)) radiation has profound effects on all living systems. Photon energy absorbed by cellular chromophores may have beneficial or detrimental effects. The wavelength dependence (action spectrum) of a given photobiological outcome is primarily dependent on the absorption spectrum of the chromophore. The skin is the major organ exposed to solar radiation. The molecular effects of this exposure are well defined, causing either direct damage to DNA, proteins or lipids, such as in the formation of DNA photoproducts [1], and indirect damage via the generation of reactive oxygen species (ROS) [2, 3] that attack a range of molecular and cellular targets. It was initially thought that direct damage was mainly responsible for the most damage; however in recent years the importance of indirect and oxidative damage has been realised, with the generation of oxidative [4] and photosensitised [5] DNA damage, and the oxidation of proteins leading to inhibition of the DNA repair machinery [6]. The main established beneficial effect of ultraviolet radiation (UVR) exposure is the production of vitamin D in the skin.

The molecular effects of sunlight are summarised in Fig. (1). These effects can lead to sunburn in the short term, and skin cancer [7] and photoageing [8, 9] in the long term. Public health advice to prevent this damage mainly advocates the use of sunscreens, along with clothing cover and avoiding the sun at the time when exposure is strongest. Modern sunscreens are formulations that are applied to the skin [10]. They contain a range of different synthetic organic and inorganic UVR

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filters with different absorbance profiles to provide broad-spectrum protection across the solar UVR spectrum.

The organic chemical filters contain a chromophore, which is typically an aromatic molecule conjugated to carbonyl groups. In general, increasing the number of conjugated double bonds and number of resonance structures stabilizes the excited state and shifts the absorption spectrum to longer wavelengths and a larger absorption cross section – leading to UVB (280-315nm) absorbers having typically smaller molecular weights compared to UVA (315-400nm) and broad spectrum (UVB + UVA) filters [11, 12].

The energy of UVR photons lies in the same magnitude as the energy of the filters’ electrons, and allows for the absorption of the photons. Once absorbed, this causes a photochemical excitation of the electrons to a higher energy ($\pi^*$) state (excited singlet state), the electrons then return to the ground state emitting the excess energy, which can occur by a number of different processes shown in Fig. (2). The preferred route, to dissipate the energy returning the excited molecule to the ground state, is by internal conversion (IC), leading to dissipation by heat, or reversible isomerisation. When this is not possible, the remaining energy can be converted into photons as fluorescence, corresponding to the difference in energy between the two levels. This energy can be emitted in either the visible, infrared or long wave UVR region. Through intersystem crossing (ISC), the singlet excited state can cross to the triplet state, which can lead to phosphorescence or photosensitisation reactions. These reactions can result in the transfer of the excess energy to oxygen molecules to form ROS, or cause bonds to break [11]. This is highly undesirable for a sunscreen molecule; as the molecules need to be stable enough to prevent further damage rather than enhance damage.

The inorganic filters that are commonly used in sunscreen formulations are titanium dioxide (TiO$_2$) and zinc oxide (ZnO), and despite popular belief also act by absorption of UVR, with only a minimal effect by reflection or scattering. A recent study demonstrated that the average reflection range of UVR by TiO$_2$ and ZnO was around 4-5%, equating to a sun protection factor (SPF) of less than 2 [14]. The UVR is absorbed by excitation of the electrons from the valence band to the

**Fig. (1). The effects of solar radiation on the skin.** There are numerous effects of solar radiation on the skin. Some of these are positive such as the production of vitamin D, however the vast majority of these is negative. The negative effects include direct damage to molecules such as DNA and the formation of cyclobutane pyrimidine dimers (CPD) and indirect damage through photosensitisation reactions resulting in the production of reactive oxygen species (ROS). Both these routes of damage induction cause secondary effects such as DNA, protein and lipid oxidation (resulting in reduced function), differential gene and protein expression (leading to photoageing, inflammation and melanogenesis) and immunosuppression. Damage to DNA can lead to mutations and eventually cancer [13].
conduction band. These molecules do not absorb in the visible range but their particulate nature results in scattering and reflecting, which accounts for the white appearance on the skin. Clearly this is highly undesirable for a sunscreen, so these molecules are increasingly micronized to improve their cosmetic properties [11].

There is evidence that suggests sunscreens may prevent a range of UVR-induced clinical outcomes including erythema (sunburn), squamous cell carcinoma (SCC), basal cell carcinoma (BCC) [15] and photoaging [16], however this is contested [17] and their ability to prevent malignant melanoma (MM) is yet to be fully established [18, 19].

