The Native and Denatured States of Ovalbumin

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The conformational state of the product of protein denaturation caused by extreme pH, heat, urea and guanidinium chloride had not been systematically investigated until 1967, when Tanford and co-workers demonstrated that proteins are fully denatured in 6M-guanidinium chloride and probably in 8–10M-urea and exist as random or cross-linked random coils. The completeness of urea denaturation has been demonstrated in only a few cases (see Tanford, 1968). It would therefore be worth while to test whether or not the urea-denatured state of a protein is the same as the one obtained in 6M-guanidinium chloride. Despite systematic studies by Frensdorff *et al.* (1953) (see also Simpson & Kauzmann, 1953) on urea denaturation of ovalbumin, the nature of the conformational state of urea-denatured ovalbumin remained uncharacterized. In the present communication we report our viscosity results on native ovalbumin and its denatured state obtained in 10M-urea and in 6M-guanidinium chloride+0.1M-2-mercaptoethanol.

For viscosity measurements at $35 \pm 0.05^\circ\text{C}$ the concentration of ovalbumin (grade V; Sigma Chemical Co., St. Louis, Mo., U.S.A.), c , in g/ml, was determined by the dry-weight method by taking isoionic protein solutions prepared by the Dintzis (1952) method in weighing bottles and heating them at $105\text{--}107^\circ\text{C}$ to constant weight. Calibrated Cannon–Fenske viscometers with flow times of 98.9 and 384.6s for distilled water at 35°C were used. For viscometers having time of fall greater than 200s the correction due to kinetic energy, if any, would be negligible. The time of fall of solvent, t_0 , and that of protein solution, t , in the viscometers were measured within $\pm 0.05\%$, by a stopwatch reading to 0.1 s. The density of solvent, ρ_0 , was determined by the standard procedure. The intrinsic viscosity, $[\eta]$, of ovalbumin was computed from a plot of $(t-t_0)/t_0 \cdot c$ versus c by using the expression (Tanford, 1955):

$$[\eta] = \lim_{c \rightarrow 0} (\eta - \eta_0)/\eta_0 \cdot c = \lim_{c \rightarrow 0} (t - t_0)/t_0 \cdot c + (1 - \bar{v}_2 \cdot \rho_0)/\rho_0 \quad (1)$$

where η_0 and η refer to the viscosity of solvent and that of solution, and $\bar{v}_2$ is the partial specific volume of the protein and was taken to be 0.75 ml/g (Neuberger & Marshall, 1966). The density of 0.1M-phosphate buffer, pH6.5, was found to be 1.0034 g/ml. The results are shown graphically in Fig. 1, in which the three lines were drawn by the method of

least squares. The value of $[\eta]$ for native ovalbumin in phosphate buffer, pH6.5 and I 0.42, at $35 \pm 0.05^\circ\text{C}$ was calculated to be 3.55 ml/g, which strongly suggests that the native ovalbumin molecule is fairly compact and globular. The intrinsic viscosity of a protein can also be expressed in terms of partial specific volumes (Tanford, 1961):

$$[\eta] = \nu(\bar{v}_2 + \sum m_i \cdot \bar{v}_i^0) \quad (2)$$

in which ν is the Simha's or shape factor and is 2.5 for spheres and larger for ellipsoids, and m_i is the g of a solvent component with partial specific volume $\bar{v}_i^0$ bound to 1 g dry wt. of protein. If the native ovalbumin in the phosphate buffer binds only water, for which $\bar{v}_i^0$ may be taken to be 1, the maximum amount of water bound to 1 g of protein may be calculated by setting $\nu=2.5$ in eqn. (2). Thus the value of m_i turns out to be 0.67 g/g of protein. The maximum value of ν showing maximum asymmetry of the ovalbumin

Fig. 1. Viscosity data for native and denatured ovalbumin

Curve A represents the plot for ovalbumin at pH6.5 and I 0.42, at 35°C . Curves B and C represent similar plots in 10M-urea, pH6.5 and I 0.49, and in 6M-guanidinium chloride+0.1M-2-mercaptoethanol, pH5.1, respectively.

