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On the lack of green light-harvesting pigments and the extremely high chlorophyll *b/a* ratio in the deep-water green alga, *Palmophyllum crassum* (Chlorosphaerales).

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Although *Palmophyllum crassum* is a green alga growing in extremely deep or shaded sites, its *in vivo* absorption spectrum is of the "shallow-water type" lacking the green band which is found in most of the green algae growing in deep waters or shaded sites. On the silica-gel thin-layer chromatogram of pigments extracted from this alga, we can find neither siphonaxanthin nor siphonein, both of which act in the chloroplasts of green algae as photosynthetic pigments harvesting the green light prevailing in deep waters and shaded sites. On the other hand, the molar ratio of chlorophyll *b/a* was determined to be 1.61 in the ether solution of pigments extracted from this alga. This is the highest value known to date among green algae. This alga, therefore, seems to adapt to deep or shaded sites through its high content of chlorophyll *b* which harvests light prevailing in such places more efficiently than chlorophyll *a* does.

Key Index Words: absorption spectra—carotenoids—chlorophyll b/a *ratios—deep waters—green algae—* Palmophyllum crassum—Udotea petiolata—Ulva rigida.

It was indicated by Yokohama and his coworkers that most green algae from deep waters or shaded sites have siphonaxanthin as a photosynthetic pigment harvesting the green light prevailing in such habitats (Yokohama *et al.* 1977, Kageyama *et al.* 1977). Siphonein, an ester of siphonaxanthin, contained in the siphonalean algae was also indicated to be a photosynthetic pigment harvesting the green light (Kageyama and Yokohama 1978). The green algae containing siphonaxanthin or siphonein are brownish dark green, while those lacking these green light-harvesting pigments are light green. The former algae are referred to as "deep-water type" and the latter as "shallow-water type". *Palmophyllum crassum* is a deep-water green alga found at great depths, up to 110 meters, or in the upper sublittoral zone at shaded sites (Lüning 1990 pp. 115–116). However, the color of this alga is light green, being similar to the species of the "shallow-water type" such as *Ulva* spp. and *Enteromorpha* spp. found at the intertidal zone. This fact could imply the lack of siphonaxanthin and siphonein in its chloroplasts.

In the present study, the *in vivo* absorption spectrum and the pigment composition of *Palmophyllum crassum* collected from the shaded rocky bottom in deep waters were investi-

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gated, and the results were compared with those obtained from two other green algae, one of which, *Udotea petiolata* of the "deep-water type", has green light-harvesting pigments while the other *Ulva rigida* of the "shallow-water type" lacks these pigments.

Materials and Methods

Fresh thalli of *Palmophyllum crassum, Udotea petiolata* and *Ulva rigida* were collected at different points near Oristano, Sardinia, Italy. The *Palmophyllum crassum* was from a shaded rocky bottom at a depth of 25 meters, while the other species were from shallow waters. Although *Udotea petiolata* has been reported to occur at the same depth of *P. crassum* and seems to be a "deep-water type" green alga (Lüning 1990 p. 115), abundant thalli of this species were found in a shallow seagrass bed at the collecting sites. The collected thalli were carried in large containers of seawater to the laboratory of the International Marine Center, near Oristano, where the present investigation was carried out.

After the *in vivo* absorption spectrum of each thallus was determined with a Beckman Du-65 Spectrophotometer, the pigments were extracted from the thalli with cold methanol. The extract was mixed with a nearly equal volume of diethyl ether. By adding 10% (w/v) NaCl solution to the mixture and shaking, the pigments were transferred to the ether layer. After washing several times with the NaCl solution, the ether layer was removed, the ether was evaporated under reduced pressure, and the residue was redissolved in a small volume of ether.

The chromatography of the pigments was carried out on a thin-layer plate of Kieselgel 60 (Merck). A mixture of petroleum ether 30°-60° and acetone (7 : 3, v/v) was used as the developing solvent.

The molar ratio of chlorophyll *b/a* was calculated from the absorbances of the pigments in the ether layer at 642.5 nm and 660 nm using the equation of Comar and Zscheile (1942) and the molecular weights of 892 for chlorophyll *a* and 906 for chlorophyll *b*.

Results

Fig. 1 shows the *in vivo* absorption spectra of *Palmophyllum crassum* and two other species used for comparison, one of which is *Udotea petiolata* with its green band being formed by the *in vivo* absorption maxima of siphonaxanthin and siphonein while the other is *Ulva rigida* lacking any green band. The spectrum of *P. crassum* lacks the green band, implying that this alga contains neither siphonaxanthin nor siphonein.

