SC 43034

Fluorescence polarisation aud location of fluorescence maxima of C-phycocyanin

Ultracentrifuge studies have shown that phycocyanin, a chromoprotein with a molecular weight of about 270000, falls apart into smaller subunits at high and low pH (SVEDBERG AND ERIKSSON[1], ERIKSSON-QUENSEL[2]). Measurements of the polarisation of phycocyanin fluorescence indicated that the degree of polarisation p is low (0.01) in the pH range at which the chromoprotein is assumed to be intact (pH 4–6), while it is appreciably higher (0.20) in the pH ranges in which phycocyanin is assumed to be disintegrated into its smallest subunits (GOEDHEER[3]). The curves for fluorescence polarisation and for particle size *versus* pH are roughly similar in shape. This phenomenon was given the interpretation, that fluorescence in the intact chromoprotein is depolarised by energy transfer from the absorbing pigment chromophore to neighbouring chromophores attached to the same protein carrier; a splitting of this carrier into subunits, with chromophores attached to each of them, decreases the probability of energy transfer, resulting in a decrease in fluorescence polarisation. Calculation showed, that with the measured values of fluorescence yield and particle size, depolarisation in aqueous suspension due to Brownian rotation is expected not to be high, even for the chromoprotein fragments. The value of p of about 0.20 at high and at low pH suggests that these fragments still contain more than one chromophore.

BRODY AND BRODY[4] calculated the number of chromophores from carefully prepared phycocyanin by measuring fluorescence lifetimes and fluorescence yield. For phycocyanin they found a value of 1–2 pigment chromophores per Svedberg unit of protein.

The intact chromoprotein thus contains a number of chromophores which, according to fluorescence depolarisation, interact with each other. Such a mutual pigment interaction may also affect the difference in wavelength between absorption and fluorescence maximum ("Stokes shift"), as well as the shape and location of the absorption spectrum. In this way, the location of the fluorescence maximum may vary as a function of pH.

To investigate these phenomena we measured fluorescence and absorption spectra of extracted phycocyanin in a pH range from 2 to 9, and determined the amount of fluorescence polarisation of the same samples. Phycocyanin was obtained by grinding the blue-green alga *Synechococcus cedrorum* with sand. Cell debris and sand were removed by two successive low-speed centrifugations (15 min, 6000 rev. per min). Chlorophyll-carrying lamellae were removed by high-speed centrifugation (60 min, 18000 rev./min) in a cooled centrifuge. Phycocyanin was not further purified, but was immediately transferred into the buffers for measurement. Citrate buffer (0.01 M) was used for pH 2–7, borax buffer for pH 7–9. To check a possible influence of type of buffer, phosphate buffer was used at pH values from 5.2 to 7.5. Absorption spectra were recorded in a Beckmann DK2 spectrophotometer. The phycocyanin concentration was such, that the peak absorbancy was 0.9 at pH 6.0. The same amount of phycocyanin was used at other pH values. To avoid reabsorption of fluorescence, the samples were diluted eight times for fluorescence measurements. For polarisation measurements, fluorescence was excited with light of the 546 and 578-mμ Hg lines, while fluorescence was separated from scattered incident light by means of a Schott

RG5 filter (transmitting light of a wavelength longer than 650 mμ). A polaroid filter type HR was used in the fluorescence beam, whereas a normal type polaroid filter was placed in the beam of exciting light.

In Fig. 1 absorption and fluorescence spectra, measured at pH 2.2, 6 and 9, are given. It is seen that the phycocyanin absorption band was broadened at high and low pH, while the peak absorbancy was markedly decreased. At all pH values except 2.2, the location of the absorption maximum did not differ by more than 1–2 mμ from the value at pH 6. At pH 2.2 the absorption maximum was shifted from 617 to 622 mμ. The fluorescence spectra remained approximately mirror-symmetrical to

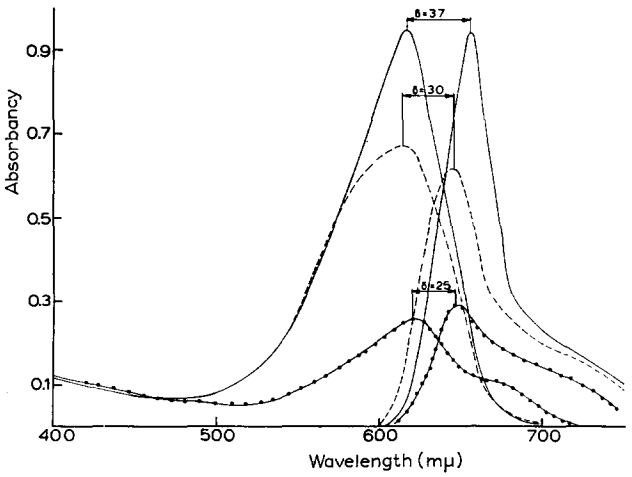

Fig. 1. Fluorescence and absorption spectra of C-phycocyanin extracted from *Synechococcus cedrorum*. Spectra are given of samples buffered at pH 6 (———), pH 9 (– – – –) and pH 2.2 (–·–·–), and were measured immediately after preparation.

