Solution Properties of Phycoerythrin. I. Characterization of Phycoerythrin

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Phycoerythrin, one of the chromoproteins, from the red alga *Porphyra yezoensis* has been characterized by physicochemical measurement. The value of the sedimentation constant was 12s and it did not change with the change in pH from 5.4 to 6.8. This was assumed from the fact that the phycoerythrin did not undergo reversible dissociation-association reaction under these conditions. The weight average molecular weight of phycoerythrin was determined to be 2.2×10^5, and the intrinsic viscosity of phycoerythrin was determined to be 2.29×10^{-2} (10^{-1} dm^3/g). From these values, the molecular shape of phycoerythrin was inferred to be globular based on the Scheraga-Mandelkern equation. The SDS-polyacrylamide gel electrophoresis of phycoerythrin showed that the phycoerythrin consisted of two subunits with molecular weights of 3.8×10^4 and 2.3×10^4. The sedimentation behavior of phycoerythrin suggested that the phycoerythrin solution was multicomponent. The phycoerythrin dissociated into two molecular species on treatment with sodium dodecyl sulfate. Their sedimentation constants were 12s and 2s. The 2s species was supposed to be phycoerythrin monomer.

Phycoerythrin, which is found widely in red and blue-green algae, is one of the photosynthetic accessory chromoproteins similar to phycocyanin. The function of phycoerythrin in the algal cell is to harvest the light energy during photosynthesis. The properties of the chromoproteins isolated from various algae have been studied extensively by several researchers in various fields of science.[1-4]

We have reported previously on the physicochemical properties, such as the molecular weight, molecular shape and size, dissociation-association behavior, and on the sedimentation and viscosity behavior of phycocyanin isolated from *Porphyra tenera* or *Porphyra yezoensis*.[5-10] Compared with phycocyanin, the physicochemical properties of phycoerythrin from *Porphyra tenera* or *Porphyra yezoensis* have not been studied as extensively, therefore, some points still remain to be clarified.

In this study, we investigated several characteristic properties of phycoerythrin isolated from *Porphyra yezoensis* based on absoption spectra measurement, sedimentation transportation and equilibrium measurements, viscosity measurements, electrophoresis measurements, and amino acid composition analysis.

Experimental

Sample Preparation. The phycoerythrin used in this study was isolated from the dried red alga, *Porphyra yezoensis*, by repeated precipitation with ammonium sulfate. The procedure was essentially the same as that used for the purification of phycocyanin.[5] The ultraviolet and visible absorption spectra of purified phycoerythrin exhibited characteristic peaks at 280, 497, and 565 nm, as shown in Fig. 1, which was the typical spectrum of phycoerythrin.[11] The ratio of the optical density at 565 nm to that at 280 nm has been used to judge the purity of phycoerythrin: The phycoerythrin which had a ratio of more than 5.0 was considered sufficiently pure.[12] The ratio was 5.0 or greater for the phycoerythrin used in this study. The purified sample was stored in a refrigerator at 5 °C as a precipitate with 40% saturated ammonium sulfate. Prior to use, the sample of phycoerythrin was dissolved in water and dialyzed against a phosphate or acetate buffer at 5 °C. Dialysis was continued for at least 48 h, with an occasional change of the external solution.

Ultracentrifugation. The sedimentation velocity measurements were performed on a Hitachi 282 ultracentrifuge. Double sectorial cells were used for all measurements. The rotor speed was set at 47000 rpm, and the temperature was maintained at 25 °C. Schlieren optics was used for the sedimentation velocity measurement. The Schlieren pattern was taken as a photograph on Fuji medical imaging film type FX. The position of the peak in the Schlieren pattern was measured using a Sinko-12 Projector. The sedimentation constant was calculated by the usual method. Phosphate buffer, pH 6.8 and I(ionic strength) 0.1, and acetate buffer, pH 5.4 and I 0.1, were used as solvents. The sedimentation equilibrium experiments were performed on the same ultracentrifuge apparatus as for the sedimentation velocity measurements. All measurements were made at a given speed for approximately 20 h, so as to ensure that an equilibrium was established in a liquid column of about 3 mm. The rotor speeds were set at approximately 9000—12000 rpm, and a temperature of 25 °C was maintained. Interference optics were used for the sedimentation equilibrium measurement. The interference pattern was taken as a photograph on the same film as was used for photographing the Schlieren pattern. The concentration at a radial distance in the cell was calculated from the concentration difference by the use of the formula

