Variation of Sedimentation Coefficient with Rotor Velocity

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The sedimentation coefficients of three proteins, which appeared homogeneous in the ultracentrifuge, have been determined at different rotor velocities. Two of the proteins, ovalbumin and human γ -globulin, showed sedimentation coefficients that were approx. 10% higher at rotor velocities below 20 000 rev./min. than at 56 100 rev./min. The third protein, haemocyanin of *Jasus lalandii*, could not be investigated over the same rotor-velocity range, but it showed a similar effect.

In ultracentrifugation it has generally been assumed that the rates of sedimentation of proteins in aqueous media are proportional to the centrifugal force acting on the protein molecules. When determining the sedimentation coefficient of a protein the rotor velocity selected is that which allows a series of schlieren diagrams to be recorded photographically while the sedimenting boundary moves a convenient distance. Thus in experiments on substances with sedimentation coefficients of 7s or less the Spinco model E ultracentrifuge is operated at 56 100 or 59 780 rev./min. When substances of higher sedimentation coefficients are examined lower rotor velocities are selected.

To verify the assumption that the calculated sedimentation coefficient is independent of the rotor velocity, a series of ultracentrifugation runs was made on three proteins each of which appeared to be homogeneous with regard to sedimentation coefficient.

Although it is known from the work of Mosimann & Singer (1944) that the hydrostatic pressure such as exists inside an ultracentrifuge cell has a very pronounced effect on the sedimentation coefficient in organic solvents, no serious systematic studies have been made of the effect of hydrostatic pressure on the sedimentation coefficients of proteins in aqueous solvents.

MATERIALS AND METHODS

Proteins. Ovalbumin was purified by crystallization from egg white according to the method of Sörensen (1917); human γ -globulin was prepared by fractionation on DEAE-cellulose by the method of Baumstark, Laffin & Bardawil (1964); the haemocyanin of *Jasus lalandii* was freed from a faster-sedimenting component by ultracentrifugation.

Ultracentrifuge cell. At low rotor velocities diffusion from the solution towards the meniscus and from the sedimented molecules at the bottom of the cell may introduce

serious errors in determining the exact position of the sedimenting boundary. When special layering cells are employed the initial boundary may be formed at a position well removed from the meniscus, so that diffusion would not transport protein to the meniscus in any significant amount to influence the calculation. At the same time an extended series of photographs can be made before the diffusion gradient from the bottom of the cell had any influence on the position of the sedimenting boundary. Pickels, Harrington & Schachman (1952) used the technique of the special layering cell for the determination of the sedimentation coefficient of sucrose. The use of this type of cell is strongly recommended by Schachman (1958, p. 85) for substances of sedimentation coefficients less than 1s.

The valve-type synthetic-boundary cell was used in the present work at all rotor velocities. A disadvantage of this cell, however, is that the freedom from disturbances of the initial boundary is greatly dependent on the density difference between the solution and the overlayering solvent. In my hands disturbances sometimes occurred when protein solutions of concentrations less than 0.6% were used, but very sharp interfaces were invariably obtained when the solvent was layered over 1% protein solutions. Most of the ultracentrifugation experiments were therefore done on solutions containing 1% of protein. Sedimentation experiments on *J. lalandii* haemocyanin were performed on 0.6% solutions. The sedimentation coefficients recorded here for ovalbumin and γ -globulin are about 5% lower than the corrected values at infinite dilution because of the concentration dependency of the sedimentation coefficient.

Ultracentrifugation. The study was carried out in the Spinco model E ultracentrifuge. All experiments were done at 20° with 0.85% NaCl solution that had been buffered to pH 7.0 with 0.01 M-phosphate. The rotor velocities were checked on the revolution counter of the instrument with a chronometer. The differences found from the selected pre-set velocities were insignificant.

The sedimentation diagrams were measured with a Precision Grinders micro-comparator and the sedimentation coefficients were calculated from the well-known equation:

$$S = \frac{2.303\Delta\log x}{t\omega^2}$$

when x is the distance of the peak from the centre of rotation, t is the time in seconds and ω is the angular velocity. $\Delta \log x$ is measured from the straight line obtained by plotting $\log x$ against t (see Schachman, 1958, p. 85).

RESULTS

Sedimentation coefficients calculated at different rotor velocities. The sedimentation coefficients calculated for 1% ovalbumin, 1% human γ -globulin and 0.6% *J. lalandii* haemocyanin at different rotor velocities are given in Tables 1, 2 and 3 respectively. All three proteins gave equally accurate $\log x$ -time plots. The sedimentation diagrams of ovalbumin

and γ -globulin are shown in Figs. 1 and 3 respectively.

Sources of error. (a) Back-diffusion gradients.

