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ORIGINAL ARTICLE

Viscometric study of lysozyme solution with sugar and urea at various temperatures

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Abstract This paper presents the results of viscosity measurement of three ternary systems i.e.

1. D (−) Glucose + lysozyme + water
2. Maltose + lysozyme + water
3. Urea + lysozyme + water

at temperatures (293.15, 303.15, 313.13 and 323.15 K) at various concentrations of glucose, maltose and urea. Change in entropy (ΔH), enthalpy (ΔS) and free energy of activation (ΔG) have also been evaluated for these systems. Value of *B*-coefficient of D (−) glucose, maltose and urea has also been calculated from viscosity data in aqueous lysozyme solution. Viscosity *B*-coefficients of glucose and maltose in aqueous lysozyme solution are positive while that of the urea–lysozyme water system it is negative due to the structure breaking effect of urea. The values of entropy of activation are negative due to attainment of transition state for viscous flow, which is accompanied by bond formation and increase in order.

1. Introduction

The native conformation of a protein is produced by a delicate balance between covalent bonds and noncovalent bonds such as hydrogen bonds, electrostatic interactions and hydrophobic interactions. Therefore, its conformation usually depends not only on temperature and pressure but also on the nature of the solvent, such as its polarity and dielectric constant. Hen egg white lysozyme is a well-known enzyme that acts as a glycoside hydrolase. This small globular protein consists of two functional domains located on each side of the active site cleft and contains both helices and regions of β sheet, together with loop regions, turns and disulfide bridges (Smith et al., 1993).

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Table 1 Viscosities (η), RTln(ηV_m/hN) and free energies of activation (ΔG) of glucose, maltose and urea in 0.15×10^{-3} molal (m) lysozyme solution as functions of concentration and temperature.

Temperature 293.15 K						
D (−) Glucose + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0610	0.0810	0.1010
$\eta \times 10^4$/Kg m^{-1} s^{-1}	1.0139	1.0351	1.0456	1.0532	1.0622	1.0713
RTln(ηV_m/hN)/kJ mol^{-1}	–	57.0905	58.2979	59.1402	59.7486	60.2414
ΔG/kJ mol^{-1}	–	57.0649	58.2733	59.1208	59.7247	60.2594
Maltose + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
$\eta \times 10^4$/Kg m^{-1} s^{-1}	1.1039	1.0869	1.1035	1.1188	1.1248	1.1339
RTln(ηV_m/hN)/kJ mol^{-1}	–	58.3924	59.8155	60.7599	61.4314	61.9666
ΔG/kJ mol^{-1}	–	58.3120	59.7367	60.6698	61.3496	61.8982
Urea + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0600	0.0800	0.1000
$\eta \times 10^4$/Kg m^{-1} s^{-1}	1.0139	1.0622	1.0450	1.0374	1.0420	1.0435
RTln(ηV_m/hN)/kJ mol^{-1}	–	55.8936	56.5842	57.1016	57.5747	57.9667
ΔG/kJ mol^{-1}	–	55.7972	56.4903	57.0413	57.5210	57.9084
Temperature 303.15 K						
D (−) Glucose + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0610	0.0810	0.1010
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.8071	0.8335	0.8421	0.8507	0.8563	0.8619
RTln(ηV_m/hN)/kJ mol^{-1}	–	58.5032	59.7521	60.6304	61.2547	64.7592
ΔG/kJ mol^{-1}	–	58.4938	59.7460	60.6255	61.2570	61.8102
Maltose + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.8071	0.8197	0.8308	0.8405	0.8563	0.8660
RTln(ηV_m/hN)/kJ mol^{-1}	–	59.6787	61.1516	62.1227	62.8506	63.4121
ΔG/kJ mol^{-1}	–	59.7378	61.253	62.2038	62.9219	63.4868
Urea + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0600	0.0800	0.1000
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.8017	0.8276	0.8019	0.7975	0.8019	0.8005
RTln(ηV_m/hN)/kJ mol^{-1}	–	57.1265	57.8209	58.3977	58.8897	59.2870
ΔG/kJ mol^{-1}	–	57.1320	57.8587	58.4367	58.9300	59.3304
Temperature 313.15 K						
D (−) Glucose + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0610	0.0810	0.1010
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.6619	0.6868	0.6937	0.7036	0.7091	0.7190
RTln(ηV_m/hN)/kJ mol^{-1}	–	59.9399	61.2293	62.1470	62.7950	63.3353
ΔG/kJ mol^{-1}	–	59.9227	61.2187	62.1301	62.7894	63.3609
Maltose + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.6619	0.6757	0.6924	0.7003	0.7218	0.7267
RTln(ηV_m/hN)/kJ mol^{-1}	–	61.1600	62.7052	63.7076	64.4897	65.0580
ΔG/kJ mol^{-1}	–	61.1636	62.7140	63.7378	64.4942	65.0754
Urea + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0600	0.0800	0.1000
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.6619	0.6676	0.6524	0.6554	0.6628	0.6584
RTln(ηV_m/hN)/kJ mol^{-1}	–	58.4625	59.2020	59.8241	60.3472	60.7449
ΔG/kJ mol^{-1}	–	58.4668	53.2272	59.8320	60.3391	60.7524
Temperature 323.15 K						
D (−) Glucose + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0610	0.0810	0.1010
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.5560	0.5685	0.5736	0.5818	0.5914	0.6010
RTln(ηV_m/hN)/kJ mol^{-1}	–	61.3556	62.6833	63.6304	64.3222	64.8857
ΔG/kJ mol^{-1}	–	61.3517	62.6915	63.6347	64.3217	64.9116

