Analysis of some Thermodynamic Properties of Bovine Serum Albumin in Aqueous Solution of some salts at a fixed Temperature

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Abstract:- Ultrasonic velocity (u), densities (ρ) and viscosities (η) of aqueous solutions of ammonium dihydrogen phosphate ($NH_4H_2PO_4$), trisodium citrate, ($Na_3C_6H_5O_7.2H_2O$), ammonium sulfate $(NH_4)_2SO_4$, potassium thiocyanate (KSCN) have been measured in the concentration range between 0.0125 mol dm^{-3} to 0.4 mol dm^{-3} at 303.15K. Such measurements have been made at concentration of these salts with fixed concentration i.e 0.02 g cm^{-3} of BSA. The isentropic compressibility (K_s) for various solutions was calculated by using the equation K, =1/u^2P.

It has been found from the literature survey that a large number of physicochemical investigation of bovine serum albumin (BSA) have been made in water and with other solvent systems under different conditions of pH, temperature, ionic strength and in the presence of varying concentration of denaturants. The interaction of BSA[1,2], metal ions [3], urea [4,5], sodium dodecyl sulphate [6-9], sulphonamide [10], guanidine hydrochloride [11]and other surfactants, has been investigated. Many physicochemical methods such as viscosity [12,13], ultrasonic absorption [4], NMR [9,17-18], ESR [19], dielectric relaxation at microwave frequency [1,20,21], pressure jump [22]are the method which require special attention. Besides this a little efforts has been made to investigate its nature in mixed solvents especially in water with inorganic metal and transition metal salts. Ultrasonic velocity studies in aqueous solutions of low molecular weight and high molecular weight polyvinylpyrrolidone have been done by G. Y.Reddy et al. , (1980) Rao formal-Ism has been applied to the polymer solutions and it was observed that there was reasonably good correspondence between theoretical and experimental value of Rao's constant. Alastair M.North at a l . . (1981), have reported the adiabatic compressibilities of solutions of Polyethyleneoxides(M-400,1000 and 1450) in water, 1- propanol, 1 , 2 -ethanediol and 1.2,3-propanetriol at 323K. Ultrasonic absorption and velocity measurements are reported on aqueous solutions of polyvinylalcohol of differcritmolecular weights, degrees of hydrolysis and concentratl , > n by Abdul Abu-Llakr et al., (1982). The absorption measurements cover a frequency range from 1-1880 MHz and thev elocity data a frequency range from 2-280 MHz, the data were obtained over a temperature range from 273 to 333K. Richard A.Pethrfck et al., (1983) have reported the ultrasonic attenuation and velocity measurements at 2 MHz over a temperature range from 293K to 353 for mixtures of Polyethyleneoxide and water for concentrations from 0% to180%.So to understand the nature and strength of interactions in bovine serum albumin in the case of mixed solvents some solvent properties such as excess thermodynamic functions, viz; excess isentropic compressibility, excess molar volume, excess enthalpy and excess heat capacity can be well understood by studying. In the present paper, ultrasonic velocity, densities and viscosity measurements of BSA have been made in some interesting salt solutions at 303.15K to get information on the interactions of this protein.

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EXPERIMENTAL

Doubly distilled conductivity water was used throughout. By using a long vertical fractionating column containing ion-exchange resins (supplied by Ion-exchange India Ltd), it was distilled twice over acidified $KMnO_4$. Ammonium dihydrogen phosphate 98%, trisodium citrate 99%, ammonium sulfate 98%, potassium thiocyanate 97% purity (all from S.D. Fine Chemicals, Mumbai). BSA fraction-V crystal was also obtained (from Sisco Laboratories, Mumbai). These salts were used without further purification. Desired concentration of BSA were prepared by weighing the protein and dissolving it in the appropriate volume of water or in desired salt solution. Vigrous stirring was avoided to prevent foam formation during preparation of protein solution in all these cases. The ultrasonic velocities (u) at 2 MHz frequency and densities (ρ) of various protein solutions were measured by using an Anton-Paar DSA 5000 density and sound analyzer instrument. DSA 5000 is the first oscillating U-tube density (accuracy, 0.000005 g cm^{-3}) and ultrasonic velocity (accuracy 0.5 m s^{-1}) meter which measures this parameter

with the highest accuracy in wide viscosity and temperature ranges. Viscosity of various protein and salt solutions were measured using an Ubbelohde suspended-level viscometer. Measurements were repeatedly made to check the reproducibility of results. The overall accuracy of viscosity measurements was ± 0.01% . The viscosity of the filled sample is measured by damping of the U-tube oscillation. The DSA 5000 automatically corrects the viscosity related errors in the density.

All physicochemical measurements were made in a water thermostat bath maintained at 303.15± 0.01K.

