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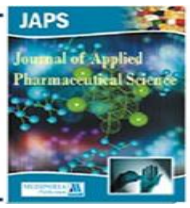

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Equilibrium dialysis studies on the binding of anionic surfactants with bovine milk casein

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ABSTRACT

The binding process of amphiphile molecules with proteins depends on the experimental conditions, such as protein concentration and surfactant type. The proteins may have different binding characteristics, such as specific and cooperative binding. Specific binding is the most important pathway observed at low surfactant concentration at levels of micromolar unity, while the cooperative process becomes the most important at higher surfactant concentration, close to the critical aggregation concentration. At the levels at which specific binding occurs there is competitivity, i.e., the presence of an additional molecules can be induced hydrophobically or electrostatically depending on the characteristics of the molecular structure and experimental conditions. For instance, electrostatic binding is pH dependent and this factor is important for ionic surfactants.

Keywords: Bovine milk casein, Equilibrium dialysis.

INTRODUCTION

Surfactants may directly interact with proteins by binding to them, which may lead to substantial changes in proteins conformation i.e. (Ananthapadmanabhan *et al.*, 1993) (Jones *et al.*, 1995). Several methods have been used to characterize protein-surfactant interaction including equilibrium dialysis (Ray *et al.*, 1966) (Reynolds *et al.*, 1970) spectroscopic (Turro *et al.*, 1995) (Chen *et al.*, 1995) light scattering (Valstar *et al.*, 2000) (Valstar *et al.*, 1999) surface tension (Santosh *et al.*, 2003) (Nishikido et al., 1982) surfactant surface selective electrodes (Oakes *et al.*, 1974) (Mokus *et al.*, 1998). A direct and effective method to study the binding between surfactant and macromolecules is measuring the binding amount of surfactant to macro moles (Luan *et al*, 2003). Equilibrium dialysis is one of the earliest methods which are used to study the interaction of surfactants with protein. Studies on the formation of soluble protein-surfactant complex have provided a better insight into the nature of protein-surfactant interactions in general. These soluble complexes are formed during the interaction of anionic surfactant with proteins above their isoelectric pH (IEP).

Arora *et al*, 2003 have been studied the interaction of surfactants to proteins above IEP by different physico-chemical methods. Recently, Singh et al. and other workers have also investigated the interaction of surfactants with proteins (George *et al*, 2009). However, the binding of surfactants to bovine milk casein is not available in the existing literature. It was though of interest to study the interaction of anionic surfactants to bovine milk casein. In this chapter of the thesis, the binding of sodium dodecyl sulphate (SDS) and sodium octyl sulphate (SOS) to bovine milk casein is described in order to explain the nature of bonding and the structural implication induced with in the molecule. A mechanism of interaction has been proposed for surfactant-protein binding. Understanding the surfactant-protein interaction at the molecular level is an important and complicated research area since proteins are complex biomacromolecules with unique primary structure expressed in term of their ammo acid sequences. A relevant point is which are the sites of proteins of portein that surfactant molecule bind to and how surfactants bind to these sites. Another relevant aspect is finding the factors of the surfactant molecular structure that determine the binding of surfactants to proteins.

MATERIALS AND METHODS

This solution was stored in a refrigerator (ii) SDS and SOS were purchased from Sigma chemical company and were pure samples. SDS and SOS solutions were prepared in double distilled water. These were not further purified and used as such for the binding studies. Their critical micelle concentration were found to be 8.2 x 10"3 M and 13.5 x 10"2M, respectively, by conductance measurements, (iii) Phosphate- sodium chloride buffers of pH 7.50 and 9.50 were prepared from reagent grade-chemicals in double distilled water using standardized Systronic pH-meter having a wide range glass electrode.

Equilibrium Dialysis

Normally the equilibrium dialysis is carried out by equilibrating macromolecule solution taken inside the bag against the ions under study outside the bag in a boiling test tube. Applying (Yang et al, 1953) a modified method was adopted which proved advantageous over the conventional method is two ways. Firstly, it was possible to cover a much wider concentration range of the surfactant due to the fact that most of the anions were immediately bound to the macromolecule of protein and thus not precipitated out. Secondly the reaction was complete for one day dialysis. This technique consists in keeping aliquots of protein-surfactant mixtures in the required buffer for atleast two days at 25°C and then dialysing against equal volumes of the same buffer for another two days. The quantity of free surfactants is determined in the dialysate.