Table 2. *Sedimentation coefficients of 1% γ -globulin at different rotor velocities, corrected for sedimentation in water at 20°.*

Rotor velocity (rev./min.)	No. of expts.	$S_{20,w}$ (s)
15220	2	7.03, 7.2
31410	1	6.61
42040	1	6.33
56100	3	6.33, 6.40, 6.33

Table 1. *Sedimentation coefficients of 1% ovalbumin at different rotor velocities, corrected for sedimentation in water at 20°*

Rotor velocity (rev./min.)	No. of expts.	$S_{20,w}$ (s)
20410	4	3.60, 3.52, 3.56, 3.59
23150	2	3.53, 3.66
31410	1	3.30
42040	1	3.15
56100	3	3.20, 3.31, 3.28

Table 3. *Sedimentation coefficients of 0.6% *Jasus lalandii* haemocyanin at different rotor velocities, corrected for sedimentation in water at 20°*

Rotor velocity (rev./min.)	No. of expts.	$S_{20,w}$ (s)
10589	2	16.6, 16.61
15220	1	16.70
20410	1	16.30
29500	1	16.14
39460	1	15.85

Fig. 1. (a) Sedimentation diagrams of 1% ovalbumin at 23100 rev./min. The exposure interval was 32 min. The temperature was 20°. The solvent was 0.85% NaCl buffered to pH 7.0. (b) Sedimentation diagrams of 1% ovalbumin at 56100 rev./min. The exposure interval was 16 min. The temperature was 20°. The solvent was 0.85% NaCl buffered to pH 7.0.

Fig. 2. (a) Plot of $\log x$ against t (in minutes) for 1% ovalbumin by using the data from Fig. 1(a) (23100 rev./min.). The broken line indicates the calculated $\log x$ -time relationship for $S_{20,w}$ 3.30s, the value found at 31410 rev./min. (Table 1). (b) Plot of $\log x$ against t (in minutes) for 1% ovalbumin by using the data from Fig. 1(b) (56100 rev./min.).

The main source of error in sedimentation experiments on proteins at low rotor velocity could be the transportation of material by diffusion to the meniscus or from the sedimented material at the bottom of the cell to the sedimenting boundary. In the present work the speed of migration of the schlieren maxima was so great that the meniscus remained free of protein in all the experiments. Material that diffused back from the bottom reached the migrating peaks only after the photographs necessary for the calculation of the sedimentation coefficient had been taken. This may be verified from the $\log x$ -time plots (Figs. 2a and 2b) in which points calculated from peak positions influenced by back diffusion would have shown a departure from linearity. For comparison the theoretical line, based on the sedimentation coefficient 3.26s that the protein appears to have at a concentration of 1% when ultracentrifuged at 56100 rev./min., is also shown (Fig. 2a). The sedimentation diagrams for γ -globulin at 15500 and 56100 rev./min. are given in Figs. 3(a) and 3(b) respectively. Their $\log x$ -time plots are shown in Figs. 4(a) and 4(b).

(b) Temperature difference between cell and centre of rotor. There was a possibility, although an extremely small one, that a temperature gradient developed across the rotor during centrifugation and that the thermistor did not record the temperature of that part of the rotor nearest the cooling shield. This would result in the cell's having a lower temperature than the centre of the rotor. To eliminate this possibility the refrigerator of the centrifuge was turned off and the rotor spun with 1% γ -globulin in the cell. The initial temperature of the rotor was 19.5° and it rose to 20.5° after 64

Fig. 3. (a) Sedimentation diagrams of 1% human γ -globulin at 15220 rev./min. The exposure interval was 64 min. The temperature was 20°. The solvent was 0.85% NaCl buffered to pH 7.0. (b) Sedimentation diagrams of 1% human γ -globulin at 56100 rev./min. The exposure interval was 8 min. The temperature was 20°. The solvent was 0.85% NaCl buffered to pH 7.0.

Fig. 4. (a) Plot of $\log x$ against t (in minutes) for 1% human γ -globulin by using the data from Fig. 3(a) (15220 rev./min.). The broken line indicates the calculated $\log x$ -time relationship for $S_{20,w}$ 6.38 s. (b) Plot of $\log x$ against t (in minutes) for 1% human γ -globulin by using the data from Fig. 3(b) (56100 rev./min.).

min. spinning at 56100 rev./min. The average sedimentation coefficient calculated from this run was 6.34 s, which is in good agreement with that found during normal temperature-controlled operation at this velocity. A temperature difference of approx. 5° between the cell and that recorded by the thermistor would be required to explain a difference of 10% in the sedimentation coefficient. The temperature regulation therefore did not appear to be at fault and a temperature gradient does not explain the phenomenon.

(c) Pressure effects. A third factor considered was the effect of pressure on the sedimentation rate. When protein sediments in the centrifuge cell the molecules pass from a region of lower into one of higher hydrostatic pressure, when they would migrate at a lower rate on account of the increased viscosity of the medium. This, however, amounts at the most to a 'few percent' for aqueous media (Schachman, 1958, p. 174). If the effect were appreciable the relationship between $\log x$ and t would not be linear and the curve would bend downwards. None of the diagrams obtained showed any departure from linearity. It should be remembered that the synthetic-boundary cell was used and the initial boundary was formed at a position where the pressure was about half that at the bottom of the cell. Further, the observations on the sedimenting boundaries were made over such a

Table 4. Sedimentation coefficients of 0.33% ovalbumin at low and high rotor velocities, corrected for sedimentation in water at 20°

Rotor velocity (rev./min.)	No. of expts.	$S_{20,w}$ (s)
23150	1	3.67
56100	1	3.38

short length of the cell that the pressure-differential effect would probably not be significant. Large departures from linearity would thus not be expected. The possibility, however, that the decrease in sedimentation coefficient with increasing rotor velocities is dependent on pressure effects cannot be completely excluded.