In recent years there has been a shift in consumer trends to prefer more natural products, rather than their synthetic equivalents, as they are perceived to be safer and better [20]. Additionally, in the case of sunscreens there is a growing body of evidence that suggests that the synthetic UVR filters such as octocrylene (OCT), benzophenone-4 (BP-4), ethyl 4-aminobenzoate (Et-PABA), 3-benzylidene camphor (3BC) and TiO2 nanoparticles may cause damage to the marine environment in which they are widely used. Several negative effects have been reported, including the bioaccumulation of filters in many different species [21, 22], hormonal changes and endocrine disruption in fish [23, 24], production of hydrogen peroxide [25] and the bleaching of corals [26]. The Environmental Effects Assessment Panel (EEAP), that answers to the United Nations Environment Programme (UNEP), recently expressed concern about sunscreen damage to fragile marine ecosystems [27].

2. MYCOSPORINE-LIKE AMINO ACIDS

One class of such natural compounds is the mycosporines and mycosporine-like amino acids (MAA), which are thought to provide photoprotection to marine species and terrestrial fungi. Many marine species are exposed to very high levels of solar radiation, particularly in shallow clear waters. Solar UVR can penetrate to depths of between 0.5-47m depending on the water clarity. DNA is very susceptible to UVR-induced damage and wavelengths that readily damage DNA can reach depths of 16.4m [28]. Organisms of all classes have evolved complex DNA repair mechanisms, but some have also evolved other strategies, one of which is by the biosynthesis and accumulation of UVR absorbing molecules such as MAA. These are compounds that are synthesized or acquired by the diet in a taxonomically diverse range of marine organisms, including protozoa, algae, seaweed, corals, other invertebrates and fish [29]. The first MAA were discovered in fungi species in 1965 [30] and a recent study found a terrestrial alga containing MAA [31].

The cellular concentration and type of MAA vary with species, geographical location and environment (e.g. nitrate concentration) in which they are found. There are also ways to increase the MAA content such as with irradiation with different visible light/UVR sources, and treatment with nitrate compounds. Table 1 shows some examples of the different MAA and their concentrations produced by different species, and some treatments used to increase the yield of MAA [32-39].

Fig. (2). Routes of excitation and dissipation of photochemical excitation of electrons.
Table 1. The reported MAA and total content of different species and treatments to induce production of MAA. (DW= Dry weight, PAR= Photosynthetically active radiation).

| Species                  | Reported MAA                  | Total MAA DW Content [ref] | MAA DW Content after Treatment |
|--------------------------|-------------------------------|----------------------------|-------------------------------|
| *Ulva lactuca*           | Porphyra-334                  | 0.005 mg/g [39]            | -                             |
| *Gymnogongrus antarcticus* | Shinorine                   | 0.1 mg/g [38]              | PAR + UVA + UVB > 0.9 mg/g    |
| *Pilayella Littoralis*   | Porphyra-334                  | 0.177 mg/g [39]            | -                             |
| *Kallymenia antarctica*  | Shinorine, Palythine, Asterina-330 | 0.3 mg/g [38]            | PAR + UVA + UVB > 1.6 mg/g    |
| *Palmaria decipiens*     | Shinorine, Porphyra-334 Palythine, Asterina-330 | 0.4 mg/g [38]            | PAR + UVA + UVB > 0.9 mg/g    |
| *Chondrus crispus*       | Palythine, Palythinol         | 0.470 mg/g [39]            | -                             |
| *Gracilaria tenuissiptitata* | Information not available    | 0.6 mg/g [33, 34]          | NO_3^- 0.5 mM + PAR + UVR > 4.29 mg/g |
| *Hydropuntia cornea*     | Shinorine, Porphyra-334, Palythine | 1.3 mg/g [32]            | Increase in nitrate availability > 2.25 mg/g |
| *Porphyra leucosticta*   | Shinorine, Palythine, Porphyra-334, Asterina | 6.99 mg/g [35]            | 300uM ammonium > 9.67 mg/g    |
| *Devaleraea ramentacea*  | Mycosporine-glycine, Shinorine, Porphyra-334, Palythine, Asterina, Palythinol, Palythene | 3.552mg/g [39]            | -                             |
| *Porphyra endiviformium* | Shinorine, Porphyra-334       | 4.2 mg/g [38]              | PAR + UVA > 8 mg/g            |
| *Porphyra columbina*     | Mycosporine-glycine, Shinorine, Porphyra-334, Palythine, Asterina, | 5.5 mg/ [36] (7.2 - 10.6 mg/g [37]) | PAR + UVA + UVB + NH_4Cl > 0.9 mg/g |
| *Gelidium pusillum*      | Shinorine, Porphyra-334, Palythine, Asterina, Palythinol | 6.506 mg/g [39]            | -                             |
| *Gymnogongrus griffithsi* | Shinorine, Porphyra-334, Palythine, Asterina, | 7.786 mg/g [39]            | -                             |

Research on the photoprotective potential of these compounds has largely been focused on the species in which they are produced or found, but more recently there has been more work investigating their possible role as photoprotective agents in human skin models.

