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Introduction

It is known that red and blue-green algae contain biliproteins. Svedberg and Katsurai (1) proposed a system of nomenclature for these biliproteins on the basis of their usual occurrence; R- or C- was prefixed to the biliproteins of *Rhodophyta* or *Cyanophyta* respectively. The R- and C- variants of phycoerythrin and phycocyanin have been isolated in crystalline states and investigated on their chemical nature by earlier workers, especially Kylin (2), Kitasato (3) and Lemberg (4-6) contributed to this research. However little is known about their physicochemical properties.

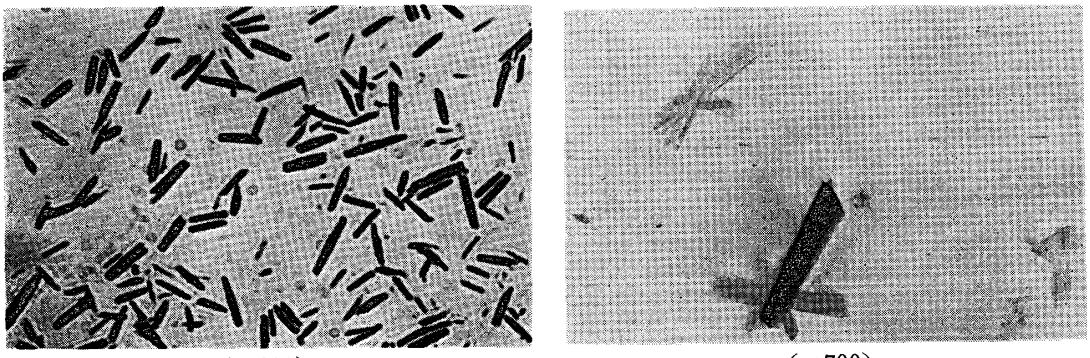
Recently, Airth, Raymond and Blinks (7-9) and Haxo *et al.* (10, 11) reported that B-phycoerythrin, allophycocyanin and biliprotein complex occur in both red and blue-green algae. On the other hand, Fujiwara (12) reported that recrystallized phycoerythrin showed positive Molisch reaction.

In the present work, we have carried out some investigations on the occurrence of several sugars in the hydrolyzates of the purified and crystallized biliproteins separated from fresh *Porphyra tenera.*

Experimental

Separation of biliproteins—Fresh *Porphyra tenera* collected from Matsushima Bay, in the northern part of Honshu, Japan, in late December, was extracted with water covering by toluene to avoid putrefaction at 5°C in a dark place. After 10 days, it was filtrated with cotton cloth and the reddish-violet filtrate was centrifuged at 3000 rpm for 10 min, then the supernatant liquid was treated with ammonium sulfate to make precipitates of biliproteins at 40 per cent saturation of ammonium sulfate. Those were separated by centrifugation and the precipitates were washed with three volumes of water, under which conditions phycocyanin dissolved much quicker in water than phycoerythrin, the phycoerythrin therefore was obtained as undissolved fraction

by centrifugation. The procedures for separation and purfication of phycoerythrin were carried out by repeating precipitation with ammonium sulfate. The purified phycoerythrin was reprecipitated with ammonium sulfate and crystallized out in long plates as shown in Fig. 1.



(×600) Phycoerythrin (×700) Phycocyanin

Fig. 1 Photomicrographs of crystalline biliproteins.

Crystallized phycocyanin was obtained by the rivanol method of Fujiwara. Namely, the precipitate from which the phycoerythrin was no longer obtained by the precipitating fractionation method was dissolved in water, centrifuged to remove impurities and dialyzed against cold water for 24 hr. Then 1 per cent rivanol solution was added to the dialyzed solution, whereon the residual phycoerythrin symplex precipitated. After centrifugation, small amounts of acid clay were added to the supernatant to remove excess rivanol. A pure solution of phycocyanin was obtained by centrifugation and this was repeatedly treated with ammonium sulfate to salt out and it was crystallized out in rhombohedric form as shown in Fig. 1. The procedure is presented in Scheme 1.

Properties of biliproteins—Phycoerythrin solution was bright red in color with yellow fluorescence which was recognized in the range of pH 3.6—11.0.

