## Miyuki Maegawa, Masayo Kunieda and Wahiro Kida: Difference of the amount of UV absorbing substance between shallowand deep-water red algae.

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The presence of a substance absorbing ultraviolet ray at around 330 nm (UVAS) in the Rhodophyta has been first reported by Tujino and Saito (1961). Physiological and ecological roles of this substance have received attention of several workers. Shibata (1969) reported that the similar UVAS exists in tropical corals and a blue-green alga as well, and suggested this substance to play the possible role of biofilter for strong UV-irradiation or precursor of the algal pigments. Iwamoto and Aruga (1973) found thereafter that most of the red and blue-green algae contain this substance and that some of the green and brown algae also contain it. Sivalingam et al. (1974a, b) observed that the compound exists ubiquitously in all algal groups and its content level fluctuates almost depending on the depth of their habitat in correlation to the level of chlorophyll and phycoerythrin. Sivalingam et al. (1976) isolated thereafter the compound from a red alga, Porphyra yezoensis, and investigated its physicochemical properties. They suggested that this substance plays some important roles as a metabolic regulator or a temporal energy transmitter at some still unknown sites in the photosynthetic pathways of algae. Recently, Sivalingam and Nisizawa (1990) observed considerable increase of UVAS in tropical marine algae with increase in UV-irradiation.

In the result of our previous work (Maegawa *et al.* 1993), it appeared that shallow-water inhabiting red algae are adapted to strong solar radiation and acquire the capacity to resist excessive UV irradiation, while deep-water species do not have such an ability. Hence, it is reasonable to assume that UVAS would be different in the content between shallow- and deep-water red algae. In the present study, we collected many species of red algae from the shallow and deep waters, and determined the contents of UVAS to compare with those obtained by Sivalingam *et al.* (1974a, b).

The red algal samples were collected around coast of the Shima peninsula, Mie prefecture in May and June 1990. Twenty one algal species from intertidal zone to 5 m in depth were regarded as shallow-water species, because they were not found in the deeper waters. Twelve species of algae collected from 25-30 m in depth were regarded as deepwater species, because they were not found in shallow waters. Also, UV ray reaches scarcely the depth more than 25 m in depth where deep-water species occur (Maegawa et al. 1993). After careful removal of microscopic epiphytes and other contamination, algal thalli were homogenized with 10 ml of 1/15 M phosphate buffer (pH 7.0) in a mortar and the homogenate was centrifuged at 2,000 gThe supernatant was filtered for 30 min. through a cellulose nitrate layer of 0.20  $\mu$ m pore size to remove suspending material and phycobiliprotein. Each solution was analyzed for UVAS using Shimazu UV-200 double beam spectro-photometer from 250 nm to 750 nm. Contents of UVAS were expressed by optical density (OD) of the absorption maxima around 330 nm per 10 ml extracts from 0.1 g wet weight samples.

Fig. 1 shows absorption spectra of *Grateloupia turuturu* Yamada from 250 to750 nm. *In vivo* absorption spectrum is composed of several peaks of chlorophyll *a* and phycobiliproteins in visible light band, and a



Fig. 1. Absorption spectra of *Grateloupia turuturu*. Straight line, *in vivo* (with air as reference); broken line, extract with 1/15 M phospate buffer solution of pH 7.0 (with water as reference); dotted line, filtrate through a cellulose nitrate layer of 0.21  $\mu$ m pore size (with water as reference). OD values of the broken and dotted line were recalculated per 10 m*l* extract from 0.1 g wet weight sample.

high peak of UVAS at 332 nm in UV band. The extract with phosphate buffer solution has also high peak at 332 nm and several peaks of phycobiliproteins. The filtration through a cellulose nitrate layer makes a high peak of UVAS at 332 nm and gives no absorbance in visible light band. Substances with the absorption maxima below 300 nm are DNA derivative, protein and amino acid, betain and other unknown ones (Tujino 1983).

Fig. 2 shows absorption spectra in UV band of extracts from four red algae collected from various depths. Intertidal alga of *Porphyra yezoensis* and upper subtidal alga of



Fig. 2. Absorption spectra of extracts from four species of red algae collected from the shallow and deep waters. Shallow-water species: (---), *Porphyra yezoensis*; (----), *Gracilaria incurvata*. Deep-water species: (----), *Meristotheca papulosa*; (.....), *Champia expansa*.

Table 1. Wavelength at absorption maxima
of UVAS and optical density (OD) per 10 ml ex-
tracts from 0.1 g wet weight samples collected from
the shallow and deep waters.

Algal species	absorption maxima	OD/0.1 g wet
	(nm)	weight
Shallow-water species		
Intertidal		
Porphyra yezoensis	334	3.811
Gloiopeltis furcata	332	1.367
Carpopeltis prolifera	332	1.038
Gigartina intermedia	327	0.949
Upper Subtidal		
Pachymeniopsis elliptica	327	1.987
Polyopes polyideoides	332	1.248
Martensia denticulata	327	1.210
Grateloupia turuturu	332	0.954
Prionitis crispata	326	0.940
Grateloupia okamurae	326	0.821
Grateloupia filicina	326	0.644
Marginisporum aberrans	332	0.684
Prionitis angusta	329	0.589
Gigartina tenella	329	0.609
Gracilaria textorii	332	0.521
Laurencia undulata	329	0.525
Champia parvula	326	0.451
Galaxaura fastigiata	332	0.407
Amphiroa zonata	331	0.361
Plocamium leptophyllum	330	0.262
Gelidium elegans	328	0.297
Deep-water species		
Meristotheca papulosa	330	0.309
Prionitis articulata	326	0.257
Prionitis patens	326	0.284
Delisea japonica	326	0.256
Gelidium linoides	326	0.146
Galaxaura falcata	326	0.086
Sebdenia yamadae	330	0.071
Peyssonnelia caulifera	*	0.044
Ptilophora subcostatum	*	0.043
Champia expansa	*	0.037
Ptilonia okadae	*	0.022
Ardissonula regularis	*	0.026

\* no OD peak around 330 nm.

