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Isolation and Spectral Characterization of Phycobiliproteins

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Several phycobiliproteins were prepared chromatographically pure and their absorption, fluorescence-emission, fluorescence-excitation and fluorescence-excitation polarization spectra determined. Changes in these spectra with ionic strength of the aqueous medium and chromoprotein concentration were interpreted in terms of inter-chromophore energy transfer and protein subunit equilibria. The complexity of the polarization spectra confirms the presence of different types of chromophore, designated sensitizing ('s') and fluorescing ('f'), in a single protein.

The phycobiliproteins (Ó hEocha, 1965*a*,*b*) are high-molecular-weight globular proteins found in three groups of algae, the red (Rhodophyta), blue-green (Schizophyta = Cyanophyta) and cryptomonad (Cryptomonadophyta). They are localized in the stroma of the photosynthetic tissue and not in the lamellae (Giraud, 1966; Fuhs, 1964). Their function in the process of photosynthesis appears to be that of accessory light-absorbers, trapped light-energy being handed on to the primary photochemical agent, chlorophyll (Duysens, 1952). Accordingly they have a very low fluorescence yield *in vivo*, which increases enormously on extraction when transfer to chlorophyll is prevented. The actual absorbing species are linear tetrapyrrole derivatives, the phycobilins. These are attached to the apoprotein covalently, PUB† by a thioether link (Ó hEocha, 1965*a*), PEB and PCB by an ester link involving a β-hydroxy amino acid (Ó hEocha, 1965*b*; Ó Carra, Ó hEocha & Carroll, 1964) and by a second linkage, probably to one of the pyrrole nitrogen atoms (Rüdiger & Ó Carra, 1969). The structures of PEB and PCB are now known (Cole, Chapman & Siegelman, 1967; Chapman, Cole & Siegelman, 1967; Rüdiger, Ó Carra & Ó hEocha, 1967; Rüdiger & Ó Carra, 1969). They are isomeric, the difference being in the extent of the conjugated double-bond systems (Fig. 1).

The number and type of chromophores found in different phycobiliproteins varies, and it appears that the same chemical species may exist in forms with different absorption spectra in one protein, probably reflecting different environments (Ó hEocha & Ó Carra, 1961; Dale & Teale, 1966). The approximate absorption maxima of the proteins studied here are shown in Table 1. The longest-wavelength-absorbing form fluoresces, the others passing on their excitation energy to it. When this sensitization process is

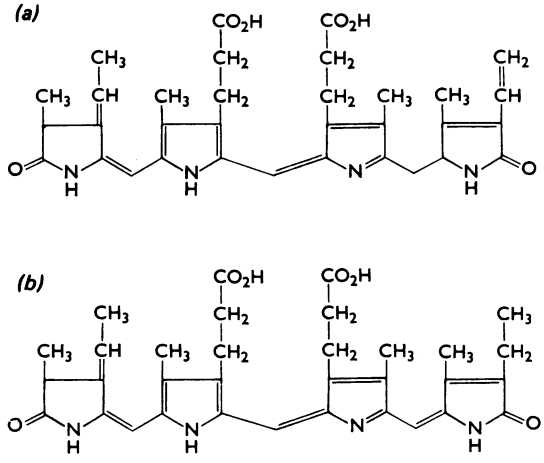

Fig. 1. Proposed chemical structures of (*a*) PEB and (*b*) PCB.

Table 1. *Absorption of phycobiliproteins*

Phycobiliprotein	Approximate absorption maxima (nm)
R-phycoerythrin	495 (PUB); 535 (PEBs); 557 (PEBf)
B-phycoerythrin	500 (PUB); 540 (PEBs); 565 (PEBf) shoulder
C-phycoerythrin	——, 530 (PEBs); 562 (PEBf) shoulder
Schizothrix C-phycocyanin	610 (PCBs); 630 (PCBf) slight shoulder
Anacystis C-phycocyanin	580 (PCBs); 620 (PCBf) slight shoulder

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† Abbreviations: PUB, phycourobilin; PEB, phycoerythrobilin; PCB, phycocyanobilin.

efficient, little direct fluorescence is seen from the shorter-wavelength-absorbing species. These shorter- and longer-wavelength-absorbing species of the same chemical structure are designated 's' (sensitizing) and 'f' (fluorescing) respectively.

Certain chemical reagents differentiate the 'f' and 's' types of protein-bound chromophore. Thus PEBf is the more readily bleached form in 1% hydrogen peroxide, whereas PEBs and PCBs absorptions disappear faster on treatment with dithionite (Jones & Fujimori, 1961; Fujimori & Quinlan, 1963). Ferricyanide in alkaline solution rapidly bleaches PEBs and both forms of PCB, but has hardly any effect on PEBf (Dale, 1967). Mercurials effect a decrease in PEBf absorption with concurrent loss of fluorescence, both being partially recovered on addition of thiols (Fujimori, 1964; Fujimori & Pecci, 1967; Pecci & Fujimori, 1967,1968). These spectral changes may reflect dissociation of the chromoproteins that also occurs on addition of mercurials, so that the suggested influence of thiol groups on chromophore spectra and fluorescence properties may not be direct. The differential effects of urea, guanidine or acid pH suggested that PEBf was hydrogen-bonded (Ó hEocha & Ó Carra, 1961), but it has since been argued that unspecified environmental influences other than hydrogen-bonding account for the absorption spectral difference between the PEB forms (Ó hEocha, 1965*b*).

