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Biophysical behavior and hydrophobic interactions of globular proteins with aqueous binary solutions

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ABSTRACT

Densities (r) and viscosities (η) for 0.5 to 2.0 mg %/100 mL aqueous solutions of Bovine Serum Albumin (BSA), Egg Albumin (E Alb), Lysozyme (Lyso), Gram and Soya Bean proteins with 0.5 mg % mL subsequent increment at temperatures from 293.1, 298.1 and 303.1 Kelvin (K) 0.05°C temperatures were obtained. The densities decrease with concentrations and temperatures except BSA, Gram and Soya with stronger structural interactions for BSA at lower temperatures. The viscosities increase with increase in conc. The Gram shows higher densities at 293.1 K with weaker hydrophobic and slightly higher hydrophilic interactions. The viscosities are higher than those of the water and infer entanglement of biopolymer molecules with drag of a solvent flow. So the proteins undergo structural unfolding with aqueous solutions due to a moderately polar (-NH~~CO-) peptide bond of the protein. Each protein showed stronger hydrophobic interactions than hydrophilic Interaction and Gram protein showed maximum densities at 293.1 K.

Key words: Structure breaking, making, hydrodynamic sphere, Newtonian flow.

INTRODUCTION

Amino acids with peptide bonds (-NH~~CO-) make large sized protein molecule known as biopolymers. Glycine (NH_2-CH_2-COOH) is model amino acid with CH_3CH_2[(COOH)NH_2] (α-alanine) and NH_2-$CH_2CH_2CH_2$-COOH (γ-butyric acid), the alpha (α) and α, omega (ω) series respectively. Thus the -NH~~CO- bonds adjoin many amino acid in chain that undergoes primary, secondary, tertiary and quaternary structures with optimization denoted as folded state of the proteins or globular proteins. Due to different electron density on NH and CO groups, the -NH~~CO- bonds remain accessible for interaction with water with distortion or the unfolding of an optimized structure with much entropic change noted as biothermodynamic[1-3] phenomenon. Due to peptide bonds with several amino acids, the globules develop many void spaces and polar sites inside the molecules. So largely the proteins remain hydrophobic and weakly hydrophilic where structured or hydrogen bonded water enters inside the molecule with exertion of pressure inside the molecules. So the biothermodynamics infer conformational state and activity in biological processes. So our data are useful for proteins interactions with water, salts, membranes, nucleic acids[4-6]. Barry and Irving[7] studied the viscosities of concentrated aqueous electrolytic solutions and Eisenberg and Pouyet[8] the electrostatic interactions of polyvalent systems with coupling approximation[9-11] with reduced viscosity. Rice and Kirkwood[12] studied the charged sites of macromolecules and a role of counter ion[13] with temperature[14] under voltage and current as external force affecting a natural behavior of proteins. The viscosities depict structure making and breaking effects on solvents, so the η_{sp}/c (reduced viscosity) is $\eta_{sp}/c=\eta_{inta}/c+\eta_{inter}/c$ and defines intramolecular hydrodynamic cage[15]. The η_{inta}/c and η_{inter}/c are the viscosities of intra and inter hydrodynamic cages[16-18] respectively. The data elucidate electronic structure[19] and an existence of

electronic unoccupied states in structural cavities caused by folding of polypeptides[20, 21]. Density functional theory with Silicon Graphics implementation of code[22] and local density approximate protein packing. Our data belong to a process where molecules freely move detaining a natural behavior and amino and carboxyl groups with intrahydrogen bonds[23-25] contribute to intrinsic viscosities[26]. Van der Waals forces often denote energy potential, as a function of distance with both the attractive and repulsion forces at close range depicted by Lennard-Jones potential[27]. The physical data on bioactive molecules are novel assistance for drug designing and protein engineering[30, 31]. The Soya lowers down cholesterol in human serum and explains a mechanism of lysine and arginine rich Soya protein with hypocholesterolemic effect. The LDC and HDC cholesterol are interrelated to physical data of the proteins[32-37] with hydrodynamic hydrate[38-41].

