Sub-units of the Algal Biliprotein Phycoerythrin

By G. A. MIERAS and R. A. WALL

Department of Chemistry, University of Edinburgh

(Received 14 December 1967)

Dissociation of the red biliprotein phycoerythrin (isolable from many species of red and blue-green algae) has been studied by Fujimori & Pecci (1967). They investigated the action of various mercurials on the intact protein and demonstrated the existence of several sub-units from which the protein (mol. wt. approx. 290 000 by sedimentation and gel filtration; cf. Eriksson-Quensel, 1938; Nolan & O'hEocha, 1967) is presumably assembled. These workers did not describe any dissociation in untreated samples of the protein and showed gel-filtration elution diagrams indicating only one component. However, Nolan & O'hEocha (1967) reported separation of a component of lower molecular weight (45 000) in R-phycoerythrin preparations from two species of algae on columns of Sephadex G-100.

During isolation and purification studies on two phycoerythrins, one from *Porphyridium cruentum* and the other from *Rhodymenia palmata*, preliminary to chemical investigations in this Laboratory, separation of three red fractions was repeatedly observed on columns of Sephadex G-100. These observations were supplemented by further work with analytical columns monitored by spectrophotometry, which showed that this separation was a constant phenomenon. Different methods of extraction and preliminary purification gave preparations that showed substantially the same elution patterns on Sephadex columns.

Experimental. (a) Preparation of protein samples. The phycoerythrin from *P. cruentum* (Agardh) Naegeli, Cambridge Culture Collection of Algae and Protozoa no. 1380A/1a (grown on the ASW medium of Jones, Speer & Kury, 1963), was extracted by treatment of an ice-cooled aqueous suspension (150 ml.) of freshly centrifuged and washed cells with a Dawes Soniprobe for 15 min. The cell debris was separated from the bright-red aqueous solution by centrifugation in the cold and the protein was precipitated from this solution by addition of saturated $(\text{NH}_4)_2\text{SO}_4$ solution in the cold. The precipitated protein was either stored under 50% saturated aqueous $(\text{NH}_4)_2\text{SO}_4$ solution or dissolved in the minimum volume of 0.01 M-sodium phosphate buffer, pH 6.4, before application to a Ca_3PO_4 -Celite column and purification by the method of O'hEocha & Haxo (1960). A small amount of insoluble material in this solution was removed by

filtration through a Celite pad before application to the column. The part of the red phycoerythrin-containing eluate from the column that did not show admixture with allophycocyanin or phycocyanin by its visible spectrum or by disc electrophoresis was used for these experiments. Samples of this protein were also prepared by the butanol extraction technique of Fujimori & Pecci (1967) and by repeated fractional precipitation with $(\text{NH}_4)_2\text{SO}_4$.

(b) Gel filtration. The analytical columns were packed with deaerated suspensions of Sephadex G-75 and G-100 according to the directions of the manufacturers (Pharmacia, 1963) to give a bed 1.25 cm. diam. $\times$ 25 cm. long. Samples (0.1 or 0.2 ml.) of protein solution, to which sucrose was added, were loaded on the columns by layering with an Agla syringe and the chromatograms were developed with 0.1 M-sodium phosphate buffer, pH 7, containing 0.1% NaN_3 at 6–12 ml./hr. The eluates were either continuously monitored at 253 m μ with a flow photometer or collected as 0.5 ml. fractions on a fraction collector actuated by an optical drop counter and their extinction at 550 m μ measured with a spectrophotometer. Ultra-violet and visible spectra of isolated fractions were determined on a Perkin-Elmer model 137 or a Unicam SP.800 spectrophotometer. The analytical chromatograms were all developed at room temperature and were all protected from the light. Larger-scale preparative separations were done in cooled (8–16°) columns, which were also protected from the light.