The fluorescence spectra of many phycobiliproteins have been reported (see Ó hEocha, 1965*b*). Those of phycoerythrins have maxima at about 570nm, those of phycocyanins at 630–660nm. The shape of the spectrum is asymmetric with a subsidiary peak or marked shoulder on the long-wavelength side of the maximum. Fully corrected fluorescence-excitation spectra have also been obtained for a number of phycobiliproteins. Their correspondence to absorption spectra is variable and, in particular, widely different efficiencies of transfer from protein aromatic groups to phycobilin chromophores have been reported (Bannister, 1954; Eriksson & Halldal, 1965; Dale, 1967; Macdowall, Bednar & Rosenberg, 1968).

R-phycoerythrin from many different sources has a molecular weight of about 290000 and does not readily dissociate (Ó hEocha, 1965*a*; Nolan & Ó hEocha, 1967), and B-phycoerythrin has been shown to dissociate in the pH range 6–6.2 into a colourless fragment of molecular weight 159000 and a smaller coloured one of molecular weight 113000 (Brody & Brody, 1961). C-phycocyanins exist as monomer or polymers having three, six or 12 subunits, the dissociation constants varying with pH and ionic strength of the solvent, the molecular weight of the hexamer being approx. 275000 (Berns, Scott & O'Reilly, 1964; Hattori, Crespi & Katz, 1965; Scott & Berns, 1967). C-phycoerythrins probably behave similarly (Pecci & Fujimori, 1968), but are of lower molecular weight, about 226000 for the hexamer (Ó hEocha, 1965*a*). Electron microscopy has shown that the hexamer of C-phycocyanin is hexagonally arranged with a central hole (Berns & Edwards, 1965). More extensive dissociation takes place at extremes of pH with all phycobiliproteins (Ó hEocha, 1965*a*,*b*).

The dissociation of C-phycocyanins is attended by changes in chromophore environment, and the consequent absorption-spectral differences have been used to observe the subunit equilibrium quantitatively (Hattori *et al.* 1965). Dissociation also produces changes in fluorescence spectra and fluorescence polarization, which have served as a qualitative index of the equilibrium (Goedheer & Birnie, 1965).

EXPERIMENTAL

Materials

The sources of phycobiliprotein were as follows: R-phycoerythrin from the large rhodophyte *Rhodymenia* sp. (supplied by the Marine Biological Station, Plymouth, U.K.); C-phycoerythrin and C-phycocyanin from the cyanophyte *Schizothrix calcicola* (Ag.) Gom. (received as a gift from Dr H. Hoogenhout, Biophysical Laboratory of the State University, Leiden, The Netherlands); C-phycocyanin from the cyanophyte *Anacystis nidulans* (supplied from the Culture Collection of Algae and Protozoa, University of Cambridge, Cambridge, U.K.); B-phycoerythrin from the unicellular rhodophyte *Porphyridium cruentum* Näg. (supplied from the Culture Collection of Algae and Protozoa, University of Cambridge, Cambridge, U.K.).

Chemicals and solvents used were of analytical grade, or that of highest purity available, from British Drug Houses Ltd., Poole, Dorset, U.K., or Fisons Scientific Apparatus Ltd., Loughborough, Leics., U.K. Whatman DE11 DEAE-cellulose for column chromatography was obtained from British Drug Houses Ltd., and Sephadex G-25 (medium grade) from AB. Pharmacia, Uppsala, Sweden.

Rhodymenia fronds were stored at −15°C until needed for extraction. The microscopic algae were cultured in 800ml batches with magnetic stirring and a good flow of air (not enriched with CO_2). After initial inoculation from slopes, the liquid cultures were propagated in a semi-continuous manner by subculturing with 10ml of the previous thick suspension produced. The media and culture conditions employed are shown in Table 2. After being harvested by centrifugation (12000*g* for 20min) and redispersed in a small volume of distilled water, the algae were frozen for storage until enough had been collected for extraction.

F. W. J. Teale (unpublished work) had shown that crystallizable R-phycoerythrin from a *Rhodymenia* could be purified by chromatography on DEAE-cellulose. The possibility of modifying this rapid and promising method to give not only a highly pure R-phycoerythrin from *Rhodymenia* but also R-phycocyanin from this and B- and C-phycobiliproteins from other sources was further investigated (Dale, 1967). Flow sheets for the preparation of *Rhodymenia* R-phycoerythrin, *Porphyridium* B-phycoerythrin, *Schizothrix* C-phycoerythrin and C-phycocyanin and *Anacystis* C-phycocyanin are given in Schemes 1–3

Table 2. *Algal culture*

Alga	Medium	Illumination	Temperature (°C)	Time before harvesting
Schizothrix calcicola	From formula supplied with slopes	Indirect daylight +Philips i.r. lamp (E106) at about 1.5m	25–35 (flasks raised off magnetic stirrer)	10–15 days
Anacystis nidulans	Modified medium C of Kratz & Myers (1955)	Indirect daylight +Philips i.r. lamp (E106) at about 1.5m	30–40	10–15 days
Porphyridium cruentum	From Brody & Emerson (1959)	Indirect sunlight	20–25	1–2 months (growth very poor)

together with approximate yields and final specific protein purity (ratio of extinction at the peak in the visible region to that in the u.v. region due to protein aromatic residues). All solutions used contained 0.1 mM EDTA to chelate any traces of metal ion present and the final solutions could be stored indefinitely at 5°C in the dark with very little change in spectral properties.