In addition to their UVR absorption characteristics, MAA appear to have other properties, such as antioxidant activity. This review reports on the mechanisms involved with the photoprotective role of MAA in nature and potential application to skin photoprotection.

2.1. MAA Structure and Biosynthesis

MAA are typically small (<400 Da) colourless, water-soluble compounds, of which over 20 are currently known. They have a similar general structure based on 4-deoxygadusol, containing cyclohexenone or cyclohexenimine rings conjugated to the nitrogen substituent of an amino acid or imino alcohol. These can undergo further carboxylation or demethylation, which changes their UVR absorption properties [40]. The general structures for mycosporines and MAA are displayed in Fig. (3).

![Fig. (3). The general structures of mycosporines and MAA.](image-url)
gae, fungi and plants and is responsible for the biosynthesis of the essential aromatic amino acids phenylalanine, tyrosine and tryptophan. This pathway is not found in animals and these amino acids must be acquired by diet. It was first implicated because the addition of the radiolabeled precursor \( [U-^{14}C]3\)-dehydroquinate to the fungus \emph{Trichothecium roseum} produced labelled glutamicol. Furthermore, the cyanobacterium species \emph{Chlorogloeopsis} successfully synthesised the MAA shinorine and mycosporine-glycine when \(^{14}\text{C}\)-pyruvate was added to the culture \cite{41, 42}. The use of the shikimate pathway inhibitors glyphosate and tyrosine has also demonstrated the ability to inhibit the production of MAA in the cyanobacteria \emph{Nostoc commune} and the coral \emph{Stylophora pistillata} \cite{43, 44}.

Further investigation has also implicated the pentose phosphate pathway in MAA biosynthesis. A four-gene cluster, linked to the pentose phosphate pathway in \emph{Anabaena variabilis} ATCC 29413, was able to produce the MAA shinorine when inserted into the heterologous host \emph{E.coli} \cite{45}. The genes in this cluster have been identified as shown in Table 2.

**Table 2. The four-gene cluster found in \emph{Anabaena variabilis} ATCC 29413 linked to the pentose phosphate pathway synthesis of MAA.**

| Designation | Name                                      | Product                        |
|-------------|-------------------------------------------|--------------------------------|
| Ava_3858    | 2-epi-5-epi-valiolone synthase (EVS)      | 2-epi-5-epi-valiolone          |
| Ava_3857    | \(\beta\)-methyltransferase (OMT)         | 4-deoxygadusol                 |
| Ava_3856    | ATP-grasp amino acid ligase                | Mycosporine-glycine            |
| Ava_3855    | NRP-like synthase                         | Shinorine                      |

This finding has also been confirmed in another cyanobacterium \emph{N. punctiforme}, which shares homologues of the first three genes found in \emph{A. variabilis} (NpR5600, NpR5599 and NpR5598), and produced mycosporine-glycine after treatment with the 2-epi-5-epi-valiolone precursor sedoheptulose 7-phosphate (SH-7P) \cite{46}. The incubation of the first two proteins of this cluster (NpR5600 and NpR5599) with SH-7P has also demonstrated the production of 4-deoxygadusol \cite{46}. Typically the EVS gene is found in genome mining of species that produce MAA and is absent in those without this ability \cite{47, 48}. There is however one known exception, in \emph{Synechocystis sp. PCC6803}, which lacks EVS but produces three novel MAA after exposure to UVR. These are mycosporine-
tau, dehydroxylusujirene and M-343 \cite{49} and suggests an alternative pathway to MAA in this cyanobacterium.

Despite experimental data that support the pentose phosphate pathway, there is an evidence that this is not the major route of MAA synthesis. \emph{A. variabilis} ATCC 29413 still produced shinorine, at levels equivalent to the wild type exposed to UVR, after a deletion of the gene encoding the enzyme EVS. This eliminates the role of the pentose phosphate pathway as the only mechanism for MAA synthesis \cite{50}. Another proteomic study of the same cyanobacterium found that UVA exposure led to an increase in expression of the enzymes DAHPS and DHQS (part of the shikimate pathway) after irradiation. There was no increase in any enzymes associated with the pentose phosphate pathway, and when shikimate inhibitors were used there was only minimal shinorine produced, suggesting any activity from the pentose phosphate pathways was minimal. Overall, this implies the shikimate route of production as the most predominant for MAA synthesis in sufficient quantities to provide photoprotection. This is confirmed in studies with shikimate inhibitors, which found expression of shinorine after UVR exposure was very low, at levels equivalent to no exposure \cite{51}. Quantities of MAA produced by the pentose phosphate pathway are likely to have other biological functions, for example in \emph{Anabaena} there is evidence of a possible phycobilisome trimming role \cite{50}.

