A DILATOMETRIC STUDY OF THE DENATURATION OF BOVINE SERUM ALBUMIN BY GUANIDINE HYDROCHLORIDE

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SUMMARY

The denaturation of bovine serum albumin by guanidine hydrochloride was studied using the dilatometric method. From dilatometric measurements the differences between the partial specific volume of the protein in denaturant solutions and water, respectively, were determined. The differences reflect the extent of unfolding as well as the binding of the denaturant. From the differences and the known partial specific volume of the native protein, the partial specific volumes at individual denaturant concentrations were obtained.

In the course of our dilatometric studies of the denaturation of human immunoglobulin G and its light chains by guanidine hydrochloride (1) the idea occurred to us that it might be wortwhile to perform also a similar study on another important serum protein, though of different origin, bovine serum albumin. A dilatometric study of this protein in guanidine hydrochloride solutions has already been reported (2) but it covered only the concentration range from 1.0 to 3.8 M. Data for volume changes accompanying the denaturation in more concentrated solutions, e.g. 6 M, at which most proteins with an ordered native structure undergo a transition to random coils (3) were thus missing. This is also the concentration at which most sedimentation equilibrium experiments for the determination of protein molecular weights are performed. From measured volume changes and the known value of the partial specific volume for the native protein, values of the volume at individual denaturant concentrations are obtained. They are needed, along with solvation parameters, for an unambiguous evaluation of the protein molecular weight in a three component system (water, protein, denaturant). This study encompasses the concentration range between 0 and 6 M denaturant. In addition, a few experiments were performed in which the protein disulfide bonds were reduced in 6 M solution by dithioerythritol.

MATERIALS AND METHODS

Two different samples of bovine serum albumin were used. One was supplied by Miles Laboratories Inc., the other by Koch and Light. Guanidine hydrochloride (GuHCl) was an ultra pure product from Schwarz/Mann. Dithioerythritol (DTE) was purchased from Sigma. Solutions for measurements were prepared by dissolving the protein in water and subsequent extensive dialysis against water. Protein concentrations were determined spectrophotometrically at 279 nm using the value 6.65 for $E^{1\%}_{1cm}$.

Volume changes were measured in Linderstrøm-Lang dilatometers at 25 °. Filling was performed by means of Hamilton syringes. One arm of the dilatometer was filled with 1.00 ml of a 5 % protein solution or $(1 - \phi)$ ml of water, the other with an appropriate GuHCl solution. ϕ is the protein displacement volume, i.e., the protein weight multiplied by its partial specific volume, $\bar{v}_2$, 0.735 ml/g (4). All other experimental details are identical with those described previously (5-7).

For each GuHCl concentration at least four measurements were made. The absolute error of single determinations is estimated at $\pm$ 60 ml/mol. pH's after mixing were around 5.7. No difference between the two samples used was observed.

In experiments with DTE 2.00 ml of protein in 6 M GuHCl were mixed with 4.00 ml of 6 M GuHCl being 0.02 M in DTE. Corrections for volume changes resulting from dilution were determined by running blanks without DTE.

RESULTS AND DISCUSSION

The relative partial molar volumes at individual GuHCl concentrations were calculated from the volume changes determined in dilatometric experiments by using the relation (5)

$$\Delta V_2 = \Delta V_{(1 - \phi)} + n_2 (\bar{V}_{2G} - \bar{V}_2) \qquad (1)$$

where ΔV_2 and $\Delta V_{(1 - \phi)}$ are the volume changes observed in mixing 1.00 ml of protein solution and $(1 - \phi)$ ml of water, respectively, with 4.00 ml of an appropriate GuHCl solution; n_2 is the amount of protein and $(\bar{V}_{2G} - \bar{V}_2)$ is the difference between the protein partial molar volume in that GuHCl solution and water. In fig. 1 the values of $(\bar{V}_{2G} - \bar{V}_2)$ are plotted as a function of denaturant concentration.