Two chromatographically distinct species of R-phycoerythrin were obtained by this method, as has previously been reported for this protein on purification by adsorption chromatography on modified calcium phosphate gels (Tiselius, Hjertén & Levin, 1956).

Methods

Absorption spectra were taken with 1 cm- or 1 mm-pathlength silica cuvettes and recorded on a Cary model 14 spectrophotometer.

Fluorescence-emission and -excitation spectra were obtained with a conventional double-monochromator apparatus. The light-source was that of an AEI 250 W d.c. xenon arc via a Bausch and Lomb monochromator with a 500mm grating of 1200 lines/mm giving a dispersion of 1.65nm/mm slit width, and blazed for first-order in the u.v. region. Fluorescence was detected by an EMI 9558B photomultiplier, and registered on a Scalamp galvanometer after passing through an observation monochromator, which was again a Bausch and Lomb grating instrument having a dispersion of 6.6nm. Excitation spectra were determined at a resolution of 3.3nm. Fluorescence spectra determined at resolutions of 5.28 and 3.96nm were the same within experimental error. In these measurements, observation and excitation wavelengths respectively were chosen to minimize the effect of excitation breakthrough while maximizing fluorescence intensity. Reproducibility of both excitation and emission spectra were better than 5% over the main range of the bands.

A 1 cm square Ultrasil cuvette was used in measurement of these spectra for dilute solutions having an extinction at the chromophore maximum of about 0.1, so that (*a*) there was negligible reabsorption of fluorescence in the overlap region and (*b*) excitation spectra (corrected for quantum output of the source) were directly comparable with those of absorption. More concentrated solutions ($E_{1cm} \simeq 1$) were observed in a diagonally placed layer 1mm thick between Perspex prisms.

Correction of the technical spectra for detector response and source output were made by first calibrating the source with a Hilger–Schwarz FT23 thermopile, giving relative energies, the product of which with the wavelength of measurement gave the relative photon flux (Lippert, Nägele, Seibold-Blankenstein, Staiger & Voss, 1959):

$$E = nh\nu \quad \therefore n = \frac{E}{h\nu} \propto E\lambda$$

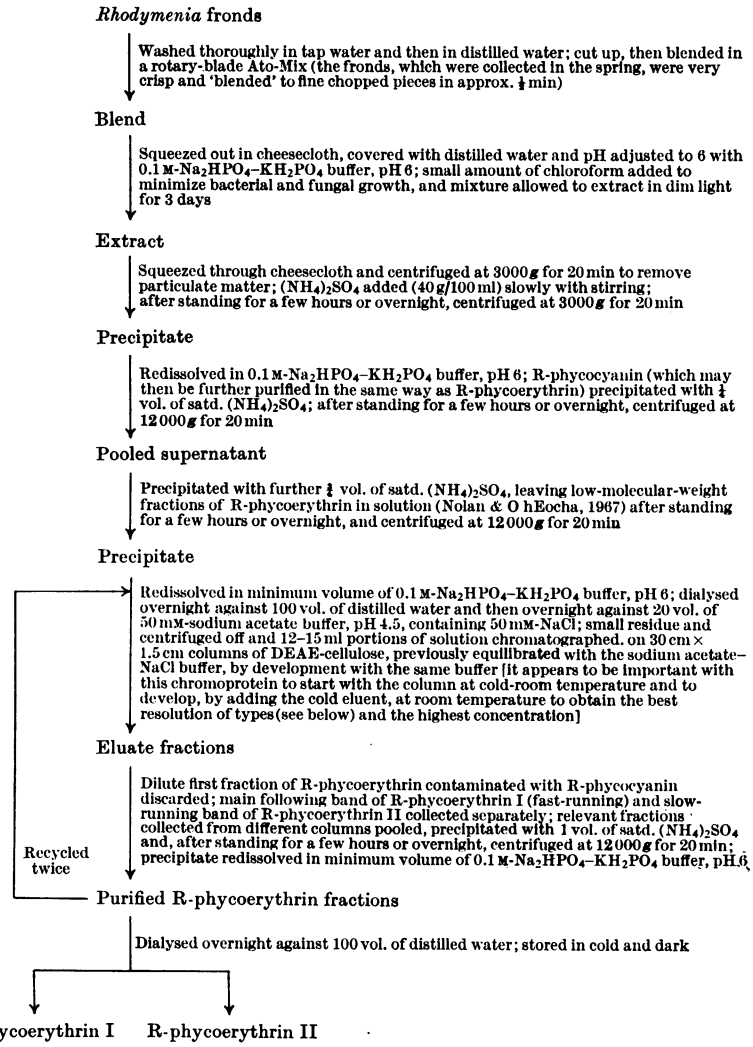
The response curve of the detector was then determined by using this calibrated source (Weber & Teale, 1958).