RESULTS AND DISCUSSION

Ultrasonic velocity (u), densities (ρ) and viscosities (η) of BSA solution in water in the concentration range 0.00125 to 0.04 g cm^{-3} have been measured at 303.15K and the results are presented in Fig.1. Such studies have also been made at different salts concentration of ammonium dihydrogen phosphate, trisodium citrate, ammonium sulfate , potassium thiocyanate in water and in 0.02 g cm^{-3} of BSA. The salt concentrationS employed in the present study was between0.0125 mol dm^{-3} to 0.4 mol dm^{-3}. By using ultrasonic velocity (u) and density (ρ) for various solutions, the isentropic compressibility (K_s) for various solutions was calculated by using the equation K, $=1/u^2P$.

The plots of K_s versus salt concentrations (c)(without BSA) are shown in Fig. 4. The K_s values for the salts investigated decreases in the order: trisodium citrate, ammonium dihydrogen phosphate, potassium thiocyanate, ammonium sulphate. In Fig. the isentropic compressibility is found to decrease linearly when salt concentration is increased in the solutions containing water and fixed amount of BSA. The decrease of Ks values with increase in salt concentration (c) indicates increased structural effect in solution.

Large decrease in Ks values with increase in salt concentrations indicates more structural changes observed in the native BSA. These structural changes arise either from the interaction of protein with the salt or protein – protein interactions. The denaturation of BSA is mainly due to the breakage of hydrogen bond, disulphide linkage or by hydrophobic interaction with anions of these salts. These results are in accord with the isentropic compressibility (K_s) data for many electrolyte.

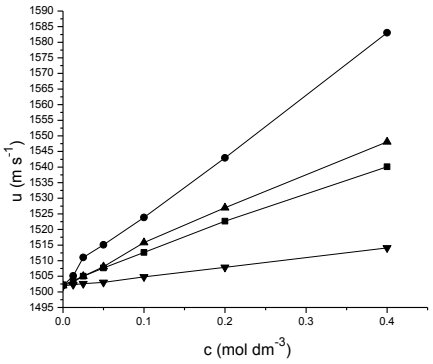

Fig. 1 Plot of ultrasonic velocity (u) vs salt concentrations (c) in water containing 0.02 g cm^{-3} of BSA at 303.15 K. ■ ammonium dihydrogen phosphate,▲ ammonium sulphate, ▼ potassium thiocyanate, ● trisodium citrate .

All plots show an increase in u, ρ and η with increase in salt concentration except in the case of potassium thiocyanate where (η) of protein is found to decrease with increase in the salt concentration. However, the interactions shown by ammonium sulphate and tri-sodium citrate with the protein are very strong as compared to the other salts when the concentration of salt is increased,keeping BSA concentration fixed (Figs.1 to 3).

It has been found that in electrolyte solutions, the behaviour of proteins depends upon the neighbouring environment of BSA. The water structure enhancement by the salts in the neighbourhood of protein will produce the denaturation of the protein.

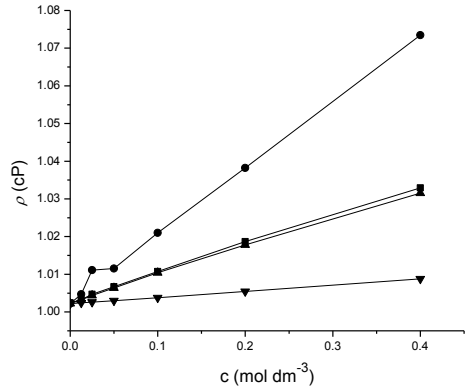

Fig. 2 Plot of density (ρ) vs salt concentrations (c) in water containing 0.02 g cm^{-3} of BSA at 303.15K. ■ Ammonium dehydrogenase phosphate, ▲ ammonium sulphate, ▼ potassium thiocyanate, ● trisodium citrate .

The breaking of hydrogen bonding of protein by interaction with anions of the added salts or the breaking of hydrogen bonding of water molecules due to presence of anions will be the probable cause of protein denaturation in these cases. But this behaviour is not observed for potassium thiocyanate (KSCN) where the viscosity (η) is found to decrease with increase in concentration of the salt in waterThis effect is probably due to the increase in the folding of the protein structure making the structure more compact and thus resulting in the stabilization of the protein BSA in the solution. The majority of the above mentioned studies of the viscosity effect.

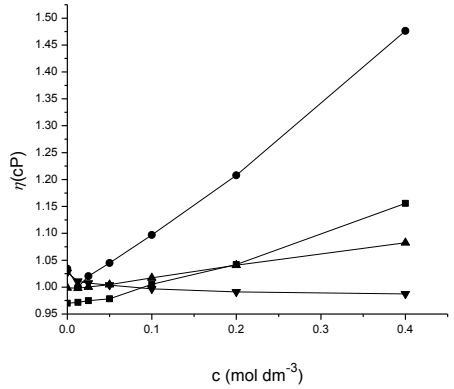

Fig. 3 Plot of viscosity (η) vs salt concentrations (c) in water containing 0.02 g cm^{-3} of BSA at 303.15 K. ■ ammonium dihydrogen phosphate, ▲ ammonium sulphate, ▼ potassium thiocyanate, ● trisodium citrate .

