CHROMOPROTEINS OF MARINE RED ALGA *PORPHYRA CRISPATA**

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Abstract

Seven chromatographically and electrophoretically homogeneous phycoerythrins were isolated from marine red alga *Porphyra crispata*, of which six belong to R-phycoerythrin. Comparison of visible absorption spectra of chromatographically separated fractions obtained in different years suggests possible environmental, seasonal or developmental variation of the relative contents of different chromoproteins. All phycoerythrins isolated are rich in amide nitrogen. They are richer in alanine, aspartic acid, glycine, leucine and serine, but have a low content of histidine. Four phycoerythrins, namely fractions IIa, IIc, IIIa and IIIb are completely free of glutamic acid. All seven phycoerythrins contain carbohydrates. Arginine constitutes the sole N-terminal amino acid of all of the phycoerythrins as determined by the polyamide thin layer chromotography of dansyl derivatives. Molecular weights of fractions Ib, IIa, IIb_1, IIb_2, IIc, IIIa and IIIb from gell filtration method are >200,000, >200,000, between 200,000 and 100,000, 94,500, 91,500 89,000 and 67,500, respectively.

Introduction

Algal biliproteins, namely phycoerythrins and phycocyanins (ÓhEocha, 1958), were shown to be the primary photosynthetic light absorber in Rhodophyta and Cyanophyta (Haxo and Blinks, 1950). The suggestion that higher plants may also contain a biliprotein, phytochrome (Siegelman *et al.*, 1964), has attracted further attention to the algal biliproteins.

This work concerns with the chemical and physical properties of phycoerythrins isolated from *Porphyra crispata*, the only known *Porphyra* from Taiwan.

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Materials and Methods

The red alga *P. crispata* used in this experiment was collected by hand-picking at the beach near Keelung during the winter. Immediately after collection, sands and shells were removed by washing in sea water. The alga was kept in ice chilled sea water and brought to the laboratory as soon as possible.

After being washed with distilled water, the fresh alga was ground with 3-fold amount (volume/weight) of 0.05 *M* tris-buffer, pH 7.5 in a ball mill at 4°. The extracts were filtered with cheese cloth and then centrifuged at 1000 xg for 10 minutes. The supernatant was concentrated to a small volume by lyophilyzation and again centrifuged at 35,000 xg for 15 minutes. The supernatant obtained was then subjected to ammonium sulfate fractionation. At first, finely ground ammonium sulfate was added to the solution until 20% saturation of the salt was reached and the precipitate was collected by centrifugation (fraction Ⅰ). The supernatant from fraction Ⅰ was next made to 40% saturation of ammonium sulfate and the precipitate thus formed was designated as fraction Ⅱ. Similarly, the supernatant from fraction Ⅱ was made to 60% saturation of the salt and fraction Ⅲ was precipitated.

Three fractions obtained were purified further by the gel filtration method. A glass column (7×45 cm) packed with Sephadex G-100 was used for this purpose. Sample applied on the Sephadex column was eluted with 0.005 *M* tris-buffer, pH 7.5, and the eluate cut into 5 *ml* fractions and collected in graduated centrifuge tubes. The optical density readings at 280 mμ and 260 mμ of the fractions were recorded. Those tubes belonging to the same peak were combined together and concentrated before determination of spectra. Fraction Ⅰ has been separated into three colored subfractions which were designated as Ⅰa, Ⅰb, and Ⅰc respectively in the order of emergence from the Sephadex column. Fraction Ⅱ consists of four colored subfractions: Ⅱa, Ⅱb_1, Ⅱb_2, and Ⅱc. Fraction Ⅲ contains only two: Ⅲa and Ⅲb.

For protein measurement, the Folin phenol reagent was used according to Lowry *et al.* (1951).

The absorption spectra of isolated chromoproteins were obtained with a Perkin-Elmer 202 recording spectrophotometer.

Electrophoretical study of each chromoprotein was made on polyacrylamide gel columns according to Nerenberg (1966).

Amino acid composition of chromoproteins was obtained with an amino acid analyzer (Model LC-5A, Yanagimoto Mfg. Co., Japan).

For the determination of approximate molecular weight of isolated chromoproteins, Sephadex columns K15/30 and K25/45 purchased from Pharmacia Fine Chemicals, Uppsala, Sweden, were used. Bovine serum albumin (M. W.