Analysis of Surfactant Solution

To 1.0 ml of solution of pararosanaline hydrochloride (4.0 x 10"4M) in a stoppered pyrex glass test tube appropriate volume of test solution not exceeding 4.0 ml was mixed. The total volume was made 5.0 ml by adding required amount of buffer. 5.0 ml of mixed solvent (50% CHC13 + 50% ethyl acetate) was added for the extraction of dye-surfactant complex into the organic phase. The tube was stoppered and was shaken by hand about 50 times. Centrifugation for one minute at 5000 r.p.m. in a centrifuse resulted in a complete separation of the organic and aqueous phases, the former containing coloured complex at the bottom. Its absorption was measured on Elico-spectrophotometer using green filter against a reference tube filled with the solvent.

RESULTS AND DISCUSSION

In the data, C_B (Bound concentration of surfactant) was determined with the help of estimated free (unbound) concentration of surfactant as C_F (because $C_B=C_T - C_F$) with the help of C_B, average number of moles of surfactant bound per 105 gm of protein (V_m) was calculated from $C_B/[P]=V_M$, where [P] is the concentration of protein in gm/litre per 10^5 gm. The data obtained from equilibrium dialysis studies are given in Tables 1 to 4. Quantitative studies on the binding of sodium dodecyl sulphate (SDS) and sodium octyl sulphate (SOS) with bovine milk casein were made by a modified equilibrium dialysis method. With a view to understanding the nature of the binding of surfactants with protein, the values of V_M, the moles of SDS and SOS bound per 10^5 gm of protein at pH 7.50 and 9.50 were plotted against log C_F where C_F, is the concentration of the unbound surfactant. These plots could be conveniently divided in three regions, viz., A, B and C. In the region A, the plots are linear indicating thereby a statistical distribution of the surfactant over the available interaction sites on the protein. This inference was further substantiated from the plots of $1/V_M$ vs. $1/C_F$ which had straight line portions. The values of binding sites (n) in form of the reciprocal of the intercept on the ordinate obtained after extrapolation corresponds of the straight line as $1/C_F$ approaches zero as a limit and slope of curve is 1/Kn. K is the average association constant with various surfactant-protein combinations, are given in Table 5. These values of n represent the maximum number of binding sites available in this region (Table 5). Beyond the region A, the values of V_M exhibit a sudden rise and the curves deviate from linearity. In all probabilities, after occupying a certain number of sites on the protein molecule (the values of which may correspond to those of V_M), the surfactant ions disrupt the once tightly folded protein molecule so that hithertofore inaccessible sites open up for occupation. With the progressive loosening of the protein structure, the potential barrier to the entry of the surfactant anions decreases resulting in the breaking of internal linkages.

In the region C, V_M increases very rapidly and apparently without limit, for exceeding the number of total cationic groups in the protein. The excessive binding occuring in this region may be explained on the assumption that the binding of one surfactant ion at a site on the protein favours the binding of additional surfactant ions in its immediate vicinity through hydrophobic interactions of the paraffin chains (similar to that in the formation of micelles). In other words, surfactant micelles or aggregates are formed on the protein molecule whereby the values of VM register a sharp increase.

Table. 1: Binding of sodium dodecyl sulphate (SDS) with bovine milk casein (concentration=6.0 gm/litre fixed) at pH 7.5O and temperatures 25°C.

Molar Conc^n of SOS X 10^5	Molar conc^n. of free SOS C_F x10^5	Molar conc^n. of bound SOS C_B x 105	Molar SOS bound per 10^5 protein (V_M)	logC_F +(5)	$\frac{1}{V_M}$	$1/C_F$ x 10^{-5}
20	2.00	18	3.0	0.3010	0.333	0.500
30	3.00	27	4.5	0.4771	0.222	0.333
40	4.00	36	6.0	0.6021	0.167	0.250
60	7.00	53	8.8	0.8451	0.114	0.143
80	14.00	66	11.0	1.1461	0.090	0.071
100	22.00	78	13.0	1.3424	0.770	0.045
120	24.00	96	16.0	1.3802	0.062	0.041
200	35.00	165	27.5	1.5441	0.0370	0.028
300	54.00	246	41.0	1.7324	0.0244	0.018
400	70.00	330	55.0	1.8451	0.0182	0.014
600	114.00	486	81.0	2.0569	0.0135	0.006

Table. 2: Binding of sodium octyl sulphate (SOS) with bovine milk casein (concentration=6.0 gm/litre fixed) by equilibrium dialysis method at pH 7.50 and temperatures 25°C.

