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SHORT COMMUNICATION

R-PHYCOCYANIN, A DISTINCT TYPE OF BILIPROTEIN*

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Abstract—It was shown by fractionation and analytical methods, spectrophotometric and denaturation studies, and microscopic examination of crystalline samples, that R-phycocyanin is a distinct type of biliprotein, contrary to the suggestion of Hattori and Fujita[1] who claimed that it is impure C-phycocyanin.

INTRODUCTION

R-PHYCOCYANIN, which has visible absorption maxima at about 553 and 615 mμ,[2] was considered by Svedberg and Katsurai[3] to be the phycocyanin characteristic of Rhodophyta (Red Algae). C-phycocyanin (single absorption maximum at 615 mμ) was later isolated from three species of Rhodophyta by Hattori and Fujita[1] and on this basis these authors stated that "the earlier claim for the occurrence of two different forms, namely C-phycocyanin in blue-green, and R-phycocyanin in red algae, was erroneous, having most probably been derived from observations made with insufficiently purified samples of the pigment".[1] The impurity was thought to be phycoerythrin. We report here evidence which refutes this suggestion.

RESULTS AND DISCUSSION

R-phycocyanin from *Porphyra laciniata* had visible absorption maxima at 553 and 615 mμ (Fig. 1), the ratio of the absorbancies (A_{553}/A_{615}) being 0·77. The spectrum of R-phycocyanin from *Ceramium rubrum* was slightly different, having λ_{max} at 553 and 618 mμ ($A_{553}/A_{618} = 0{\cdot}72$). Phycocyanin preparations having such properties were obtained from many samples of the fresh algae, collected on different occasions. Dried samples of *Porphyra laciniata* also yielded R-phycocyanin. C-phycocyanin was not detected in either species.

The R-phycocyanin from both species crystallized as well-defined rhombohedral platelets. In each case, careful microscopic examination revealed only one type of crystal, of a uniform violet-blue color, easily distinguishable both by crystal form and color from crystals of C-phycocyanin and of the various known types of phycoerythrin. Repeated calcium phosphate chromatography, ammonium sulfate fractionation, and crystallization did not change the spectral properties of the purified R-phycocyanin preparations or reveal any tendency towards resolution into spectrally different chromoproteins. On electrophoresis

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[1] A. HATTORI and Y. FUJITA, *J. Biochem.* (*Tokyo*) **46**, 903 (1959).
[2] C. Ó hEOCHA, in *Chemistry and Biochemistry of Plant Pigments* (Ed. T. W. GOODWIN), pp. 175–196. Academic Press, London (1965).
[3] T. SVEDBERG and T. KA SURAI, *J. Amer. Che Soc.* **51**, 3573 (1929).

in starch gels at pH 5, 7 and 8·5. R-phycocyanin migrated at each pH as a single narrow zone of uniform color. On chromatography on DEAE-cellulose, it was eluted as a spectrally homogeneous band in 0·2 M phosphate buffer, pH 6·5.

Subtraction of the absorption spectrum of C-phycocyanin from that of R-phycocyanin leaves a difference spectrum with a single positive maximum at 549 mμ (Fig. 1). This does not correspond with the spectrum of any known phycoerythrin or other known constituent of algae and indicates that the spectral difference between C- and R-phycocyanin cannot be attributed to an impurity, as Hattori and Fujita claimed to have demonstrated.[1] These

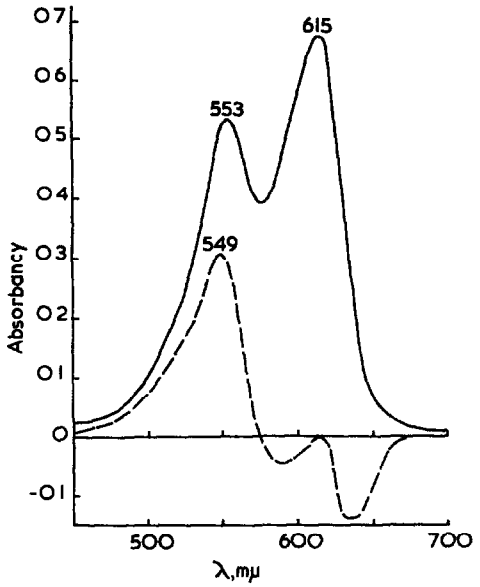

FIG. 1. (——) VISIBLE ABSORPTION SPECTRUM OF R-PHYCOCYANIN (FROM *Porphyra laciniata*) IN 10 mM SODIUM PHOSPHATE BUFFER, pH 6·5; (– – –) DIFFERENCE SPECTRUM, R-PHYCOCYANIN MINUS C-PHYCOCYANIN.