$$\Delta c = \Delta J/K$$

where Δc is the concentration difference, ΔJ the number of fringe counted from the meniscus, K the apparatus constant. The number of fringes were measured using the projector. The value of K was determined from the interference pattern of a phycoerythrin solution of known concentration. The weight average molecular weights were calculated by the usual method. The partial specific volume of phycoerythrin, $\bar{v}$, was calculated after ascertaining the

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amino acid composition by the Cohn and Edsall method;[13] it was also calculated from the density of the phycoerythrin solutions which were measured by using the Sibayama Model SS-D-200-EXT precision densimeter at various concentrations of phycoerythrin. The value of 0.73 $cm^3 g^{-1}$ was obtained for the partial specific volume from the amino acid composition as shown in Table 1, and the value of 0.72 $cm^3 g^{-1}$ as the partial specific volume was obtained from the density measurement. These values are similar to the value of 0.73 $cm^3 g^{-1}$ reported by MacColl et al.[12] The value of 0.73 $cm^3 g^{-1}$ was used for the calculation of the molecular weight.

Determination of Concentration. The protein concentrations were determined by weighing the substances which were obtained from the aliquots of protein solution by drying at 105 °C until a constant weight. They were also determined by the semi-micro-Kjeldahl method.

Viscosity Measurement. Viscosity measurements were made for phycoerythrin in phosphate buffer solutions of pH 6.8 and I 0.1, at 25 °C. An Ubbelohde dilution type viscometer with a flow time for water ca. 120 s was used for the measurements.

Acrylamide Disc Gel Electrophoresis. Disc electrophoresis of native phycoerythrin was performed according to the method of Davis.[14] A 0.005 mol dm^{-3} Tris-0.0038 mol dm^{-3} glycine system adjusted to pH 8.3 was used for the running buffer solution. The phycoerythrin sample was applied to 4.5 to 7.0% acrylamide gels with the constant ratio of *N,N'*-methylenebis[acrylamide] to acryamide contents. The gel was calibrated by using the following reference proteins: bovine albumin (molecular weight, M_w, 6.8×10^4), catalase (M_w, 2.4×10^5), and ferritin (M_w, 4.5×10^5).

SDS-Polyacrylamide Gel Electrophoresis. The SDS-polyacrylamide gel electrophoresis procedure of Weber and Osborn[15] was employed to measure the molecular weight of the phycoerythrin subunit. The phycoerythrin sample was incubated overnight at room temperature in a 0.01 mol dm^{-3} phosphate buffer solution with 1% 2-mercaptoethanol. The following proteins were used as molecular weight calibration markers: cytochrome c (M_w, 1.25×10^4), chymotrypsinogen (M_w, 2.5×10^4), trypsin inhibitor (M_w, 2.8×10^4), egg albumin (M_w, 4.5×10^4), bovine serum albumin (M_w, 6.8×10^4).

Amino Acid Analysis. The amino acid analysis of phycoerythrin was performed on a JEOL JLC-6AS amino acid analyzer with a Hokusin Laboratory recorder R140-O-D.

