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CHLOROPHYLL AND PEPTIDE COMPOSITIONS IN THE TWO PHOTOSYSTEMS OF MARINE GREEN ALGAE

KEIJI NAKAMURA, TERUO OGAWA and KAZUO SHIBATA

Laboratory of Plant Physiology, The Institute of Physical and Chemical Research (Rikagaku Kenkyusho), Wako-shi, Saitama (Japan)

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SUMMARY

The molar ratios of chlorophyll *a* to *b* in the thalli of marine green algae were between 1.5 and 2.2, being appreciably lower than the ratio between 2.8 and 3.4 found for the leaves of higher plants and the cells of fresh-water green algae. The ratio of chlorophylls to *P*-700 in these marine algae was also lower than that in higher plants. The *a*/*b* ratios in the pigment proteins of Photosystems 1 and 2 separated by polyacrylamide-gel electrophoresis from sodium dodecyl sulfate-solubilized chloroplasts of four species of marine green algae, *Bryopsis maxima*, *Cheatomorpha spiralis*, *Enteromorpha compressa* and *Ulva conglobata*, were approximately 5 and 1, which are considerably smaller than the ratios, 7 and 2, respectively, found for the pigment proteins of the two photosystems of higher plants separated by the same technique. The chloroplasts of *Bryopsis maxima* and *Cheatomorpha spiralis* lacked two of the peptides associated with Photosystem II, which are present in the chloroplasts of *Spinacia oleracea* and *Taraxacum officinale*.

INTRODUCTION

Leaves of higher plants and cells of fresh-water green algae contain chlorophylls *a* and *b* in an *a*/*b* ratio of approximately 3. These chlorophylls are distributed in two photochemical systems in different *a*/*b* ratios; a higher ratio in Photosystem 1 and a lower ratio in Photosystem II [1–4]. For example, the *a*/*b* ratios determined for the two photosystems of spinach chloroplasts were 7 and 2 and the ratios found for those of *Chlorella pyrenoidosa* were 5.6 and 1.8, respectively [5]. The data available for marine green algae seem, however, to indicate significantly different *a*/*b* ratios. Willstätter and Stoll [6] reported low ratios between 1.3 and 2.2 for *Ulva lactuca*, and the data obtained by Jeffrey [7] indicated low ratios of 2.0 and 2.2 for *Nannochloris atomus* and *Dunaliella tertiolecta*, respectively. Because of this low *a*/*b* ratio, *Ulva* had once been classified into a group of "shadow plants" [8].

Attention was paid in the present study to these lower *a*/*b* ratios to see if the lower ratios are a general trend in common to marine green algae and to determine

how chlorophylls *a* and *b* in the lower ratios are distributed in the two photosystems. The *a*/*b* ratio was determined for the thalli of nine species of marine green algae.

The pigment proteins of the two photosystems were isolated from four species (*Enteromorpha compressa*, *Cheatomorpha spiralis*, *Ulva conglobata* and *Bryopsis maxima*) to determine the *a*/*b* ratios in the two photosystems, and the results were compared with those obtained by the same procedure on higher plants; *Brassica vulgaris*, *Spinacia oleracea*, *Brassica rapa* and *Taraxacum officinale*.

The peptide compositions of the chloroplasts of *Bryopsis maxima* and *Cheatomorpha spiralis* were also studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and compared with the compositions in those of *Spinacia oleracea* and *Taraxacum officinale*.

MATERIALS AND METHODS

Preparation of samples

The eight species of marine green algae, *Cheatomorpha spiralis*, *Ulva conglobata*, *Bryopsis maxima*, *Enteromorpha compressa*, *Cheatomorpha crassa*, *Codium fragile*, *Boodlea coacta* and *Cladophora wrightiana*, were harvested on the seashore at Choshi and Kominato in Chiba prefecture and a higher plant, *Zostera marina*, was harvested on the same seashore in the wavefront. A marine green alga, *Caulerpa okamurai*, was harvested on the seashore at Tamano in Okayama prefecture. Leaves of *Spinacia oleracea*, *Brassica vulgaris*, *Brassica rapa* and *Hydrilla verticillata* studied for comparison were purchased at a market, and leaves of *Taraxacum officinale*, *Oenothera lamarchiana*, *Gingo bioloba* and *Equisetum arvense* were harvested on our campus. Cells of *Scenedesmus obliquus* were supplied by Dr. A. Ichimura at the Institute of Applied Microbiology in Tokyo University.