Fluorescence-excitation polarization spectra were measured with solutions in 1 cm square cuvettes of controlled temperature. The apparatus was based on a previous design (Weber, 1956), but employed electrical compensation to give a direct reading of the degree of polarization or of the anisotropy (Martonosi & Teale, 1965; F. W. J. Teale, unpublished work). The light-source and monochromator were the same as used for excitation spectra. Observation was made through sharp-cut filters (Ilford 206 for phycoerythrins, Kodak K70 for phycocyanins) by EMI 9592B photomultipliers. In the range of total photocurrent 1–40 μA the degree of polarization or the anisotropy could be read to ± 0.001, and even at very low intensities (0.3–0.5 μA) the reproducibility was still ± 0.003–0.005. Both negative and positive values of these parameters could be determined.

The maximum limiting value of emission of anisotropy A_F, obtained by excitation at the absorption edge, was calculated by correcting the observed anisotropy $A_{obs.}$ for the effects of excitation breakthrough by applying the equation:

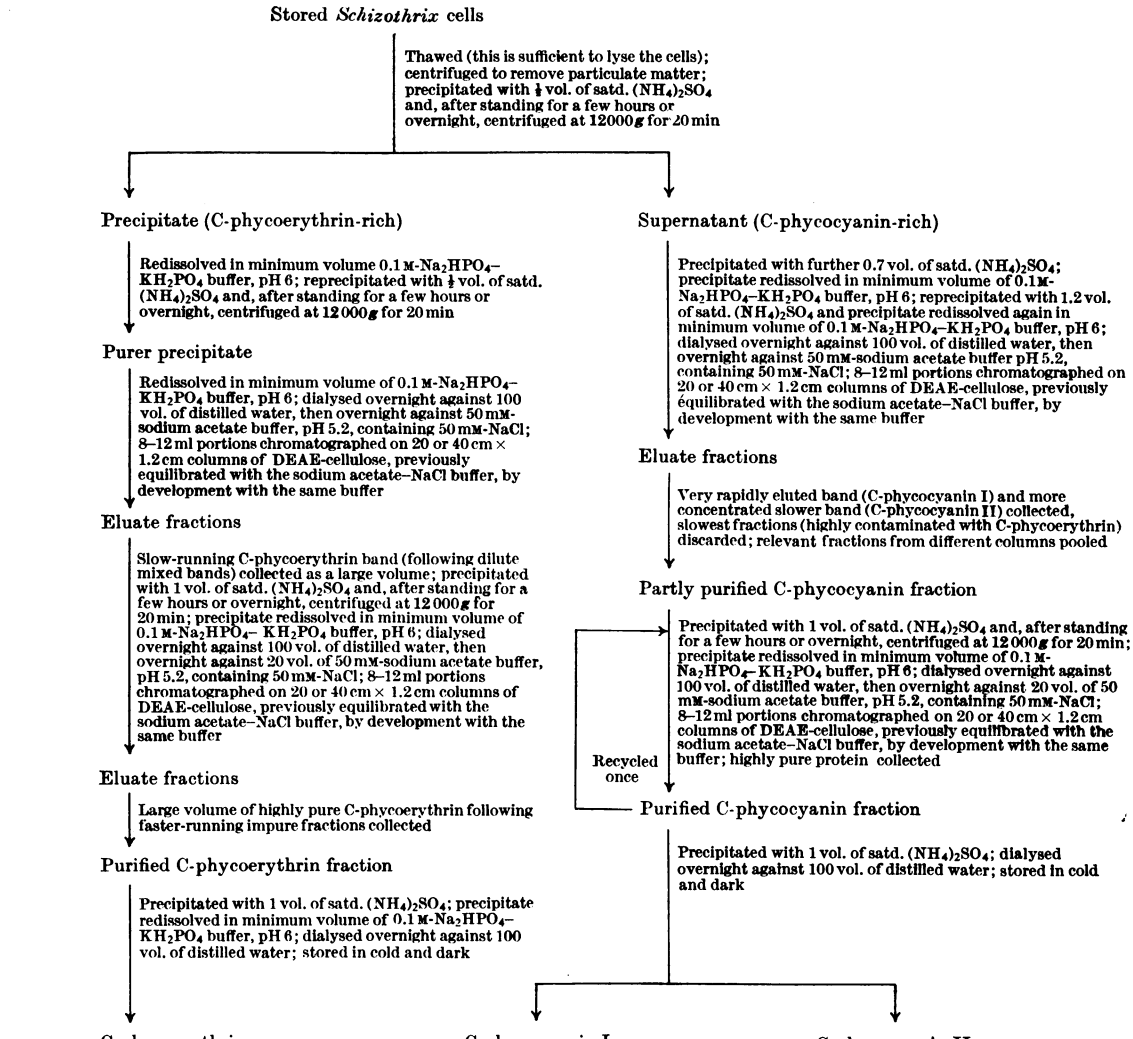
$$A_F = A_{obs.} - \frac{I_B}{I_F}(A_B - A_{obs.})$$

where I_B and A_B are respectively the intensity and anisotropy of the scattered excitation and I_F is the fluorescence intensity. Values of I_B and A_B were obtained from non-fluorescent blank solutions of glycogen or plasma albumin. The ratio I_B/I_F was also checked by comparing the observed average fluorescence lifetime $\tau_{obs.}$ with the maximum value $\tau_{max.}$ measured at slightly shorter excitation wavelengths that were completely excluded by



