A DILATOMETRIC STUDY OF THE DENATURATION OF CHYMOTRYPSINOGEN A BY GUANIDINE HYDROCHLORIDE

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SUMMARY

The denaturation of chymotrypsinogen A by guanidine hydrochloride was followed using the dilatometric method. From dilatometric measurements the differences between the partial molar volume of the protein in guanidine hydrochloride solutions and water, respectively, have been obtained. The differences reflect the extent of unfolding as well as the binding of the denaturant to the protein. Finally, comparison of the dilatometric results with those obtained from optical rotation measurements is made.

Dilatometry is one of the methods by which the extent of denaturation, i. e., unfolding, of globular proteins can be followed (1, 2). In a previous paper (3) it has been shown that volume changes accompanying the denaturation of chymotrypsinogen A by urea, a relatively strong denaturant, reflect various processes taking place during that denaturation. In this communication we report on a dilatometric study of the denaturation of the same protein by guanidine hydrochloride (GuHCl), which is an even stronger denaturant for globular proteins, since it produces complete denaturation at a lower concentration than urea. The purpose of this investigation was to determine volume changes accompanying the denaturation of chymotrypsinogen A by GuHCl and to explain them in terms of molecular processes composing this denaturation. Furthermore, comparison of the results obtained in GuHCl solutions with those for urea solutions could also lead to a qualitative explanation why GuHCl is a stronger denaturant. In addition, the extent of denaturation was followed by measuring the optical rotation at 436 mμ of the same solutions. Comparison of results obtained by two independent methods has proved to be very instructive in the case of the denaturation of β - lactoglobulin by urea (4).

MATERIALS AND METHODS

Chymotrypsinogen A (5 x crystd.) was obtained from Worthington Biochem. Corp. Prior to use, it was deionized using a mixed bed ion exchange column and then lyophilized. The GuHCl used in this study was purchased from Dr. T. Schuchardt G.m.b.H. & Co. (Munich, Germany) and was purified as described by Nozaki and Tanford (5). The pH of solutions was between 7.0 and 7.5.

Volume changes were measured at 25° C in Linderstrøm - Lang dilatometers. The volume of each arm was about 10 ml, the volume of the graduated capillary 10 µl. Four dilatometers were used in each experiment. The experimental procedure described earlier (3) was modified. Into one arm of two dilatometers 1.000 ml of chymotrypsinogen A in water or in 3.2 M GuHCl was pipetted, and into the other 4.00 ml of an appropriate GuHCl solution. In the other two dilatometers (1.000 - ϕ) ml of water or 3.2 M GuHCl solution was mixed with 4.00 ml of GuHCl solution. ϕ is the protein displacement volume which is equal to the protein weight multiplied by its partial specific volume. All other details are the same as described previously (3).

Optical rotation measurements at 436 m and 25° C were performed with the Perkin - Elmer Model 141 polarimeter. Readings were below 1° with a reproducibility of ± 0.002°. Usually they were taken 1 h after preparing solutions. Results are reported in terms of the mean residue rotation $[m']$, obtained from the specific rotation, $[\alpha]$, by the relation

$$[m'] = [\alpha] \frac{3 M_o}{100(n^2 + 2)}$$

where M_o is the mean residue weight of the protein and n the refractive index of the solvent. Values of the refractive index were obtained by using the Brice-Phoenix differential refractometer and, for references, urea solutions of known refractive index (6).

RESULTS AND DISCUSSION

The results of dilatometric measurements are summarized in Table 1. The terms $\Delta V_{(1 - \phi)}$ and ΔV_2 represent the volume changes produced upon mixing (1.000 - ϕ) ml of water (or 3.2 M GuHCl) or 1.000 ml of 8 % protein solution with 4.00 ml of appropriate GuHCl solutions. In our experiments ϕ was equal to 0.059 ml. The value was obtained by multiplying the protein

Table 1

Volume changes at 25° C as a function of guanidine hydrochloride concentration.

Equil.GuHCl concentration (mole/l)	$-\Delta V_{(1-\phi)}$ (µl)	$-\Delta V_2$ (µl)	$(\bar{V}_{2G} - \bar{V}_2)$ (ml/mole protein)
3.2	5.31 ± 0.03	5.26 ± 0.03	16 ± 20
4.0	6.98	6.88	32
3.5	0.16	0.18	10
4.0	0.26	0.17	45
5.0	0.97	0.71	100
6.0	1.90	1.41	173

$\Delta V_{(1-\phi)}$ in the first two rows refers to the addition of 0.941 ml of water to 4.00 ml of GuHCl solutions, in other rows to the addition of 0.941 ml of 3.2 M GuHCl to 4.00 ml of GuHCl solutions. ΔV_2 in the first two rows refers to the addition of 1.000 ml of 8 % chymotrypsinogen A in water to 4.00 ml of GuHCl solutions, in other rows to the addition of 1.000 ml 8 % protein in 3.2 M GuHCl to 4.00 ml of GuHCl solutions. In the calculations the partial specific volume of chymotrypsinogen A in water and 3.2 M GuHCl, respectively, was taken to be 0.734 and 0.735 ml/g, molecular weight 25,700.

weight, 0.08 g, with its partial specific volume in water, 0.734 ml/g (3), or in 3.2 M GuHCl, 0.735 ml/g (7). The term $(\bar{V}_{2G} - \bar{V}_2)$ is the difference between the partial molar volume of the protein in a GuHCl solution of defined concentration and the same quantity in water. The values of $(\bar{V}_{2G} - \bar{V}_2)$ have been obtained from the following relation

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

where n_2 is the number of moles of protein (3). In cases where the protein in 3.2 M GuHCl was mixed with GuHCl solutions the differences $(\bar{V}_{2G} - \bar{V}_{2G}^{3.2})$ were first determined and from them by adding $(\bar{V}_{2G}^{3.2} - \bar{V}_2)$ the differences $(\bar{V}_{2G} - \bar{V}_2)$. Such a procedure was neccessary since it is not possible to perform transfers from water to GuHCl concentrations higher than 4 M in one step owing to very large volume changes accompanying mixing of water

and GuHCl solutions. The reason for choosing 3.2 M GuHCl as the second reference solution is due to the fact that at 25° C chymotrypsinogen A is not soluble in the GuHCl concentration range from nearly 0 to 3.2 M. In Fig. 1 the differences $(\bar{V}_{2G} - \bar{V}_2)$ are plotted as a function of GuHCl concentration.