There are however clear links between the pentose phosphate and shikimate pathways. SH-7P, an intermediate of the pentose phosphate pathway is easily converted to the shikimate intermediate erythrose-4-phosphate by a transaldolase enzyme. The enzymes 3-dehydroquinate synthase (DHQS), from the shikimate pathway, and EVS, from the pentose phosphate pathway, are both part of the sugar phosphate cyclase family of enzymes. These enzymes have also remarkably similar amino acid sequences and carry out very similar reactions \cite{52}. A knockout of the gene encoding the enzyme O-methyltransferase (OMT) (linked to the pentose phosphate pathway) in \emph{A. variabilis} ATCC 29413 completely prevented shinorine synthesis \cite{51} implying that both pathways must be linked at this point.

Evaluating this evidence, one proposed scheme for MAA synthesis is that SH-7P of the pentose phosphate pathway is fed into the shikimate pathway to form erythrose-4-phosphate (which is also formed from the earlier stages of the shikimate pathway). This then reacts with phosphoenolpyruvate (PEP) to form 3-deoxy-D-arabinoheptulosinate phosphate (DAHP) and 3-dehydroquinate (DHQ). This explains the upregulation
of the enzymes DAHPS and DHQS. The involvement of OMT implies that DHQ cannot be the direct precursor of 4-deoxygadusol, and it must feed back into the pentose phosphate pathway and undergo conversion to an intermediate (by an unidentified enzyme(s)), which is then converted by OMT into 4-deoxygadusol. A suggested biosynthesis scheme is depicted in (Fig. 4), which incorporates both pathways.

The synthesis of mycosporine-glycine from 4–deoxygadusol has been shown to occur via ligase, and shinorine through NRP-like synthase of mycosporine-glycine. The biosynthesis routes of many other MAA are yet to be established [53]. However, a new five-gene cluster has recently been discovered in the soil dwelling cyanobacteria *Cylindrospermum stagnale* sp. PCC 7417, which when cloned into *E. coli* produced mycosporine-ornithine and mycosporine-lysine, giving insight into the synthesis of other MAA and a possible route to large scale production [54].

3. PHOTOPROTECTIVE ROLE OF MAA IN NATURE

3.1. Structural Evidence for Photoprotection

The UVR protective properties of MAA are largely inferred from their absorption spectra and high molar extinction coefficients. Typically they have a peak absorption wavelength (λ_max) between 268-362nm, covering much of the spectral range of UVR (~295-400nm) that reaches the earth’s surface [29].

The photochemistry of MAA is poorly understood, with only a select few compounds having been investigated. The MAA that have been most studied are porphyra-334, shinorine and mycosporine-glycine. The photo-excited states of these molecules have been shown to relax by intersystem crossing from the singlet excited state to the triplet excited state and by subsequent non-radiative decay, resulting in a controlled dissipation of the energy as heat without the production of ROS [55]. Porphyra-334 and shinorine dissipate 96-98% of absorbed energy in this way [56, 57]. This pathway is consistent with the strong photostability of MAA. Palythine in particular has been shown to be extremely photostable in a saturated aqueous solutions [55], as well in the presence of seawater and the strong photosensitising agents riboflavin and rose Bengal [58]. The increased photostability of palythine over other MAA (such as shinorine and asterina-330) has been attributed to the substitution of the nitrogen atom (R1=H), in relation to the geometrical isomerisation around the C=N double bond [55]. Displayed in (Fig. 5) are data generated demonstrating the photostability of MAA as (a) a mixture and (b) palythine as a single molecule. Both examples demonstrate excellent photostability, with palythine as a single molecule demonstrating only a 3% degradation after an exposure
of 40 standard erythema doses (SED) of solar simulated radiation (SSR), equivalent to around a full day of UK summer sun on an unshaded surface (a dose of around 60J/cm²). Many synthetic filters rely upon additional filters to provide photostability; this demonstrates that this is not necessary with MAA.

MAA appear to be preferentially accumulated in tissues that receive the greatest UVR exposure – the epidermis of coral reef holothuroids, sea urchin eggs and the eggs and lenses of freshwater and marine teleosts [29, 59, 60]. In other circumstances, such as with corals, the MAA are transferred through symbiosis with algae (e.g. zooanthellae). In this relationship, the host also receives organic carbon in the form of carbohydrates, lipids and amino acids and the symbiont is nourished by the host’s waste products such as nitrogen, phosphorus, sulphur and carbon dioxide for photosynthesis [61, 62].

The MAA concentration of a species is directly related with its UVR exposure level that is dependent on latitude and altitude. Zooplankton sampled from lakes at different altitudes showed increasing MAA concentration with increasing altitude [63]. Species found in tropical waters have a greater concentration of MAA than those found in cooler climates. High levels of MAA are also found in species in the Antarctic Ocean, possibly due to high irradiances from the ozone layer hole [64]. Species show seasonal variation in MAA concentration. In winter months, they have a lower concentration compared to summer months when UVR exposure is highest as demonstrated in plankton growing in lake environments [65, 66]. There is also a strong negative correlation between the coral depth and MAA concentration that reflects the attenuation of UVR by water. This has been demonstrated experimentally by Dunlap et al, who relocated corals, with low MAA concentration, from deep to shallow waters [67]. This resulted in an increased in MAA content that supports a role in photoprotection. An increase in extracellular nitrogen concentration has also demonstrated an increase in MAA production, suggesting a potential role in intracellular nitrogen storage [68].