The same R-phycocyanin sample was used for both spectra, and the C-phycocyanin (from *Nostoc muscorum*),[2,4] in 10 mM sodium phosphate buffer, pH 6·5, was adjusted to the same absorbancy (0·67) at 615 mμ before subtraction.

authors illustrated their point with an absorption spectrum of a mixture of C-phycocyanin and a phycoerythrin, but the spectrum is not identical with that of R-phycocyanin; in particular a pronounced shoulder at about 500 mμ betrays the presence of R-phycoerythrin (λ_{max} *c.* 495 and 565 mμ). We find that even a trace of R-phycoerythrin, the only type of phycoerythrin detected in either *Ceramium rubrum* or *Porphyra laciniata*, is detectable by spectrophotometry when mixed with either R-phycocyanin or C-phycocyanin. The bright orange fluorescence of phycoerythrins lingers for at least 30 hr in 4 M urea at 3 and pH 7. Under the same conditions the fluorescence of solutions of R-phycocyanin is completely quenched within half an hour, indicating absence of any type of phycoerythrin.

On denaturation of R-phycocyanin in 3 M urea, the rates of decrease of its absorbancy at both 553 and 615 mμ coincide (Fig. 2), suggesting that the chromophores responsible for the two absorption peaks are part of the same chromoprotein.

All these data indicate that R-phycocyanin is a distinct type of biliprotein and not a mixture of C-phycocyanin and a phycoerythrin. However, comparative *N*-terminal studies,[4] and other evidence (Ó Carra, to be published) indicate that R-phycocyanin is composed of two types of subunits which may be related to C-phycocyanin and the phycoerythrins.

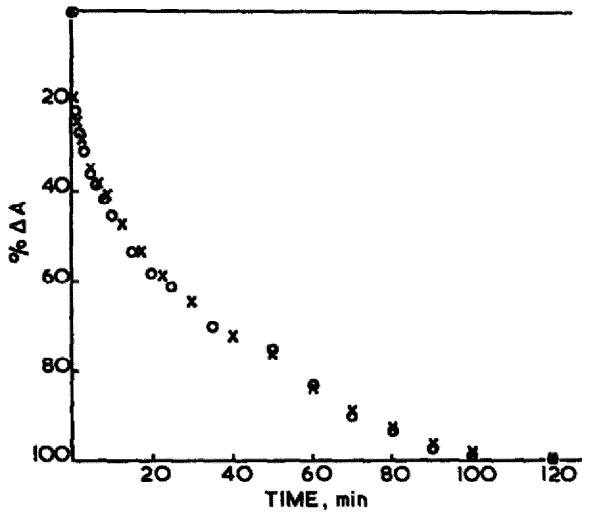

FIG. 2. PERCENTAGE DECREASE OF ABSORBANCY ($\%\Delta A$) AT 553 mμ (CIRCLES) AND 615 mμ (CROSSES) OF R-PHYCOCYANIN (FROM *Porphyra laciniata*) IN 3 M UREA, pH 7, 25°, PLOTTED AGAINST TIME (SEE TEXT).

EXPERIMENTAL

R-phycocyanin preparations from *Porphyra laciniata* and *Ceramium rubrum* (both freshly collected), and other biliproteins used for comparative purposes, were isolated and purified as described elsewhere.[4]

Horizontal starch-gel electrophoresis was carried out as described by Smithies[5] using 0·01 M sodium phosphate buffers to make the gels at pH 5 and 7, and 0·026 M sodium borate buffer at pH 8·5. R-phycocyanin (1% solutions) in the buffers was pipetted into slots in the gel to which starch grains were then added, and a potential gradient of 7 V/cm was applied for 15 hr.

For chromatography on DEAE-cellulose, R-phycocyanin was applied in 0·05 M sodium phosphate buffer, pH 6·5, and elution was effected with a 0·05 M–0·2 M gradient of the same buffer. Chromatography on calcium phosphate, ammonium sulphate fractionation, and crystallization were carried out as described elsewhere.[4]

The denaturation of R-phycocyanin from *P. laciniata* in 3 M urea was followed spectrophotometrically at the wavelengths 553 and 615 mμ. 1 ml of a 0·1% solution of R-phycocyanin in 0·01 M sodium phosphate buffer (pH 7) and 3 ml of 0·01 M sodium phosphate buffer in 4 M urea (pH 7), both solutions at 25°, were mixed rapidly and incubated at 25° in a thermostated spectrophotometer cell. The absorbancies at 553 and 615 mμ were read at intervals

[4] P. Ó CARRA, *Biochem. J.* **94**, 171 (1965).
[5] O. SMITHIES, *Biochem. J.* **61**, 629 (1955).

and the decrease in absorbancy at each wavelength, calculated as a percentage of the final decrease in absorbancy (when no further decrease occurred), was plotted against time (Fig. 2). For these calculations the absorbancy at zero time was taken as that of a solution of R-phycocyanin at the same concentration (0·025 $^{o}_{o}$) in 0·01 M sodium phosphate buffer, pH 7.