The Volume and Compressibility Changes Associated with Protein Denaturation

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The changes in the apparent specific volume and apparent specific adiabatic compressibility associated with thermal and chemical denaturations were studied for hen egg white lysozyme solution. No transition was found in the temperature dependence of the apparent quantities, whereas the addition of a certain amount of guanidine hydrochloride seems to decrease slightly the volume and compressibility.

So far the volumetric behavior associated with protein denaturation has been widely studied by means of densimetric, dilatometric or pressure dependence of protein stability.[1,2] It is now generally recognized that the denaturation is accompanied by more or less negative volume change.[3-5] Such a volume decrease has been interpreted in terms of the loss of holes in the native structure and the exposure of hydrophobic groups to water with unfolding process.[1] A few authors, on the other hand, have reported positive volume changes for some proteins[6,7] or no volume change.[8] Thus, in our opinion, the volume change accompanying the protein denaturation process may be still open to question.

It is often said that the pressure dependence of volume, i.e., compressibility, is more sensitive to hydration phenomena rather than volume itself. Therefore, the study on the compressibility behavior is of interest in order to elucidate solute–solute and solute–solvent interactions in connection of protein unfolding process. There is, however, relatively little information available for the partial specific compressibilities of biopolymers. In this communication we report the effects of temperature and denaturant on the apparent specific volume and apparent specific adiabatic compressibility of lysozyme in solution.

Hen egg white lysozyme was purchased from Sigma Chem. Co. and used without further purification. Guanidine hydrochloride was purchased from Nacalai Tesque Inc. and other chemicals from Wako Pure Chemical Industries. Ltd. The water used for preparing solutions was deionized. Densities (ρ) and sound velocities (u) were measured with a vibrational tube density meter (DMA 60/601, Anton Paar),[9-11] and a sing-around velocity meter constructed in our laboratory,[9,12] respectively.

The apparent specific quantities (ϕ_q) of the solute in solution can be calculated from the corresponding specific quantity of solution (q) and of solvent (q_0) by the relation[13]

$$\phi_q = (q - q_0)/w + q_0$$

where w is weight fraction of sample solution. In the present study, ϕ_q refers to the apparent specific volume (ϕ_v) or the apparent specific adiabatic compressibility (ϕ_k) of lysozyme and q refers to the specific volume ($v = 1/\rho$) or specific adiabatic compression ($k = -(\partial v/\partial P)_S = (v/u)^2$).

Figure 1 shows the temperature dependences of the apparent specific volume (A) and the apparent specific adiabatic compressibility (B). The lysozyme concentration is 0.78 %wt and the solvent used is Glycine-HCl buffer at pH 1.50. For these experiments the temperature was increased step by step from 15 to 60 °C at 5 °C intervals and the density and sound velocity values were measured after thermal equilibrium was established at each temperature. It is evident that both apparent quantities increase linearly with the increase in temperature. For this system the thermal denaturation was confirmed by means of a differential scanning microcalorimeter; that is, the lysozyme begins to denature at about 40 °C and the temperature of the denaturation midpoint was found to be about 47 °C. Thus it is noticeable that no marked transition was found in the vicinity of the denaturation temperature. This finding may be rather reasonable since it has been known that protein denaturation occurs with only a marginal decrease in volume from the studies of the influence of high pressure on protein stability.[1,8]

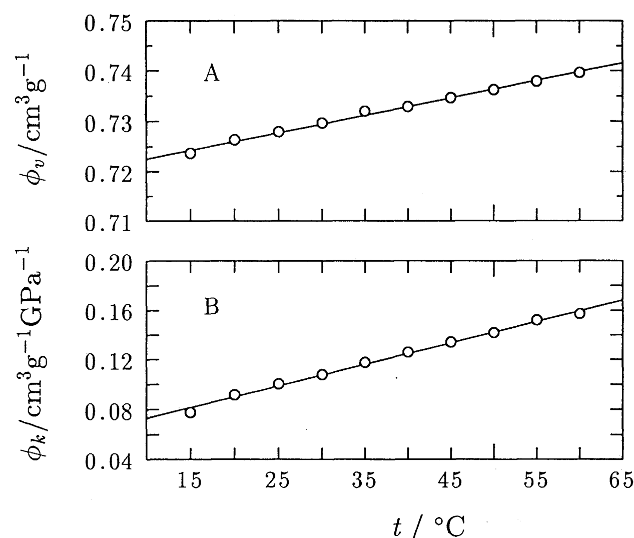

Figure 1. Temperature dependences of the apparent specific volume (A) and apparent specific adiabatic compressibility (B) of lysozyme in Gly–HCl buffer solution (pH 1.50).