66,500, tetramer), hemoglobin (M. W. 68,000), papain (M. W. 20,900), carboxypeptidase (M. W. 34,300) and cytochrome-c (M. W. 13,000, dimer) were used as the standard proteins to calculate the molecular weight of the chromoproteins. The column eluate was cut into 0.5 to 1.0 *ml* fractions. Optical density reading at the absorption maximum for the chromoprotein examined was taken for each tube to locate its peak position.

N-terminal amino acid of each purified chromoprotein was deermined by the polyamide thin layer chromatography of dansyl derivatives (Woods and Wang, 1966). Chromoproteins were dialyzed against distilled water for 36 hours before N-terminus determination.

Results

An extract from 170 *gm* fresh red alga contained 675 *mg* proteins which amounted to 0.397% of fresh weight. Much of fraction I denatured soon after ammonium sulfate precipitation, thus, lowered the value of fraction I listed in Table 1.

Table 1. *Protein content of the three major fractions and the relative amounts of their subfractions*

	I			II				III		Supernatant
Total* protein content	69.12mg			120.40 mg				32.90 mg		149.96 mg
Relative**	Ia	Ib	Ic	IIa	IIb$_1$	IIb$_2$	IIc	IIIa	IIIb	
amount	1.0	2.6	0.8	2.1	2.0	0.9	1.0	2.5	1.0	

* It is reasonable to assume that there are colorless proteins in each major fraction, hence chromoproteins do not constitute the total amount of each fraction.

** These values show the intrafractional ratios only and can not be compared among the different major fractions.

Sephadex column chromatographic pattern of fractions I, II and III are shown in Fig. 1, 2 and 3, respectively. The O. D. of both 260 mμ and 280 mμ of the eluates were determined. In Fig. 2 and 3, the absorption at 560 mμ were also recorded. By adjusting the flow rate (1 ml/5 min.) and the amount of sample applied, IIb could be further separated into two components, namely IIb$_1$ and IIb$_2$, on a Sephadex G-200 column.

All the UV spectra of the nine chromoproteins isolated from *P. crispata* showed slight differences. But visible spectra of the compounds revealed characteristic peaks clear enough for identification. Ia and Ic showed no O. D. maximum at 400–650 mμ region but did have a maxium at about 300–350 mμ which indicated that these two chromoproteins could be assumed to be certain kinds of flavoproteins.