Table 1 (*continued*)

Maltose + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0610	0.0820	0.1030
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.5560	0.5633	0.5780	0.5988	0.6122	0.6197
$\underline{RTln}(\eta V_m/\underline{hN})$/kJ mol^{-1}	–	62.6348	64.2319	65.3308	66.1161	66.7170
ΔG/kJ mol^{-1}	–	62.5893	64.2026	65.2717	66.0664	66.6641
Urea + aqueous lysozyme						
m/mol kg^{-1}	0.0000	0.0200	0.0400	0.0600	0.0800	0.1000
$\eta \times 10^4$/Kg m^{-1} s^{-1}	0.5560	0.5528	0.5467	0.5482	0.5484	0.5488
$\underline{RTln}(\eta V_m/\underline{hN})$/kJ mol^{-1}	–	59.8320	60.6272	61.2642	61.7738	62.2021
ΔG/kJ mol^{-1}	–	59.8015	60.5956	61.2274	61.7481	62.1743

Very little attention has been paid to the viscosity of lysozyme aqueous solutions (Lefebure, 1982) and data of viscosity of lysozyme in mixed aqueous solutions are rare. Recently, attention has been paid, in particular, to the rich conformational variety of carbohydrates (Gabius, 2000; Hindley et al., 2005; Waris et al., 2001). Viscosity of egg-white lysozyme was measured in the presence of carbohydrate additives in reaction medium. These additives show a significant affinity for water. They depress water activity and increase the viscosity of the medium (Lamy et al., 1990). Solute–solvent interactions in aqueous solutions of the additives are characterized by *B*-coefficient.

The present work is a continuation of our research program on the thermodynamic studies on ternary systems (Siddique and Naqvi, 2010, 2011a). In this work viscosity measurements have been carried on sugars (D-glucose and maltose) and urea + aqueous lysozyme solutions (keeping the concentration of aqueous lysozyme solution (0.15 milli-molal) constant) at different temperatures (293.15, 303.15, 313.15 and 323.15 K) for different concentrations of sugar and urea to understand the increased or decreased stability of lysozyme in the presence of sugars and urea, respectively.

Heating of protein in solution can lead to aggregation, gelation, denaturation and thermal expansion, etc; depending upon the temperature range. The solute–solvent, solvent–solvent and solute–solute interactions in a protein solution undergo substantial changes upon exposure to different temperatures that bring about the observable physical change in the protein solution. As the thermal environment is altered, the Gibbs free energy, ΔG^* of the system changes, altering the physical state of the protein for which ΔG^* is minimized.

2. Materials and methods

Lysozyme (⩾99%) obtained from SIGMA–ALDRICH CHEMIE Gmbh Steimhein, Germany, was used for sample preparation. Sugars viz. D-glucose (⩾99%) and maltose (⩾99%) were obtained from Qualigans fine chemicals (a division of Glaxo Smith Kline Pharmaceuticals Limited, Mumbai). Urea crystal (⩾98%) extra pure was obtained from Merck Limited Worli, Mumbai. All solvents and chemicals were of analytical grade. These chemicals were used without further purification. The triply distilled water (with the specific conductivity of $1.29 \times 10^{-6}\ \Omega^{-1}\ cm^{-1}$) was used for making lysosome, sugars and urea stock solutions. All the solutions were stored in special airtight bottles to avoid exposure of solutions to air and evaporations.