### 3.3. Biological Evidence for Photoprotection

The role of MAA in protecting marine species from UVR damage is a widely researched area. One study has shown an inverse relationship between the production of DNA photolesions, especially the cyclobutane pyrimidine dimer (CPD), and total MAA concentration in the coral *Montipora verrucosa* (found in Kaneohe Bay, Oahu, Hawaii) which contains mycosporine-glycine, shinorine and porpyra-334 [69]. Reduction of CPD and 6,4 pyridimine-pyrimidone photoproducts by MAA has also been demonstrated, acting by attenuating the UVR before it reaches critical cellular targets in addition to their quenching effects (discussed in DNA Damage section) the photo-excited state thymine base
Photoprotection has also been demonstrated in green sea urchin embryos by preventing UVB induced abnormalities [71, 72]. There are numerous studies investigating the effect of increased MAA content on UVR resistance for a range of species and environmental stressors such as UVR and desiccation [73-77].

Initially it was thought that MAA acted solely by absorbing UVR before it could reach the critical cellular targets, but they also appear to have antioxidant properties. This is an extremely desirable characteristic for a photoprotective molecule, as much of the damaging effect of UVR is due to ROS. This has been demonstrated with different MAA from a large variety of species [78-81]. MAA have also been shown to block specific consequences of oxidative damage preventing lipid peroxidation and superoxide radicals [82]. An extensive review of MAA antioxidant abilities has been carried out by Wada et al [83].

### 3.4 Additional Protective Roles of MAA in Nature

Apart from their photoprotective properties, MAA exhibit additional protective effects, particularly to other environmental stressors. These roles are summarised below and are reviewed in more detail by Oren and Gunde-Cimerman [77].

#### 3.4.1. Osmotic Stress

One such stressor the MAA appear to have an action against is osmotic stress, where a change in the solute concentration surrounding an organism, causes a loss or gain of cellular solvents. One halotolerant unicellular cyanobacterium, inhabiting in a gypsum crust in a hypersaline saltern pond, has an extremely high concentration of MAA (≥98mM), accounting for >3% of its mass. A reduction of the salinity of its surroundings was accompanied with a rapid expulsion of MAA, suggesting a role in osmotic stabilisation [84]. This hypothesis has since been investigated and the role of MAA in prevention from osmotic stress supported [77, 85-88].

#### 3.4.2. Desiccation Stress

There is also evidence that MAA can protect against desiccation. Cyanobacteria under desiccation stress contain high concentrations of water stress protein (WSpA) and MAA in a 1:1 ratio (around 4-5% of dry mass each), along with other compounds including scyttonemin (another UVR filter), superoxide dismutase and glycan. This group of compounds is thought to act by modifying the structure of the extracellular matrix. Upon rehydration there is an expulsion of MAA. Overall, this supports a role for MAA comparable to that for osmotic stress [76]. Another study found that cyanobacteria experimentally stressed by desiccation increased their total MAA content. When these pre-stressed cells were placed under desiccation conditions, they had better viability compared to control cyanobacteria (with a lower MAA content) [73]. Many different bacteria have shown this property in a range of environments [77].

#### 3.4.3. Thermal Stress

In the above mentioned desiccation study, cyanobacterium survival was also measured under different temperatures. Pre-stressed cells had a higher survival rate than the controls between -20°C and 40°C but this difference was lost at 50°C [73]. There are also other examples of thermal stress protection by an increase in MAA production in a range of species [77].

#### 3.4.4. Photosynthesis Accessory Pigments

There are other reported properties of MAA but these are much less researched. There is evidence that porphyra-334 may act as a photosynthetic accessory pigment due its UVA absorption and subsequent production of small amounts of fluorescence in the Soret band of chlorophyll. This has been debated due to the relatively low amount of fluorescence that is produced in this way and that MAA are produced in environments of significant irradiance, suggesting photosynthetic wavelengths are in abundance [77, 89].

### 4. PHOTOPROTECTION OF THE SKIN

Despite the evidence that MAA are prime candidates for use as biocompatible photoprotective molecules for human use, there has been surprisingly little work carried out in skin models to demonstrate potential for human use. The reported effects in skin models are described below.

