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Mycosporine-like Amino Acids as Natural Scavengers of Singlet Oxygen in Marine Organisms: Photoprotection of Biological Systems

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This report concerns a putative role of mycosporine-like amino acids (MAA) as natural scavengers of singlet oxygen ($^1O_2$) in marine organisms. MAA prepared from the ascidian *Lissoclinum patella* were found to protect biological systems against detrimental effects of the type II photosensitization *in vitro*. *L. patella* MAA were resolved into five components, and the relative $^1O_2$ quenching efficiencies were measured for three major components in aqueous media. It turned out that they were all effective in scavenging $^1O_2$, to different degrees albeit. The results suggest that physiological relevance of MAA in marine organisms may be found in a 'built-in' defense against photooxidative effects of sunlight.

Key words: mycosporine-like amino acids, photoprotection, photosensitization, singlet oxygen quenching

INTRODUCTION

Diverse marine organisms contain large amounts of mycosporine-like amino acids (MAA) as secondary metabolites that exhibit strong UV absorption peaks over 310-360 nm [1]. Although their physiological function remains unclear, it has been associated with a protection mechanism against harmful sunlight effects in organisms, acting as a UV screen [1,2].

Some MAA have been shown to inhibit lipid peroxidation [1,3]. Since this inhibition is thought to result from eliminating a peroxyl radical, an activated oxygen species, it would also be a possibility that MAA remove other reactive oxygen species (ROS) that can be produced in organisms by certain endogenous sensitizers in strong light. If such is the case, photoprotection function of MAA could have connection with their reactivity toward photogenerated ROS. This point was subjected to experimental scrutiny in the present study, focusing on, in particular, whether MAA protects biological systems from photodynamic effects mediated by singlet oxygen ($^1O_2$) and, if so, how effectively MAA can eliminate $^1O_2$ from the systems.

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MATERIALS AND METHODS

MAA preparation. Flesh of ascidian *L. patella* was homogenized in 80% aqueous methanol, kept shaking (5 h, 4°C) and filtered. Methanol in the filtrate was removed under reduced pressure, and the aqueous extract was re-filtered and washed with chloroform. The clear extract was freeze-dried, re-dissolved in methanol and then loaded onto a column packed with Baker's Bakerbond™ Amino Sorbent (40 μm) in methanol. The loaded column was washed with methanol, and then total MAA were eluted with a medium containing ammonium acetate (40 mM) and acetic acid (17.5 mM) in 80% methanol. Total MAA were

subjected to reverse phase HPLC in an YMC-Pack ODS-A column with an aqueous mobile phase of 0.1% acetic acid, obtaining 3 major components of *L. patella* MAA.

Biological and biochemical assays. Susceptibility of *Escherichia coli* to photodynamic inhibition was tested in agar culture plates by the filter paper disc method [4]. Lipid peroxidation of biological membrane was assessed by the malone dialdehyde (MDA) method as in Buege and Aust [5], using microsomes prepared from rat liver. Photosensitized inactivation of enzyme was studied using lactate dehydrogenase (LDH), for which the enzyme activity was measured as described by Chen *et al.* [6].

Detection of $^1O_2$ by ESR. $^1O_2$ was trapped with a trap compound 2,2,6,6-tetramethyl-4-piperidone (TMPD), and the resulting formation of TMPD-N-oxyl was monitored using ESR at an operating frequency of approximately 9.42 GHz as in Moan and Wold [7]. Changes in the ESR signal at 336.92 mT were then monitored during photolysis. UV-filtered light (>430 nm, 450 W/m^2) from a halogen lamp was used for irradiation.

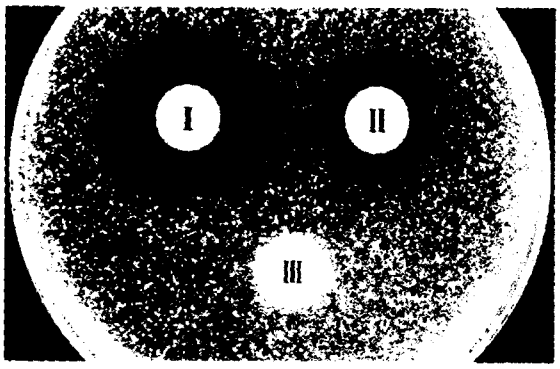

Figure 1. MAA Effect on EY-sensitized inhibition of *E. coli* growth in visible light (50 W/m^2). I, EY (40 nmole); II, EY (40 nmole) + MAA (160 nmole); III, blank test

RESULTS

The growth of *E. coli* was inhibited as a result of the combined action of eosin Y (EY) and light. Inhibition of the bacterial growth was indicated with a clear zone on agar plate circled around a filter paper dics loaded with EY. The zone of inhibition was diminished by loading MAA on the disc together with the photosensitizer, as shown in Fig. 1.

