THE MONOMER MOLECULAR WEIGHT OF C-PHYCOCYANIN*

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Calculations of the size and shape of phycocyanin aggregates have been reported recently (Jennings, 1968; Bloomfield et al., 1967). Hydrodynamic models and the ratio of volume of polar to nonpolar constituent amino acids have been used to justify the assignment of molecular weights and axial ratios to aggregates and subunits. These calculations rely on data from this and other laboratories (Berns et al., 1964; Berns and Edwards, 1965; Scott and Berns, 1965; Hattori et al., 1965) and incorporate no new experimental information. There is some apparent confusion in the literature concerning the size and molecular weight of the monomer subunit of C-phycocyanin as to whether it is 15,000, 30,000, or 46,000 (Jennings, 1968; Bloomfield et al., 1967; Scott and Berns, 1965; Hattori et al., 1965). This confusion has also permeated the attempted calculation of molecular weights of higher aggregates and shape factors (Jennings, 1968; Bloomfield et al., 1967). Instead of suggesting further calculations, we would like to clarify the matter by reporting three independent experimental determinations of the monomer molecular weight of C-phycocyanin.

MATERIAL AND METHODS

Sedimentation velocity measurements in 5 M guanidine hydrochloride, pH 6.0 phosphate buffer, 0.01 molar in dithiothreitol at 25°, were performed by the method of Tanford et al. (1967). A double sector synthetic boundary

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cell was used for all measurements in the Spinco Model E analytical ultracentrifuge at a rotor speed of 59,780 rpm. All the phycocyanin used was extracted and purified by ammonium sulfate fractionation (Berns and Morgenstern, 1966) or by a hydroxylapatite batch procedure (Bogorad, 1962). Phycocyanin was also treated with _p_-chloromercuribenzoate and the resulting monomeric phycocyanin was collected and used in guanidine hydrochloride sedimentation experiments.

Electrophoresis in the presence of 0.1% sodium dodecyl sulfate at pH 7.0 in 10% polyacrylamide gels was carried out by the method of Shapiro _et al_. (1967). Prior to fixation in sulfosalicylic acid, the gel was examined under a long wave length UV lamp and the position of the red fluorescing phycocyanin zone was measured in relation to the tracking dye. In many cases the intense blue color of the band made this observation superfluous. The position of the tracking dye was also fixed by inserting a small piece of wire into the gel.

Purified C-phycocyanin samples from _Plectonema calothricoides_, _Phormidium luridum_, _Tolypothrix tenuis_, and _Synechococcus lividus_ were examined, as were crude extracts from _Cyanidium caldarium_, _Porphyridium aerugenium_, _Anacystis nidulans_, _Anabaena_ sp., _Porphyridium cruentum_, and _Calothrix membranacea_. In all purified phycocyanins the blue band that fluoresced red was the only band staining for protein. Coomasie blue (Mann Research) was used as a protein stain. In the crude preparations there was only one blue absorbing and red fluorescing band, although other proteins were present. The assignment of the single phycocyanin monomer band was unequivocal. To calibrate the system, samples (Mann Research) of ovalbumin (2X crystallized), pepsin (3X crystallized), and trypsin (3X crystallized) were electrophoresed simultaneously with the phycocyanin specimens.

Purified C-phycocyanin samples, approximately 100 mg each, were made 0.1 N in HCl and then allowed to react with at least a 30-fold excess of cyanogen bromide for 24 hours at room temperature (Gross and Witkop, 1962;

Neufeld, 1966). Controls without cyanogen bromide were examined simultaneously. The reaction mixture was lyophilized and small amounts of the samples were used to perform polyacrylamide electrophoresis at pH 8.1 with monitoring of migration to both anode and cathode. Peptides were also analyzed by the combination of paper chromatography and electrophoresis as indicated by Bennett (1967). Most of the cyanogen bromide-treated sample and the controls were dissolved in 0.2 N acetic acid and applied to a Sephadex G-75 column. The effluent was monitored with a UV scanner and by reaction of aliquots with ninhydrin.

RESULTS

The sedimentation coefficients of the 5M guanidine hydrochloride experiments are plotted as a function of protein concentration in Figure 1.