Molar Conc^n of SOS X 10^5	Molar conc^n. of free SOS C_F x10^5	Molar conc^n. of bound SOS C_B x 105	Molar SOS bound per 10^5 protein (V_M)	logC_F +(5)	$\frac{1}{V_M}$	$1/C_F$ x 10^{-5}
20	5.0	15.0	2.50	0.6990	0.400	0.200
30	7.5	22.5	3.75	0.8751	0.266	0.133
40	11.0	29.0	4.80	1.0414	0.208	0.091
60	18.0	42.0	7.00	1.2553	0.143	0.055
80	29.0	51.0	8.50	1.4624	0.117	0.035
100	37.0	63.0	10.50	1.5682	0.095	0.027
120	45.0	75.0	12.50	1.6532	0.080	0.022
200	68.0	132.0	22.00	1.8325	0.045	0.014
300	108.0	192.0	32.00	2.0334	0.031	0.009
400	142.0	258.0	43.00	2.1492	0.023	0.007
600	228.0	372.0	62.00	2.3579	0.016	0.004

Table. 3: Binding of sodium dodecyl sulphate (SDS) with bovine milk casein (concentration=6.O gm/litre fixed) by equilibrium dialysis method at pH 9.50 and temperatures 25°C.

Molar Conc^n of SOS X 10^5	Molar conc^n. of free SOS C_F x10^5	Molar conc^n. of bound SOS C_B x 105	Molar SOS bound per 10^5 protein (V_M)	logC_F +(5)	$\frac{1}{V_M}$	$1/C_F$ x 10^{-5}
30	9.0	21	3.5	0.9542	0.285	0.1110
40	13.0	27	4.5	1.1139	0.222	0.0770
60	21.0	39	6.5	1.3222	0.154	0.0476
80	32.0	48	8.0	1.5051	0.125	0.0312
100	43.0	57	9.5	1.6335	0.105	0.0232
120	54.0	66	11.0	1.7324	0.091	0.0185
200	74.0	126	21.0	1.8692	0.0476	0.0135
300	102.0	198	33.0	2.0086	0.0303	0.0093
400	130.0	270	45.0	2.1139	0.0222	0.0077
600	192.0	408	68.0	2.2833	0.0147	0.0052

Table. 4 : Binding of sodium octyl sulphate (SOS) with bovine milk casein (concentration - 6.0 gm/litre) by equilibrium dialysis method at pH 9.50 and temperatures 25°C.

Molar Conc^n of SOS X 10^5	Molar conc^n. of free SOS C_F x10^5	Molar conc^n. of bound SOS C_B x 105	Molar SOS bound per 10^5 protein (V_M)	logC_F +(5)	$\frac{1}{V_M}$	$1/C_F$ x 10^{-5}
30	13.5	16.5	2.75	1.1303	0.36	0.0740
40	19.0	21.0	3.50	1.2788	0.286	0.0526
60	30.0	30.0	5.00	1.4771	0.200	0.0333
80	42.5	37.5	6.25	1.6284	0.160	0.0236
100	55.0	45.0	7.50	1.7404	0.133	0.0182
120	63.0	57.0	9.50	1.7993	0.105	0.0159
200	98.0	102.0	17.00	1.9912	0.059	0.0102
300	144.0	156.0	26.00	2.1584	0.038	0.0070
400	190.0	210.0	35.00	2.2788	0.029	0.0052
600	288.0	312.0	52.00	2.4594	0.019	0.0034

Table. 5 : Binding constants of surfactant-bovine milk casein system.

Constant	SDS- Bovine milk casein system		SOS- Bovine milk casein system	
	pH 7.50	pH 9.50	pH 7.50	pH 9.50
Binding site	Ĥ5	19.4	25	19.2
Intrinsic association constant (K)	6000	1933	5600	1017

These aggregates may consist of a single palisade layer of surfactant molecule clustered about a binding site on the protein. These micelles on the protein may be further stabilized through interactions of paraffin tails with hydrophobic residues of the amino along the polypeptide chain. The values of V_M in the region A are significantly lower at the higher pH. However, the values at both pH 7.50 and 9.50 approach close in the region B and still closer in the region C. This is because the binding of the surfactant in the region A, depends on the total number of surface cationic groups available on the protein molecule which decreases at higher pH. The binding in the regions B and C involves hithertofore inaccessible sites now presented by the unfolded protein molecules and tends to become increasingly independent of the surface cationic groups on the protein molecules.

