Studies on phycobiliproteins in *Algae*. VI. Light-harvesting phycobiliprotein pigments in some *Rhodophyta* from the Adriatic Sea

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Abstract

The phycobiliprotein content in 5 species of red algae from the coast of the Adriatic Sea was studied by chromatography on Sephadex G-100. The phycobiliproteins, R-phycoerythrin, C-phycocyanin and allophycocyanin were identified. The total content of phycobiliproteins ranged from 0.152 (*Phyllophora nervosa*) to 1.874 mg·g⁻¹ dry wt. (*Plocamium cartilagineum*). The dominant phycobiliproteins were found to belong to the phycocyanin group, this resulting from complementary chromatic adaptation.

Key words: *Rhodophyta*, *phycobiliproteins*, *chromatic adaptation*

INTRODUCTION

In addition to the chlorophyll and carotenoid pigments present in all algae, some algae also contain phycobiliprotein complexes (Goodwin 1974). It has been found that the most common of these pigments in various *Rhodophyta* species are two types of phycoerythrin (B and R) and phycocyanin (C and R) together with allophycocyanin (Rudiger 1975).

Experimental studies shown, that when blue-green and red algae are grown in green light, a decrease in the content of phycobiliprotein pigments from the phycoerythrin group occurs, whereas red light gave an increase in phycocyanin (Sudina et al. 1978). This effect is called complementary chromatic adaptation. The studies made by Tyler and Smith (1970) showed that red rays penetrate only a few meters into the sea, the deeper layers being penetrated only by green and blue rays. If complementary chromatic adaptation occurs in red algae in their natural environment,

a predominance of phycobiliproteins of the phycoerythrin group should be observed in the thalli of red algae collected from the deeper layers (10-25 m), whereas in those from the surface layers, phycobilins of the phycocyanin group should predominate. In the red algae collected from the deeper layers (15-25 m) of the coastal waters of New Zealand into which only green and blue rays penetrate, phycoerythrins were found to dominate (Czeżuga and Taylor 1983). The presence of different phycobiliprotein pigments in red seaweeds has been found useful in the taxonomy of these algae (Hirose et al. 1969). Attempts have also been made to substantiate the hypothesis presented some time ago on the evolution of red algae from blue-green algae (Sudina et al. 1978) by studying their phycobiliprotein content. In this connection, all information on the presence of phycobiliproteins in the representatives of these two types of algae may be contributory to the confirmation of the validity of this theory.

MATERIAL AND METHODS

In order to determine whether, in the layers penetrated by red rays, the content of phycobiliproteins of the phycocyanin group increases in red algae thalli, we carried out investigations into the content of these pigments in thalli collected from depths of water penetrated by red rays, that is, at ebb tide in a one-meter surface layer.

The red algae were collected from the low water zone of the Island of Sipan near Dubrownik (Yugoslavia) according Boudouresque (1971) and Czeżuga (1979). The following species were investigated: *Nemalion helminthoides* (Vellay) Bot., *Plocamium cartilagineum* (L.) Dixon, *Phyllophora nervosa* (Dc.) Grey., *Digenea simplex* (Wulf.) Ag. and *Laurencia obtusa* (Huds.) Lam.

The phycobiliproteins were extracted from homogenized material (about 250 g dry mass) at 2-3°C for 1 hour with 0.01 M phosphate buffer with 0.15 M NaCl at pH 7.0. The extract was then centrifuged at 4°C in a K-24 Janetzki centrifuge at 20000 × g for 20 min. The supernatant obtained was saturated to 35% with ammonium sulphate and left for 2 h after which it was centrifuged again at 22300 × g for 15 min (fraction I). The supernatant was saturated to 70% with ammonium sulphate, left for 1 h, and the precipitate thus formed was centrifuged at 22300 × g for 15 min (fraction II). Both fractions were then suspended in 6 cm³ of 0.1 M phosphate buffer (pH 7.0) and dialyzed overnight in a Dialysis

Membrane (Union Carbide Corporation, Chicago, U.S.A.) at 4 °C in the presence of the same phosphate buffer at pH 7.0

Both fractions were then applied to a Sephadex G-100 column (Pharmacia Fine Chemicals AB, Uppsala, Sweden) that had been previously washed in 0.1 M phosphate buffer (pH 7.0). Fraction I contained phycoerythrin. The phycoerythrin was eluted from the column with a 0.1 M phosphate buffer, then saturated to 30% with ammonium sulphate, left for 1 h, and centrifuged at 4 °C and $22\,300\times g$ for 30 min. The precipitate was dissolved in 0.1 M phosphate buffer (pH 7.0) to which a few drops of 0.01% sodium azide were added to prevent bacterial growth. After overnight dialysis at 4 °C in the presence of the same buffer, measurements of extinction were made in a Spektromom-203 spectrophotometer. Fraction II is containing phycocyanin and allophycocyanin. After passing through the column, phycocyanin was washed off with 400 cm³ of phosphate buffer in a linear concentration gradient of 0.005-0.1 M. Allophycocyanin remain at the highest point. The phycocyanin was saturated with ammonium sulphate to 65%, left for 1 h and then centrifuged at $14\,000\times g$ for 15 min. The precipitate was suspended in a small volume of 0.1 M phosphate buffer (pH 7.0) containing 0.01% of sodium azide and dialyzed overnight at 4 °C in the presence of the same buffer. After dialysis, the extinction was measured.

