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The Molecular Weight of Myeloperoxidase

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The molecular weight of myeloperoxidase with an iron content of 0.074 % has been determined to be 149 000, which means that there are two atoms of iron per enzyme molecule. The calculations are based upon experimental results indicating a sedimentation coefficient at infinite dilution of 7.93 *S*, a diffusion coefficient of 4.81 *F* and a partial specific volume of 0.731 ml/g.

Myeloperoxidase [1] (MPO) has recently been obtained as a highly purified and crystalline preparation with an iron content of 0.074 % [2]. Therefore, it has been of interest to determine the sedimentation and diffusion coefficients of the enzyme as well as its partial specific volume and by means of these values to calculate the molecular weight.

METHODS

Sedimentation. The sedimentation studies were carried out in a Spinco analytical ultracentrifuge, model E, at a rotor speed of 59 780 rpm. The centrifuge was equipped with an automatic rotor temperature control. The inclined bar was used as schlieren diaphragm in most of the experiments; in a few cases the bar was replaced by a phase-plate [3], that facilitated the evaluation of the exposures but did not effect the results.

The sedimentation velocity was determined for enzyme concentrations from 0.8 up to 30 mg per ml. Temp. 20°C. The MPO was in these experiments as well as in the diffusion experiments stated below dissolved in phosphate buffer, 0.05 N, pH 7.0, containing also 1 % NaCl.

Diffusion. The diffusion experiments were made in a Spinco diffusion and electrophoresis instrument at a temperature of 1.17° C. Boundary sharpening was obtained by suction with a long hypodermic syringe needle cut perpendicular to its axes, and the diffusion process was followed over a period of 43 h. The optics were adjusted for studying the Rayleigh-Calvet-Philpot-interferogram at 546 mμ, and the number of fringes and the position of maxima and minima were measured out visually by means of the optical arrangements of a Hilger densitometer for spectrograms, using the slit plane as a projection screen. The cell magnification was 1.007. The diffusion coefficients were evaluated according to the "height-area" method as described by Svensson [4] and according to the moment method also described by Svensson [5] but slightly modified here.

Partial specific volume. The partial specific volume was determined for solutions of MPO, extensively dialysed against distilled water. The density was determined in a pycnometer of Sprengel-Ostwald type at 24°C.

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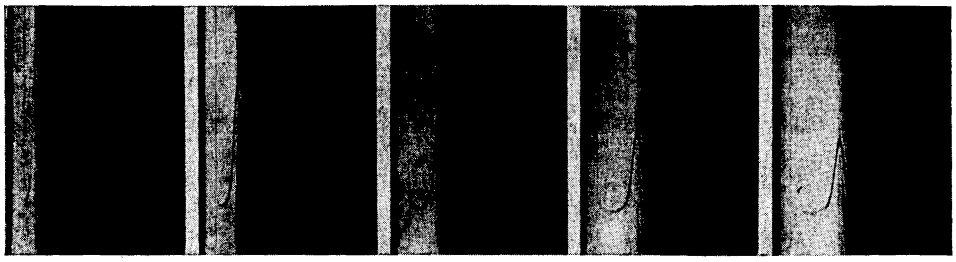

Fig. 1. Photographs taken in the ultracentrifuge during sedimentation of a myeloperoxidase solution of 10 mg/ml. First exposure taken 7 min after full speed was attained. Exposure intervals 8 min.

RESULTS

Sedimentation coefficient. The enzyme appeared to be homogeneous as far as could be judged from the photographs of the ultracentrifuge experiments, Fig. 1. The sedimentation coefficients were evaluated both from drawings made from magnified pictures and from measurements made directly on the plates in the Hilger densitometer. By means of the least square method the two sets of data were separately fitted to the equation

$$S^0_{20} = S_\infty - \beta a$$

where S_∞ is the sedimentation coefficient extrapolated to infinite dilution and a is the mean concentration of myeloperoxidase during the sedimentation in

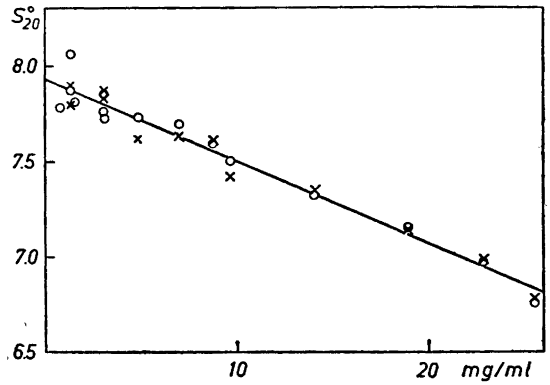

Fig. 2. Concentration dependence of the sedimentation coefficient of myeloperoxidase. Abscissa: mean protein concentration during observation in the ultracentrifuge. Ordinate: Sedimentation coefficients S^0_{20} calculated from enlarged drawings of the photographs O, and from comparator readings directly on the photographs, ×. The drawn line has been fitted to the points by the least square method.