Chloroplasts and lamellae were prepared from the thalli of *Enteromorpha* and *Ulva* by the following procedure. The thalli were immersed in 50 mM Tris · HCl buffer (pH 7.8) containing 0.4 M sucrose and ground with a mortar and pestle. The homogenate was filtered through two layers of cheese cloth. The filtrate was centrifuged at $1000 \times g$ for 1 min, and the supernatant was further centrifuged at $10\,000 \times g$ for 15 min. Chloroplasts and lamellae precipitated after centrifugation were suspended in 0.04 M borate buffer (pH 9.4) to be subjected to electrophoresis. In the case of *Bryopsis* and *Cheatomorpha*, the thalli were cut with a pair of scissors into small pieces about 2 cm long, and chloroplasts were squeezed out into 50 mM Tris · HCl buffer (pH 7.8) containing 0.4 M sucrose through two layers of cheesecloth. The filtrate was centrifuged at $1000 \times g$ for 1 min, and the supernatant was further centrifuged at $3000 \times g$ for 10 min to isolate chloroplasts. Chloroplasts were suspended in 0.04 M borate buffer (pH 9.4). Chloroplasts of higher plants were prepared from leaves according to the method described previously [9].

Disc electrophoresis

The technique used for the analysis of pigment proteins in chloroplasts was essentially the same as that reported previously [2], except that disc electrophoresis was used in the present study for better separation. Chloroplasts were solubilized with 0.025–0.2 M sodium dodecyl sulfate and subjected to disc electrophoresis in polyacrylamide gel (0.5 cm diameter and 5.0 cm in length). Electrophoresis was

carried out with a current of 3 mA, 100 pulses/s fed by a power supply, Toyo model HP-10. Proteins in the gel were stained with amidoblack 10B and destained electrophoretically.

In the experiment to isolate pigment proteins in a freeze-dried state, ten aliquots (0.1 ml each) of sodium dodecyl sulfate-solubilized chloroplasts in buffer were subjected to the gel electrophoresis. Ten segments of each separated pigment protein in the gel were collected, and the pigment protein was eluted from the gel into 0.04 M borate buffer (pH 9.4) using a glass homogenizer. The pigment protein in the solution thus obtained was freeze-dried, and pigments were extracted from the dried sample with methanol.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of membrane polypeptides was carried out according to Weber and Osborn [10]. Chloroplasts extracted with acetone were solubilized with sodium dodecyl sulfate by the procedure reported by Klein and Vernon [11]. Electrophoresis was carried out at 8 mA, 100 pulses/s. Proteins in the gel were stained with Coomassie brilliant blue and destained electrophoretically.

Photometry

Absorption spectra of chloroplasts or their extracts were measured with a Shimadzu multipurpose recording spectrophotometer model MPS-5000. The gel after electrophoresis was scanned with a Shimadzu dual-wavelength thin layer chromato-scanner model CS-900 to record the distribution of pigments and proteins in terms of absorbance difference between two wavelengths along the direction of electrophoresis.

The content of *P*-700 was estimated from the absorbance change at 700 nm upon illumination for a clear solution of sonicated chloroplasts (20–25 μM in terms of chlorophyll concentration) in 0.04 M Tris · HCl buffer (pH 7.8) containing 0.4 M sucrose. Sonication was made with a Toyo Riko sonicater model VF-50-6 at 10 kcycles for 5 min, and *P*-700 was reduced by ascorbate before illumination. The sample was illuminated with blue light (20 mW/cm^2) from a projector lamp through a fan cooled heat absorbing filter and a Corning 4–96 filter. The absorbance change, $\Delta A = A_{700} - A_{750}$, between 700 nm and 750 nm was measured in an expanded full scale of ΔA = 0–0.005 with a Shimadzu dual wavelength spectrophotometer UV-300. The errors included in this determination of *P*-700 were within 10 %. The molar extinction coefficient of $6.4 \cdot 10^4$ cm$^{-1} \cdot$ M^{-1} at 700 nm obtained by Hiyama and Ke [12] was used for the calculation.