#### 4.1. Cell Viability/Proliferation

Cell viability and toxicity are critical endpoints for MAA assessment. One study, done according to the International Organization for Standardization (ISO) ‘extracted media’ recommended short-term toxicity assay (ISO 10993-12), showed no toxicity of MAA including shinorine, porphyra-334 and mycosporine-glycine in murine fibroblasts. This was confirmed in a second longer-term direct incubation assay in the same cell line. After 14 and 21 days of incubation, with the different MAA, there was no significant toxicity and only minor effects on cell morphology for some of the
MAA tested [90]. The same three MAA were shown to be non-toxic in human TIG-114 lung fibroblast cells at concentrations between 0-100μM at 48 hours, and actually increased cell proliferation [91]; an effect confirmed in by Kim et al studying cell viability [92]. Porphyra-334 has also shown no effect on cell viability of human skin fibroblasts at concentrations up to 200μM [93]. In contrast with these findings, is work by Choi et al who found shinorine, porphyra-334 and mycosporine-glycine all significantly reduced cell viability in HaCaT keratinocytes, to differing extents, at concentrations from 0.1-mg/ml (around 0.301mM for shinorine) and above [94]. As mentioned MAA have demonstrated cell proliferation properties and have potential wound healing applications [94].

There are several studies that show that MAA prevent UVR induced toxicity. This protective effect has also been demonstrated in other MAA such as collem A (a compound with a structure related to MAA), where UVB exposure of HaCat keratinocytes through a collem A coated quartz plate produced an increase in cell viability, demonstrating a filtering effect [95]. Post 20J/cm² UVA exposure, application of porphyra-334 at concentrations 10-40μM to skin fibroblasts also prevented reduction in cell viability and induction of senescence [93], confirmed with UVB irradiation with a greater effect with increasing MAA concentration [91]. Application of MAA post exposure contributing to increased cell viability compared to control suggests a significant effect outside of UVR filtering.

4.2. Oxidative Stress

Oxidative stress is a major consequence of UVR exposure [2, 3]. This results from photosensitization reactions, which can produce highly reactive molecules (such ROS). UVR-induced ROS may also be generated post-UVR exposure [96]. As previously mentioned, oxidative DNA damage can lead to mutations, and recently oxidative damage to proteins has shown to inhibit DNA repair, exacerbating the effect of UVR induced DNA damage [6].

Many studies using non-biological chemical assays have shown that MAA are antioxidants [78, 81, 97, 98]. A DPPH radical scavenging assay demonstrated that mycosporine-glycine had significant, dose dependent radical scavenging ability, but that porphyra-334 and shinorine had no effect. The authors concluded that this was because mycosporine-glycine has an oxocarbonyl structure whereas porphyra-334 and shinorine have an imino structure [99]. However, many other chemical and biological studies have reported that porphyra-334 and shinorine have antioxidant properties, and it is possible that MAA act in multiple ways.

Studies have also been carried out in biological models. Porphyra-334, the most widely studied MAA, has also demonstrated a dose dependent reduction in oxidative stress in skin fibroblasts, when added post exposure, again suggesting antioxidant capability [93]. These studies measured oxidative stress immediately post irradiation suggesting a ROS quenching role of MAA. The results from the biological and chemical assays are not always in agreement suggesting the further investigation is required to elucidate the mechanism of the anti-oxidant effects. Catalase and superoxide dismutase (SOD) showed reduced post-irradiation activity over time in unprotected mouse skin. However, the application of a reference sunscreen, or a porphyra-334 and shinorine formulation offered complete protection, along with a decrease in the expression of 70 kilodalton heat shock (stress) protein (Hsp70) [100]. For the most part, studies demonstrate that MAA efficiently prevent oxidative stress though filtering, direct and indirect quenching mechanisms, however the exact mechanism are yet to be elucidated.

4.3. NRF-2 Activation

Closely linked to prevention of environmental damage to the skin is the Kelch-like ECH-associated protein 1 (Keap1) and nuclear factor erythroid-2-related factor 2 (Nrf2) complex. Under conditions of stress (particularly oxidative stress), this complex dissociates to release Nrf2 which subsequently binds to the antioxidant response element (ARE), leading to the transcription of over 200 cytoprotective genes linked to DNA repair, inflammation anti-oxidant response (among others). This is an area of emerging interest for photoprotection, using Nrf2 activators to boost the skin’s natural responses to UVR damage [101, 102].

Recently, a bioinformatics based protein modelling and virtual screening approach has been applied to investigate potential compounds that interact with Keap1-Nrf2 complex. This approach identified 75 promising compounds that activated Nrf2, of which 25 were experimentally known to be potent activators. Eleven of these compounds were known to have antioxidant activities but had not been previously linked to Nrf2 activation, of which three were MAA: mycosporine-glycine, mycosporine-glycine-valine and porphyra-334 [103]. This in silico approach has been confirmed experimentally with porphyra-334, which demonstrated potent Nrf2 activation activity through the prevention of UVA induced markers of inflammation.
and cell death. Skin fibroblasts were incubated with increasing concentrations of porphyra-334 (0-40μM) after UVA irradiation. This resulted in a significant reduction of gene and protein expression of IL-6, IL-1β, TNF-α and nuclear expression of NF-κB. In addition there was sustained Nrf2 activation, leading to the expression of a number of cytoprotective genes such as (HMOX-1), glutathione (GSH) and NAD(P)H dehydrogenase [quinone] 1 (NQO1) and the direct scavenging of reactive oxidative entities and their conversion to less harmful and inert products [104].

