Behaviour of bovine serum albumin in aqueous solutions of some sodium salts of organic acids, tetraethylammonium bromide and dextrose investigated by ultrasonic velocity, viscosity and density measurements

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Ultrasonic velocity (\(\nu\)), viscosity (\(\eta\)) and density (\(p\)) of aqueous solutions of disodium succinate (DSS), trisodium citrate (TSC), disodium ethylenediaminetetraacetate (DSEDTA), tetraethylammonium bromide (TEAB) and dextrose (DT) have been measured in the concentration range 0.01 to 0.40 mol dm\(^{-3}\) at 308.15 K. Such measurements have also been made at different concentrations of these salts and DT in 0.02 g cm\(^{-3}\) aqueous bovine serum albumin (BSA) and also at varying concentrations of BSA in the range 0.003 to 0.040 g cm\(^{-3}\) in 0.1480 mol dm\(^{-3}\) solution of these salts or DT in water. The isentropic compressibilities (\(K_s\)) of all the solutions have been calculated from the relation: \(K_s = \frac{\nu_2}{\eta p}\). The isentropic compressibility contribution (\(\Delta K_s\)) and viscosity contribution (\(\Delta \eta\)) due to BSA in various salt solutions as well as in pure water have been calculated at different concentrations of the protein and plotted against BSA concentration (\(C\)). The results show that BSA interacts strongly with all these salts and DT. TEAB, TSC and DT stabilize the protein by interaction at all BSA concentrations. At low concentrations of BSA, 0.1480 mol dm\(^{-3}\) DSS, TSC and DSEDTA denature or dissociate (destabilize) the protein by interaction. At high concentrations of protein only DSEDTA denaturates or dissociates the protein while all other salts and DT have stabilizing effect.

Materials and Methods

Doubly distilled water with conductivity 2.4 \(\times\) 10\(^{-7}\) S cm\(^{-1}\) was used for all measurements. Disodium succinate hexahydrate (DSS) 99% and tetraethylammonium bromide (TEAB) >98% (both from Sisco Research laboratories, Bombay), disodium ethylenediaminetetraacetate dihydrate (DSEDTA) 98%, dextrose (DT) >98%, trisodium citrate dihydrate (TSC) >99% (all from S.D. Fine Chemicals, Boisar) and BSA (Fraction V, Fluka) were used as received. Desired concentrations of BSA were prepared by weighing the protein and dissolving it in the appropriate volume of water or in the desired salt or dextrose solutions. Vigorous stirring was avoided to prevent foam formation during preparation of protein solutions in all cases. Ultrasonic velocities were measured at 2 MHz frequency using an ultrasonic time intervalometer model UTI-101 from Innovative Instruments, Hyderabad by a pulse echo overlap technique. The absolute accuracy of sound velocity measurements was 2 parts in 10\(^4\). Viscosities of the salt solutions as well as of the protein solutions were measured using an Ubbelohde suspended bulb viscometer. Measurements were repeat-
Results and Discussion

Ultrasonic velocity ($u$), viscosity ($\eta$), density ($\rho$) and electrical conductivity ($\kappa$) of BSA solutions in water in the concentration range 0.003 to 0.040 g cm$^{-3}$ have been measured at 308.15K and the results are presented in Fig.1. Such studies have also been made at different salt concentrations to check reproducibility of results. The overall accuracy of viscosity measurements was ± 0.1%. Densities of all solutions were measured with reproducibility of ±1 x 10$^{-5}$ g cm$^{-3}$ by Anton Paar digital densitimeter model 60 and a calibrated cell type 602.

All physicochemical measurements were made in a water thermostat bath maintained at 308.15 ± 0.01K.
concentrations of DSS, TSC, DSEDTA, TEAB and DT in water and in 0.02 g cm⁻³ BSA solutions in water and also in 0.1480 mol dm⁻³ salt solutions in water with varying BSA concentrations. The salt or DT concentration employed in the present study was between 0.01 and 0.40 dm⁻³ and the BSA concentrations between 0.003 and 0.04 g cm⁻³. By using ultrasonic velocity (υ) and density (ρ) data for various solutions, the isentropic compressibility (Kₛ) for various solutions was calculated by using the equation