Owing to limited solubility of the protein in GuHCl solutions, comparison of the differences $(\bar{V}_{2G} - \bar{V}_2)$ in these solutions with those found for urea solutions is possible only for the concentration range above 3.2 M. From Fig. 1 it can be inferred that $(\bar{V}_{2G} - \bar{V}_2)$ decreases until a minimum appears around 3.6 M GuHCl; then it increases again. In urea solutions (3) the minimum was observed around 7 M. These are the concentrations of GuHCl and urea, respectively, at which complete unfolding occurs. This is in agreement with the experimental finding that, on the molar scale, GuHCl is about twice as effective a denaturant as urea (2). The increase of $(\bar{V}_{2G} - \bar{V}_2)$ after minimum can, as in the case of urea solutions, be ascribed to continuous binding of GuHCl (or rather the constituent ions) to protein molecules which increases with GuHCl concentration. We can again assume that only binding to the ionic groups of the protein results in an increase of volume which is reflected in higher values of $(\bar{V}_{2G} - \bar{V}_2)$. The increase is due to the competitive displacement of some of the water bound to the ionic groups of the protein by denaturant ions (2). Dilatometric experiments with KCl in GuHCl and urea solutions support such an interpretation (8). It is interesting to note, furthermore, when comparing the values of $(\bar{V}_{2G} - \bar{V}_2)$ after minimum with the corresponding values in urea solutions (3) that the latter are lower. This agrees with the experimental finding that GuHCl is a more potent dehydrating agent than urea (9). This could be one of the reasons why GuHCl is more efficient a denaturant than urea. On the other hand, it is very likely that at equal concentration the overall binding of GuHCl is larger, which is another, perhaps decisive reason for GuHCl being a more potent denaturant.

We can only speculate on the course of the curve for $(\bar{V}_{2G} - \bar{V}_2)$ in the concentration range from 0 to 3.2 M GuHCl in Fig. 1. However, we can assume, as in the case of urea solutions, that as long as there is no unfolding, i. e., up to 2 M, the values of $(\bar{V}_{2G} - \bar{V}_2)$ would increase owing to binding of GuHCl.

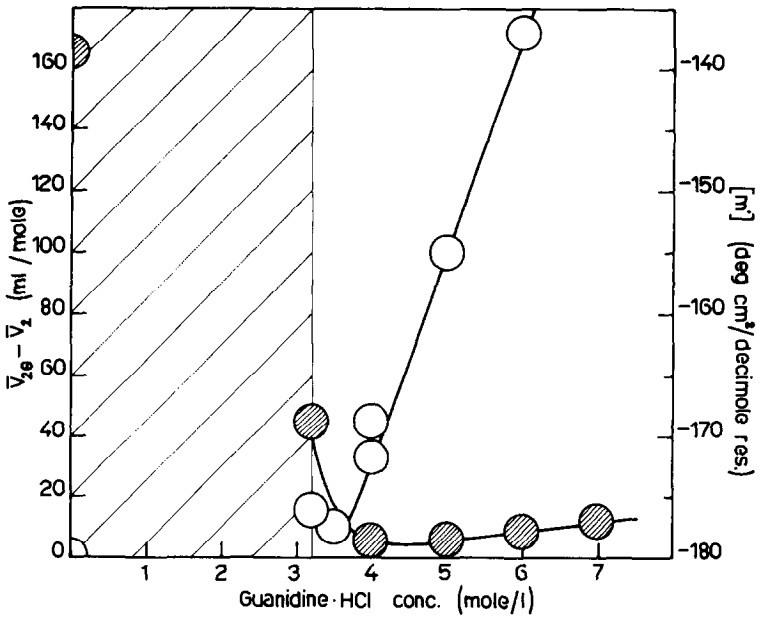

Fig. 1. Differences of the partial molar volume of chymotrypsinogen A in GuHCl solutions and water, respectively, O, and the mean residue rotation of chymotrypsinogen A at 436 mμ ●, as a function of GuHCl concentration.

Since unfolding is accompanied by a decrease of volume (3) one would expect decreasing values of $(\bar{V}_{2G} - \bar{V}_2)$ at higher GuHCl concentrations until unfolding is completed. This is in accord with the finding that $(\bar{V}_{2G} - \bar{V}_2)$ is higher in 3.2 M GuHCl than at 3.5 M GuHCl.

In Fig. 1 the plot of $[m']$ of chymotrypsinogen A at 436 mμ as a function of GuHCl concentration is also presented. Comparison of the curves for $(\bar{V}_{2G} - \bar{V}_2)$ and $[m']$ is very instructive too. The minima of both curves practically coincide, i. e., they occur around 3.6 M GuHCl where unfolding is complete. However, the increase of $[m']$ after that is relatively small but it also represents a solvent effect (10). We can therefore conclude that in the values of $(\bar{V}_{2G} - \bar{V}_2)$ unfolding and binding are reflected to about the same extent, whereas the change of $[m']$ with GuHCl concentration is mainly due to unfolding.

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