	R-phycoerythrin I	R-phycoerythrin II	
Specific protein purity	5.4	5.1	(5.3 for R-phycoerythrin in Hattori & Fujita, 1959)
Yield (% of first redissolved precipitate)	14.9	1.0	

Scheme 1. Flow sheet for the preparation of *Rhodymenia* R-phycoerythrin. All operations were carried out in a cold-room at 4°C, except for the special conditions used for chromatography. All solutions (including 'distilled water') contained 0.1 mM-EDTA. *Porphyridium* B-phycoerythrin was prepared similarly. In this case, however, the thawed cells were lysed over 2 days, releasing almost all the chromoprotein. A smaller total volume was available and a single 20 cm × 1.2 cm column of DEAE-cellulose was sufficient to separate the B-phycoerythrin as one narrow band. A second cycle did not improve this product, which had a specific protein purity of 4.6 (3.92 for *Porphyridium* B-phycoerythrin in Ó hEocha, 1960), the yield being 42% of the initial redissolved precipitate.



Scheme 2. Flow sheet for the preparation of *Schizothrix* C-phycoerythrin and C-phycocyanin. Allope rations were carried out in a cold-room at 4°C. All solutions (including 'distilled water') contained 0.1 mM-EDTA. C-phycocyanin I and C-phycocyanin II behaved virtually identically in the experiments described in this paper, and results are presented for C-phycocyanin I only.

the emission filter from the detector. Assuming the scattered excitation has zero lifetime, we have:

$$\frac{I_B}{I_F} = \frac{\tau_{max.}}{\tau_{obs.}} - 1$$

RESULTS

The chromoproteins prepared by the methods outlined in Schemes 1–3 could be rechromatographed without further resolution or change in the u.v.- and

Stored *Anacystis* cells

↓ Thawed and serially lysed several times over 2–3 week periods with chloroform as biostatic agent, with centrifuging between each lysis and redispersion of the packed cells in water for the next extraction; supernatants pooled

Supernatant

↓ Precipitated with 1.2 vol. of satd. $(NH_4)_2SO_4$ and, after standing for a few hours or overnight, centrifuged at 12 000*g* for 20 min; precipitate redissolved in 50 mM-sodium acetate buffer, pH 5, containing 50 mM-NaCl; dialysed overnight against 20 vol. of the same sodium acetate–NaCl buffer; pH lowered to 4 by addition to the dialysis solution of 50 mM-acetic acid containing 50 mM-NaCl, and equilibrated overnight; 8–12 ml portions chromatographed on 20 or 40 cm × 1.2 cm columns of DEAE-cellulose, previously equilibrated with the sodium acetate–NaCl buffer, pH 4, by development with the same buffer

Eluate fractions

Recycled once ↓ Rapidly eluted band of C-phycocyanin collected, slow-moving band discarded

Purified C-phycocyanin fraction

↓ Precipitated with 1.2 vol. of satd. $(NH_4)_2SO_4$ and, after standing for a few hours or overnight, centrifuged at 12 000*g* for 20 min; precipitate redissolved in minimum volume of 0.1 M-Na_2HPO_4–KH_2PO_4 buffer, pH 6; dialysed overnight against 100 vol. of distilled water; stored in cold and dark

C-phycocyanin

Specific protein purity	3.7
Yield (% of extract)	50

Scheme 3. Flow sheet for the preparation of *Anacystis* C-phycocyanin. All operations were carried out in a cold-room at 4°C. All solutions (including 'distilled water') contained 0.1 mM-EDTA.

visible-absorption spectra, suggesting that progressive changes in properties did not result from the column treatment, and that the products were chemically well defined. The very low quantum yield (<0.01) of tryptophan fluorescence in the purified fractions indicated the probable absence of non-conjugated tryptophan-containing proteins, and this agreed with the observation that the specific protein purities were comparable with or higher than those previously published in the literature for the same or closely related material.

To compare the absorption and excitation spectra, the former were normalized to unit extinction at the absorption maxima, and the excitation spectra were normalized to be maximally coincident throughout the main visible-absorption bands. Within experimental error, coincidence was virtually complete in all cases (Figs. 2, 3 and 4). The polarization spectra, expressed in terms of emission anisotropy, were complex and showed low absolute values, both positive and negative (Fig. 4), within the absorption bands, rising steeply to maximum positive values at the absorption limit. In contrast, the free chromophore PEB, complexed with Zn^{2+} in glycerol (Dale, 1967), showed a nearly constant maximal value of anisotropy throughout the visible-absorption bands

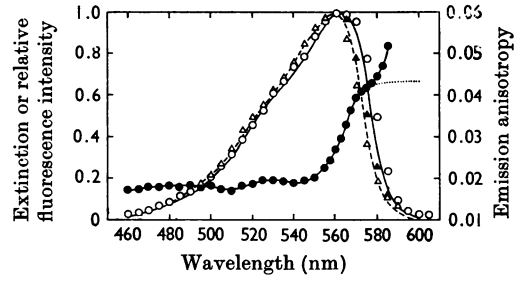


Fig. 2. Absorption, fluorescence-excitation and fluorescence-excitation polarization spectra of C-phycoerythrin in sodium acetate buffer, pH 6. ——, Absorption in 3.5 M buffer; – – – –, absorption in 20 mM buffer; ○, excitation in 3.5 M buffer (observation wavelength 590 nm); △ and ▲, excitation in 20 mM buffer (observation wavelengths 590 and 640 nm respectively); ●, polarization spectrum in 3.5 M buffer, $E_{max.}$ (visible) ≃ 0.8; · · · · · · ·, limiting anisotropy.