$$K_s = 1/\rho \cdot \eta / \rho$$

The plots of Kₛ versus salt concentration (M) (without BSA) are shown in Fig. 2. The Kₛ values for the salts (solutes) investigated decrease in the order: TEAB > DT > DSS > DSEDTA > TSC (Fig.2). The plots of Kₛ versus M for salts or DT in 0.02 g cm⁻³ BSA solution in water are shown in Fig.3. In Fig.3, the Kₛ values at each salt concentration is lower in 0.02 g cm⁻³ BSA as compared to the corresponding values for the salts or DT in pure water in Fig. 2. From the Kₛ values for BSA in 0.1480 mol dm⁻³ salt solution in water, the Kₛ values for 0.1480 mol dm⁻³ salt solution in water in each case obtained from Fig. 2 were substrated to get isentropic compressibility contribution (ΔKₛ) due to BSA at different BSA concentrations. 0.1480 mol dm⁻³ salt concentration was only a selected value and is close to midway concentration for most of the salt concentrations used in the present study. These ΔKₛ values are plotted as a function of BSA concentration (C) to examine the effect of different salts on BSA. The plots of ΔKₛ versus protein concentration(C) are shown in Fig. 4. The ΔKₛ...
values for BSA without salt at different concentrations of protein were obtained by subtracting the isentropic compressibility of pure water from that of BSA solutions in water at the respective BSA concentrations. These \( \Delta K_s \) values without any salt are also plotted against BSA concentrations in Fig. 4 as a reference plot for comparison with other plots for various salts. It is shown that a very interesting effect is obtained from Fig. 4. \( \Delta K_s \) values for DSEDTA and DSS fall on the more negative value side of the reference plot up to about 0.015 g cm\(^{-3} \) of BSA, while they fall on the more positive value side or less negative value side for all salts in the higher protein concentration region. More negative \( \Delta K_s \) values are due to more structural effects. When BSA denaturates or dissociates it occupies more volume of space and due to increased volume more structure is produced. On the other hand when BSA interacts with ions and the protein structure becomes more compact only in certain regions of solutions and the \( \Delta K_s \) value will become more positive or less negative. This result indicates that all the salts and dextrose interact with BSA and the protein shows denaturation or dissociation only with DSEDTA and DSS up to 0.015 g cm\(^{-3} \) of the protein. In the higher protein concentration range, all salts show stabilization effect on BSA with the result protein shows no denaturation effect. Similar result is also obtained from viscosity measurements. In Fig. 5 is shown the variation of viscosity (\( \eta \)) of various salts and DT with salts concentration (\( M \)) without BSA. Here the order of change of viscosity is: TSC>DSEDTA>DSS>DT>TEAB. In Fig. 6 is shown the plot of variation of \( \eta \) with salt concentration (\( M \)) in the presence of 0.02 g cm\(^{-3} \) BSA. The viscosity of the salt solutions in the presence of 0.02 g cm\(^{-3} \) BSA significantly increases from the corresponding values of the salts without BSA. The order of viscosity in Fig. 6 becomes: DSEDTA>TSC>DSS>DT>TEAB which shows that DSEDTA affects the viscosity of BSA much more than any other salt. In Fig. 7, the plot of variation of \( \eta \) of BSA as a function of protein concentration (\( C \)) in 0.1480 mol dm\(^{-3} \) salt solution of each salt and DT is shown. Like in Fig. 4 where \( \Delta K_s \) was plotted as a function of BSA concentration, the contribution of viscosity due to BSA (\( \Delta \eta \)) for each salt have been calculated and plotted against protein concentrations (\( C \)). Viscosity contribution due to BSA in water at different concentrations of protein is also plotted in Fig. 8 as a reference plot.

At low BSA concentrations (upto 0.015 g cm\(^{-3} \)) \( \Delta \eta \) values for BSA in the presence of DSEDTA and DSS fall on higher side of the reference plot. High \( \Delta \eta \) values indicate more strong structure which can arise due to denaturation or dissociation of BSA. In the viscosity effect plots (Fig. 8), DSEDTA throughout the protein concentration range gives \( \Delta \eta \) value which is higher than the corresponding value of protein in water (reference plot). Viscosity results show that DSEDTA over the whole protein concentration range studied and DSS up to 0.015 g cm\(^{-3} \) BSA denaturate the protein TEAB and DT both show protein stabilising effect at all the concentrations of BSA. The viscosity results are also in agreement with the isentropic compressibility results.

DSS and TSC are the salts of acids of slightly different chain length and differing in some functional groups but they produce almost similar effect on BSA. The effect of DSEDTA on BSA is different due to its long chain and different nature of anion involved. Na\(^+ \) is strongly solvated in water. It is structure promoter in water. If only Na\(^+ \) ions are involved to produce the effect on BSA, then the effect of DSS and DSEDTA should have been similar because of the same concentration of Na\(^+ \) ions produced from a specific concentration of salt in both cases. Since DSEDTA strongly produces denaturation of BSA over the whole protein concentration range, while DSS and TSC produce only in a limited range, therefore, the ethylenediaminetetraacetate ion also plays major role than Na\(^+ \) ion towards denaturation of BSA. In the neighbourhood of protein will produce the denaturation or stabilization of the protein. The breaking of hydrogen bonding of protein by interaction with anion...
of the added salt or the breaking of hydrogen bonding of the neighbouring water molecules due to presence of anion will be the reason for protein denaturation in the case of DSEDTA. Two ionisable hydrogens which are present in DSEDTA may also be responsible in producing strong denaturation of BSA due to lowering of pH of the solution. Such pH effect, however, is not present in DSS or TSC. Similarly in the case of TEAB, Et₄N⁺ ion plays important role for interaction with BSA. This ion has hydrophobic interaction with protein and thus has strong effect on BSA due to hydrogen bonding enhancement. Dextrose interacts with BSA predominantly by hydrogen bonding and thus enhances hydrogen bonding in BSA either by direct interaction or interaction through neighbouring water molecules with the result the protein remains very stable in this solution.

The ultrasonic velocity, viscosity and density data for salt solutions, at varying concentrations in 0.02 g cm⁻³ BSA have also been utilized to obtain information on the interactions of protein with these salts and DT. The results obtained from these measurements are also in agreement with the results explained above from ultrasonic velocity and viscosity measurements in water and in 0.1480 mol dm⁻³ salt solutions. When the isentropic compressibility and viscosity of the salt solutions were subtracted from the corresponding values of the salts in 0.02 g cm⁻³ BSA, the contribution of 0.02 g cm⁻³ protein was obtained. For illustration, such viscosity contribution effect of 0.02 g cm⁻³ BSA in different salt solutions is plotted in Fig. 9. The effect of 0.02 g cm⁻³ BSA in water is also plotted as a reference plot for comparison with other plots. In the case of DSEDTA, the Δν values for BSA are higher than the reference plot for BSA in pure water. These results also confirm that DSEDTA destabilizes BSA and produces higher viscosity of protein in salt solution as compared to that in pure water.

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**References**