TABLE I

LOCATION OF FLUORESCENCE AND ABSORPTION MAXIMUM, "STOKES SHIFT" AND DEGREE OF FLUORESCENCE POLARISATION AS A FUNCTION OF pH AND TIME OF STORAGE

pH	*0 h*				*1 h*				*24 h*			
	Abs. max. (*mμ*)	*Fluor. max.* (*mμ*)	*Stokes shift* (*mμ*)	*p*	*Abs. max.* (*mμ*)	*Fluor. max.* (*mμ*)	*Stokes shift* (*mμ*)	*p*	*Abs. max.* (*mμ*)	*Fluor. max.* (*mμ*)	*Stokes shift* (*mμ*)	*p*
2.2	621	647	26	0.14	621	646	25	0.18	622	646	24	0.23
3	617	647	30	0.08	617	646	29	0.18	620	647	27	0.23
4	617	651	34	0.05	627	651	34	0.06	617	651	34	0.05
5	617	654	37	0.02	617	651	34	0.02	617	652	35	0.03
5.3	617	653	36	0.01	617	653	36	0.01	617	653	36	0.03
5.9	617	654	37	0.02	617	654	37	0.03	617	654	37	0.04
6	617	654	37	0.01	617	654	37	0.03	617	654	37	0.04
6.5	617	653	36	0.01	617	654	37	0.06	617	654	37	0.07
7	617	653	36	0.02	617	653	36	0.05	617	653	36	0.09
7.4	617	653	36	0.03	617	652	35	0.06	617	651	34	0.10
8	617	651	34	0.07	616	652	36	0.10	614	647	33	0.18
9	616	646	30	0.13	615	645	30	0.16	612	643	31	0.16

the absorption spectra in the red. However, the broadening of the absorption band at high and low pH was not reflected in the fluorescence band.

In Table I the location of absorption bands, the "Stokes shift" and the fluorescence polarisation is given *versus* pH, at different times after preparation (stored in the dark at 20°). The table shows that the location of the fluorescence maximum of freshly prepared C-phycocyanin from Synechococcus was at about 654 mμ at pH 5 and 6 (Stokes shift 37 mμ and $p = 0.01$). At pH 9 and 3 it was at about 646 mμ (Stokes shift 30 mμ and $p = 0.13$). Fluorescence polarisation and "Stokes shift" thus appear to be correlated with pH in a way similar to that found for particle size.

If we assume that the high values of fluorescence polarisation and low values of Stokes shift are indicative of the smallest fragments of phycocyanin, it follows also that at pH 8 the chromoprotein was partly broken after 2 h while after 24 h a splitting occurred at all pH values except 5 and 6. After 72 h even at these values the chromoprotein was affected. As the preparations were not purified, it might be that certain enzymic and uncoloured cell components were responsible for acceleration, or even effectuation of the splitting phenomena.

A relation of "Stokes shift" and fluorescence depolarisation with size of pigmented unit may be of importance in a study of other fluorescing pigment systems, such as the far-red chlorophyll forms (*cf.* refs. 5–7).

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Protein precipitation by uncharged water-soluble polymers

Lipids and lipoproteins are precipitated by polyelectrolytes such as dextran sulfate and heparin, which probably act as liquid ion exchangers. Several proteins are thus irreversibly precipitated. ALBERTSSON[1] described a novel type of protein precipitation by uncharged water-soluble polymers. Polyethyleneglycol precipitated proteins depending on the concentration of the polymer and the type of ions present in the reaction mixture. While this manuscript was in preparation POLSON *et al.*[2] reported on the precipitation of γ-globulin and fibrinogen by linear polymers. These findings have been confirmed and extended in the present report.

Several uncharged water-soluble polymers, such as polyethyleneglycol of mol. wt. 500, 4000, 6000, 20000 and 10^6 (ref. 3), polypropyleneglycol of mol. wt. 400–500,