Chlorophyll a/b ratio

Thalli of marine green algae and leaves of higher plants were ground in hot methanol to extract pigments. The extraction was repeated three times, and the extracts were combined. The chlorophyll *a/b* ratio in the extract or in the pigment extract from pigment proteins was determined by the hydroxylamine method of Ogawa and Shibata [13].

RESULTS

The ratio of chlorophyll a to b in intact leaves, thalli and cells

Table I summarizes the ratios of chlorophyll *a* to *b* determined for the thalli of marine green algae in comparison with those obtained for the leaves of higher plants and the cells of two species of fresh-water green algae, *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. The ratios found for marine green algae were between 1.5 and 2.2 which are appreciably lower than the ratios between 2.8 and 3.4 obtained for higher plants. The average of the ratios for marine green algae was 1.9. It is interesting to note that the *a*/*b* ratio for *Zostera marina*, which is a higher plant growing in sea water, is 2.8, being in the ratio range for higher plants. The fact that the *a*/*b* ratios found for fresh-water green algae, *Chlorella* and *Scenedesmus*, are close to 3 indicates that the lower *a*/*b* ratio is a character specific to marine green algae.

Curves A in Figs 1 and 2 show the absorption spectra of the chloroplasts of *Enteromorpha compressa* and *Brassica vulgaris*, respectively, as the representative spectra of marine green algae and higher plants. The spectrum of *Enteromorpha* chloroplasts (curve A in Fig. 1) shows a marked shoulder at 655 nm due to chlorophyll *b* absorption on the red band of chlorophyll *a* at 678 nm, consistent with the high *b* content, while the shoulder on the spectrum of *Brassica* chloroplasts (curve A in Fig. 2) is less distinct. The high *b* content is also reflected in the absorption in the Soret region. The shoulder at 470 nm is more marked on the spectrum of *Enteromorpha* chloroplasts.

The ratio of chlorophylls to P-700 in whole chloroplasts

The ratio of chlorophylls to *P*-700 was determined for eight species of marine green algae and higher plants. The result is summarized in Table II which indicates appreciably lower ratios of chlorophylls/*P*-700 for marine green algae.

TABLE I

THE RATIOS OF CHLOROPHYLL *a* TO *b* IN THE THALLI OF MARINE GREEN ALGAE AND THE LEAVES OF HIGHER PLANTS AND THE CELLS OF FRESH-WATER GREEN ALGAE

Marine green algae	*a*/*b*	Higher plants and fresh-water green algae	*a*/*b*
Ulva conglobata	1.5	*Zostera marina*	2.8
Caulerpa okamurai	1.7	*Hydrilla verticillata*	3.0
Cheatomorpha spiralis	1.7	*Brassica vulgaris*	3.1
Bryopsis maxima	1.8	*Brassica rapa*	3.2
Enteromorpha compressa	1.9	*Spinacia oleracea*	3.2
Cheatomorpha crassa	2.0	*Taraxacum officinale*	3.2
Cladophora wrightiana	2.0	*Equisetum arvense*	3.3
Codium fragile	2.0	*Ginkgo bioloba*	3.4
Nannochloris atomus	2.0[a]	*Oenothera lamarchiana*	3.4
Boodlea coacta	2.2	*Chlorella pyrenoidosa*	2.8[b]
Dunaliella tertiolecta	2.2[a]	*Scenedesmus obliquus*	3.0

[a] Ratios calculated from the data reported by Jeffrey [7].
[b] Average of the ratios determined by Grimme and Boardman [16].