molecule, calculated by eqn. (2) by using $\sum m_i \cdot v_i^0 = 0$, turns out to be 4.7, which corresponds to an axial ratio, a/b , of 4 for the prolate ellipsoid. The two extreme behaviours probably do not represent the true hydrodynamic behaviour of the protein. In fact, Bull & Breese (1970) have shown that 1g of ovalbumin binds about 0.29g of water in the absence of electrolyte, so that in the presence of electrolyte, sodium phosphate, the binding of water by ovalbumin may be even less. By using the value of 0.29g for m_i in eqn. (2) the values of ν and a/b were calculated to be 3.4 and 2.7 respectively. This, together with the known frictional ratio, $f/f_{\min.}$, of 1.17 (Tanford, 1961) for ovalbumin, indicates the compact and globular nature of ovalbumin. The equivalent hydrodynamic sphere calculated from viscosity data turns out to be 2.9nm (29Å) for molecular weight, M , equal to 43000.

It may be pointed out that a given ovalbumin preparation may consist of three electrophoretically separable components, A_1 , A_2 and A_3 , having respectively two, one and no phospho groups. The three components of ovalbumin were, however, found to be indistinguishable in the ultracentrifuge (Tanford, 1961) and are similar in immunological behaviour (Durieux & Kamiski, 1956; A. A. Ansari & A. Salahuddin, unpublished work). It can therefore be reasonably assumed that the presence or absence of phospho groups does not affect the hydrodynamic behaviour of ovalbumin. Further, at a relatively higher ionic strength of 0.42, the presence of charge on the protein molecule contributed by phospho groups will not affect the value of the intrinsic viscosity of the protein.

By using 1.0907 and 1.4470g/ml for ρ_0 in 10M-urea and in 6M-guanidinium chloride+2-mercaptoethanol, the values of intrinsic viscosity of ovalbumin in 10M-urea and in 6M-guanidinium chloride+0.1M-2-mercaptoethanol were computed by setting $\bar{v}_2 = 0.75$, from the curves B and C of Fig. 1, and were found to be 37.9 and 39.9ml/g respectively. A small discrepancy in the value of $[\eta]$ may very well be due to a solvent effect. The intrinsic viscosities of several reduced proteins have been found (Tanford, 1968) to be lower in 8M-urea than in 6M-guanidinium chloride. For example, the intrinsic viscosity of reduced β -lactoglobulin in 8M-urea, in which the random-coil nature of the protein had been clearly demonstrated (Pace & Tanford, 1968), was found to be 21.6ml/g, which is more than 5% lower than that (22.8ml/g) in 6M-guanidinium chloride.

From the dependence of $[\eta]$ of several randomly coiled proteins in 6M-guanidinium chloride on the chain length and hence the number of amino acid residues, n , of a protein, Tanford (1968) found an empirical relationship: $[\eta] \cdot M_0 = 77n^{0.666}$, in which $M_0 = M/n$. By using values of 111.1 and 387 respectively for M_0 and n , the value of $[\eta]$ of ovalbumin in 6M-guanidinium chloride+0.1M-2-mercaptoethanol was calculated to be 36.7ml/g, which is about 8% lower than the experimental value. The root-mean-square end-to-end distance, $\langle L^2 \rangle^{1/2}$, calculated from viscosity data by using the appropriate equation (Tanford, 1968), was calculated to be 20.1nm (201 Å). From the amino acid composition the value of the unperturbed dimension, $\langle L^2 \rangle^{1/2}$, of randomly coiled ovalbumin was computed by the empirical equation of Tanford (1968) to be 16.5–18.1nm (165–181 Å). Thus the unperturbed end-to-end distance is 10–18% lower than the actual one calculated from the viscosity data. Such discrepancies have also been noted for other proteins in the random-coil state (Tanford, 1968). We therefore conclude that native ovalbumin, which is a fairly compact and globular molecule, exists as a random coil devoid of any native structure in 6M-guanidinium chloride+0.1M-2-mercaptoethanol. The protein probably exists in the same conformational state in 10M-urea, where one or two disulphide bonds/molecule (Neuberger & Marshall, 1966; Fothergill & Fothergill, 1970) remain intact. The presence of disulphide bonds does not seem to impose any detectable physical constraint affecting the hydrodynamic volume.

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