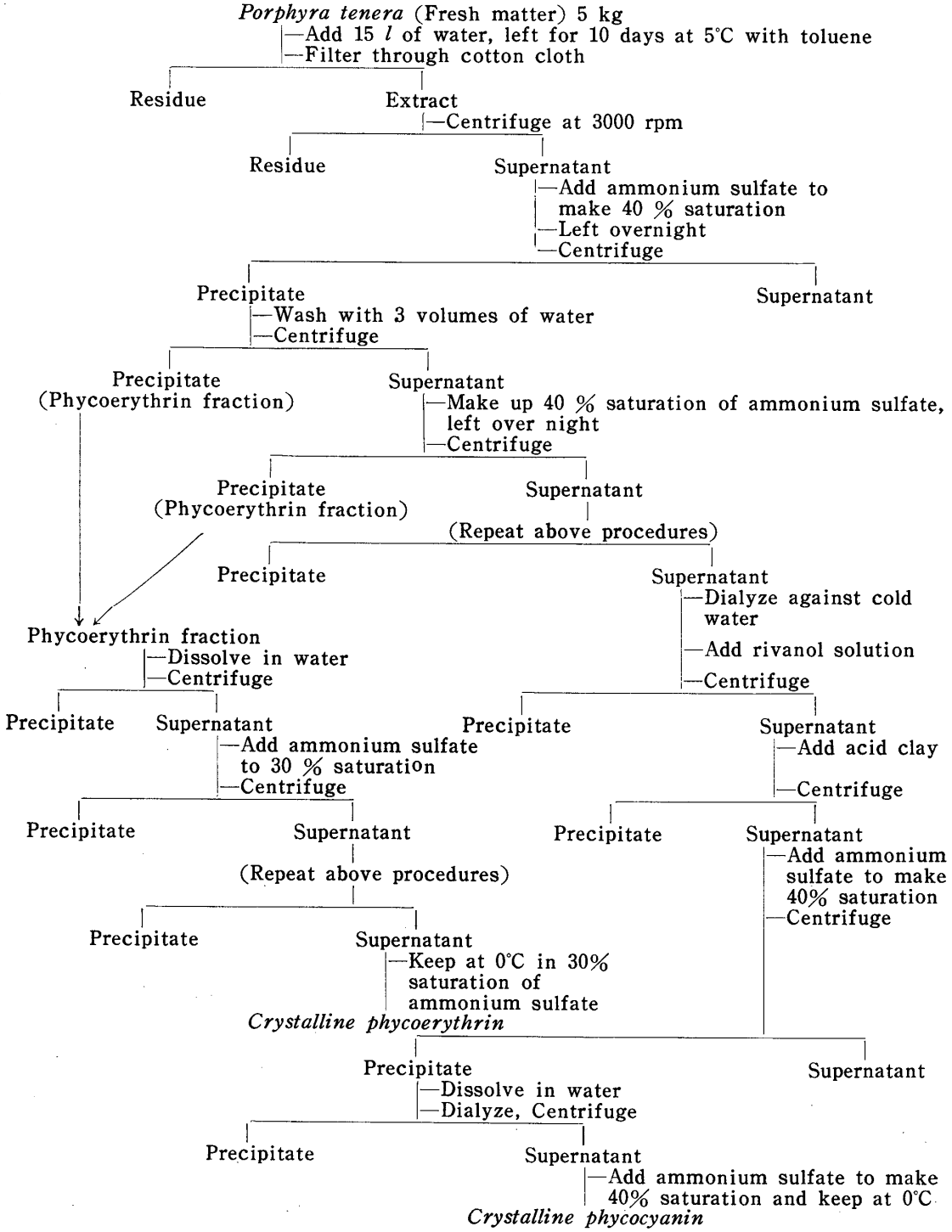
On the other hand, blue aqueous solution of phycocyanin showed red fluorescence which was stable in the pH 5.6—9.0 range.

The biliproteins were found to be pure so far as the electrophoretic and chromatographic examinations we concerned. That is, electrophoretic behaviours of both biliproteins were tested in phosphate buffer of ionic strength $\mu=0.1$ at pH 7.3, 6.7 and 5.6 using the Tôyô C-type paper electrophoretic apparatus and the patterns obtained were found to be homogenous. The chromatographic behaviours were also examined on tricalcium phosphate columns developing with 0.01*M* phosphate buffer. These experiments will be reported in detail at another opportunity.

Absorption spectra—The light absorptions of both biliproteins were measured at pH 6.8 with the Hitachi photoelectric spectrophotometer and their spectra are shown in Fig. 2. Phycoerythrin showed the absorption maxima at

Scheme 1.

Flow-sheet of preparation of biliproteins.



498 mμ and 568 mμ in the visible spectrum region and phycocyanin revealed a mono-peaked absorption curve at 615 mμ.