(Fig. 5). The emission spectra showed single sharp maxima with another lower and almost unresolved band at longer wavelengths. The Stokes shifts were small (≃15 nm) in phycoerythrins and somewhat

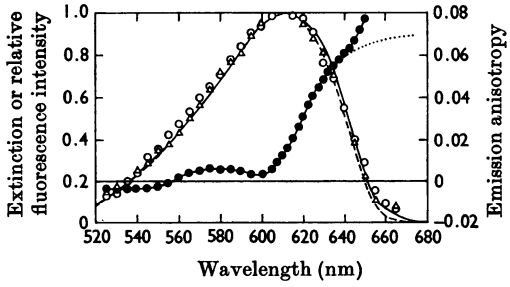


Fig. 3. Absorption, fluorescence-excitation and fluorescence-excitation polarization spectra of *Schizothrix* C-phycocyanin in sodium acetate buffer, pH6. ——, Absorption in 3.5M buffer; – – – –, absorption in 20mM buffer; ○ and △, excitation in 3.5M and 20mM buffer respectively (observation wavelength 670nm); polarization spectrum in 3.5M buffer, $E_{max.}$ (visible) ≃ 0.8; ·····, limiting anisotropy.

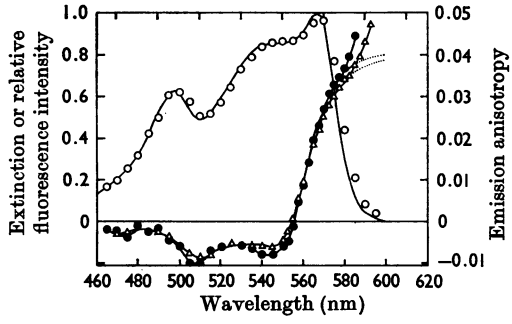


Fig. 4. Absorption, fluorescence-excitation and fluorescence-excitation polarization spectra of R-phycoerythrin I in sodium acetate buffer, pH6. ——, Absorption and excitation respectively in 20mM buffer (excitation wavelength 540nm); ●, polarization spectrum in 20mM buffer, $E_{max.}$ (visible) ≃ 0.08; △, polarization spectrum in 3.5M buffer, $E_{max.}$ (visible) ≃ 0.8; ·····. limiting anisotropies.

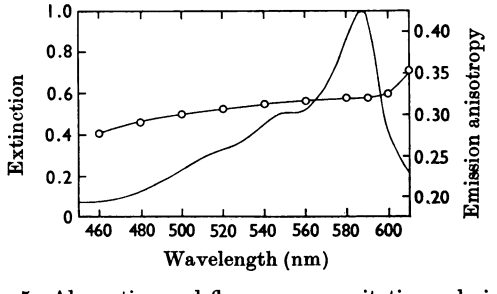


Fig. 5. Absorption and fluorescence-excitation polarization spectra of a Zn^{2+}–PEB complex. ——, Absorption (from Ó Carra *et al.* 1964); ○, polarization spectrum in glycerol.

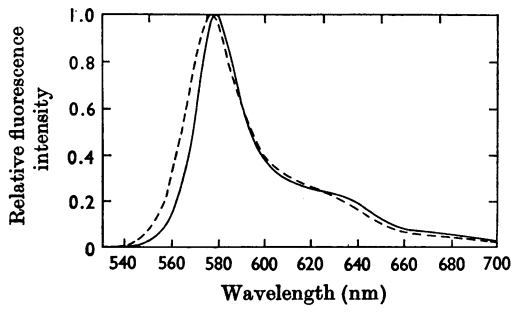


Fig. 6. Fluorescence-emission spectra of C-phycoerythrin in sodium acetate buffer, pH6 ——, 3.5M buffer; – – – –, 0.02M buffer (excitation wavelengths 510 and 560nm).

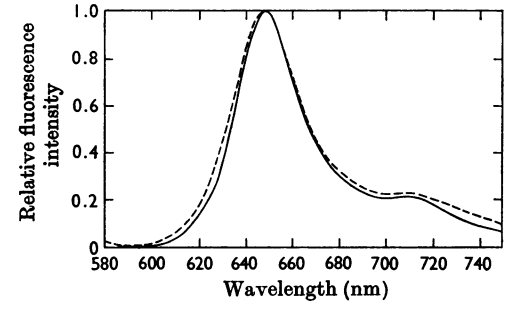


Fig. 7. Fluorescence-emission spectra of *Schizothrix* C-phycocyanin in sodium acetate buffer, pH6. ——, 3.5M buffer; – – – –, 20mM buffer (excitation wavelengths 570 and 620nm).