The viscosity measurements were performed using an Ubbelohde-type capillary viscometer (Tanford, 1961). The working procedure is described elsewhere (Siddique and Naqvi, 2011b). The uncertainties in viscosity measurements have been found to be within ±0.003 mPa s. The densities required for the calculation of viscosity values of the solutions were taken from our earlier studies (Siddique et al., communicated) (unpublished data).

The triplicate reproducibility was established during the entire experimental work. The thermostatic paraffin bath (JULABO, Model-MD Germany) used during the measurements of density and viscosity was maintained at desired temperature (±0.02 K) for about 30 min prior to the record of reading at each temperature of study. The weighing was done on electronic balance (model: GR-202R, AND, Japan) with the precision of ±0.01 mg. The uncertainty in molal concentration values is found to be within 1.0×10^{-4} mol kg^{-1}.

3. Results

The experimental values of viscosity (η) are measured at different temperatures for lysozyme in aqueous and in sugar and urea solutions. These data are used to calculate the relative viscosity, (η_r) by the relation given below; (Jones and Dole, 1929; Tyrell and Kennerly, 1968; Devine and Lowe, 1971).

$$\eta_r = \eta/\eta_0 = 1 + BC \quad (1)$$

where C is the concentration (mol kg^{-1}), B is Jones–Dole viscosity coefficient, η and η_0 are the viscosities of solution and solvent, respectively, η_r is the relative viscosity of the solution.

The *B*-coefficient values of the solute are obtained by the least-squares procedure. *B*-Coefficient is the measure of order or disorder introduced by the solute into solvent structure. This constant is specific and is an approximately additive property of ions of an electrolyte at a given temperature, although no satisfactory theoretical treatment has yet been given.

Viscosity data have also been used for the calculation of solute activation parameters (Pandey and Prakash, 1982). The free energy of activation (ΔG^*) for viscous flow is given by Eyring viscosity equation (Eyring and John, 1969),

$$\eta = (hN/V_m)e^{(\Delta G^*/RT)} \quad (2)$$

where h is the Planck's constant, N is Avagadro's number, R is the universal gas constant and V_m is the molar volume of the mixture. Molar volume of the mixture has been calculated from the corresponding mixture densities (Siddique et al., communicated) (unpublished data) by the following relation;

$$V_m = \sum X_i M_i/\rho, \quad i = 1, 2, 3, \ \ldots \ ! \tag{3}$$

The energies of activation (ΔG^*) for viscous flow of the solute at different temperatures are obtained by using following equation;

$$\Delta H^* = \Delta G^* + T\Delta S^* \tag{4}$$

where ΔH^* and ΔS^* are the enthalpy and entropy of activation for the viscous flow of solute, respectively. From equations below we get the value of ΔG^*,

$$\Delta G^* = RT\ ln(\eta V_m/hN) = \Delta H^* - T\Delta S^* \tag{5}$$

The values of ΔH^* and ΔS^* can be obtained by least squares fitting. ΔS^* is the corresponding experimental slope of RTln (ηV_m/hN) vs temperature plots.

4. Discussions

Viscosities of sugars + aqueous lysozyme and urea + aqueous lysozyme systems are shown in Table 1 for different molalities of solute at different temperatures. The increase in concentration of solute increases the viscous behavior of the solution due to an increase in number of solute molecules, which causes more frictional resistance to the flow. But when we observed in case of urea in lysozyme solution, viscosity first decreases for lower concentration of urea (from 0.02 to 0.06 mol/kg) and then it gradually increases on further increase in concentration of urea. Therefore, we may conclude that at lower concentration of urea, its structure breaking effect is more pronounced while at higher concentrations it shows opposite behavior.

The increase in the concentration of solute in solution contributes positively to the viscosity *B*-coefficient. On the other hand, breaking of the solvent structure by solute causes a decrease in the viscosity. This contributes negatively to the *B*-coefficient. Thus, *B*-coefficient is the resultant of these two opposite forces (Mason et al., 1952). Therefore, the urea molecules exhibiting negative *B*-coefficient have been assumed to exert a structure breaking effect on the solvent while glucose and maltose exhibit effect on the solvent with positive *B*-coefficient and, thus, have structure-making effect on the solvent.