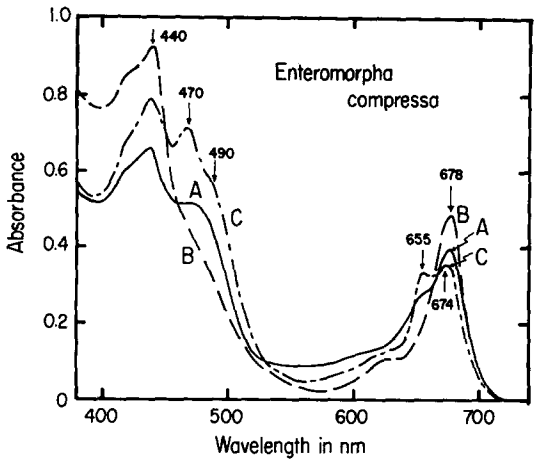

Fig. 1. Absorption spectra of chloroplasts (curve A), components I(B) and II(C) of *Enteromorpha compressa* obtained at the sodium dodecyl sulfate/chlorophyll ratio (the molar ratio in sample chloroplasts) of 114.

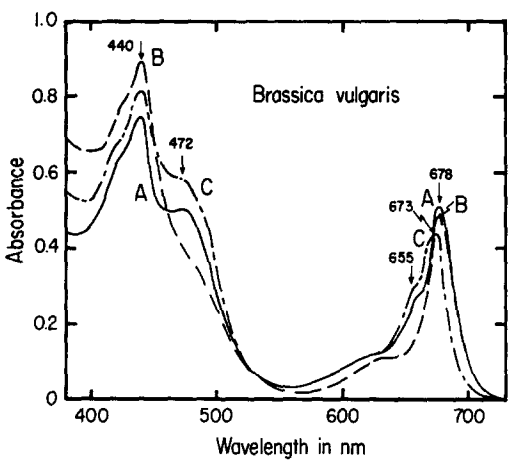

Fig. 2. Absorption spectra of chloroplasts (curve A), components I(B) and II(C) of *Brassica vulgaris* obtained at the sodium dodecyl sulfate/chlorophyll ratio of 150.

Pigment proteins of Photosystems 1 and 2

The electrophoretic patterns obtained for the sodium dodecyl sulfate-solubilized chloroplasts of *Enteromorpha compressa, Ulva conglobata* and *Cheatomorpha spiralis* are shown in Figs. 3A, 3B and 3C, respectively. The solid curves with three peaks show the distribution of chlorophyll *a* recorded in terms of $\Delta A = A_{675} - A_{750}$ (the absorbance difference between 675 nm and 750 nm), and the broken curves in the two peaks show the distribution of stained proteins recorded in terms of $\Delta A = A_{600} - A_{700}$. These distribution curves for marine green algae and the curves obtained for the chloroplasts of *Bryopsis maxima* were similar in band position to the distribu-

TABLE II

THE RATIOS OF CHLOROPHYLLS TO *P*-700 IN THE CHLOROPLASTS OF MARINE GREEN ALGAE AND HIGHER PLANTS

Plants	Chlorophylls/*P*-700
Marine green algae	
Bryopsis maxima	408
Cheatomorpha spiralis	425
Codium fragile	524
Ulva conglobata	512
Higher plants	
Brassica rapa	635
Hydrilla verticillata	617
Spinacia oleracea	724
Taraxacum officinale	571

tion curves obtained by the same procedure for the chloroplasts of higher plants such as *Brassica vulgaris*, *Brassica rapa*, *Spinacia oleracea* and *Taraxacum officinale*. An example for higher plants is shown by the curves in Fig. 3D which are the distribution curves obtained for the chloroplasts in *Brassica* leaves. The three peaks due to chlorophyll absorption found previously for spinach chloroplasts were designated components I, II and III in the order from the origin to the anode [2]. Components I