Results and Discussion

The sedimentation velocity pattern of phycoerythrin obtained by using Schlieren optics showed a single boundary in phosphate and acetate buffer solutions, respectively. The plot of the sedimentation coefficient versus the concentration of protein was linear over the concentration region of 0.025—0.44 g/10^{-1} dm^3 as shown in Fig. 2. As is seen in this figure, the sedimentation coefficient did not significantly change despite the change in pH of the solution. The concentration dependence of the sedimentation coefficient was not observed at any of the concentrations that were studied. The sedimentation constant of this protein was determined to be 12.2 s at pH 6.8 and 11.9 s at pH 5.4, respectively. These values were

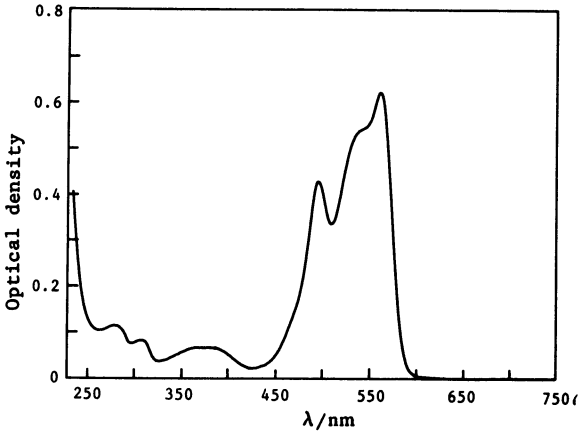

Fig. 1. Typical absorption spectrum of phycoerythrin solution at pH 6.8 and I 0.1.

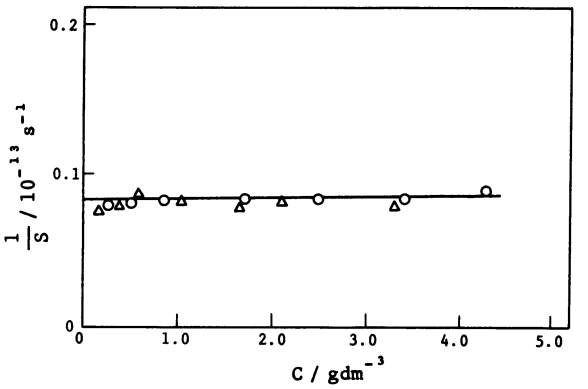

Fig. 2. Plot of the reciprocal of sedimentation coefficients versus concentration in phosphate and acetate buffer solutions.
△: phosphate buffer solution, pH 6.8 and I, 0.1,
○: acetate buffer solution, pH 5.4 and I 0.1.

considered to be almost the same and were in good agreement with the value of 10.8 s for phycocyanin in acetate buffer solutions at pH 5.4 and I 0.1.[5] It is well known that the sedimentation constant of phycocyanin changes with the change in pH of the solution because of the self-dissociation-association reaction.[5,16] Namely, the phycocyanin existed predominantly in the form of a hexamer at pH 5.4, and predominantly in the form of a trimer or tetramer at pH 6.8. The sedimentation constant of the hexamer is 11.3 s, and that of the trimer or tetramer is 6.3 s.[5] Therefore, it seems from the result of this study that the phycoerythrin solution is a single component solution over the pH range of 5.4 to 6.8. The phycoerythrin does not undergo any reversible self-dissociation-association reactions under these pH conditions.

Figure 3 shows the plot of the logarithm of concentration of the protein versus the square of the radial distance in the sedimentation equilibrium cell. It was drawn from the analysis of the sedimentation equilibrium pattern for the phycoerythrin at pH 5.4 and I 0.1. As is clearly seen from the figure, the plot

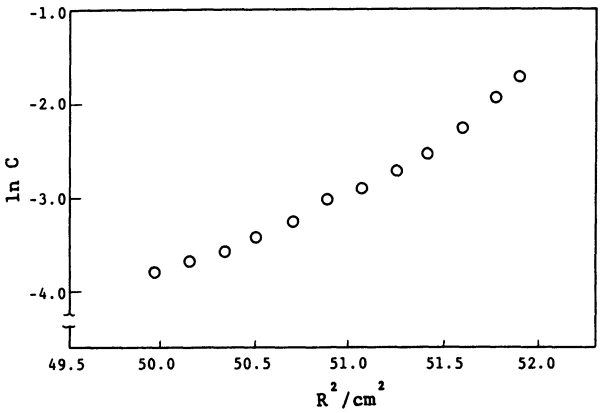

Fig. 3. Plot of the logarithm of concentration versus the square of radial distance in sedimentation equilibrium cell.