CONCLUSION

In the present studies, the average number of binding sites and their association constants are given in Table 5. These values indicated that binding occurrs strongly for SDS in comparison to SDS at pH 7.50 as well as at 9.50 with bovine milk casein. In these binding constants decrease from pH 7.50 due to deprotonation of cationic groups of protein molecules. These data of interaction of SDS and SOS with bovine milk casein resembled to the binding of SDS with BSA and HSA. In interaction of SDS with milk casein at pH 7.50 and 9.50, the values of K are 6.0 x 103 and 1.933 x 103, respectively which are closure to SDS-BSA system during spectroscopic studies.

REFERENCES

Ananthapadmanabhan K.P. (Eds.) Interaction of surfactants with polymers and protein, Boca Raton, CRC (1993) 319-366.

Arora J.P.S., Vineeta and Singh R.P., Lauryl Trimethyl Ammoniums with New Type Counteranion Tenside Surfact. Detergents, 1993; 30 (2), 136-141.

Azzam E.M.S., Study of Interfacial Tension in an Acid-Free Oil/Alkali/ Surfactant System. Journal of Surfactant and Detergent, 2007; 10, (1), 13-17.

Chen A., Wu D. and Johnson C.S., Synthesis, Structural Properties and Applications of Gemini Surfactants. J. Phys. Chem., 1995; 99, 828-834.

Dubey R.K. and Tripathi D.N., Lauryl Trimethyl Ammoniums with New Type Counteranions. Ind. J. Biochemistry and Biophysics, 2005; 49, 301-306.

George J., Sudheesh PO. and Neelakanta P., Reddy J. Influence of Salt on Cationic Surfactant-Biopolymer Interactions in Aqueous Media. Solution Chem., 2009; 38, 373-381.

Jones M.N. and Champman M.N., Design principles for self-assembly with short-range interactions Monolayers and Biomembrances, New York, Wiley (1995) 58.

Luan X.Y., Xu G.Y. and Chen A.M., Applications of Surfactant-selective Membrane Electrode in the Study of the Interactions between Surfactants and Polymers. Acta Phys-Chim Sin, 2003; 19 (2), 185-192.

Madaeni S.S. and Rostami E., Spectroscopic Investigation of the Interaction of BSA with Cationic Surfactants. Chem. Eng. Technol., 2008; 31 (9), 1265-1271.

Mokus M., Kragh-Hansen U., Letelher P., Maire P. Le. and Moller J.V., Construction and Use of a Detergent-Sensitive Electrode to Measure Dodecyl Sulfate Activity and Binding. Anal. Biochem., 1998; 264,1, 34-40.

Nishikido N., Takahara T., Kobayashi H. and Tanak Interaction between Hydrophilic Proteins and Nonionic Detergents Studied by Surface Tension Measurements. Bull. Chem. Soc. Japan, 1982; 55, 3085-3088.

Oakes J., Protein–surfactant interactions. Nuclear magnetic resonance and binding isotherm studies of interactions between bovine serum albumin and sodium dodecyl sulphate. J. Chem. Soc., Faraday Trans. 1974; I, 70, 2200-2209.

Reynolds J.A., Gallagher J.P., Steinhardt J., Effect of pH on the binding of N-alkyl sulfates to bovine serum albumin. Biochemistry, 1970; 9, 1232-1238.

Singh R.P., Arora P., Arora S.K. and Chaudhary R. Interaction of Transfusion Gelatin Molecule with Cetylpyridinium Chloride. Asian Journal of Chemistry, 2008; 20 (6), 4647-4658.

Singh R.P., Arya S.V. and Chaudhary R., Peptides of a biologically active tryptic digest of bovine growth hormone. Journal of Acta Ciencia Indica, 2008; 34C (2), 223-227.

Turro N.J., Lei X.G., Ananthapadmanabhan K.P. Spectroscopic Probe Analysis of Protein-Surfactant Interactions: The BSA/SDS System. Langmuir, 1995; 11, 2525-2533.

Valstar A., Alingren M., Brown W. and Vasilesae M., The Interaction of Bovine Serum Albumin with Surfactants Studied by Light Scattering. Langmuir, 2000; 16, 922-927.

Valstar A., Brown W. and Almgren M.,The Lysozyme−Sodium Dodecyl Sulfate System Studied by Dynamic and Static Light Scattering. Langmuir, 1999; 15, 2366-2374.

Yang J.T. and Foster J.F., Statistical and All-or-None Binding of Alkylbenzenesulfonate by Albumins. J. Am. Chem. Soc., 1953; 75, 22, 5560-5567.