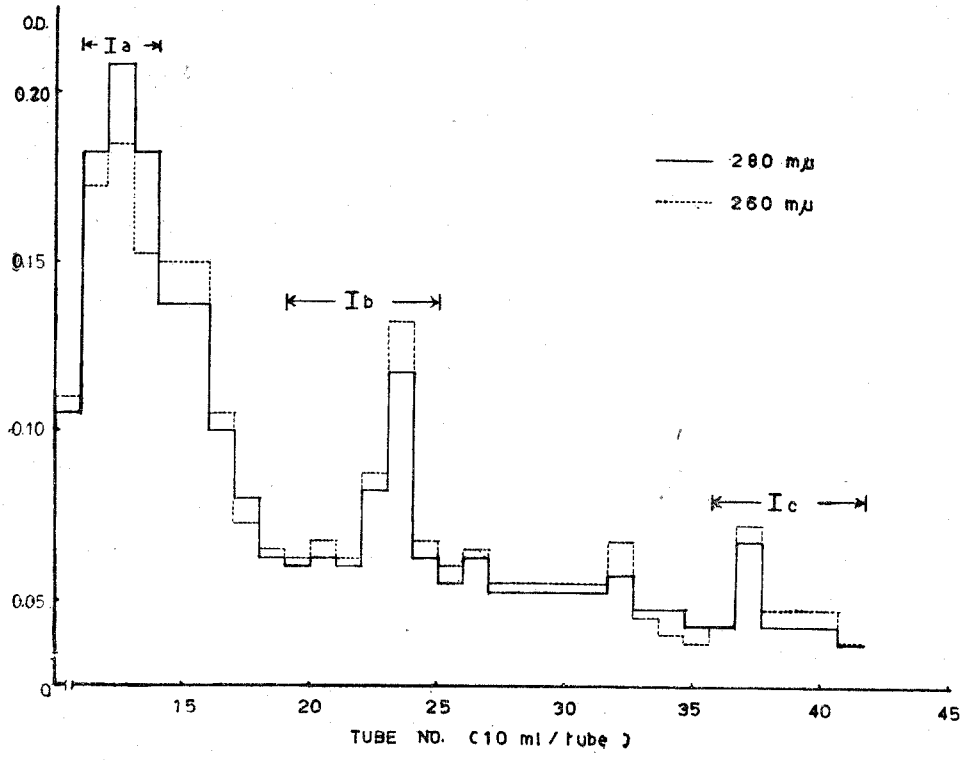

Fig. 1. Sephadex G-200 Column Chromatographic Pattern of Fraction I

The absorption properties of other chromoproteins are summarized in Table 2.

Table 2. *Absorption property of chromoproteins*

Fraction	Absorption maxima (mμ) (in tris-buffer, pH 7.5)		
	Main	Shoulder	Small
Ib	500	515	542, 564(v. s.)*, 640(v. s)
IIa	500, 544	405, 515	562, 641
IIb_1	499, 564(499>564)	549	373(v. s.), 643(v. s.)
IIb_2	499, 564(499<564)	549	643(v. s.)
IIc	513, 544	404, 494, 585	645(v. s.)
IIIa	498, 562	549	644(v. s.)
IIIb	498	403, 504	540, 645(v. s.)

* v. s.=very small

In an earlier work (Jan. 17, 1966) an extract from *P. crispata* was applied directly on a Sephadex G-200 column. Three *ml* fractions were collected with a flow rate of 3 ml/30 min. The absorption property of the five peaks

obtained is summarized in Table 3 arranged in the order of emergence from the column.

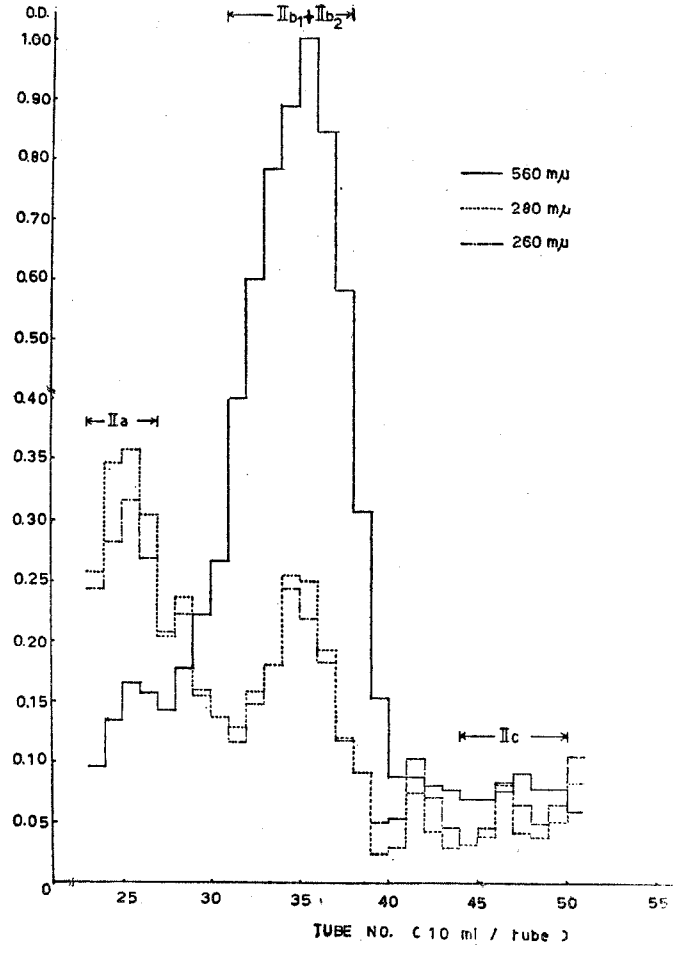

Fig. 2. Sephadex G-200 Column Chromatographic Pattern of Fraction II

Table 3. *Absorption property of fractions separated by Sephadex column without previous ammonium sulfate fractionation*