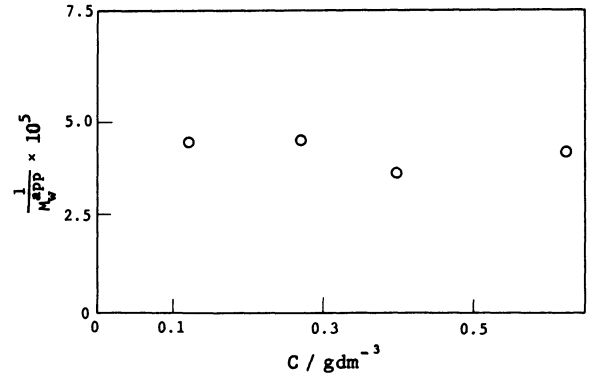

Fig. 4. Plot of the reciprocal of apparent weight average molecular weight versus concentration.

has a curvature. This sedimentation equilibrium behavior means that the phycoerythrin solution is a multicomponent solution at pH 5.4 and I 0.1. This result is inconsistent with that from the sedimentation velocity measurements. It may be reasonable to consider that the minor components in the phycoerythrin solution, probably phycoerythrin monomer and its associated species, were not found in the Schlieren pattern under our experimental conditions because the concentration of these minor components is very low.

Figure 4 shows the plot of the reciprocal of the apparent weight average molecular weight of phycoerythrin, $1/M_w^{app}$, versus concentration. The weight-average molecular weight of phycoerythrin was determined to be 2.2×10^5 from the plot. This value agrees with that reported by Svedberg et al.[1] The second virial coefficient, A_2, was estimated to be 1.1×10^{-5} ($cm^3\,mol/g^2$). Therefore, the phycoerythrin solution may be considered to be a pseudo-ideal solution similar to a phycocyanin solution. This property is characteristic of globular proteins.

Figure 5 shows the result of the viscosity measurements for phycoerythrin performed at pH 6.8 and I 0.1. The intrinsic viscosity of phycoerythrin was found

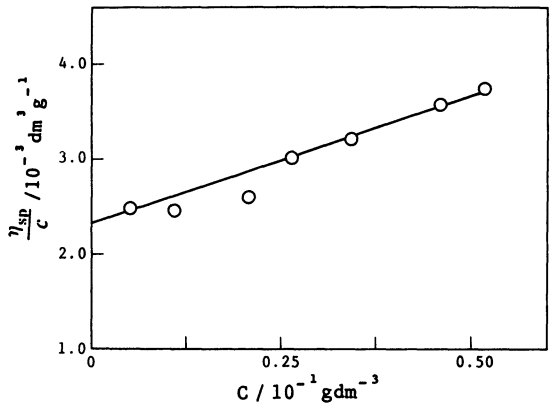

Fig. 5. Plot of the reduced viscosity of phycoerythrin in phosphate buffer versus concentration.

to be 2.29×10^{-2} ($10^{-1}\,dm^3/g$). Tanford has stated that the values of the intrinsic viscosity of globular proteins lie between 3.3×10^{-2} ($10^{-1}\,dm^3/g$) and 4.0×10^{-2} ($10^{-1}\,dm^3/g$) regardless of the molecular weight of the protein.[17] The value of the intrinsic viscosity of phycoerythrin did not differ greatly from that of the globular proteins.