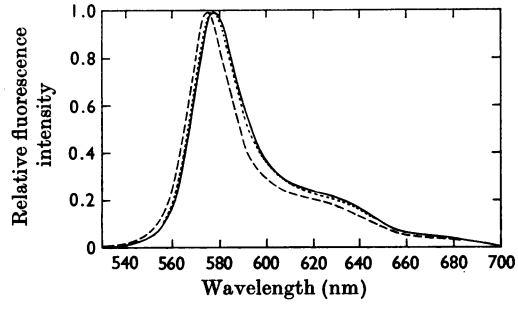


Fig. 8. Fluorescence-emission spectra of phycoerythrins in 20mM-sodium acetate buffer, pH6. ——, R-phycoerythrin I; ·····, R-phycoerythrin II; – – – –, B- phycoerythrin (excitation wavelength 540nm).

larger (≃ 35nm) in the phycocyanins (Figs. 6, 7 and 8).

In the non-dissociating R-phycoerythrin I, the polarization spectrum, as well as the absorption, excitation and emission spectra, were hardly altered by concentration (Fig. 4). This observation indicated

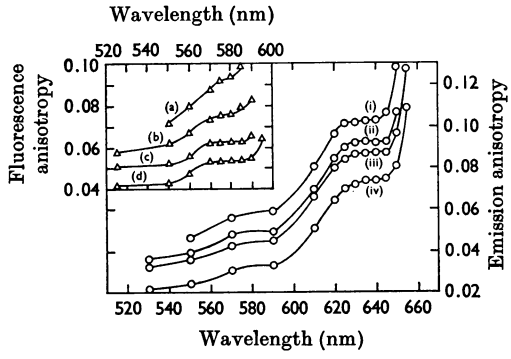


Fig. 9. Fluorescence-excitation polarization spectra of C-phycobiliproteins in 20mM-sodium acetate buffer, pH 6. Main graph: *Schizothrix* C-phycocyanin, $E_{max.}$ (visible) (i) 0.02, (ii) 0.04, (iii) 0.06, (iv) 0.08. Insert: C-phycoerythrin, $E_{max.}$ (visible) (a) 0.02, (b) 0.04, (c) 0.06, (d) 0.08.

that reabsorption of fluorescence in concentrated solutions was not an important source of depolarization. Concentration effects were, however, shown in the emission and polarization spectra of the dissociating phycobiliproteins C-phycoerythrin and *Schizothrix* C-phycocyanin. Concentrated solutions displayed sharper emission maxima at longer wavelengths (Figs. 6 and 7). Anisotropy values decreased with increasing concentrations (Figs. 2, 3 and 9), even becoming negative in some spectral regions with *Schizothrix* C-phycocyanin (Figs. 3 and 9), whereas the general shape of the polarization spectra changed progressively with concentration.

DISCUSSION

Except in very special cases (Feofilov, 1961) fluorescent dye molecules exhibit constant high anisotropy across the absorption band of least frequency, owing to the presence of a single electronic transition. The polarization spectrum of the PEB–Zn^{2+} complex (Fig. 5) is evidently of this type, even though the conjugation path of this molecule is not linear since the methene bridges are in the *cis* rather than the *trans* configuration depicted in Fig. 1. Constant anisotropy can then be expected from whichever form, either extended or closed, appears in the chromoprotein. Unfortunately the PCB–Zn^{2+} complex was non-fluorescent and it has been assumed that its electronic structure is similar to that of PEB. In contrast, the complex polarization spectra of the native phycobiliproteins, in particular the regions of negative anisotropy (Figs. 3 and 4), indicate that in the macromolecule different chromophore types are present, those that absorb at shorter wavelength ('s' type) sensitizing those that absorb and emit ('f' type) at longer wavelengths. The relative orientations of the 's' and 'f' chromophores and their relative absorption intensities determine the detailed shape of the polarization spectrum. Quantitative analyses of the spectra (not presented in detail here) assign the 's' absorption maxima to the region 540–555nm (phycoerythrin) and 600–615nm (phycocyanin), whereas the 'f' chromophores absorb at 565–570nm (phycoerythrin) and 630–635nm (phycocyanin).

The coincidence of the excitation and absorption spectra throughout the visible-absorption spectral range (Figs. 2, 3 and 4) means that energy transfer from 's' to 'f' types is very efficient, probably as a consequence of close proximity between donor and acceptor chromophores.

The absence of significant absorbing impurities is also confirmed by this observation.

Earlier work had suggested that the excitation and absorption spectra were not coincident, and this was attributed to the presence of a non-fluorescent fraction of 'f' chromophores (Eriksson & Hallden, 1965; Macdowall *et al.* 1968). This conclusion is not supported by the present findings. That the 's' residues are capable of some fluorescence when the efficiency of energy transfer to the 'f' type is decreased is suggested by the different effects of concentration on the dissociating C-phycoerythrin and *Schizothrix* C-phycocyanin and on the non-dissociating R-phycoerythrin I. Whereas the last chromoprotein exhibits little change in fluorescence properties, the first two show a blue shift of fluorescence spectra as the protein concentration is lowered (Figs. 6 and 7), probably reflecting the presence of some 's'-type fluorescence. In dilute solutions of C-phycoerythrin excitation spectra differ for two observation wavelengths that favour 's' and 'f' emission respectively (Fig. 2), whereas no differences were detected in concentrated solution. The appearance of 's' fluorescence is presumably due to less efficient transfer from 's' residues to the smaller total number of 'f' residues present in the monomer or trimer compared with the hexameric forms.