It has been observed (Table 2) that all the values of viscosity *B*-coefficient for saccharides are positive and in aqueous lysozyme solution, these values are greater for maltose than for glucose. *B*-Coefficient depends directly on size, shape and charge of the solute molecules, and maltose has two glucose units joined by α-1,4-glucosidic linkage. Therefore, the order is *B* (glucose) < *B* (maltose). It is noteworthy that the *B* (maltose) is not twice as large as that of is *B* (D-glucose), indicating that the formation of α-1,4-linkage reduces the structure making effects of saccharides.

Table 2 *B*-Coefficient ($B/dm^3\ mol^{-1}$) of glucose, maltose and urea in 0.15×10^{-3} molal lysozyme as functions of temperature.

T/*K*	D-Glucose	Maltose	Urea
293.15	0.4400	0.5675	−0.2000
298.15	0.5895	0.8420	−0.2215
303.15	0.4390	0.7320	−0.3350
308.15	0.6235	0.9525	−0.0750
313.15	0.6030	0.9940	−0.0600
318.15	0.5975	1.0885	−0.0775
323.15	0.7445	1.3215	−0.0685

Table 3 Entropy (ΔS/kJ mol^{-1} K^{-1}) and enthalpy (ΔH/kJ mol^{-1}) of glucose, maltose and urea in 0.15×10^{-3} molal lysozyme solution as a function of concentration.

m/mol kg^{-1}	ΔS	ΔH
D (−) Glucose		
0.0200	−142.8914	15.1763
0.0400	−147.2714	15.1007
0.0610	−150.4643	15.0122
0.0810	−153.2364	14.8034
0.1010	−155.0729	14.7998
Maltose		
0.0200	−142.5779	16.5153
0.0400	−148.8643	16.0971
0.0610	−153.3957	15.7019
0.0820	−157.2271	15.2585
0.1030	−158.8636	15.3273
Urea		
0.0200	−133.4771	16.6684
0.0400	−136.8436	16.3746
0.0600	−139.5357	16.1364
0.0800	−140.9043	16.2149
0.1000	−142.1950	16.2240

According to Feakin's model (Feakins et al., 1974) greater the value of ΔG^*, the greater is the structure making ability of solute. A perusal of Table 1 shows that ΔG^* increases with increase in temperature. This, thereby, indicates that the structure making ability of solute increases with temperature. Negative values of ΔS^* (Table 3) suggest that the attainment of transition state for viscous flow is accompanied by bond formation and increase in order.

References

Devine, W., Lowe, B.M., 1971. J. Chem. Soc. A, 2113–2116.

Eyring, H., John, M.S., 1969. Significant Liquid Structure. John Wiley and Son, New York.

Feakins, D., Freemantle, J.D., Lawrence, K.G., 1974. J. Chem. Soc. Faraday Trans. 70, 795–806.

Gabius, H.J., 2000. Naturewissenschaften 87, 108–121.

Hindley, M.L., Lee, K.C., Davis, J.T., 2005. J. Carbohydr. Chem. 24 (3), 261–273.

Jones, G., Dole, M., 1929. J. Am. Chem. Soc. 51, 2950–2964.

Lamy, L., Portmann, M.O., Mathlouthi, M., Larreta-Garde, V., 1990. Biophys. Chem. 36 (1), 71–76.

Lefebure, J., 1982. Rheol. Acta 21, 620–625.

Mason, L.S., Kampmeyer, P.M., Robinson, A.L., 1952. J. Am. Chem. Soc. 74, 1287–1290.

Pandey, P., Prakash, O., 1982. J. Pure Appl. Ultrason. 14, 12–15.

Siddique, J.A., Naqvi, S., 2010. J. Chem. Eng. Data 55, 2930–2934.

Siddique, J.A., Naqvi, S., 2011a. Chin. J. Chem. 29 (4), 669–678.

Siddique, J. A., Naqvi, S., 2011b. Int. J. Thermophys. (Accepted for publication). doi:10.1007/s10765-011-1111-y.

Siddique, J. A., Sharma, S., Naqvi, S., (Communicated).

Smith, L.J., Sutcliffe, M.J., Redfield, C., Dobson, C.M., 1993. J. Mol. Biol. 229 (4), 930–944.

Tanford, C., 1961. Physical Chemistry of Macromolecules. John Wiley and Sons, Inc., New York, N.Y.P., 329.

Tyrell, J.V., Kennerly, M., 1968. J. Chem. Soc. A, 2724–2728.

Waris, B.N., Hasan, U., Srivastava, N., 2001. Thermochim. Acta 375, 1–7.