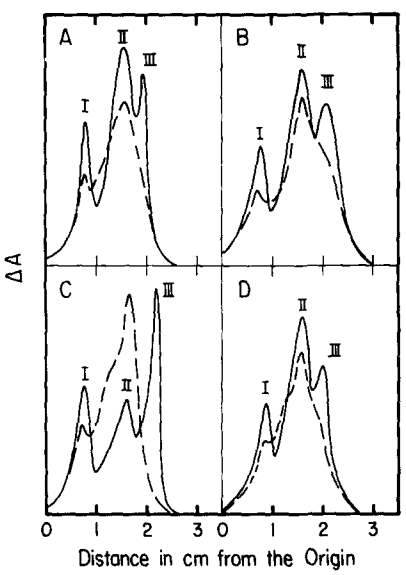

Fig. 3. Electrophoretic patterns of sodium dodecyl sulfate-solubilized chloroplasts of *Cheatomorpha spiralis* (A), *Enteromorpha compressa* (B), *Ulva conglobata* (C) and *Brassica vulgaris* (D) obtained at the sodium dodecyl sulfate/chlorophyll ratios of 75, 56, 56 and 75, respectively. The solid curves recorded in terms of $\Delta A = A_{675} - A_{750}$ show the distribution of chlorophyll *a* and the broken curves recorded in terms of $\Delta A = A_{600} - A_{700}$ show the distribution of proteins stained with amidoblack 10B.

and II were shown to be pigment proteins derived from Photosystems I and II, respectively, and component III was free chlorophylls solubilized out of these pigment proteins by the action of sodium dodecyl sulfate. The distribution of proteins shown by broken curves in Figs. 3A, 3B and 3C confirmed this conclusion for marine green algae. The solubilization of chloroplasts for the data in these figures was made at a critical concentration of sodium dodecyl sulfate below which the solubilization was incomplete. Incomplete solubilization was noticed by colored materials at the origin after electrophoresis.

Table III summarizes the *a/b* ratios determined for the two components of marine green algae which were isolated by polyacrylamide-gel electrophoresis at a critical ratio of sodium dodecyl sulfate/chlorophyll (the molar ratio of sodium dodecyl sulfate to chlorophylls in sample chloroplasts) for complete solubilization. Different critical ratios used for various species are listed in the second column. The comparative data for marine green algae listed in the upper part of this table indicate *a/b* ratios of 4.6–4.9 and 0.8–1.1 for Photosystems 1 and 2, which are considerably smaller than the ratios, 6.5–7.1 and 1.8–2.0, respectively, found for the two photosystems of higher plants. The fact that the ratio for Photosystem 2 of marine green algae is approximately one half the ratio in Photosystem 2 of higher plants indicates that the lower *a/b* ratio found for the whole cells of marine green algae is mostly due to this lower *a/b* ratio in Photosystem II.

The data in Table III may include some errors due to the formation of free chlorophylls (component III) from pigment proteins (components I and II). The deviation from the true *a/b* ratio by the formation of free chlorophylls may be large when chlorophylls *a* and *b* are detached in a different *a/b* ratio from that in the pigment protein. In order to estimate this systematic error, the *a/b* ratio was determined for the two pigment proteins of *Enteromorpha compressa* and *Brassica vulgaris* solubilized at three different sodium dodecyl sulfate/chlorophyll ratios. As seen from the result summarized in Table IV, the increase of component III to about 40 to 50 % of the total chlorophyll content at higher sodium dodecyl sulfate/chlorophyll ratios

TABLE III

CHLOROPHYLL *a/b* RATIOS IN COMPONENTS I AND II OF MARINE GREEN ALGAE AND HIGHER PLANTS OBTAINED AT THE CRITICAL SODIUM DODECYL SULFATE/CHLOROPHYLL RATIOS

Plants	Sodium dodecyl sulfate/chlorophyll	*a/b*	
		I	II
Marine green algae			
Bryopsis maxima	75	4.8	1.1
Cheatomorpha spiralis	75	4.6	1.0
Enteromorpha compressa	56	4.8	0.9
Ulva conglobata	56	4.9	0.8
Higher plants			
Brassica rapa	75	7.1	2.0
Brassica vulgaris	75	6.5	2.0
Spinacia oleracea	114	6.8	1.9
Taraxacum officinale	150	7.0	1.8

TABLE IV

Chlorophyll *a*/*b* ratios and the fractional chlorophyll *a* contents in components I, II and III of *Enteromorpha compressa* and *Brassica vulgaris* as a function of the molar ratio, sodium dodecyl sulfate/chlorophyll.