Table1: Densities, ρ in10^3kg m^{-3} and viscosity, η in kg $m^{-1}s^{-1}$ at different temperatures, in Kelvin K

			293.15 K			
mg %/ 100mL^{-1}	**BSA**		**E-Alb**		**Lyso**	
	ρ	**η**	**ρ**		**ρ**	
0.5	0.99788	1.0181	0.99865	1.0139	0.99861	1.0107
1.0	0.99862	1.1054	0.99863	1.0213	0.99849	1.0231
1.5	0.99854	1.1061	0.99855	1.0277	0.99847	1.0245
2.0	0.99835	1.1059	0.99839	1.0269	0.99831	1.0241
			298.15 K			
0.5	0.99698	0.8929	0.99699	0.8774	0.99700	0.8785
1.0	0.99686	0.8956	0.99696	0.8836	0.99686	0.9068
1.5	0.99673	0.8991	0.99684	0.8876	0.99684	0.9317
2.0	0.99668	0.8989	0.99679	0.8859	0.99679	0.9312
		303.15 K				
0.5	0.99641	0.7895	0.99625	0.7614	0.99634	0.8039
1.0	0.99622	0.7992	0.99623	0.7769	0.99621	0.8041
1.5	0.99619	0.8085	0.99623	0.7917	0.99607	0.8065
2.0	0.99617	0.8079	0.99619	0.7913	0.99601	0.8059
			29315 K			
0.5	Gram		Soya			
1.0	1.00015	1.0011	0.99702	1.0528		
1.5	1.00019	0.9970	0.99702	1.0520		
2.0	1.00027	0.9963	0.99704	1.0505		
0.5	1.00032	0.9961	0.99707	1.0475		
		298.15 K				
0.5	0.99543	0.8691	0.99546	0.8774		
1.0	0.99544	0.8684	0.99547	0.8773		
1.5	0.99545	0.8670	0.99548	0.8770		
2.0	0.99547	0.8640	0.99551	0.8765		
		303.15 K				
0.5	0.99379	0.7358	0.99436	0.7386		
1.0	0.99381	0.7351	0.99404	0.7376		
1.5	0.99386	0.7338	0.99406	0.7357		
2.0	0.99395	0.7308	0.99407	0.7322		

Table 2: Regression constants of densities and viscosities data.

Temp K	$\rho^0 \times 10^3$ kg m^{-3}	$S_d \times 10^3$ kg $m^{-3} mol^{-1}$	$B \times 10^{-3}$ m^3 kg^{-1}	$D \times 10^{-3}$ m^3 mol^{-1}	D
		BSA			
293.15	0.99721	0.8207	-23.63	52046	
298.15	0.99729	-0.3149	-1.10	3459	
303.15	0.99639	-0.2764	-32.58	22606	
		E-Alb			
293.15	0.99878	-0.1291	9.38	2856	
298.15	0.99719	-0.1892	-30.46	16279	
303.15	0.99631	-0.0220	-96.01	51987	
		Lyso			
293.15	0.99879	-0.1843	5.64	4651	
298.15	0.99720	-0.1999	-60.86	49260	
303.15	0.99671	-0.3383	8.45	-1698	
		Gram			
293.15	1.00438	0.7783	3174.08	-203	329
298.15	1.00123	0.1197	2268.33	-144	231
303.15	0.99808	0.4698	512.88	-33	54
		Soya			
293.15	0.99825	0.1380	4693.58	-296	477
298.15	0.99655	0.1348	2531.79	-159	256
303.15	0.99485	0.1445	617.17	-40	65