The thin-layer chromatograms of the pigments extracted from these three algae shown in Fig. 2 indicate that *P. crassum* lacks both siphonaxanthin and siphonein, which are contained in *U. petiolata* of the "deep-water type". From the carotenoid composition, *P. crassum* seems to belong to "shallow-water type", containing carotene(s), lutein, violaxanthin and neoxanthin. In *P. crassum*, however, carotene(s) and violaxanthin are found in trace amounts and the amount of lutein seems to be much less than in *U. rigida* of the "shallow-water type". The chromatograms further indicate that *P. crassum* contains two kinds of yellow pigments supposed to be

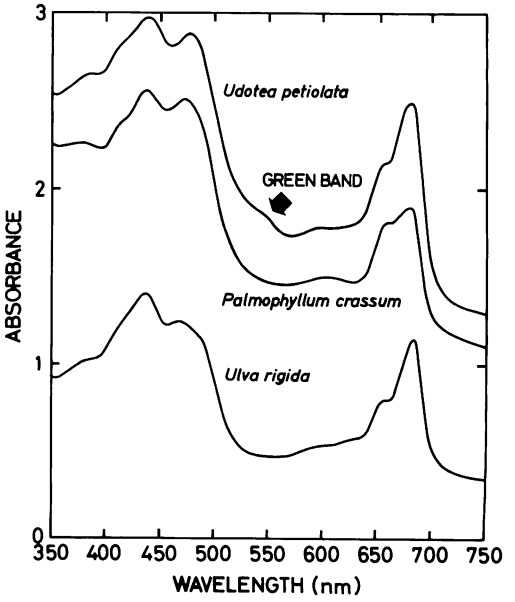

Fig. 1. *In vivo* absorption spectra of *Palmophyllum crassum, Ulva rigida* and *Udotea petiolata*.

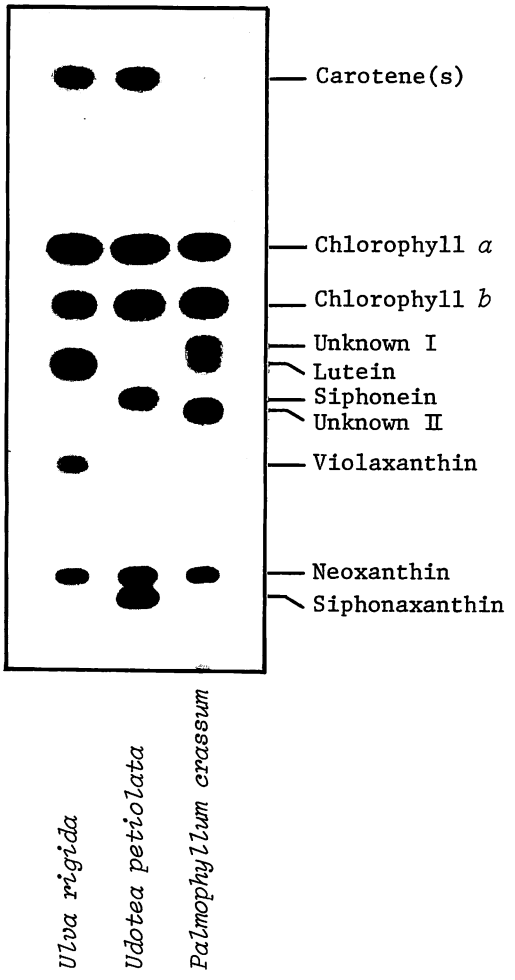

Fig. 2. Kieselgel thin-layer chromatograms of pigments extracted from *Ulva rigida, Udotea petiolata* and *Palmophyllum crassum*. Developing solvent: petroleum ether 30°–60° and acetone (7 : 3, v/v).

carotenoids, neither of which is detected in the other two species.

Fig. 3 shows the absorption spectra of the ether solutions of pigments from these three species in the wavelengths from 550 to 750 nm, each of which is drawn with relative values to the absorption maximum of chlorophyll *a* at 660 nm. As can be seen, the maximum of chlorophyll *b* at 642.5 nm in the absorption spectrum of *P. crassum* is much higher than those of the other species. The molar ratios of chlorophyll *b*/*a* of the three species calculated from absorbances at 642.5 nm and 660 nm are shown in Table 1. The value

Table 1. Molar ratios of chlorophyll *b*/*a* in *Ulva rigida, Udotea petiolata* and *Palmophyllum crassum*.

Species	Habitat	Chl. *b*/*a*
Ulva rigida	Upper sublittoral zone	0.55
Udotea petiolata	Shallow seagrass bed	0.82
Palmophyllum crassum	Shaded rocky bottom at the depth of 25 m	1.61