In general the spectral differences between concentrated and dilute solutions of C-phycobiliproteins confirms previous results obtained with different C-phycocyanins (Hattori *et al.* 1965; Goedheer & Birnie, 1965).

The increase in anisotropy with dilution can be attributed to subunit dissociation, which decreases the depolarization that results from intra-chromoprotein energy migration (Goedheer, 1957).

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REFERENCES

Bannister, T. T. (1954). *Archs Biochem. Biophys.* **49**, 222.

Berns, D. S. & Edwards, M. R. (1965). *Archs Biochem. Biophys.* **110**, 511.

Berns, D. S., Scott, E. & O'Reilly, K. T. (1964). *Science, N.Y.*, **145**, 1054.

Brody, M. & Emerson, R. (1959). *Am. J. Bot.* **46**, 433.

Brody, S. S. & Brody, M. (1961). *Biochim. biophys. Acta*, **50**, 348.

Chapman, D. J., Cole, W. J. & Siegelman, H. W. (1967). *J. Am. chem. Soc.* **89**, 5976.

Cole, W. D., Chapman, D. J. & Siegelman, H. W. (1967). *J. Am. chem. Soc.* **89**, 3643.

Dale, R. E. (1967). Ph.D. Thesis: University of Birmingham.

Dale, R. E. & Teale, F. W. J. (1966). In *Currents in Photosynthesis*, p. 169. Ed. by Thomas, J. B. & Goedheer, J. C. Rotterdam: Ad. Donker.

Duysens, L. N. M. (1952). Ph.D. Thesis: University of Utrecht.

Eriksson, C. E. A. & Halldal, P. (1965). *Physiologia Pl.* **18**, 146.

Feofilov, P. P. (1961). *The Physical Basis of Polarized Emission*, pp. 137–141. New York: Consultants Bureau Enterprises Inc.

Fuhs, G. W. (1964). *Bact. Proc.* **64**, 15.

Fujimori, E. (1964). *Nature, Lond.*, **204**, 1091.

Fujimori, E. & Pecci, J. (1967). *Archs Biochem. Biophys.* **118**, 448.

Fujimori, E. & Quinlan, K. (1963). *Publ. natn. Acad. Sci. U.S.A. no.* 1145: *Photosynthetic Mechanisms of Green Plants*, p. 519.

Giraud, G. (1966). In *Currents in Photosynthesis*, p. 329. Ed. by Thomas, J. B. & Goedheer, J. C. Rotterdam: Ad. Donker.

Goedheer, J. C. (1957). Ph.D. Thesis: University of Utrecht.

Goedheer, J. C. & Birnie, F. (1965). *Biochim. biophys. Acta*, **94**, 579.

Hattori, A., Crespi, H. L. & Katz, J. J. (1965). *Biochemistry, Easton*, **4**, 1225.

Hattori, A. & Fujita, Y. (1959). *J. Biochem., Tokyo*, **46**, 633.

Jones, R. F. & Fujimori, E. (1961). *Physiologia Pl.* **14**, 253.

Kratz, W. A. & Myers, J. (1955). *Am. J. Bot.* **42**, 282.

Lippert, E., Nägele, W., Seibold-Blankenstein, I., Staiger, U. & Voss, W. (1959). *Z. anal. Chem.* **170**, 1.

Macdowall, F. D. H., Bednar, T. & Rosenberg, A. (1968). *Proc. natn. Acad. Sci. U.S.A.* **59**, 1356.

Martonosi, A. & Teale, F. W. J. (1965). *J. biol. Chem.* **240**, 2888.

Nolan, D. N. & Ó hEocha, C. (1967). *Biochem. J.* **103**, 39P.

Ó Carra, P., Ó hEocha, C. & Carroll, D. M. (1964). *Biochemistry, Easton*, **3**, 1343.

Ó hEocha, C. (1960). In *Comparative Biochemistry of Photoreactive Systems*, p. 196. Ed. by Allen, M. B. New York: Academic Press Inc.

Ó hEocha, C. (1965*a*). In *Chemistry and Biochemistry of Plant Pigments*, p. 175. Ed. by Goodwin, T. W. New York: Academic Press Inc.

Ó hEocha, C. (1965*b*). *A. Rev. Pl. Physiol.* **16**, 415.

Ó hEocha, C. & Ó Carra, P. (1961). *J. Am. chem. Soc.* **83**, 1091.

Pecci, J. & Fujimori, E. (1967). *Biochim. biophys. Acta*, **131**, 147.

Pecci, J. & Fujimori, E. (1968). *Biochim. biophys. Acta* **154**, 332.

Rüdiger, W. & Ó Carra, P. (1969). *Eur. J. Biochem.* **7**, 509.

Rüdiger, W., Ó Carra, P. & Ó hEocha, C. (1967). *Nature, Lond.*, **215**, 1477.

Scott, E. & Berns, D. S. (1967). *Biochemistry, Easton*, **6**, 1327.

Tiselius, A., Hjertén, S. & Levin, Ö. (1956). *Archs Biochem. Biophys.* **65**, 132.

Weber, G. (1956). *J. opt. Soc. Am.* **46**, 962.

Weber, G. & Teale, F. W. J. (1958). *Trans. Faraday Soc.* **54**, 640.