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Cytochrome *c* Peroxidase

2. The Size and Shape of Cytochrome *c* Peroxidase of Baker's Yeast

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The molecular weight of crystalline cytochrome *c* peroxidase of baker's yeast has been determined as 34 100 on the basis of sedimentation and diffusion studies. The value is derived from a sedimentation coefficient of 3.55 S at infinite dilution, a diffusion coefficient of 9.44 F and a partial specific volume of 0.733. ml per g. The molar frictional ratio is equal to 1.03.

The molecular weight of cytochrome *c* peroxidase (CcP) of baker's yeast has been estimated on the basis of its hemin content. Abrams *et al.*[1] obtained a minimum molecular weight of 60 000, whereas Yonetani reported a somewhat lower value of 49 000,[2] which was corrected to that of 46 500.[3] A crystalline preparation, the hemin content of which was found to remain constant through several re-crystallization steps, was found to have a value of 37 000.[4,5]

In the present study the sedimentation and diffusion coefficients of CcP, as well as its partial specific volume, have been determined and by means of these values the molecular size and shape of CcP have been calculated. A preliminary report was recently published on these results.[4]

EXPERIMENTAL

Material. Crystalline CcP was prepared by a procedure that has recently been published.[4,5] The ratio of E_{407}/E_{280} of the different preparations used in this study was equal to 1.28–1.29. For the sedimentation and diffusion measurements, the protein solutions were dialyzed for 48 h at +4°C against 0.283 μ buffer of pH 7.0 which contained 0.05 M sodium phosphate and 0.171 M sodium chloride.

Sedimentation. All sedimentation analyses were made in a Spinco analytic ultracentrifuge model E fitted with Schlieren optics and phase plate according to Philpot-Svensson. All analyses were performed at 59 780 rpm. Ten photographs of the sedimenting boundary position were obtained in each experiment. The sedimentation constants were evaluated from enlarged images of the photographs projected onto millimeter graph paper. In estimates of the distance from the rotation center to the reference line, the stretching of the rotor was not taken into consideration. The sedimentation constants

obtained in the different experiments were reduced to pure water at 20°C by applying the usual corrections for the density and viscosity of the medium,[6] using a partial specific volume equal to 0.733 ml/g. The different sedimentation constants have also been corrected for the adiabatic cooling of the rotor.[7] The results are given in Svedberg units ($1\ S = 10^{-13}$ cm sec^{-1} $dyne^{-1}$).

Diffusion. The diffusion experiments were conducted in the analytical ultracentrifuge. A synthetic boundary cell of the valve type was used. The diffusion experiments were performed at 4059 rpm and at 12 590 rpm, both rotor speeds at which, according to Lamm [8] or Fujita,[9] no correction is necessary. The evaluation of the diffusion constant was performed on magnifications projected onto millimeter graph paper. The areas were determined with a planimeter. The diffusion constant was evaluated according to the "height-area" method:

$$D_A = \frac{1}{4\pi t}\left(\frac{\int_{-\infty}^{+\infty}\frac{\delta c}{\delta x}\,dx}{H}\right)^2 = \frac{1}{4\pi t}\left(\frac{A}{H}\right)^2$$

where A = area in cm^2, H = maximum height in cm, t = time in seconds. All the diffusion experiments were performed in the 0.283 μ sodium phosphate-sodium chloride buffer of pH 7.0.

The diffusion constants were reduced to pure water at 20°C by applying corrections for the density and viscosity of the medium. The results are given in Fick's units ($F = 10^{-7}$ cm^2 sec^{-1}).

Partial specific volume. Samples of crystalline CcP obtained by extensive dialysis against distilled water were dissolved by addition of a minimum amount of 0.1 N NaOH to give a pH of 6.0–6.5. The densities of the solutions and of the solvent were measured at 23.85°C, using a pycnometer of the Sprengel-Ostwald type having a capacity of 5 ml. The protein concentration was determined from samples of the solutions by dry weight at 105°C. The calculated amount of sodium present was subtracted in calculating the dry weight of the protein.

RESULTS

Sedimentation coefficients. Crystalline cytochrome *c* peroxidase, as far as could be judged from photographs obtained in the ultracentrifuge experiments, appeared to be homogeneous throughout the concentration ratios studied. All sedimentation patterns consisted of single, symmetric peaks showing no indications of more than one component. The sedimentation constant for CcP was concentration-dependent, increasing slightly in value with dilution. Values of the sedimentation constant at different protein concentrations are shown in Fig. 1. The line drawn through the data was calculated by the method of least squares and fits the equation

$$S_{20}^{\circ} = S_{\infty} - \beta a$$

where S_{∞} is the sedimentation coefficient extrapolated to infinite dilution and a is the concentration of the protein in mg/ml. S_{∞} was found to be 3.55 S. The equation for the sedimentation coefficient of cytochrome *c* peroxidase was:

$$S_{20}^{\circ} = 3.55 - 0.019\ a$$

Diffusion coefficient. Several experiments were performed on the crystalline preparations from different batches. The protein concentrations of the different experiments were 4.4 and 12.9 mg/ml. Two runs were made at lower concentrations, giving the values 9.21 and 9.72 F, and one at the higher one,

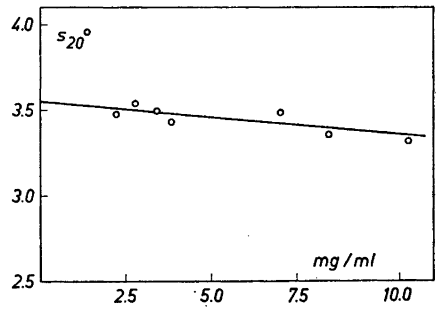

Fig. 1. Concentration dependence of the sedimentation constant of CcP. Abscissa: mean protein concentration during observation in the ultracentrifuge. Ordinate: sedimentation coefficients, $s_{20,w}$, of crystallized CcP. The drawn line has been fitted to the points by the least square method.