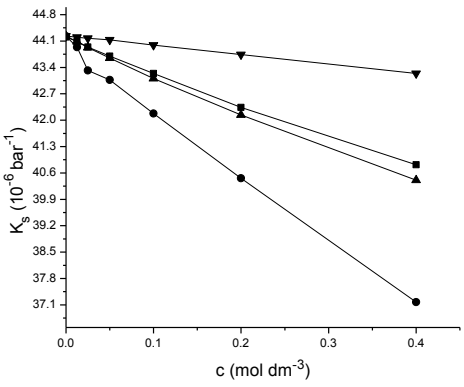

Fig. 4 Plot of isentropic compressibility (K_s) vs salt concentrations (c) in water containing 0.02 g cm^{-3} of BSA at 303.15 K. ■ ammonium dehydrogenase phosphate, ▲ ammonium sulphate, ▼ potassium thiocyanate, ● trisodium citrate.

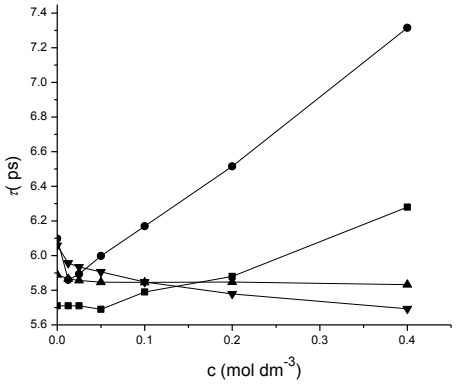

Fig. 5 Plot of relaxation time (τ)vs salt concentrations (c) in water containing 0.02 g cm^{-3} of BSA at 303.15 K. ■ ammonium dihydrogen phosphate, ▲ ammonium sulphate, ▼ potassium thiocyanate, ● trisodium citrate .

the relaxation times (τ) increases in all the cases with increase in BSA concentration in the presence of fixed concentration of protein. This indicates strong interaction of the salts with the protein BSA. The results o indicate that trisodium citrate and ammonium sulphate show stronger interaction with BSA. The larger value indicates more structural effect due to strong interaction among protein and solvent molecule in these systems.

REFERENCES

[1] Barnes, J.A. Evans, J. Anthony and T.J. Lewis, J. Accost. Soc

[2] E. Ayranci and A. Kaya, Doga. Turk, Kim. Derg., 1990, **14**, 339.

[3] M. Janado, Y.Yano, H. Nishida and T. Nishida, J. Solution Chem., 1986, **15**, 839.

[4] A.A. Zaretskii, O.M. Zorina, K.P. Fursov and I.E. Elpiner, Akust. Zh., 1971, **17**, 470.

[5] J. Oakes and M.C. Café, Eur. J. Biochem., 1973, **36**, 559.

[6] J. Steinhardt and J.A. Reynolds, Multiple equilibria in proteins (Academic Press, New York), 1969.

[7] M.M. Breuer, and U.P. Stranes, J. Phys. Chem. and Ind., 1972, **52,** 530.

[8] M.M. Breuer, and U.P. Stranes, J. Phys. Chem., 1960, **64**, 228.

[9] J. Oakes, J. Chem. Soc. Faraday Trans. 1, 1974, **70**, 2200.

[10] A.R. Torres and K.H. Meining, Arzenemittal Forsch., 1965, **15**, 443.

[11] M.E. Neolken and S.N. Timasheff, J. Biol. Chem., 1968, **242**, 5080.

[12] D. W. Kupke, M. G. Hodgins and J.W. Beams, Proc. Nat. Acad. Sci. (U.S.A.), 1972, **69**, 2258.

[12] D. W. Kupke, M. G. Hodgins and J.W. Beams, Proc. Nat. Acad. Sci. (U.S.A.), 1972, **69**, 2258.

[13] W.S. Bery, T.E. Evans and L. H. Hitzrot, J. Colloid Interfac. Sci., 1968, **26**, 306.

[14] C. Barnes, J.A. Evans, J. Anthony and T.J. Lewis, J. Accost. Soc. Am., 1986, **81**, 1291.

[15] S. Bone, P.R.C. Gascoyne and R. Pething, J. Chem. Soc. Faraday Trans. 1, 1977, **73**, 1605.

[16] I.E. Elpiner, A.A. Zaretskii and K.P. Fursov, BioFizika, 1970, **15**, 585.

[17] J. Oakes, Eur. J. Biochem., 1973, **36**, 553.

[18] R.S. Menon and P.S. Allen, Biophys. J., 1990, **3**, 57.

[19] J. Oakes, Eur. J. Biochem., 1973, **36**, 558

[20] E.H. Grant, S. E. Keefe and S .Takaschima, J. Phys. Chem., 1968, **72**, 4373.

[21] E.H. Grant, J.Mol. Biol., 1966, **19**, 133.

[22] K.Takeda, Bull. Chem. Soc. (Japan), 1982, **55,** 2547.