Microsomes were readily susceptible to EY-sensitized lipid peroxidation in light. This photosensitization was suppressed by *L. patella* MAA that was apparently more efficient in protecting microsomes compared with histidine. Similarly, MAA were found to be effective in preventing photosensitized inactivation of enzyme (Fig. 2).

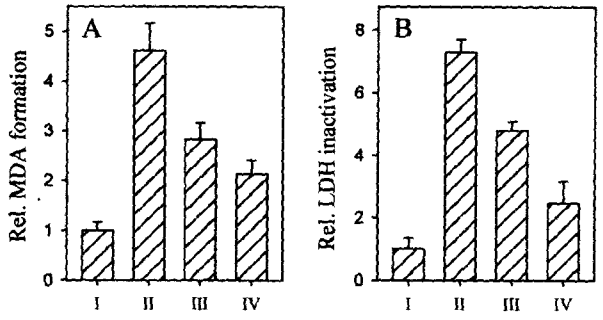

Figure 2. EY-sensitized microsomal peroxidation (A) and LDH inactivation (B) in visible light (300 W/m^2, 7 min for microsomes and 55 W/m^2, 6 min for LDH). I, EY + Dark; II, EY + Light; III, EY + Histidine + Light; IV, EY + MAA + Light

The time-courses of TMPD-N-oxyl production in aqueous solutions of TMPD plus EY under illumination, as traced by ESR, are shown in Fig. 3. The rate of the nitroxyl radical formation was decreased by the presence of either MAA (ca. 0.1 mM) or histidine (ca. 0.1 mM). Estimated on an equal concentration basis from the degrees of decrease in the rate, however, MAA appeared far more efficient in removing $^1O_2$ in the photolysis systems compared with histidine, a well-known scavenger of $^1O_2$.

Three major components of *Lissoclinum patella* MAA were identified as mycosporine glycine (MG), shinorine

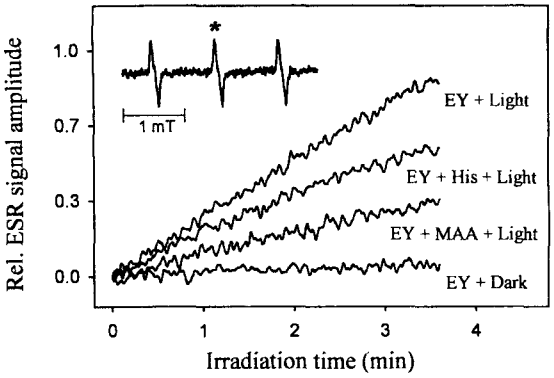

Figure 3. EY-photosensitized production of TMPD-N-oxyl in air-saturated solutions of TMPD at ambient temperature

and P-334 by LC-ESI mass spectrometry and proton NMR.

Among these, MG was found to be the most effective in removing $^1O_2$: its efficiency was much higher than that of histidine. The other two also scavenged $^1O_2$ with relatively lower efficiencies, as summarized in Table 1.

Table 1. Relative rates of TMPD-N-oxyl formation in the presense of histidine and MAA components

Additive	Rel. formation rate	$^1O_2$ quenching efficiency
None	1.00	–
Histidine	0.59	1.00
MG	0.21	1.93
Shinorine	0.86	0.34
P-334	0.83	0.41

DISCUSSION

We have here demonstrated that some MAA are effective in inhibiting photodynamic reactions in biological systems. UV screening by MAA is apparently not associated with the protection effect at least under our experimental conditions, because the model systems have been irradiated only with visible light. It may therefore be concluded that MAA as a class of abundant secondary metabolites play a role in protecting marine organisms *in vivo* against photodamaging effects of sunlight mainly by eliminating $^1O_2$ generated from certain endogenous sensitizers under illumination rather than by screening energetic UV itself.

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