Calcutta, for the electrical resistivity measurements. This communication is published by the permission of the Director, Central Building Research Institute, Roorkee.

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Sedimentation of β-Lactoglobulin (*A*,*B*) under Dissociating Conditions in Acid Solution

Evidence that β-lactoglobulin molecules dissociate into halves in the *p*H region 1·6–4 has been presented by Timasheff, Townend and Weinberger[1-3], and they have shown[3] that there is reasonable agreement between the results obtained by the complementary methods of ultracentrifugation and light-scattering. The overall picture which emerges is of a dumb-bell-shaped[4] lactoglobulin molecule, of axial ratio 2, dissociating reversibly at 25° below *p*H 4 into two spherical sub-units of equal size. According to light-scattering data, and on the assumption of a molecule of weight 36,000, the dissociation constant at *p*H 1·6 is $2{\cdot}5 \times 10^{-4}$ mol./l. and at *p*H 3·5 is $4{\cdot}3 \times 10^{-6}$ mol./l.[3].

We have now attempted to carry a comparison of the two methods a stage further, and to calculate the implications for sedimentation of accepting these values from light-scattering of the dissociation constants.

The main effect of dissociation on sedimentation is seen in a departure of the sedimentation velocity–concentration curve from the normal form. Weak dissociation leads to a high maximum, whereas strong dissociation leads to a lower maximum, or if sufficiently strong, to no maximum and only a change of slope[5]. In Fig. 1 theoretical curves are shown for sedimentation at *p*H 1·6 and 3·5. The sedimentation velocity–concentration curves marked *a* and *b* have been constructed on the basis of the light-scattering dissociation constants, together with the following minimal assumptions: (1) The limiting sedimentation coefficient of the sub-unit (monomer) is 1·89 svedbergs. (2) The whole molecule (dimer) moves relatively faster than the sub-unit by the factor $2^{2/3}$ because of its weight, and 1/1·044 because of its shape[6], that is, $s_2 = 1{\cdot}52\, s_1$. (3) The sedimentation coefficient for each species considered alone obeys the relation $s = s_0\ (1/1 + gc)$, where g is a constant. (4) At *p*H 1·6, the maximum value of the sedimentation coefficient, measured using the boundary median, is 2·34 svedbergs. This fixes the value of g as 0·1 (gm./100 ml.)$^{-1}$.

Whether the curves are based on a weight average[7], or the position of the median of the boundary[5,8], is not very material for this simple monomer–dimer system, as was shown earlier[5].

The experimental points in Fig. 1 are taken from the work of Townend, Weinberger and Timasheff[3], and include one check point not hitherto published (Timasheff and Townend, personal communication). It is seen that they lie fairly near the theoretical curves *a* and *b* when considered as a whole, but that there is an obvious discrepancy at low concentrations at *p*H 3·5. It is difficult to see how a dissociation constant as great as $4{\cdot}3 \times 10^{-6}$ mol./l. can be reconciled with the sedimentation results at the two lower concentrations there. The effect on the position of the theoretical curve of reducing the value of the dissociation constant ten-fold is shown in Fig. 1 (curve *c*). It will be interesting to see whether future work on purified *A* and *B* lactoglobulin eliminates this conflict between the interpretation of light-scattering and ultracentrifuge data.

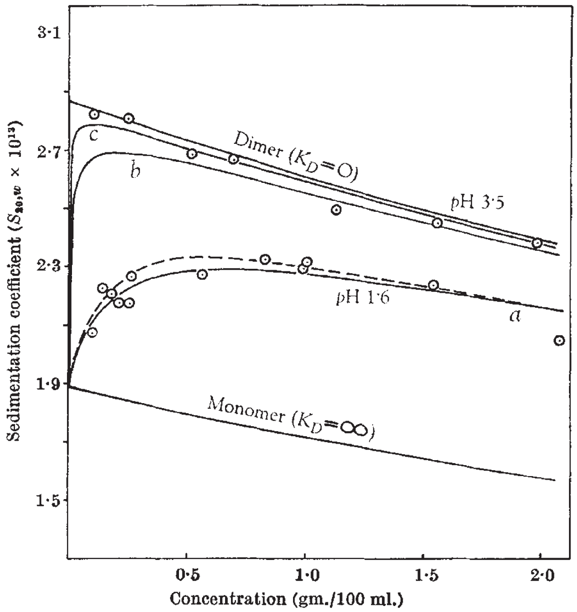

Fig. 1. Sedimentation of lactoglobulin (*A*,*B*) under dissociating conditions. *a*, $K_D = 2{\cdot}5 \times 10^{-4}$ mol./l.; *b*, $K_D = 4{\cdot}3 \times 10^{-6}$ mol./l.; *c*, $K_D = 4{\cdot}3 \times 10^{-7}$ mol./l. ——, Weight average (theory); – – –, median of boundary (theory); ⊙, experiment (Townend, Weinberger and Timasheff, ref. 3)

The value of g is in itself of some interest, since it is about twice that to be expected for a non-reacting protein near its isoelectric point. This may be a reflexion of the fact that the lactoglobulin molecules are sedimenting in a highly charged condition, and that electrostatic repulsion is reducing the number of near neighbours, and hence their contribution to the net velocity of sedimentation[9].

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