The elements of phycoerythrin and phycocyanin were analyzed but sulfur was not determined. The results in Table 1 agree with those of earlier reports.

Table 1. Elemental analysis of biliproteins.

Phycoerythrin (%)

C	H	N	S	Ash	References
51.78	8.07	15.41		1.75	(Authors)
50.80	7.99	15.13	2.06	1.06	(Fujiwara)
50.87	7.04	15.31	1.76		(Kitasato)

Phycocyanin (%)

C	H	N	S	Ash	References
51.58	7.94	15.13		0.72	(Authors)
51.01	6.82	15.81	1.12	1.52	(Fujiwara)
50.60	6.90	15.76	1.69		(Kitasato)

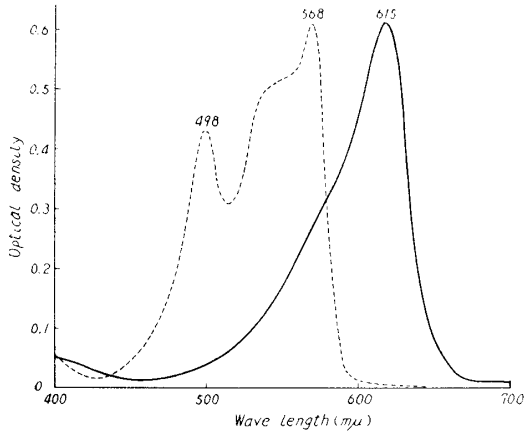


Fig. 2 Spectral absorption curves of biliproteins.
Phycoerythrin (……),
Phycocyanin (——)

Detection of sugar—The pure phycoerythrin and phycocyanin, 100 mg of each sample, were hydrolyzed with 20 *ml* of 0.6N hydrochloric acid at 100°C for 24 hr. in sealed glass tubes. Both hydrolyzates showed positive Molisch reaction but glucosamine was not recognized by Elson-Morgan reaction (13). After passing through the columns of Amberlite IR (H^+ form) and IR-410 (COO^- form) of ion exchange resins, the hydrolyzates were concentrated and spotted on Tôyô filter paper No. 52. The size of the filter paper employed was 40 cm in length. Pyridine-butanol-water mixture (4 : 6 : 3) was used as the developing solvent and the multiple development was performed by the ascending method at 25°C. Anilinehydrogenphthalate was used for the development of the color. The mixture of equal volumes of 1 per cent alcoholic resolcinol and 0.2*N* hydrochloric acid was used for the identification of mannose, fructose and arabinose, following the method of Rachniskii *et al.* (14), because the three sugars are very close in the Rf values. Six spots were clearly detected from the hydrolyzates of phycoerythrin

and seven from those of phycocyanin as shown in Fig. 3. Among them, four spots were identified as xylose, mannose, glucose and galactose and the two spots which were high in Rf values seemed to be rhamnose and tetrose respectively and the spots of low Rf values seemed to be a kind of be oligosaccharides.

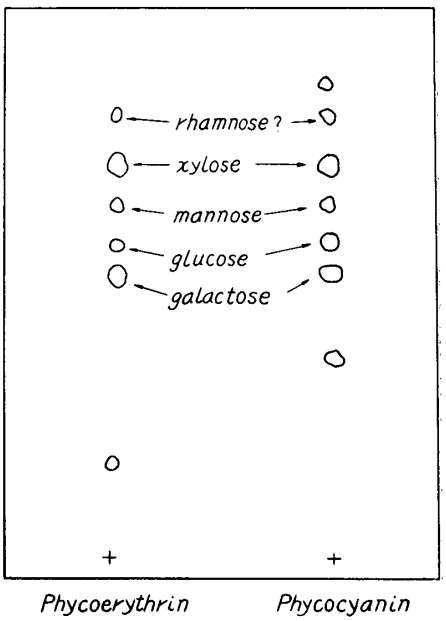


Fig. 3 The chromatograms of hydrolyzates of biliproteins developed twice by pyridine-butanol-water mixture.

Summary

Phycoerythrin and phycocyanin of *Porphyra tenera* were isolated and crystallized. Both biliproteins were ascertained to be pure chromatographically and electrophoretically. The results of elemental analysis agreed with those of earlier reports. From the absorption spectra of phycoerythrin and phycocyanin, it was observed that phycoerythrin has two absorption maxima and phycocyanin has one.

Eight sugars were detected from hydrolyzates of phycoerythrin and phycocyanin and among them four sugars were identified as xylose, mannose, glucose and galactose.

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