Gracilaria incurvata show OD higher than 1.5 around 330 nm of wavelength. The absorbancies of deep-water species of Meristotheca papulosa and Champia expansa collected from 25 to 30 m are very small or show no peak around 330 nm. In Table 1, UVAS contents of 21 shallow-water species and 12 deep-water species are listed. The wavelength of maximal absorption around 330 nm and OD maxima of filtrates through a cellulose nitrate layer are shown. Intertidal algae are higher in OD values than 0.949, and many upper subtidal algae also show the higher in OD values ranging from about 0.3 to 2. As compared with those of shallow-water species, the OD maxima of deep-water ones are less than 0.3. Particularly, some of the deep-water species exibit no peak around 330 nm, the absorption of UVAS.

It has been thought that UVAS exists ubiquitously in all the Rhodophycean algae (Sivalingam et al. 1974a, b). Our present result (Table 1) almost coincided with that of Sivalingam et al. In the present study, however, several algae collected from the deep waters showed no peak around 330 nm, suggesting the absence of UVAS in these species. As mentioned in our previous paper (Maegawa et al. 1993), photosynthesis of deep-water species was inhibited seriously by exposure to direct sunlight, while that of shallow-water species was affected little. It was strongly suggested that at least a part of the inhibition on the photosynthesis of deepwater species is attributed to UV radiation. Thus, the solar UV radiation might be related to determine the vertical distribution of red algae through its effect on the photosynthetic activity of these algae. In this respect, the UVAS existing in red algae may act as a biofilter for solar UV radiation. Since solar UV radiation reaches scarcely the depth more than 25 m (Maegawa et al. 1993), there seems to be no need to protect it for deep-water red algae.

From the results obtained in our previous

and present study, it may be postulated that shallow-water red algae have an ability to produce UVAS to resist excessive UV irradiation while deep-water ones have lower or no ability to produce it. The fact that drastic decrease in UVAS content of red algae coincident with the increasing depth (Table 1) would partially verify the above postulation.

## References

- Iwamoto, K. and Aruga, Y. 1973. Distribution of the UV-absorbing substance in algae with reference to the peculiarity of *Prasiola japonica* Yatabe. J. Tokyo Univ. Fish. 60: 43-54. (in Japanese)
- Maegawa, M. Kunieda, M. and Kida, W. 1993. The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. Jpn. J. Phycol. 41: 207-214.
- Shibata, K. 1969. Pigments and a UV-absorbing substance in corals and blue-green alga in the Great Barrier Reef. Plant Cell Phisiol. 10: 324-335.
- Sivalingam, P. M., Ikawa, T. and Nisizawa, K. 1974a. Possible physiological roles of a substance showing characteristic UV-absorbing patterns in some marine algae. Plant Cell Physiol. 15: 583-586.
- Sivalingam, P. M., Ikawa, T., Yokohama, Y. and Nisizawa, K. 1974b. Distribution of a 334 UVabsorbing substance in algae, with special regard of its possible physiological roles. Bot. Mar. 17: 23-29.
- Sivalingam, P. M., Ikawa, T. and Nisizawa, K. 1976. Physiological roles of a substance 334 in algae. Bot. Mar. 19: 9-21.
- Sivalingam, P. M. and Nisizawa, K. 1990. Ozone hole and its correlation with the characteristic UVabsorbing substance in marine algae. Jpn. J. Phycol. 38: 365-370.
- Tujino, I. and Saito, T. 1961. Studies on the compounds specific for each group of marine algae. I Presence of characteristic ultraviolet absorbing material in Rhodophyceae. Bull. Fac. Fish., Hokkaido Univ. 12: 49-58.
- Tujino, I. 1983. UV-absorbing compounds. p. 78-89. In Japanese Soc. Sci. Fish. [ed.] Biochemistry and Utilization of Marine Algae. Koseisha-Koseikaku, Tokyo. (in Japanese).

## 前川行幸\*・国枝昌代\*\*・喜田和四郎\*:浅所産および深所産紅藻の紫外線吸収物質

潮間帯から水深 5 m までの浅所および水深 25-30 m の深所から採取された紅藻について,紫外線吸収物質(U-VAS)を測定し比較・検討した。紫外線吸収物質の量は 330 nm 付近の最大吸収を示した吸光度で表した。潮間帯の紅藻は0.949以上の高い吸光度を示し,多量の紫外線吸収物質を含んでいた。水深 5 m までの潮下帯の紅藻 も0.5以上の高い吸光度を示す種が多く見られた。これらの浅所産紅藻に対し,深所産紅藻では吸光度は0.309以 下で,紫外線吸収物質の量は少なかった。また,いくつかの深所産紅藻では吸収極大は見られず,紫外線吸収物 質が含まれていないものと思われた。これらのことから,紫外線吸収物質の生態学的な役割として,従来考えら れているように,紫外線に対する生体防御物質として働いていることが示唆された。(\*514 三重県津市上浜町 1515 三重大学生物資源学部藻類増殖学研究室,\*\*465 名古屋市東区猪子石2-710 ㈱東海技術センター)

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