4.4. Accumulation in Human Skin Models

A key property of MAA is their accumulation in the food chain in marine species. This is a poorly researched area in non-marine species. In one study, investigators fed SKH-1 hairless mice a standard daily diet or the same diet with a freeze-dried red alga that contained a mixture of MAA that was known to accumulate in medaka fish. They found no MAA accumulation in the eyes, skin or liver after 14-130 days, apart from small amounts in the small intestine, suggesting no route for accumulation in mammals [60]. As part of the same study, the uptake of the MAA shinorine by human skin cancer A431 cells was also investigated. A dose dependent increase in shinorine (1-1.5mM) was observed after 48 hours of incubation, but saturation occurred at concentrations from 1.5-2.5mM. Raman confocal spectroscopy has shown that MAA incorporated into polymer gels applied to the skin in vivo penetrate and accumulate at depths of 2μm in the Stratum corneum at a concentration 103.4% higher than at the surface. These results suggest that MAA may accumulate in the skin, if not through the diet but further research is needed [105].

4.5. DNA Damage/Erythema

It is generally accepted that the most damaging consequence of UVR exposure to the skin is the formation of DNA photoproducts, which can subsequently lead to genomic mutations [106]. The CPD is the predominant and most important photoproduct induced by both UVA and UVB radiation, however oxidative photoproducts such as 8-oxo-7,8-dihydroguanine (8-oxoGua) are proving to be of increasing importance [107]. Closely related to the formation of DNA photoproducts, particularly the CPD, is the development of erythema in the skin, with DNA absorption and erythema sharing very similar action spectra [1].

In terms of photoprotection, the most widely used metric of the efficacy of sunscreen products is the SPF, which is a measure of their ability to prevent erythema (and presumed causal DNA damage). Despite this, the investigation of MAA to prevent DNA damage and/or erythema in human models is hugely under-researched.

Collemin A significantly reduced UVB-induced CPD in HaCat human keratinocytes cells in vitro compared to an irradiated control [95]. In the same study, a crude formation of collemin A was made by mixing with olive oil and then applied to the skin (6μg/cm²) of one volunteer. This formulation was estimated to have an SPF of at least 4. Little can be concluded from this pilot study other than it requires confirmation [95]. A more robust study in SKH-1 hairless mice, with a galenic formulation of 2% porphyra-334 and shinorine (ratio of 88:12) applied to the dorsal skin, prevented solar simulated UVR induced erythema, stratum corneum thickening, edema and sun burn cell formation (apoptosis) comparable with a reference sunscreen (the reference sunscreen is the standard used in sunscreen testing according to COLIPA guidelines). The calculated SPF was 3.71±0.78 [100]. One criticism of this study is the formulation was applied at a thickness of 4mg/cm², double the thickness at which sunscreens are tested suggesting that the real SPF would be at least half of this value, and used at a concentration significantly thicker than sunscreens are typically applied in real life situations [108].

Studies in a chemical model have shown that UVR-induced CPD can be inhibited by an MAA extract containing porphyra-334, shinorine and palythine. Thymine monomers were irradiated through the MAA extract with, no direct contact (in manner similar to a sunscreen application to the surface of the skin), and also irradiated with the extract and monomers mixed together. There was a greater protective effect in the mixed samples than those with no contact, suggesting an effect beyond the absorption properties of MAA. Further investigation established this was through quenching of the triplet state of UVR-excited thymine [70]. This shows that MAA may have even greater potential for photoprotection over current filters, especially with the recent discovery of delayed (also known as ‘dark’) CPD formation, which suggests that CPD can form for hours after exposure through a triplet photexcitation mechanism [5].

4.6. Inflammation

The ability of MAA to inhibit biomarkers of skin inflammation is poorly studied. Over expression of these markers is linked to a range of inflammatory skin con-
ditions such as psoriasis. Expression of cyclooxygenase-2 (COX-2) mRNA, widely associated with inflammation, was also prevented by topical application of mycosporine-glycine to HaCat keratinocytes at the highest concentration tested (0.3mM) and with only at the lowest concentration of shinorine (0.03mM) having a statistically significant effect (questioning the validity of the result), and with porphyra-334 having no effect [99]. This used a broad-spectrum UVR source and the results could possibly be explained by the peak absorbance of each of the MAA, with mycosporine-glycine (λmax =310nm) and shinorine (λmax =334nm) absorbing in shorter wavelengths and porphyra-334 (λmax =344nm) absorbing at slightly longer wavelengths, suggesting COX-2 expression is linked to shorter wave UVR, however the lack of dose-response relationship is unclear.