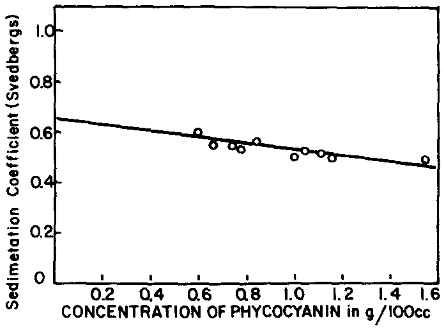

Figure 1. Sedimentation data in 5 M guanidine hydrochloride for C-phycocyanin from P. calothricoides.

Tanford et al. (1967) have shown that $s°/(1 - \phi'\rho) = 0.286n^{0.473}$ (1), where ϕ' is the apparent partial specific volume, ρ is the solvent density, and n is the number of residues in the polypeptide chain. With the data in Figure 1 and the least squares procedure, the extrapolated s° for phycocyanin from P. calothricoides is s° = 0.65. Substituting this s° = 0.65 and the appropriate parameters in (1) gives n = 238. Berns et al. (1964) reported the number of residues for phycocyanin from P. calothricoides to be 249 for the monomer molecular weight of 28,080.

In Figure 2 we have plotted typical data for the sodium dodecyl sulfate polyacrylamide gel electrophoresis of C-phycocyanin from P. calothricoides. The molecular weights determined by this method for phycocyanin from a number of sources are shown in Table I.

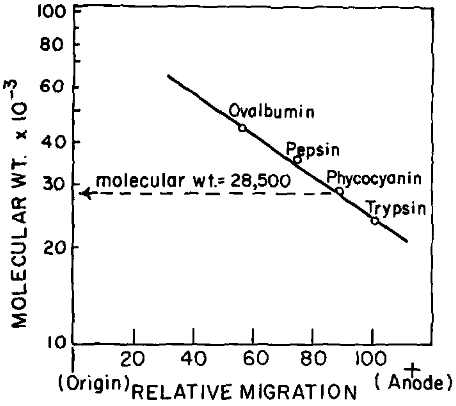

Figure 2. A plot of typical sodium dodecyl sulfate polyacrylamide gel electrophoresis data.

A final check of the monomer molecular weight of phycocyanin from P. calothricoides was performed by the analysis of the products of cyanogen bromide cleavage. Sephadex gel filtration procedures and also a combination of paper chromatography and electrophoresis indicated that there are at least six distinguishable peptide bands on cyanogen bromide-treated material. Additional work with polyacrylamide disc electrophoresis procedures confirmed this finding and may resolve one or two additional peptides. These chemical data are in general agreement with the eight methionine residues per 28,080 molecular weight we reported (Berns et al., 1964). The cyanogen bromide peptide study would not support a monomer molecular weight higher than 28,000.

Table I. Molecular weight of C-phycocyanins from sodium dodecyl sulfate polyacrylamide gel electrophoresis procedure.

Source of phycocyanin	Molecular weight
P. calothricoides	28,500
P. luridum	28,000
T. tenuis	28,500
S. lividus	27,000
*C. caldarium**	25,500
Anabaena sp.*	30,000
*A. nidulans**	25,500
*P. aerugenium**	25,500
*P. cruentum**	28,700
*C. membranacae**	26,000

*From extracts, not highly purified.

An additional method employed to confirm the monomer molecular weight of phycocyanin from *P. calothricoides* was the evaluation of both the sedimentation and diffusion coefficients from a synthetic boundary sedimentation experiment on monomer prepared by treatment with *p*-chloromercuribenzoate. The diffusion coefficient was determined with the boundary spreading analysis of Baldwin (1957). This analysis at two pH values (pH = 6.0 and pH = 8.0), but not extrapolated to infinite dilution, resulted in a molecular weight of 23,000-24,000.

DISCUSSION

The experimental data presented in this publication agree well with the proposed molecular weight of 28,000-30,000 for the C-phycocyanin monomer (Berns *et al.*, 1964; Scott and Berns, 1965), but not with that of 46,000 or 14,000 (Jennings, 1968; Bloomfield *et al.*, 1967; Hattori *et al.*, 1965). Indeed, no direct experimental evidence has ever been presented to support a monomer molecular weight other than 30,000. All other reported monomer molecular weights were calculated with diffusion coefficients determined in our laboratory, but assigned to species in contradiction to our original designations (Scott and Berns, 1965), which were based upon careful experimental tests (Berns, 1967). At least one other report of experimental data suggests a monomer molecular weight for phycocyanin of 30,000 (Neufeld, 1966). Hopefully, the present data will clarify the literature so that we may now elucidate the exact state of aggregation of all higher phycocyanin aggregates.

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