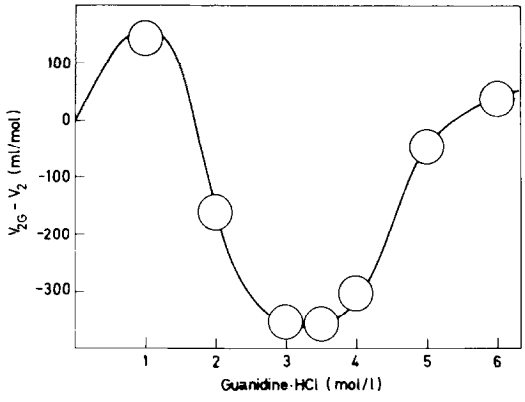

Fig. 1. Differences of the partial molar volume of bovine serum albumin in guanidine hydrochloride and water at 25 °, as a function of guanidine hydrochloride concentration.

Examination of the curve in fig. 1 reveals that its form is similar to those observed with other proteins (5-7) and therefore similar interpretation can be applied. The initial increasing of $(\bar{V}_{2G} - \bar{V}_2)$ may be attributed to changes of solvation of ionic groups at the surface of protein molecules, i.e., to the gradual displacement of water molecules in hydration sheaths by guanidinium or chloride ions. The maximum is reached just before protein molecules start to unfold. Unfolding is accompanied by volume decrease due to gradual filling up of the hollows in protein molecules with solvent which prevails over the volume increase due to changing solvation. The minimum, around 3.5 M, thus corresponds to a more or less complete unfolding. After that the difference $(\bar{V}_{2G} - \bar{V}_2)$ becomes less and less negative and eventually positive again.

Comparison of our curve with that obtained by Katz (2) shows that its form is similar to ours; however, all his values of $(\bar{V}_{2G} - \bar{V}_2)$ are higher. Thus his values in 2.0 M and 3.8 M GuHCl are 90 and - 10 ml/mol, while ours are - 160 and - 350 ml/mol. An explanation for the disagreement is not apparent (8).

The value of $(\bar{V}_{2G} - \bar{V}_2)$ in 6 M GuHCl is 40 ml/mol from which we deduce that the value of $\bar{v}_2$ in 6 M GuHCl is practically the same as in water. Reisler and Eisenberg (9) found for the same protein in 6 M GuHCl - 0.1 M 2-mercaptoethanol by

a combination of their density data and the solvation parameters of Hade and Tanford (10) the value 0.728 ml/g, and by using the solvation parameters of Noelken and Timasheff (11) the value 0.741 ml/g which they considered "too high to be correct". As we will see later, the volume change due to the rupture of disulfide bonds by DTE is extremely small. Ullmann et al. (12), on the other hand, using the Archibald method arrived at the conclusion that reasonable molecular weights could be obtained for homogeneous proteins in 6 M GuHCl by assuming identical values for $\bar{v}_2$ in water and denaturant. We would not agree with such a generalization, especially if accurate values are wanted. On the basis of this and previous studies it can be claimed that no safe predictions of $\bar{v}_2$ for a protein in 6 M GuHCl, or, for that matter, any other concentration, are possible; the values of $\bar{v}_2$ have to be determined experimentally, the method of choice depending on the accuracy wanted. The following is a good example: $\bar{v}_2$ in 6 M GuHCl for pooled human immunoglobulin G is 0.739 ml/g as compared to 0.733 ml/g for the native protein (1). The difference is due to the fact that the volume change accompanying unfolding is quite small compared to that in bovine serum albumin. Objections could be raised to our comparing of dilatometric data, obtained at high protein concentrations, with those found for dilute protein solutions by other methods. However, it has been shown for several proteins that in the concentration range used in dilatometric experiments the protein partial specific volume is independent of concentration (4,5,13).

The difference between the partial molar volume of the protein with reduced disulfide bonds and that of the unreduced form in 6 M GuHCl was found to be - (16 $\pm$ 3) ml/mol or - (0.9 $\pm$ 0.2) ml per mol of disulfide bonds. The same value per disulfide bond was obtained for human immunoglobulin G and oxidized glutathione (1). This clearly proves that bovine serum albumin in 6 M GuHCl with intact disulfide bonds is randomly coiled which is in agreement with previous findings (3).

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