EXPERIMENTAL

Aqueous protein solutions were prepared with Millipore water, w/v, the densities and viscosities were measured with $20 \times 10^{-3} dm^3$ bicapillary pyknometer and Survismeter[42, 43] to ± 0.05°C, with Beckman thermometer. The BSA, E-Alb and Lyso were procured from Sigma and the Gram and Soya were extracted from raw dried seed powder of Soya and Gram, respectively and purified with standard methods. The measurements were carried out in a thermostatically controlled water bath with ± 0.05°C temperature accuracy, read on Beckman thermometer. Pyknometer and Survismeter were calibrated with aqueous[42] NaCl solutions at 298.15 K, with 1×10^{-5} mol kg^{-1} accuracy of solution concentration. Densities for water were used from literature[43]. Kinetic corrections to energy of Survismeter were with negligible shear on natural flow.

RESULTS AND DISCUSSION

The ρ values were calculated with equation 1.

$$\rho = ((w - w_e)/(w_0 - w_e))\rho_0 + 0.0012(1 - (w - w_e)/(w_0 - w_e)) \quad ...(1)$$

The ρ solution, ρ_0 solvent and 0.0012×10^3 kg mol^{-1} air densities, respectively. The $(1-(w-w_e)/(w_0-w_e))$ is buoyancy correction for air, m molality, w_e, w_0 and w are weights of empty, solvent and solution filled pyknometer, respectively. Errors in the densities were with standard statistical methods[43]. The viscosities (h) are calculated with equation 2.

$$\eta_r = [(\rho \times t)/(\rho_0 \times t_0)]\eta_0 \quad ...(2)$$

The ρ and ρ_0 densities of solution and solvents, t and t_0, the flow times, respectively. The ρ data were regressed with equations 3.

$$\rho = \rho_0 + S_d\, c \quad ...(3)$$

The ρ^0 is limiting density at infinite dilution $c \rightarrow 0$, the S_d is slope. An extended Jones Dole equation was used for viscosities data with equation 4.

$$(\eta_r - 1)/c = B\,c + D\,c + D'c \quad ...(4)$$

The B (kg mol^{-1}) Jones-Dole coefficient, D (kg mol^{-1})^2 and D' (kg mol^{-1})^3 are slopes. The D is conc. for protien-protien interactions. The $\eta_r = \eta/\eta_0$ is relative viscosity, the regression constant data are given in table 2.

The densities at 293.1, 298.1 and 303.1 K are as Gram > E Alb > Lyso > BSA > Soya, Lyso > E Alb > BSA > Soya > Gram and BSA > Lyso > E Alb > Soya > Gram respectively. The Gram, Lyso and BSA at 293.1, 298.15 and 303.1 K respectively infer stronger internal pressure with Gram on water molecules associated with –NH- and >CO polar groups due to a compact hydrated structure with higher densities.

The densities at 293.1 K are as Gram > E Alb > Lyso > BSA > Soya. So Gram and E-Alb-water interactions at 293.1 K are stronger which strengthen with increase in composition with a prominent caging of water around protein molecules. But the Lyso, BSA and Soya predict comparatively less internal pressure of polar groups with the weaker interactions and caging. With increase in compositions the interacting strength of the E-Alb remains similar but the strength of BSA, Gram and Soya slightly enhanced than those of the Lyso. With concentrations, the densities at 298.1 K are as Lyso > E Alb > BSA > Soya > Gram. Their densities with increase in compositions decrease except BSA, Gram and Soya but at 293.1 K, the densities for dilute solutions increase and then decrease for subsequent compositions. At 303.1 K, the densities of E Alb for 1.8 and 2.0 mg % are equal and also lower than those of 0.5 mg % (table1).