Table 1. Diffusion data obtained by the "height area" method.

Conc.	t_0	$D_{1.17}$	D^0_{20}
mg/ml	sec	F	F
2.50	−2 170	2.467	4.813
1,30	−2 000	2.474	4.826

mg/ml. S_∞ was found to be 7.935 and 7.915 S, respectively, and β was 0.04359 and 0.04215 S ml.mg^{-1}, respectively. The following equation

$$S^0_{20} = 7.93 - 0.0429 \cdot a$$

may thus be accepted as the equation of the line that best fits the experimental data, Fig. 1.

Diffusion coefficient. Two concentrations were studied, 2.50 and 1.30 mg/ml, corresponding to the total fringe numbers of 38.50 and 20.31. When the "height-area" method was applied, the fringe interval Δn used in calculating the maximum fringe density, n'_{max}, was accordingly chosen as 10 and 5, respectively [4]. In the former experiment 6 exposures were evaluated and in the latter 8. In Fig. 3 plots have been made of $(1/n'_{max})^2$ *versus* the time and the best fitting lines, as calculated by the least square method, have been drawn. The numerical data are given in Table 1. The apparent starting time, t_0, is slightly negative because of the impossibility to produce a completely sharp boundary.

Three exposures from each experiment were also evaluated according to the moment method. The equation to be used has the following form [5]

$$D_m = \frac{\int_{n_0}^{n_c} x^2 dn}{2(t-t_0)(n_c-n_0)} = \frac{a}{t-t_0} \qquad (1)$$

where n denotes the refractive index increment measured in fringe numbers ($n_0 = 0$ and $n_c = n_{tot}$), x is the fringe coordinate measured from the center of the boundary, and t is the time of the exposure. Since the myeloperoxidase is strongly coloured, only dilute solutions could be studied, and n_{tot} was small. It thus appeared difficult to estimate the limiting parts of the integral, since x approaches infinity when n approaches zero or n_{tot} and it had to be extrapolated over relatively large intervals. This difficulty was avoided by taking the integral only over the interval that really was covered by the fringes that could be measured in the comparator. For instance, if the first minimum, $n = 0.5$, could be read, and Simpsons formula was used for the numerical integration, the lower limit, μ, of the integral equals 0.5; if instead the trapets

formula was used, $\mu = 0.25$. The same is valid for the upper limit. To compensate for the smaller interval of integration, a of eqn. (1) must be changed into the form

$$a = \frac{\int_{\mu}^{\nu} x_2 \mathrm{d}n}{2n_{tot} \int_{z_\mu}^{z_\nu} z^2 \frac{1}{\sqrt{2\pi}} \cdot e^{-\frac{1}{2}z^2} \mathrm{d}z} \qquad (2)$$

The two integrals are proportional as long as the diffusion is ideal. In the denominator integral the normalized error function $f(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}z^2}$ has been used. The corresponding limits of integration are readily found from any table of that function including its integral, since $\int_0^{z_n} f(z)dz = \frac{n_{tot} - 2n}{2n_{tot}}$. A table of the integral $\int_{-\infty}^{z} z^2 f(z)\, dz$ is easily constructed from the data of the error function table, and the denominator integral of eqn. (2) is calculated by means of the formula

$$\int_{z_\mu}^{z_\nu} z^2 f(z)dz = 1 - \int_{-\infty}^{z_\mu} z^2 f(z)dz - \int_{z_\nu}^{+\infty} z^2 f(z)dz$$

after interpolation of the values of the two later integrals in the constructed table.

Table 2. Diffusion data obtained by the moment method. t_o is taken from Table 1.

Conc.	t	$t-t_o$	$a \times 10^2$	$D_{1.17}$	D°_{20}
mg/ml	sec	sec	cm²	F	F
2.50	23 640	25 810	0.6406	2.482	4.84
	66 840	69 010	1.696	2.457	4.79
	154 200	156 370	3.845	2.459	4.80
			Mean value		4.81
1.30	20 820	22 820	0.5643	2.473	4.82
	64 020	66 020	1.644	2.490	4.86
	93 120	95 120	2.302	2.420	4.72
			Mean value		4.80