Plants	Sodium dodecyl sulfate/chlorophyll	Chlorophyll *a* content (%)			*a*/*b*			$(a/b)_{cal}$*
		I	II	III	I	II	III	
Enteromorpha compressa	56	26	52	22	4.8	0.9	(3.6)	1.5
	114	24	43	32	4.2	0.9	(2.8)	1.6
	300	20	28	52	4.6	0.8	(2.2)	1.6
Brassica vulgaris	75	27	52	21	6.5	2.0	(2.9)	2.7
	150	29	42	29	6.2	1.9	(3.7)	2.9
	300	25	33	42	6.8	1.9	(3.0)	2.9

* The ratio in whole chloroplasts calculated from the *a*/*b* ratios observed for the three components and their fractional chlorophyll *a* contents. The *a*/*b* ratios determined experimentally for whole chloroplasts of *Enteromorpha* and *Brassica* were 1.9 and 3.1, respectively. The values in parentheses contain some errors due to partial pheophytization of chlorophylls in component III.

practically did not affect the *a*/*b* ratios of chlorophylls remaining on the pigment proteins of these two species. These data as well as the similar *a*/*b* ratios found for the two photosystems of marine green algae may support the above conclusion on the significant differences in the pigment compositions of the two photosystems between marine green algae and higher plants.

The absorption spectra of components I and II of *Enteromorpha compressa* obtained at sodium dodecyl sulfate/chlorophyll = 114 are shown by curves B and C, respectively, in Fig. 1 and those of components I and II of *Brassica vulgaris* obtained at sodium dodecyl sulfate/chlorophyll = 150 by curves B and C in Fig. 2 for comparison. The red band of chlorophyll *b* appeared as a distinct peak at 655 nm in the spectrum of component II of *Enteromorpha* (curve C in Fig. 1), and the height of this peak is close to the height of the red peak at 673 nm. The same band appeared as a shoulder in the spectrum of component II of *Brassica* (curve C in Fig. 2). The high *b* content in component II of *Enteromorpha* is thus reflected in the absorption spectrum as the distinct peak of chlorophyll *b*. By contrast, the chlorophyll *b* shoulder on the red band can not be recognized in the spectrum of component I of *Enteromorpha* (curve B in Fig. 1) as well as in the spectrum of component I of *Brassica* (curve B in Fig. 2). The high content of chlorophyll *b* in component II of *Enteromorpha* and its low content in component I are seen also in the absorption in the Soret region as the high peak at 470 nm and a low shoulder in the same region, respectively. It may be worth noting that the red peak of component I of both *Enteromorpha* and *Brassica* is located at 678 nm, which is longer than the red maxima at 673–674 nm of component II.

Peptide compositions of two pigment proteins

Peptide analysis was made by sodium dodecyl sulfate-polyacrylamide g

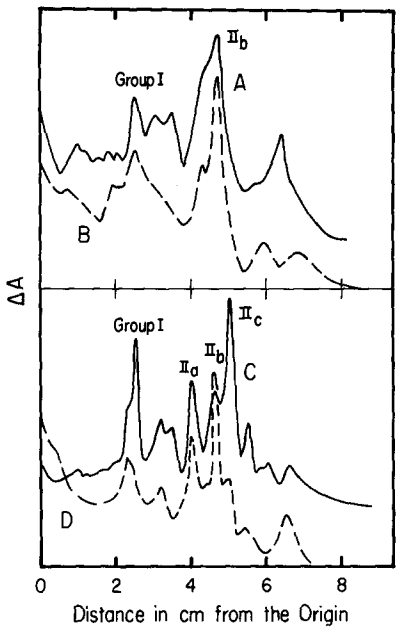

Fig. 4. Electrophoretic patterns of membrane polypeptides (stained with Coomassie brilliant blue) solubilized with sodium dodecylsulfate from acetone-treated chloroplasts of *Bryopsis maxima* (curve A), *Cheatomorpha spiralis* (B), *Spinacia oleracea* (C) and *Taraxacum officinale* (D). These curves were recorded in terms of $\Delta A = A_{600} - A_{700}$.