Tube number	Absorption maxima (mμ), in tris-buffer pH 7.5		
	Main	Shoulder	Small
No. 13	438, 680	420	382, 496, 630
No. 35	499, 566, 620	415	380, 440, 653, 678
No. 46	499, 565, 618	650	370, 440(v. s.)
No. 54	499, 562, 618	650	366
No. 72	500, 560		416, 618

Table 4. *Amino acid compositions of* ***P. crispata*** *phycoerythrins*

Amino acid / Fraction	Mole ratio in each fraction																
	Lys.	His.	Ammo.	Arg.	Asp.	Thr.	Ser.	Glu.	Pro.	Gly.	Ala.	Cys.	Val.	Met.	Ileu.	Ieu.	Tyr.	Phe.
Ⅰb	2.80	1.36	7.38	2.44	7.62	4.08	5.07	5.82	4.30	6.78	8.54	1.00	5.41	1.41	3.18	6.66	3.00	2.82
Ⅱa	7.19	6.03	12.28	7.12	16.30	22.30	10.52	0	8.26	20.60	21.90	33.01	1.00	3.89	10.25	15.60	4.41	4.67
$Ⅱb_1$	5.73	1.00	10.44	6.63	13.71	5.43	9.73	9.00	5.68	11.31	17.60	2.88	13.62	6.54	4.37	10.24	4.70	3.37
$Ⅱb_2$	5.42	1.00	11.60	5.74	18.90	5.87	11.86	9.68	5.67	13.56	22.05	3.19	15.95	2.45	4.93	10.68	5.32	3.68
Ⅱc	6.66	1.00	15.87	6.43	18.40	7.23	22.23	0	10.43	17.30	23.60	13.30	4.33	3.56	6.90	15.40	5.70	5.27
Ⅲa	3.74	1.00	6.90	3.08	9.26	8.56	8.72	0	5.76	8.89	12.57	0	6.03	1.87	3.69	9.32	3.26	3.32
Ⅲb	4.91	v. s.	16.65	v. s.	18.10	14.75	18.40	0	1.08	17.00	20.08	21.70	2.43	3.57	6.23	13.60	1.00	5.48

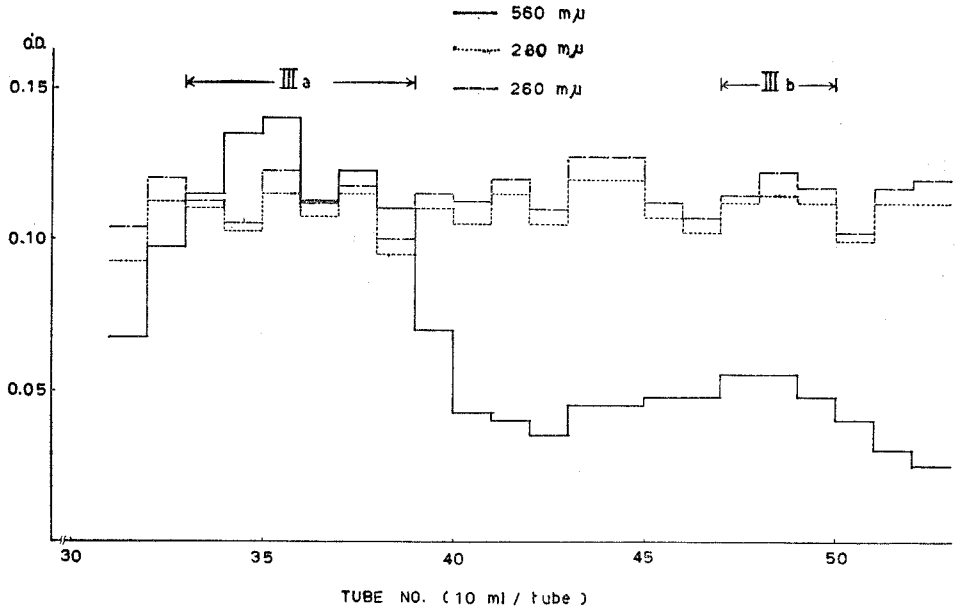

Fig. 3. Sephadex G-100 Column Chromatographic Pattern of Fraction Ⅲ

Each chromoprotein listed in Table 2 moved as a single band when subjected to polyacrylamide gel disc electrophoresis.