The trends predict a compact conformational structure at 1.6 mg % with the BSA due to stronger intramolecular interactions with higher densities. The densities of Gram and Soya slightly increase with increase in compositions with stronger protein-protein interactions. The E Alb shows weaker hydrogen bonding with water so the concentration hinders the protein-water interactions and develops protein-protein-water interactions rather than protein-water interactions (table1). The densities at 303.1 K are as BSA > Lyso > E Alb > Soya > Gram, with lowest densities for Gram and maximum densities for BSA with stronger peptide bond disruption with BSA and least with the Gram. Perhaps unfolded peptide bond develops stronger interaction with water dipoles while the water molecules enter inside void spaces of the Gram molecule and exert higher pressure with larger expansion and lower densities. Probably behavior of the Soya is near Gram while of the Lyso is near BSA. The E Alb shows moderate interaction with water. The densities decrease with temperature that weakens the protein-water interactions.

Limiting densities (ρ^0) of proteins with temperature are as 293.1 > 298.1 > 303.1 K except BSA. The values at 293.1 K are as (Lyso = E Alb) > BSA but at 298.1 K the Lyso and E Alb show equal and lower values than that of the BSA. The temperature weakens the intermolecular forces with comparatively lower internal pressure that lead to produce lower densities with temperature increase (table 2). The ρ^0 data at 293.1, 298.1 and 303.1 K are as Gram > Lyso > E Alb > Soya > BSA, Gram > BSA > Lyso > E Alb > Soya and Gram > Lyso > BSA > E Alb > Soya, respectively. It infers stronger interaction with Gram and weaker with Soya, respectively.

It infers almost similar interacting strength of the Lyso and E Alb at 293.1 and 298.1 but both the BSA at 298.1 K show slightly stronger strength. The densities as Lyso > BSA > E Alb at 303.1 K, show temperature effect on enzymatic activities of Lyso (table1). The ρ^0 data for Gram > Soya, infer stronger intermolecular forces with the Gram than of the Soya (table 2). The ρ^0 data are higher than of water with stronger hydrogen bonding where the hydrophilic interactions are weaker than those of the hydrophobic due to their amino acid residues. The S_d values are as Gram > Soya, and Gram with concentration infers Gram-Gram intermolecular interactions. The proteins tend to optimize a state and undergo several conformational changes with solvent with stronger Gram-Gram hydrophobic intermolecular interactions. The ρ^0 data decrease with K due to weakening in residual forces. Proteins

develop weaker London/dispersive forces with several interactions as per Fort and Moore observations[40, 41].

The S_d data at 293.1, 298.1 and 303.1 K are as BSA > Gram >Soya> E Alb > Lyso, Soya> Gram > E Alb > Lyso > BSA and Gram >Soya> E Alb > BSA > Lyso, respectively. With higher concentration effects with BSA, Soya and Gram at 293.1, 298.1 and 303.1 K respectively. An increase in concentrations does much disruption in structured water causing stronger interaction with BSA, Soya and Gram at lower, normal and slightly higher temperatures, respectively.

The S_d values are as BSA>E Alb>Lyso, E Alb>Lyso>BSA and E Alb > BSA > Lyso at 293.1, 298.1 and 303.1 K, respectively, and infer composition effect on the protein–water and protein-protein linkages. The higher S_d values at 293.1 K infer higher concentration effect on water structure disruption and the lower values at 298.1 and 303.1 K with slightly weaker composition effects[29] (table 2). Attractive forces multipoles of proteins are weaker than those of the ions and dipoles of water. The proteins with multipoles form intrahydrogen bonds between the -NH- and >CO groups to contribute to the interactions.