4.7. Photoageing

Skin photoageing is a consequence of long-term solar UVR exposure. This is different from chronological skin ageing and is associated with deep wrinkles and sagging. It is generally accepted that photoageing is the consequence of UVR induced activation of a group of proteins known as the matrix metalloproteinases (MMPs), which degrade the structural extracellular matrix proteins of the dermis such as elastins and collagens [9].

The incubation of fibroblasts with porphyra-334 (0-40μM) after UVA exposure inhibited MMP-1 and MMP-8 gene expression, but had no effect on MMP-13. Elastase activity was dose dependently reduced by porphyra-334, with an increase in collagen and elastin mRNA and protein expression, and procollagen secretion [93]. Shinorine, porphyra-334, and palythine significantly inhibited MMP-2 activity in an in vitro fluorogenic assay, which was hypothesised to be due to competitive inhibition by binding to the active site determined using computer modelling [109].

In addition to their photoprotective properties, mycosporine-glycine, porphyra-334 and shinorine have been shown to be procollagen C proteinase enhancers (PCOLCE) and induced elastin mRNA upregulation in a largely dose dependent manner after UVA exposure, whereas only porphyra-334 showed an upregulation of involucrin, another skin protein [99].

Overall, relatively limited data suggest that MAA have multiple actions in the prevention of photoageing.

4.8. Potential Human Use of MAA

The evidence reviewed above demonstrates that MAA have huge photoprotection potential in traditional optical ways as well as in with new photomolecular strategies. Many studies have suggested the widespread use of MAA as sunscreens [110-113], however they have yet to been be exploited on a large scale, with only a few products currently available. One MAA product currently available called Helioguard 365, which contains MAA porphyra-334 and shinorine (11.5:1 ratio) extracted from the red alga Porphyra umbilicalis [114]. This product however mainly provides protection in UVA region with minimal protection in the more damaging UVB range, and the final concentration of MAA in the product is extremely small when compared to the concentration of UVR filters in most sunscreen products. One product contains a final MAA concentration of 0.0005%, whereas most sunscreen formulations contain filters at 0.5-10% w/v. This suggests the addition of a very low MAA concentration to a formulation will have a negligible influence on the SPF claims of the product.

There are numerous reasons for the lack of widespread use of MAA, one of which is the poor understanding of biosynthesis pathways involved to make specific MAA in an industrially economic manner. This makes the production process more complex, for example the need to farm vast amounts of seaweed. Further understanding of these pathways could lead to easier large-scale commercial biosynthesis, for example in a heterologous bacterial host e.g. E. coli, which is easier to cultivate. The chiral centres of MAA compounds make them highly difficult to synthesis chemically; again meaning large-scale synthesis is difficult, with unrealistic costs associated to production. One way that has been proposed to overcome this issue is via the synthesis of ‘MAA-like’ compounds, which are structurally similar and retain the chromophore of MAAs, but are simpler and cheaper to synthesize [115].

MAAs are highly water soluble, which makes it more difficult to formulate sunscreens intended for beach use. Water-soluble filters would however be much better for day-care products, because aqueous formulations have better sensorial properties than those based on oils. These properties improve compliance and therefore photoprotection. It is important to note MAA photostability would also have to be assessed in sunscreen formulations because photostability may vary with solvent.

Finally, the European Chemicals Agency (ECHA) has recently responded to environmental and human health concerns about some sunscreen filters by adding them to its Community Rolling Action Plan (CoRAP)
list that includes 8/16 UVR filters that are commonly used in European sunscreen formulations [116]. These concerns, especially for marine environments, support the development of spectrally equivalent MAA as alternative biocompatible sunscreens. Fig. (6) shows the similarity of the relative UVR absorbance spectra of 4 four CoRAP filters: (a) isoamyl p-methoxycinnamate, (b) octocrylene, (c) ethylhexyl methoxycinnamate and (d) diethylhexyl butamido triazone with those of palythine, shinorine and porphyra-334.

CONCLUSION

MAA are natural compounds that are thought to offer photoprotection to marine species. Initially thought of as protective based on their absorption properties in the solar UVR region, it is clear that they have additional activities, some of which may be also useful after exposure to UVR, such as anti-oxidant capacity and Nrf2 activation. This suggests that MAA offer a novel approach to photoprotection, which is usually focused on attenuation of UVR before it reaches cellular targets. Most MAA studies have been in vitro and concentrated on porphyra-334, shinorine and mycosporine-glycine, as these are some of the most abundant in nature, but systematic in silico and in vitro screening of all MAA may identify other compounds. Current in vitro data strongly suggest that MAA have potential for the protection of human skin from a diverse range of adverse effects of solar UVR.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare the following interests: The results of our research into MAA compounds are subject of a patent application by King’s College London, UK (PCT/GB2016/052227).

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