electrophoresis for the chloroplasts of *Bryopsis maxima* and *Cheatomorpha spiralis* which are chosen as representatives of marine green algae. Intact whole chloroplasts could be easily prepared from these algae by cutting the thalli. The peptide patterns were compared with those obtained by the same method for the chloroplasts of *Spinacia oleracea* and *Taraxacum officinale*. Curves A and B in Fig. 4 are the electrophoretic patterns of membrane polypeptides solubilized with sodium dodecyl sulfate from acetone-treated chloroplasts of *Bryopsis* and *Cheatomorpha*, respectively. For comparison, acetone-treated chloroplasts of spinach (curve C) and *Taraxacum* (curve D) were analyzed by the same procedure. The pattern obtained for spinach chloroplasts (curve C) was in good agreement with that reported previously [11, 14, 15]. According to Anderson and Levine [14], the polypeptides with molecular weights of about 60 000 (denoted as Group I) are associated with a membrane fraction enriched in Photosystem I activity and those denoted as II_a, II_b and II_c with molecular weights in the range of 25 000 to 30 000 are associated with a membrane fraction enriched in Photosystem II activity. All of these polypeptides were identified in the pattern (curve D) of *Taraxacum* chloroplasts, although the relative contents of these groups were considerably different for these two species of higher plants. The highest band in the pattern of *Taraxacum* was the band of II_b while the band of II_c was highest in the pattern of spinach. The patterns of *Bryopsis* and *Cheatomorpha* chloroplasts (curves A and B) are qualitatively different from curves C and D. The bands of II_a and II_c were missing and a different band appeared on the left side of the II_b band as a

shoulder or a small peak. It may be concluded from these data that Photosystem II of these marine green algae lacks the polypeptides of II_a and II_c which are present in Photosystem II of spinach and *Taraxacum* chloroplasts.

DISCUSSION

It was demonstrated in the present study that the ratio of chlorophyll *a* to *b* in the thalli of marine green algae is approximately 2, being lower than the ratio of 3 in the leaves of higher plants and the cells of fresh-water green algae. *Ulva lactuca*, a marine green alga, had once been classified into a group of "shadow plants" because of its lower *a*/*b* ratio close to 2. This view is, however, inconsistent with the fact that the *a*/*b* ratio in *Zostera marina*, which is a higher plant growing in sea water under the same light conditions as for some of the marine green algae examined in the present study, is 2.8. The lower *a*/*b* ratio is, therefore, not due to the low light intensity for marine green algae.

The chlorophyll composition in leaves of shadow plants has recently been reported by Boardman et al. [16] and Brown et al. [17]. They found that the chlorophylls/*P*-700 ratio in these plants is higher than that in the chloroplasts of the sun species of plant. By contrast, the chlorophylls/*P*-700 ratio in the chloroplasts of marine green algae was demonstrated in the present experiments to be lower than the ratio in the chloroplasts of higher plants. This change of the chlorophylls/*P*-700 ratio to a lower value also supports the above conclusion that the lower *a*/*b* ratio in marine green algae is a characteristic of marine green algae but not a characteristic in common with the plants growing under low light intensity.

The *a*/*b* ratio of the Photosystem II pigment proteins of *Enteromorpha compressa*, *Cheatomorpha spiralis*, *Ulva conglobata* and *Bryopsis maxima* was approximately 1 which is one half the ratio found for the pigment proteins of higher plants. The *a*/*b* ratio of the Photosystem I pigment proteins in these marine green algae was approximately 5 which is also lower than the ratio of 7 found for the Photosystem I pigment proteins of higher plants. This indicates that the lower chlorophyll *a*/*b* ratio of marine green algae is mostly due to the lower *a*/*b* ratio in Photosystem II. Similar data were drawn by Anderson et al. [18] and Boardman et al. [16], who showed that the low *a*/*b* ratio close to 2 in the chloroplasts of shadow plants is due to the lower *a*/*b* ratios in the two photosystems but not due to an increase of the total chlorophyll content in Photosystem II relative to Photosystem I.

The protein analysis of the chloroplasts of *Cheatomorpha spiralis* and *Bryopsis maxima* indicated that these chloroplasts lack the peptides of II_a and II_c which are present in the chloroplasts of spinach and *Taraxacum* but contain a different polypeptide which appeared between II_a and II_b bands in the profile. Further experiments are required to assign this peptide to one of the two photosystems.

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