The effects of chemical denaturation on the volumetric properties were examined by the addition of denaturant guanidine hydrochloride (GuHCl). The preliminary measurements of circular dichroism at 280 nm established that the lysozyme in KCl-HCl buffer at pH 3.00 begins to unfold by the addition of about 2 M GuHCl. The dependences of ϕ_v and ϕ_k of 0.44 %wt lysozyme on GuHCl concentration at 25 °C are shown in Figures 2A and 2B, respectively. Both apparent quantities, as a whole, increase with increasing GuHCl concentration. It is, however, interesting to note that these quantities seem to decrease slightly in the vicinity of the onset of unfolding.

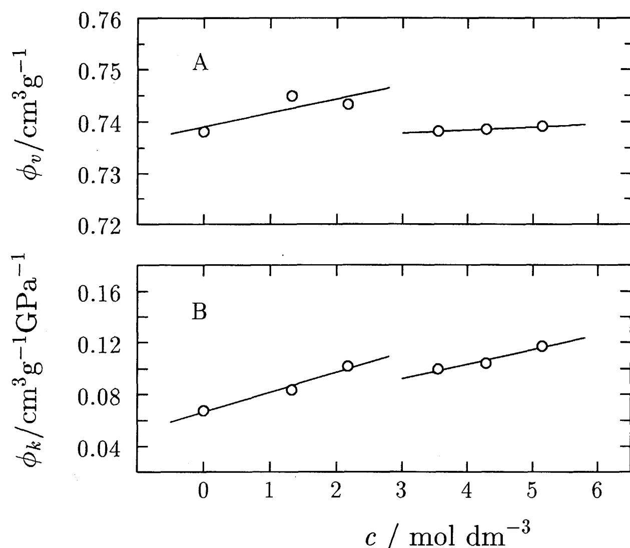

Figure 2. GuHCl concentration dependences of the apparent specific volume (A) and apparent specific adiabatic compressibility (B) of lysozyme in KCl–HCl buffer solution (pH 3.00) at 25 °C.

The different volumetric feature caused by thermal and chemical denaturations may be attributed to the difference in nature and/or extent of two denaturation reactions.[14–16] In the study of the effects of chemical denaturation, however, it should be noted that some difficulty was encountered in estimating the density and sound velocity values with sufficient precision because of separate preparation of sample solutions. Such an experimental disadvantage and lower lysozyme concentration, compared with the temperature scanning measurements, inevitably cause somewhat larger uncertainty in the ϕ_v and ϕ_k values. Further careful experiments must be required in order to ascertain the effects of chemical denaturants on the volumetric behavior.

In any event, the volume change associated with protein denaturation may be very small or, in certain cases, cannot be detected by direct volumetric methods. On the other hand, it is well-known that the denaturation process is undoubtedly accompanied by positive heat capacity change, which has been interpreted in terms of the hydration of internal non-polar groups.[8] Taking into account that the compressibility behavior is also believed to be affected sensitively by the solute–solvent interactions, a detectable change in the apparent specific compressibility should be expected for the process. The present results, however, reveal that this is not really the case. Although we cannot definitely conclude such a discrepancy, it compels us to suspect that the thermodynamics of protein denaturation is discussed only from the point of view of the hydration behavior. In this regard we shall call attention to the fact that characteristic volume and compressibility behavior in aqueous solutions can be observed for the solutes which contain both polar and non-polar groups, such as short-chain alcohols, and not for hydrophobic solutes, such as benzene or long-chain alcohols.[17, 18] More precise and comprehensive volumetric studies must be done on biopolymers and low-molecular model compounds for the decisive description of the nature and properties of protein denaturation.

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