The viscosities at 293.1, 298.1 and 303.1 K are as Soya > BSA > E Alb > Gram > Lyso, BSA > Lyso > E Alb > Soya > Gram and Lyso > BSA > E Alb > Soya > Gram, respectively, with higher viscosities for Soya, BSA and Lyso at 293.1, 298.1 and 303.1 K. The biopolymers do cause entanglement of the solvents that drag down a flow with higher viscosities. Probably a primary hydration sphere of proteins detains its identity with increase in viscosities with concentration and decrease with temperature (table 1). As the protein-protein sphere of larger size hinders a viscous flow with torsional forces. An increase in the viscosities of polyelectrolyte with dilutions[30] could be attributed to an expansion effect of polyionic chains. The solutions show an alignment of counter ions that weakens a screening effect with concentration[31] with an increase in molecular size. This increases intramolecular forces with an increase in viscosities which increase with increase in concentrations causing stronger structural reorientation. But at 293.1 K, the disparity is noted with the BSA and Lyso where it first increases and then decreases as 16.2 < 73.8 > 57.8 and 8.8 < 15.1 > 12.5. The viscosities of a Lyso at 303.1 K, decrease from 0.5 to 1.6 mg % and then increase for 2.0 mg % as 7.1 > 5.3 < 5.8. The E-Alb develops weakly non-Newtonian solutions with viscosities at 293.1 K for 0.5 mg % as BSA > E Alb > Lyso, 1.8 mg % BSA > E Alb < Lyso and 2.0 mg % BSA > E Alb > Lyso (table1). The viscosities at 298.1 K are positive but at 298.1 and 303.1 K, are negative and increase with compositions, the BSA and Lyso show positive values with a weaker cage around the protein molecules[32]. Molecular size enhances the viscosities[33].

The B values are as Soya > Gram > E Alb > Lyso > BSA, Soya > Gram > BSA > E Alb > Lyso and Soya > Gram > Lyso > BSA > E Alb at 298.1, 293.1, 303.1, respectively (table 2). It infers lager sized hydrodynamic sphere with both the Soya and Gram while small sized with BSA, Lyso and E Alb at 298.1, 293.1, and 303.1, respectively. The B values are as 298.1 > 293.1> 303.1, 293.1 > 298.1 > 303.1 and 303.1 > 293.1 > 298.1 K for the BSA, E Alb and Lyso (table 2). The protein-water interactions are indirect because with time and temperature, the viscosity changes. The hydration is temperature dependent and varies with size of hydrated sphere. For example at 293.1, 298.1 and 303.1 K, the B values are as E Alb > Lyso > BSA, BSA > E Alb > Lyso and Lyso > BSA > E Albumin. It infers a state of hydration and disruption of hydrogen bonds with structural spontaneity.

The B data are as Soya>Gram with higher decrease in temperature are from 112 to $2162/10^{-3}$ kg mol^{-1}, with higher hydrodynamic sphere than of the Gram. The D values are as BSA> Lyso > E Alb > Gram > Soya, Lyso > E Alb > BSA > Gram > Soya and E Alb > BSA > Lyso > Gram > Soya at 298.1, 293.1, 303.1, respectively. It infers stronger interactions with BSA, Lyso and E Alb at 298.1, 293.1 and 303.1, respectively. The D values with compositions denote higher structural tendency of BSA and E-Alb at lower and higher temperatures respectively. The D values for BSA, E Alb and Lyso are as 293.1 > 303.1 > 298.1, 303.1 > 298.1 > 293.1 and 298.1 > 293.1 > 303.1 K, respectively. The B values at 298.1 are higher for BSA and at 298.1

and 293.1 for E Alb and 298.1 and 303.1 K for Lyso, respectively. It infers a larger sized protein-water sphere at 298.1 K that develops stronger torsional forces with higher B values for BSA, E Alb and Lyso at 298.1, 293.1 and 303.1 K. The higher D values with BSA, E-Alb and Lyso at 293.1, 303.1 and 298.1 K illustrate higher hydrodynamic volume contribution at respective temperatures. The D' values are Soya > Gram at 3 temperatures and illustrate interaction dynamics (table 2) and structural reorientations.

CONCLUSION

The ρ^0 data decrease with temperature weakening in van der Waals forces, and higher internal pressure shrinks a protein hydrate size. The compositions affect the proteins interaction. Hydrophobic structure making or hydrophilic breaking tendency was rationalized with the densities and viscosities. The ρ^0 and B data denote solute-solvent interactions with Soya-Soya stronger interactions than that of the Gram-Gram.

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