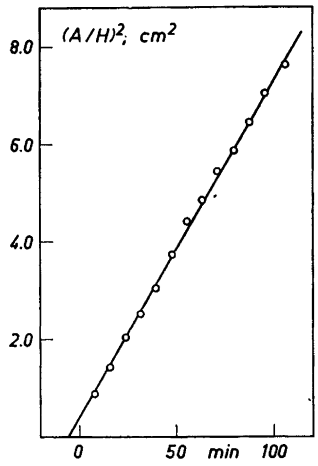

Fig. 2. A plot of $(A/H)^2$ against time, from which the diffusion coefficients of crystalline CcP have been calculated. Time measured in minutes from the end of boundary formation. Protein concentration was 12.9 mg/ml in sodium phosphate-sodium chloride buffer (pH 7.0, ionic strength 0.283). The rotor speed was 12 590 rpm. The drawn line correspond to $D_{20}^{0} = 9.40$ F.

giving the value 9.40 F. One representative plot of data of one experiment is shown in Fig. 2. The extrapolated t_0-values were all between 2—9 min. The value of 9.44 F obtained as an average is used in the calculations.

Partial specific volume. The partial specific volume was determined in two experiments. A solution of 11.2 mg/ml gave the value 0.733 ml/g.

Molecular weight. The value of the molecular weight of CcP was calculated by means of Svedberg's formula.[6]

$$M = \frac{RTs}{D(1 - V\varrho)}$$

From the values of s, D, and V determined, CcP was found to have a molecular weight of 34 144.

Molecular shape. The molecular frictional ratio f/f_0 was calculated from the following formula [6]

$$f/f_0 = \left[\frac{1 - V\varrho}{D_{20}^{2} \cdot s_{20} \cdot V}\right]^{1/3} \times 10^{-8}$$

The frictional ratio was found to be equal to 1.03 for CcP. If the entire molar frictional ratio is assumed to be due to asymmetry only, this value would represent axial ratios of about 2 for both prolate and oblate ellipsoids.[6]

Following the procedure of Oncley,[6] f/f_0 can be represented as a product of two factors, f/f_e and f_e/f_0. The first factor, f/f_e, represent the effect of hydra-

tion, while the second factor f_e/f_0, represents the influence of the asymmetry of the molecule. Kramer [6] has shown that the hydration factor is related to the grams of water per gram of protein, w, by the formula

$$f/f_e = \left[1 + \frac{w}{V\varrho}\right]^{1/3}$$

in which ϱ is the density of the water solvating one gram of pure solute of the partial specific volume V. If the molecular frictional ratio, f/f_0, of CcP were due entirely to hydration, the value for w would be 0.07.

DISCUSSION

The value of 34 100 for the molecular weight of CcP presently determined conforms fairly well with the minimum molecular weight of 37 000 calculated on the basis of the hemin content of repeatedly crystallized CcP. Confirmative molecular weights were also obtained by using some amino-acids as the calculation basis. The content of half cystine, histidine, and arginine was found to give values equal to 34 354, 33 435, and 33 687, respectively, the average of these molecular weights being 33 825.[10] This value is somewhat lower than that obtained from the iron analysis but conforms well with the molecular weights obtained from ultracentrifugal data. Since the iron content is determined by the pyridine hemochrome method, the low iron content might be due to slight degradation of the hemin ring during the lengthy process of crystallization. The earlier values of 60 000, 49 000, and 46 500 reported by Abrams *et al.*[1] and by Yonetani,[2,3] respectively, might also be explained on this basis.

It is important to note that the value for the frictional ratio calculated for CcP is one of the lowest described. This low value is also reflected in the rather small effect of the protein concentration on $s_{20,w}$ observed for CcP (Fig. 1). It is evident that the protein must be highly symmetrical in shape.

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REFERENCES

1. Abrams, R., Altschul, A. R. and Hogness T. R. *J. Biol. Chem.* **142** (1942) 303.
2. Yonetani, T. *J. Biol. Chem.* **240** (1965) 4503.
3. Yonetani, T. *J. Biol. Chem.* **241** (1966) 2981.
4. Ellfolk, N. *Acta Chem. Scand.* **20** (1966) 1427.
5. Ellfolk, N. *Acta Chem. Scand.* **21** (1967) 175.
6. Svedberg, T. and Pedersen, K. O. *The Ultracentrifuge*, Oxford University Press, London 1940.
7. Schachman, H. K. *Ultracentrifugation in Biochemistry*, Academic, New York, London 1959.
8. Lamm, O. *Z. physik. Chem. (Leipzig)* **A 143** (1929) 177.
9. Fujita, H. J. *J. Phys. Chem.* **63** (1959) 1092.
10. Ellfolk, N. *Acta Chem. Scand. To be published.*

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