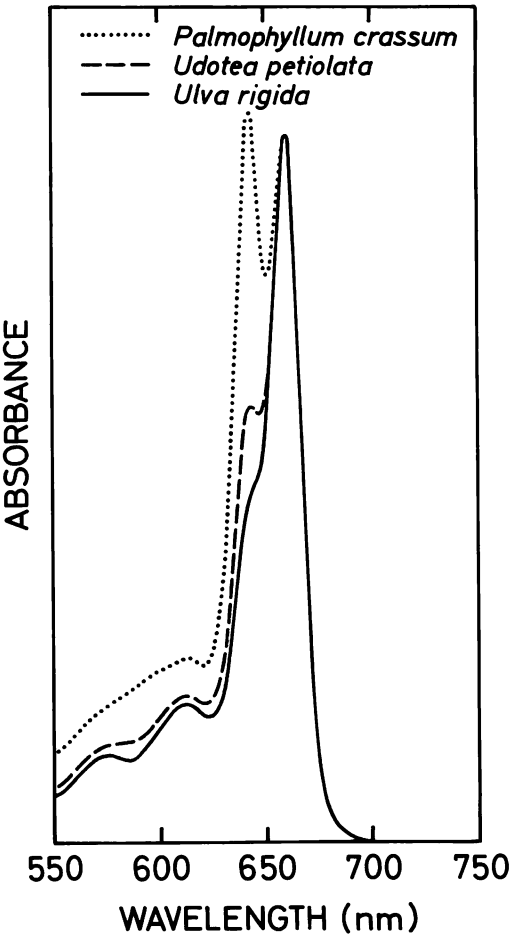

Fig. 3. A longer wavelength part of the absorption spectra of pigments extracted from *Ulva rigida, Udotea petiolata* and *Palmophyllum crassum* in diethyl ether.

of *P. crassum* is 1.61, while those of *U. petiolata* and *U. rigida* are 0.82 and 0.55, respectively.

Discussion

It is remarkable that a deep-water alga *Palmophyllum crassum* contains neither

siphonaxanthin nor its ester siphonein, which are photosynthetic pigments harvesting the green light prevailing in deep waters. Although two kinds of unknown carotenoids were detected on the chromatogram of pigments from this alga, they cannot be regarded as green light-harvesting pigments since the *in vivo* absorption spectrum of this alga lacks the distinct green band. However, this alga seems to adapt to deep or shaded sites through its high content of chlorophyll *b*. The high proportion of chlorophyll *b* can be regarded as one of the characteristics being favorable for growth in deep waters or shaded sites since chlorophyll *b* absorbs light prevailing in such places more efficiently than chlorophyll *a* does (Yokohama and Misonou 1980). Such high proportions of chlorophyll *b* were reported for several deep-water species of the Cladophorales and the Siphonocladales lacking green light-harvesting pigments, but they were around 1.0 (Yokohama 1983). The value 1.61 as a chlorophyll *b/a* molar ratio determined in this alga is the highest one known to date among green algae (cf. Jeffrey 1965, Keast and Grant 1976, Yokohama and Misonou 1980, Yokohama 1981, Yokohama *et al.* 1992). Thus, *P. crassum* may have an ability to grow at great depth, up to 110 meters or more (Lüning 1990 p. 115), by an extreme increase in chlorophyll *b/a* ratio.

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Gianfranco Sartoni* ・ Francesco Cinelli ・ 平田　徹*** ・ 片山舒康**** ・ 横浜康継***** :**

緑色光捕獲色素を欠く深所性緑藻 *Palmophyllum crassum* の クロロフィル *b/a* 比について

緑藻でも，深所に分布する種のほとんどは褐色がかった暗褐色をしている。それはシホナキサンチンやシホネインを含有しているためであること，更にそれらの色素は緑色光を捕獲する光合成色素として機能していることなどが判明している。これらの色素を含有する緑藻を深所型緑藻とよび，浅い所に分布しているアオサ類やアオノリ類のようにこれらの色素をを持たない鮮緑色を呈した緑藻を浅所型緑藻とよぶことが提唱されている。

Palmophyllum crassum は水深110メートルあたりまで分布する深所性緑藻であるにもかかわらず，その体色は浅所型緑藻に近い鮮緑色であることから，シホナキサンチンやシホネインを欠くものと予想された。実際に生体吸収スペクトルにはそれらの色素の存在を示す緑色部吸収帯はみられず，藻体から抽出した色素のシリカゲル薄層クロマトグラフィーによる分析でも，それらの色素は検出されなかった。しかし，抽出色素のエーテル溶液の642.5 nm および 660 nm における吸光度から計算されたクロロフィル *b/a* のモル比は，1.61という高い値となった。これは，これまでシホナキサンチンなどを欠く他の深所性緑藻で知られていた1.0前後という値をはるかにしのぐものであった。これらのことから，*P. crassum* は，深所まで透過される光をクロロフィル *a* よりも効率よく吸収できるクロロフィル *b* を多量に含むことによって，深所の光環境に適応していると考えられる。(*Department of Botany, University of Firenze, Via la Pira 4, 50121 Firenze, **Department of Environmental Science, University of Pisa, Via Volta 6, 56121 Pisa, Italy, ***400 山梨県甲府市武田4-4-37　山梨大学教育学部生物学教室，****184 東京都小金井市貫井北町4-1-1　東京学芸大学生物学科，*****415 静岡県下田市5-10-1　筑波大学下田臨海実験センター)

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