Scheraga and Mandelkern derived the following equation for a revolutional ellipsoid:[18]

$$s_0[\eta]^{1/3} = \frac{M^{2/3}(1-\bar{v}\rho)\beta}{N_A\eta_0}, \quad (1)$$

where s_0 is the sedimentation constant, $[\eta]$ the intrinsic viscosity, M the molecular weight, $\bar{v}$ the partial specific volume of the solute, ρ the density of the solvent, N_A Avogadro's number and η_0 the viscosity of the solvent. The quantity β is a function of the axial ratio of ellipsoid, and has a value of $2.12—2.20\times10^6$ for a prolate ellipsoid when the axial ratio is less than 5.

The β value for the phycoerythrin was calculated to be 2.16×10^6 using Eq. 1. Therefore, it is reasonable to assume from the values of A_2, $[\eta]$, and β, that the phycoerythrin is a globular protein.

Disc gel electrophoresis for phycoerythrin exhibited two bands. The molecular weight of each band was calculated to be 2.3×10^5 and 2.4×10^5 from the calibration plot as shown in Fig. 6. Although the values of the molecular weights of the two bands are nearly the same, the result suggests that the phycoerythrin solution is a multicomponent solution. Also, the result corresponds to the sedimentation equilibrium behavior observed for phycoerythrin in this study. It may be assumed that the appearance of two bands is due to some denaturation occurred during the disc electrophoresis or some difference in their structure. However, these points are not clear.

SDS-polyacrylamide gel electrophoresis for phycoerythrin exhibited two bands, with the molecular weights of 3.8×10^4 and 2.3×10^4 as is shown in Fig. 7. Those molecular weights might correspond to

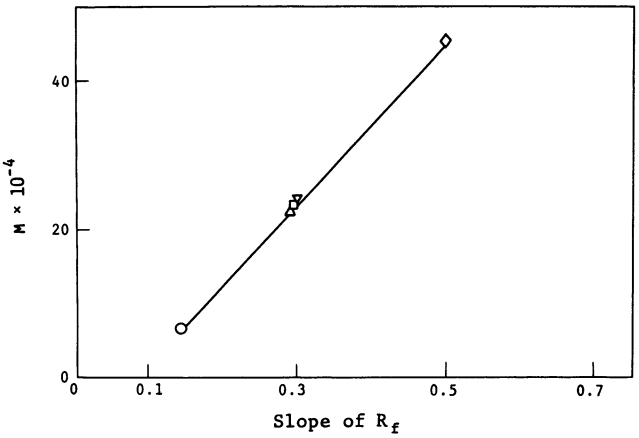

Fig. 6. Molecular weight determination of native phycoerythrin by disc electrophoresis. Three marker proteins used were, bovine serum albumin, catalase and ferritin. All proteins were run on various different concentrations of gel, and the slope (axis of abscissa) was obtained from the plot of the R_f-value versus concentration.
○: Bovine serum albumin, □: catalase, ◇: ferritin, △ and ▽: phycoerythrin.

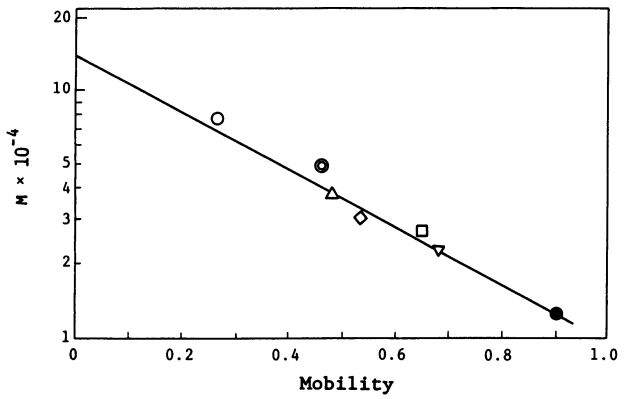

Fig. 7. Determination of molecular weight of denatured phycoerythrin by SDS-polyacrylamide gel electrophoresis. The mobilities on 10% gel were plotted against the known molecular weight expressed on a semi-logarithmic scale. The five proteins used as the marker.
●: Cytochrome c, □: chymotrypsinogen, ◇: trypsin inhibitor, ◎: egg albumin, ○: bovine serum albumin, △ and ▽: phycoerythrin.