Results and discussion. The ratio of the void volume, V_0 , to the elution volume (of a peak), V_e , was plotted against the logarithm of the molecular weight of several reference proteins (Andrews, 1964) for each analytical column used and on the Sephadex G-100 column the three peaks given by the phycoerythrin from *P. cruentum* had ratios 1.01 (mol. wt. > 85 000), 1.66 (mol. wt. 36 000) and 1.99 (mol. wt. 23 000). The minimum molecular weight of a possible sub-unit of this protein was calculated from amino acid analysis results (assuming 1 histidine residue/sub-unit) to be about 18 000, which suggests that a monomer-dimer-polymer system was what was observed. A less detailed investigation of the phycoerythrin isolated

by Ca_3PO_4 chromatography from aqueous extracts of *Ceramium rubrum* gave similar but not identical results. On Sephadex columns this biliprotein also showed three components, which had apparent molecular weights of 35 000, 70 000 and 85 000, suggesting a possible dimer-tetramer-polymer system (minimum sub-unit mol. wt. by amino acid analysis 19 000; O'hEocha, 1965). No traces were observed of a lower-molecular-weight component with the *C. rubrum* protein.

When solutions of the lowest-molecular-weight fractions of the phycoerythrins of *P. cruentum* and *C. rubrum* were treated with sufficient solid $(\text{NH}_4)_2\text{SO}_4$ to precipitate all the red solute, a solution of the precipitated material in 0.1M-sodium phosphate buffer, pH 7, demonstrated only one component on Sephadex G-100 columns. The elution volume of this 'reaggregated' component was the same as that of the 'heavy' fractions in the original preparations.

A decrease in the intensity of the longest-wavelength absorption peak of the visible spectrum was observed with each isolated 'light' fraction, just as reported by Nolan & O'hEocha (1967). Visual observations suggested that the fluorescence was also decreased, although not completely quenched as is the case after thermal denaturation of biliproteins. The 'reaggregated' protein produced by $(\text{NH}_4)_2\text{SO}_4$ precipitation of the 'light' fraction from *P. cruentum* showed at least partial restoration of the long-wavelength absorption towards that of the 'heavy' fraction, although the shoulder at 500 m μ present in the spectrum of the 'heavy' fraction was not recovered.

These results indicate that phycoerythrin, like phycocyanin (Scott & Berns, 1965; Hattori, Crespi & Katz, 1965), forms an associating system in aqueous solution. The quantitative aspects of

the association will need detailed study by ultracentrifugal and diffusion techniques. The sub-unit peaks on the analytical columns were very diffuse, a condition that became very serious when the elution rate was decreased in an attempt to increase resolving power. It would appear that the kinetics of the association process are not negligibly slow, at least not for periods of several hours. This means that these values may not be accurate, although fully reproducible, so that it may be that all biliproteins follow the monomer-trimer-hexamer-dodecamer pattern of dissociation observed in the C-phycocyanin system (Scott & Berns, 1965; Hattori *et al.* 1965). It is hoped that the Gilbert-Kellett (Kellett, 1967) steady-state approach to this type of gel filtration problem will yield more reliable answers.

We thank Professor C. O'hEocha and his staff at the Department of Biochemistry, University College, Galway, Irish Republic, for much assistance and good advice during a working visit (by R. A. W.). This work was supported by the Browne Research Fund of the Royal Society and the Science Research Council.

- Andrews, P. (1964). *Biochem. J.* **91**, 222.
 Eriksson-Quensel, I.-B. (1938). *Biochem. J.* **32**, 585.
 Fujimori, E. & Pecci, J. (1967). *Arch. Biochem. Biophys.* **118**, 448.
 Hattori, A., Crespi, H. L. & Katz, J. J. (1965). *Biochemistry*, **4**, 1225.
 Jones, R. F., Speer, H. L. & Kury, W. (1963). *Physiol. Plant.* **16**, 336.
 Kellett, G. L. (1967). *J. Lab. Pract.* **16**, 857.
 Nolan, D. N. & O'hEocha, C. (1967). *Biochem. J.* **103**, 39P.
 O'hEocha, C. (1965). *Annu. Rev. Plant Physiol.* **16**, 425.
 O'hEocha, C. & Haxo, F. T. (1960). *Biochim. biophys. Acta*, **41**, 515.
 Pharmacia (1963). *Sephadex in Gel Filtration*. Uppsala: Pharmacia.
 Scott, E. & Berns, D. S. (1965). *Biochemistry*, **4**, 2597.