An attempt to isolate pigments from purified chromoproteins according to the method of ÓhEocha (1963) failed beacuse only a small amount of purified chromoproteins was obtained.

The amino acid compositions of the purified chromoproteins are summaried in Table 4.

The chromoprotein fractions obtained showed positive Molisch's α-naphthol-H_2SO_4 reaction (for carbohydrate in general) and aniline acetate reaction (for pentose). The minimal molecular weight unit of each phycoerythrin was

Table 5. *Molecular weights of phycoerythrins*

Fraction	Mol. Wt. from gel filtration method		Minimal mol. wt. from total amino acid analysis
	Elution volume	Mol. Wt.	
Ⅰb	—	>200,000	7,700
Ⅱa	—	>200,000	23,660
$Ⅱb_1$	—	between 200,000 and 100,000	I5,540
$Ⅱb_2$	3.0 ml	94,500	15,410
Ⅱc	3.5 ml	91,500	18,510
Ⅲa	4.0 ml	89,000	9,240
Ⅲb	8.0 ml	67,500	14,890

calculated on the basis of its content of histidine or valine or tyrosine, its least abundant amino acid. The results together with those obtained by gel filtration method are shown in Table 5. Molecular weights from gel filtration method were calculated from a semi-log plot of molecular weight vs. elution volume.

Arginine constitutes the sole N-terminal amino acid of all the seven phycoerythrins examined. Blank of chromophore(s) of phycoerythrins was simultaneously made in order to exclude the interference of the derivatives of pigment(s).

Discussion

In Table 2. all chromoproteins, except IIc which showed a peak at 513 mμ, belong to R-hycoerythrin according to the review of ÓhEocha (1965). No chromoprotein of phycocyanin type was found.

The solutions and lyophilized products of both Ib and IIa looked alike, but the possibility that these two fractions might be the same component was excluded by the following criteria: difference of absorption spectra, different 280/260 O. D. ratios, and different amino acid compositions.

Because of either presence in small amount or lower visible/280 mμ O. D. ratio, IIc and IIIb was detected only under careful separation and examination followed by lyophilyzation.

Comparing with Fujiwara's work (1955, 1956), *P. crispaia* seems to contain much more chromoprotein components than *Porphyra tenera.* However it is possible that some other chromoproteins exist in small amount in *P. tenera.* It is interesting to note that no chromoprotein of *P. crispata* corresponds to that of *P. tenera.*

In spite of the possible overlapping of some chromoproteins in the earlier study (Jan. 17, 1966), some absorption maxima listed in Table 3, especially those corresponding to phycocyanin types, were completely absent in Table 2. This suggests that environmental, seasonal or developmental variation of relative amounts of differet chromoproteins may exist.

Fujiwara (1956) reported that phycoerythrin from *P. tenera* contained small amounts of glutamic acid, phenylalanine, leucine, and isoleucine, but they were rich in amide nitrogen. As shown in Table 4, all phycoerythins from *P. crispata* are also rich in amide N. With the exception of Ib, IIb$_1$, and IIb$_2$, they are completely free of glutamic acid. They are richer in alanine, aspartic acid, glycine, leucine, and serine, but have a low content of histidine. Both histidine and arginine are very small in IIIb.

According to Fujiwara (1961), *P. tenera* chromoprotein contain considerable amount of carbohydrates. The chromoprotein fractions obtained in this study

also showed positive Molisch's α-naphthol-H_2SO_4 reaction (for carbohydrate in general) and aniline acetate reaction (for pentose) indicating that they also contained carbohydrates. To what extent the carbohydrate residues contribute to their molecular size has not been determined.

All results indicate that the *P. crispata* phycoerythrins can be distinguished from each other not only by their absorption spectra, amino acid compositions and electrophoretic mobilities but also molecular sizes.

Qualitative analysis of R-, B-, and C-phycoerythrins showing methionine to be the sole N-terminal amino acid residue in all three biliproteins has been reported by Ó Carra and ÓhEocha (1962). However, arginine constitutes the sole N-terminal amino acid of all the seven phycoerythrins examined in this work. That all of the peptide chains of the phycoerythrins isolated from *P. crispata* have identical N-terminus, viz. argine, may have certain evolutional significance, since the alga belongs to Bangiaceae, one of the most primitive families of red algae.

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