(d) Protein-concentration effects. The results recorded here were obtained on protein solutions at relatively high concentration and the sedimentation coefficients have not been corrected for concentration effects. To test the influence of protein concentration on the rotor-velocity effect, two centrifugation runs were made on 0.33% ovalbumin in buffered 0.85% sodium chloride. The synthetic-boundary cell was used as in the previous experiments. On account of the low protein concentration several attempts had to be made to obtain initial boundaries that were free from disturbances due to imperfect layering of the solvent over the protein solution. The results obtained from two successful experiments appear in Table 4, and the $\log x$ -time plots are shown in Figs. 5(a) and 5(b).

Table 4 shows that the sedimentation coefficient at low rotor velocity is still appreciably higher than that obtained at high rotor velocity. The smallness of the scatter of the $\log x$ values about the straight line is an indication of the accuracy of the calculated sedimentation coefficients at the two speeds. The change in sedimentation coefficient with rotor velocity is thus not due to concentration effects.

DISCUSSION

It has been found that the rotor velocity in ultracentrifugation has an appreciably larger effect on the sedimentation coefficient than the 'few percent' (Schachman, 1958, p. 174) that had hitherto been assumed for aqueous solutions. The sedimentation coefficients reported in this work for human γ -globulin and also ovalbumin at high rotor velocities agree well with those reported if the concentration-dependency of the sedimentation coefficient is taken into account. Thus Pedersen (1945) calculated sedimentation coefficients of 7.1 and 6.51 s for γ -globulin at zero and 1% protein concentration respectively, and Cann (1953) reported the values 6.51 and 6.05 s for this protein at

Fig. 5. Plots of $\log x$ against t (in minutes) for 0.33% ovalbumin spun at 23150 rev./min. (a) and 56100 rev./min. (b), with 16 min. and 8 min. exposure intervals respectively. The temperature was 20°. The solvent was 0.85% NaCl buffered to pH 7.0.

Fig. 6. Graphical presentation of the variation of the sedimentation coefficients with rotor velocity. Curve A, *J. lalandii* haemocyanin; curve B, γ -globulin; curve C, ovalbumin.

the same concentrations. Numerous determinations have been made by other authors and the values reported fall in general between the limits 6.50 and 7.1 s.

The sedimentation coefficient of ovalbumin at zero g./100 ml. is 3.55 s (Lamm & Polson, 1936; Svedberg & Pedersen, 1940).

Molecular weights of proteins are calculated from ultracentrifugation data by: (a) the sedimentation-equilibrium method or the approach-to-equilibrium method; (b) the velocity method in which use is made of sedimentation and diffusion coefficients determined independently.

In (a) the protein is spun at low rotor velocities that are of the order of those used in the present work in the determination of sedimentation coefficients at low velocities. In (b) it is customary to determine the $S_{20,w}$ at high velocities and $D_{20,w}$ under the influence of unit gravity. It would thus seem to be erroneous to combine $S_{20,w}$ and $D_{20,w}$ determined under different gravitational stresses in the calculation of the molecular weights, as $S_{20,w}$ would be different at high and low rotor velocities. $S_{20,w}$ obtained by extrapolation to zero rev./min. (Fig. 6) should be used in conjunction with $D_{20,w}$ determined separately. Molecular weights deter-

mined by the sedimentation-equilibrium method are usually obtained at rotor velocities below 20000 rev./min., and therefore are probably closer to the true values and higher than those determined by the velocity method.

The change of sedimentation coefficient with velocity is possibly due to a combination of several factors. It is known that the density of water increases with pressure. At 56100 rev./min. the density would be approx. 1.02 g./ml. at the bottom and 1.01 g./ml. in the centre of the centrifuge cell. The viscosity would also increase correspondingly. The overall effect of this pressure would result in the lowering of $S_{20,w}$ by 2–3%.

Additional factors that may influence the sedimentation coefficient are the effect of pressure on the partial specific volume and hydration of the protein molecules. The contributions from these sources cannot be estimated at the moment.

Koenig & Perrings (1952a) have reported that $S_{20,w}$ of bovine serum albumin decreased slightly but progressively over the velocity range 39466–59733 rev./min. Koenig & Perrings (1952b) also found that bovine fibrinogen behaved in the reverse manner by showing a slight but progressive increase of $S_{20,w}$ with increase of rotor velocity. The behaviour of this substance they ascribed to partial orientation of the rigid rod-shaped molecules at higher centrifugal stress. It is difficult, however, to find a reason why molecules that are in rapid Brownian motion should be oriented in the absence

of an orienting force gradient. This observation requires some other explanation.

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