For elution of allophycocyanin from the column, 0.5 M phosphate buffer (pH 7.0) was used. The eluent was then saturated to 75% with ammonium sulphate, left overnight, and then centrifuged at 4 °C and $22\,300\times g$ for 30 min. The precipitate obtained was suspended in a small volume of 0.1 M phosphate buffer (pH 7.0) containing 0.01% sodium azide and dialyzed overnight in the presence of the same buffer after which the extinction measurements were made.

Fluorescence emission spectra were obtained at room temperature essentially as described by Ray et al. (1978).

The total content of phycobiliprotein pigments in the red seaweed thalluses and the percentages of the various pigments were determined by the method described in the paper by Bennett and Bogorad (1973).

Statistical elaboration was done using Student's *t* test.

RESULTS AND DISCUSSION

Three phycobiliproteins were identified in the material studied, that is R-phycoerythrin, C-phycocyanin and allophycocyanin (Table 1). These phycobiliprotein pigments were found in all species under investigation. The total phycobiliprotein content varied between 0.152 (*Phyllophora nervosa*)

and $1.874 \text{ mg} \cdot \text{g}^{-1}$ of dry mass (*Plocamium cartilagineum*). The comparatively high content of phycocyanins in the material studied, comparing as it did from 64.5 to 85.0% (mean) of all of the phycobiliproteins, is of particular interest.

In our studies on the phycobiliprotein pigment content of several red algae species from the New Zealand coast (Czeżuga and Taylor 1983), we found that pigments of the phycoerythrin group predominated in all five species investigated. Their content ranged from 42.7 to 64.1%. The explanation for this high percentage of phycoerythrin pigments lay, in our opinion, in the fact that the algae had been taken from comparatively deep water penetrated mainly by green light rays which, as we know cause an accumulation of phycoerythrin in blue-green algae (Jevner et al. 1965, Fujita and Hattori 1960). We assumed that a similar phenomenon took place in red algae also. Blue-green algae grown under red light accumulate mainly phycocyanins. If the same applies to red algae, the specimens collected from the surface layer of the water penetrated mainly by red light rays should contain mainly phycobiliprotein pigments of the phycocyanin group. As the findings of these investigations on the red algae of the Adriatic Sea have shown, this supposition was correct. The thalli of all five red algae species were found to contain mainly pigments of the phycocyanin group, C-phycocyanin and allophycocyanin, which constituted from 64.5 to 85.0% of all of the phycobiliprotein pigments contained in the algae, whereas R-phycoerythrin comprised only 15.0 to 35.5%.

This phenomenon supplements the chromatic adaptation of algae of the *Rodophyta*, enabling them to live in ecological niches into which light rays of various wave-lengths penetrate. Phycobiliprotein pigments act as additional absorbing light rays of shorter wave-lengths than those proper to chlorophylls. The energy absorbed is then transferred to the various photosystems of the alga photosynthesizing system. As was demonstrated in studies of red algae, *Porphyridium cruentum* (Ley and Butler 1980), it is transferred from photosystem II to photosystem I (Kursar et al. 1983).

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Table 1

The phycobiliproteins content in certain species of red algae (n = 5)

Family and species	Fraction	Visible absorption maxima, nm	Fluorescence emission maximum, nm	Identified compound	Amounts, % of all of the phycobiliproteins (mean)	Total content of phycobiliproteins, mg · g ⁻¹ dry weight
<i>Helminthocladiaceae</i>						
<i>Nemalion helminthoides</i>	I	496, 544, 568	578	R-phycoerythrin	35.5	0.444 ± 0.012
	II	612	637	C-phyococyanin	2.0	
	III	650	660	allophycocyanin	62.5	
<i>Plocamiaceae</i>						
<i>Plocamium cartilagineum</i>	I	496, 544, 564	578	R-phycoerythrin	22.1	1.874 ± 0.028
	II	614	637	C-phyococyanin	29.9	
	III	648	660	allophycocyanin	48.0	
<i>Phyllophoraceae</i>						
<i>Phyllophora nervosa</i>	I	494, 534, 560	578	R-phycoerythrin	24.0	0.152 ± 0.008
	II	608	638	C-phyococyanin	32.0	
	III	652	660	allophycocyanin	44.0	
<i>Rhodomelaceae</i>						
<i>Digenea simplex</i>	I	496, 546, 562	577	R-phycoerythrin	19.6	0.548 ± 0.024
	II	614	638	C-phyococyanin	28.6	
	III	646	660	allophycocyanin	51.8	
<i>Laurencia obtusa</i>	I	496, 546, 566	578	R-phycoerythrin	15.0	0.245 ± 0.016
	II	622	638	C-phyococyanin	35.0	
	III	648	660	allophycocyanin	50.0	

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Badania barwników fikobiliproteinowych u glonów. VI. Barwniki fikobiliproteinowe pochłaniające światło u niektórych krasnorostów z morza Adriatyckiego

Streszczenie

Stosując chromatografię na Sephadex-100 badano zawartość barwników fikobiliproteinowych u 5 gatunków krasnorostów z morza Adriatyckiego. Ustalono obecność R-fikoerytryny, C-fikocjaniny oraz allofikocjaniny. W plechach wszystkich badanych gatunków krasnorostów w największych ilościach (64,5-85,0%) wystąpiły fikobiliproteiny należące do frupy fikocjanin (C-fikocjanina, allofikocjanina) jako wynik uzupełniającej adaptacji chromatycznej tych glonów do warunków świetlnych panujących w powierzchniowych warstwach Adriatyku. Ogólna zawartość barwników fikobiliproteinowych u badanych glonów wahała się od 0,152 (*Phyllophora nervosa*) do 1,874 mg·g⁻¹ suchej masy (*Plocamium cartilagineum*).