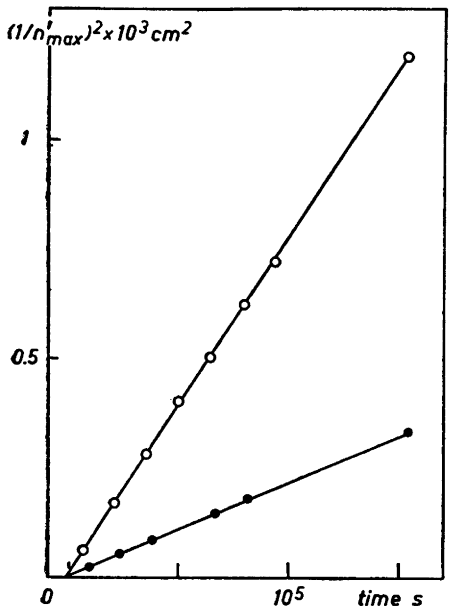

Fig. 3. Graphs from which the diffusion coefficients have been calculated according to the "height-area" method. Abscissa: time of exposure measured from end of boundary sharpening procedure. Ordinate: inverted square of maximum fringe density. Protein concentrations 2.50 mg/ml, ●, and 1.30 mg/ml, O.

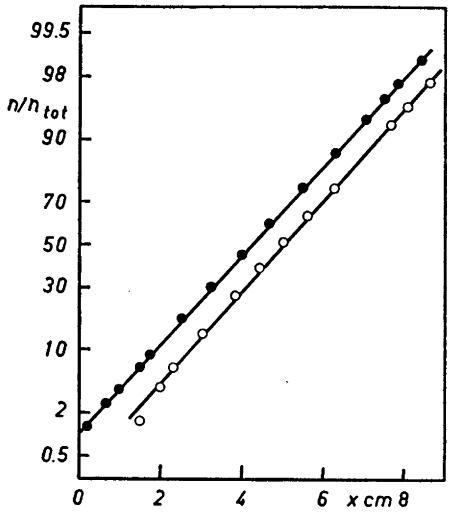

Fig. 4. Plots on a normal distribution graph showing the fit of boundary shape to that required by the ideal law of diffusion. Abscissa: measured fringe coordinates. Ordinate: relative fringe numbers. Exposures for 2.50 mg/ml at 66 840 sec, ●, and for 1.30 mg/ml at 64 020 sec, O. The drawn lines correspond to $D^{\circ}_{20} = 4.81\ F$.

The results obtained by the moment method are collected in Table 2. The zero time corrections as found by the "height-area" method have been used. For the protein concentration 2.50 mg/ml the mean value of D°_{20} is 4.81 F and for 1.30 mg/ml it is 4.80 F. It is not necessary to use the zero time corrections obtained by the "height-area" method, since according to eqn. (1) a plot of α *versus* time directly would give D_m as the slope of the line. This would, however, necessitate the evaluation of several exposures, which is rather time consuming.

The close agreement between the values obtained by the two methods might be considered as indicating that the diffusion is ideal and follows Fick's law. No dependence on protein concentration could be detected, and the mean value of all the data, $D^{\circ}_{20} = 4.81\ F$, is taken as the best value of the diffusion coefficient for the concentration range studied.

How closely the concentration distribution of the boundary fits the requirements of the law of ideal diffusion can be studied in detail in the way that has been described by Svensson [4]. A convenient and rapid method, that was

suggested to us by Lindqvist *, makes use of commercially available normal distribution graphs. On these graphs the ordinate is adjusted so that a straight line is obtained, when the integral

$$\int_{-\infty}^{z} \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}z^2} dz$$

is plotted as a function of z. In Fig. 4 the relative fringe numbers n/n_{tot} have been plotted *versus* the fringe coordinates for two exposures, one for each concentration. The drawn lines correspond to $D^{\circ}_{20} = 4.81\ F$, and minor deviations of the points from the line is only seen in case of the lower concentration.

Partial specific volume. The apparent partial specific volume was determined in three experiments at the protein concentrations 26.17, 36.39 and 27.90 mg/ml. The following values were found: 0.732, 0.730 and 0.730. Mean value 0.731 ml/g.

DISCUSSION

Insertion of $S^{\circ}_{20} = 7.93\ S$, $D^{\circ}_{20} = 4.81\ F$ and $V = 0.731$ ml/g in the Svedberg formula yields a molecular weight of 149 000 for myeloperoxidase. The iron content of the enzyme has been determined to be 0.074 % which corresponds to an equivalent weight of 75 500 per iron atom. Each molecule of myeloperoxidase contains thus two atoms of iron — in contrast to other hitherto analyzed heme proteins which contain either one or four iron atoms per molecule.

The fricitional ratio f/f_0 is calculated to be 1.26. If the molecule is an unhydrated rotational ellipsoid the axial ratio would thus be about 5: 1.

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