the subunits which have been reported for phycoerythrin from *Porphyridium cruentum* by Glazer et al.[3] O'Carra and Killilea have observed two polypeptides for phycoerythrin from *Phormidium persicinum* by polyacrylamide gel electrophoresis in SDS solution. They suggested that the phycoerythrin existed as a polypeptide dimer composed of two different types of chain with a molecular weight of 1.97×10^4 and 2.20×10^4.[19] MacColl et al. have proposed that the phycoerythrin from *Rhodomonas lens* was composed of two pairs of subunits ($\alpha_2\beta_2$), and the molecular weights of α and β are 9.8×10^3 and 1.7×10^4, respectively.[12] It may be said that the phycoerythrin

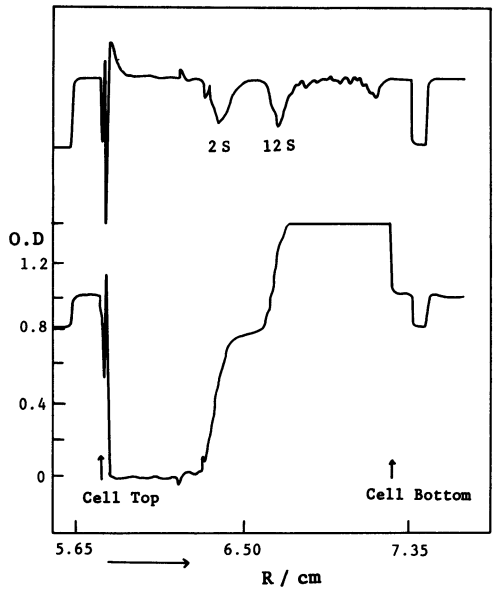

Fig. 8. Absorbance distribution and absorbance gradient distribution corresponding to the concentration distribution in the sedimentation velocity measurement for the denatured phycoerythrin. The curve in the under side and in the upper side represents the absorbance distribution and absorbance gradient distribution, respectively. The abscissa represents the radial distance, R, in the ultracentrifuge cell.

from *Porphyra yezoensis* is composed of two subunits similar to the other phycoerythrin from various different algae.

Figure 8 represents the result of the sedimentation velocity measurement for the denatured phycoerythrin treated with sodium dodecyl sulfate at a concentration of 0.005 mol dm^{-3}. The curves of absorbance distribution (under side) and of its differentiation (upper side) in the ultracentrifuge cell were drawn by the recorder connected with the absorption scanner. In this figure, two boundaries with a sedimentation coefficient of approximately 12 and 2 s were observed for the denatured phycoerythrin. The value of 12 s is close to that of phycoerythrin in the phosphate and acetate buffers obtained in this study. It is supposed that the 12 s component is probably a native phycoerythrin which was not affected by the sodium dodecyl sulfate treatment. It is also supposed that the appearance of the 2 s component can be attributed to the dissociation of the phycoerythrin. The 12 s component may correspond to the band in the disc electrophoresis. Hattori et al. have reported the value of 3.2 s for the phycocyanin monomer.[16] Although the value of 2 s is somewhat lower than that of the phycocyanin monomer, it may be said that the 2 s component corresponds to the phycoerythrin monomer composed of two subunits which appeared in the SDS electrophoresis observed in this study. The 2 s component may expand a little by interaction with the sodium dodecyl sulfate so that the sedimentation velocity decreases.

Thus, it is likely that the phycoerythrin from *Porphyra yezoensis* is a globular protein and its solution is a multicomponent solution although the concentration of the minor component is very low. Phycoerythrin seems to be stable compared with phycocyanin in solution over the pH range of 5.4 to 6.8. Unlike phycocyanin, there is no evidence that phycoerythrin undergoes a reversible self-associating reaction with change of pH from 5.4 to 6.8.

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