Oriental Journal of Chemistry Vol. **24**(3), 977-982 (2008)

The Interaction of surfactants to gliadin (Wheat Protein) by dialysis equilibrium method

R.P. SINGH[1]*, S. KUMAR[1], POONAM C. KUMAR[2] and R. CHAUDHARY[2]

[1]Chemistry Department, S.G.P.G. College, Sarurpur Khurd, Meerut (India).
[2]Chemistry Department, R.K. Goel Institute of Technology, Ghaziabad - 201 001 (India).

(Received: April 12, 2008; Accepted: June 04, 2008)

ABSTRACT

A study on the binding of sodium dodecyl sulphate (SDS) and sodium octyl sulphate to gliadin was made by employing dialysis equilibrium method. The binding data reveals that the rising molar concentration of the surfactants correspond to the changes in the binding process. In the lower concentration range (region A) of the surfactants, the plot V_M Vs log C_f represented linear behaviour. This region was found to have maximum number of binding sites (n) available. Beyond region B, the way of binding is found to change and binding appears to be more statistical in nature. In region C of binding isotherm V_M is found to increase apparently without limit. The linking is probably be much weaker in this region than the region A due to the involvement of the non-specific forces. In the region C which is the region of relatively low proportion of protein to surfactant, the value of V_M increases apparently without limit and its value for exceed the number of total positively charged groups in the protein molecule. The data show that either any conformation change or binding of surfactant with gliadin gives abnormal binding isotherms.

Key words : Sodium dodecyl sulphate, Sodium octyl sulphate, Gliadin.

INTRODUCTION

A lot of work on the binding of surfactants to proteins has been done by Arora *et al.*[1-16] and others[17-34]. However the binding of surfactants to seed proteins is not available in the existing literature. It was thought of interest to study the interaction of anionic surfactants to gliadin (a fraction of wheat gluten). In this paper, the binding of sodium dodecyl sulphate (SDS) and sodium octyl sulphate (SOS) to gliadin is described in order to explain the nature of bonding and the structural implications induced within the molecule. A mechanism of interaction has been proposed for surfactant-protein binding.

EXPERIMENTAL

Protein solution

Gluten powder (BDH) was kept over night immersed in light petroleum ether to extract out fats and oils. The process was repeated several times to extract all the gliadin fraction of the powder. The fractions were isolated as following.

´ Extract in 50% alcohol contained the protein gliadin. Alcohol (solvent) was paractically distilled off and the residue left behind was washed by double distilled water and dried. It was then dissolved in a very dilute KOH solution and dialysed to get an isoionic solution of gliadin protein. Its concentration

was determined by colorimetric biuret method as well as gravimetrically. It was stored in a refrigerator under toluene to check and avoid the surface denaturation.

The residue left behind after alcohol seperation was dried and dissolved in a minimum amount of very dilute KOH solution. The protein was then precipitated by the addition of dilute HCl. It was again dissolved in dilute HCl. The process was repeated many times to get a sample of pure glutenin. It was finally dissolved in dilute KOH solution and dialysed against double distilled water to get an isoionic protein solution. Its concentration was also determined by colorimetric biuret method.

Surfactants solutions

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 (CMC) were found to be 0.0082 and 0.135 moles per litre, respectively, by conductance measurements.

Buffer solution

Phosphate-sodium chloride buffer of pH 7.50 was prepared from reagent grade chemicals in double distilled water using the composition: 0.0321M K_2HPO_4, 0.0036M KH_2PO_4 and 0.10M NaCl. Ionic strength of this buffer was 0.2M.

Technique

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 and Foster (25), a modified method was adopted which proved advantageous over the conventional method in 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 needed 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.

Procedure

20.0 ml aliquots of gliadin surfactant mixture in which the concentration of SDS and SOS vaired from 0.15×10^{-3}M to 20.0×10^{-3}M at fixed concentration of protein (6/105g) were obtained by mixing required amounts of buffer, 6% protein, 0.05M SDS and SOS solutions. These were stored for two days at 25°C. 5.0ml protein from each aliquot was then transferred in a dialysis tubing and equilibrated against a 5.0 ml solution of the phosphate buffer in stoppered boiling tubes for two days at 25°C in a thermostat. The boiling tubes containing the bags were gently shaken by means of an electrical shaker. The dialysates were then analysed spectrophotometerically (36).

Analysis of surfactant solution

To 1.0 ml solution of pararosanaline hydrochloride (4×10^{-4}M) in a stoppered pyrex glass test tube appropriate volume of test solution not exceeding 4.0 ml was mixed. The total volume was made upto 5.0 ml by adding required amount of the phosphate buffer. 5.0 ml of mixed solvent (50% CHCl3 + 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 centrifuge resulted in a complete separation of the organic and aqueous phases, the former containing coloured complex at the bottom. Its absorption was measured on Klett summersion photoelectric calorimeter using green filter against a reference tube filled with the solvent.

RESULTS AND DISCUSSION

The effect of the addition of SDS and SOS to gliadin is shown in Fig. 1, where the average number of moles of surfactants to per 10^5 gm of protein, V_M is plotted against the log of free concentration of the unbound surfactant, i.e. log C_F. An insight on the plots of V_M Vs Log C_F (Fig. 1) reveals that the mode of linking changes with the rising molar concentration of the surfactants, corresponding to these changes in the binding process, each plot has been roughly divided into

three zones A, B and C respectively. This is necessary to have some demarcation in a veiw to consider the mechanism of the surfactant-gliadin combination.

In the lower concentration range of the surfactants (region A), the plot of V_M Vs Log C_F represented linear behaviour. It may be concluded that in region A the surfactant is distributing itself over all the available protein molecule in a more or less statistical manner. Similar results have been reported by Klotz *et al*. (37) for dye-protein interactions. The applicablity of simple statistical theory can be tested in this study from the plots of $1/V_M$ against $1/C_F$ when a linear plot is obtained (Fig. 2) upto a certain limit. The values of the

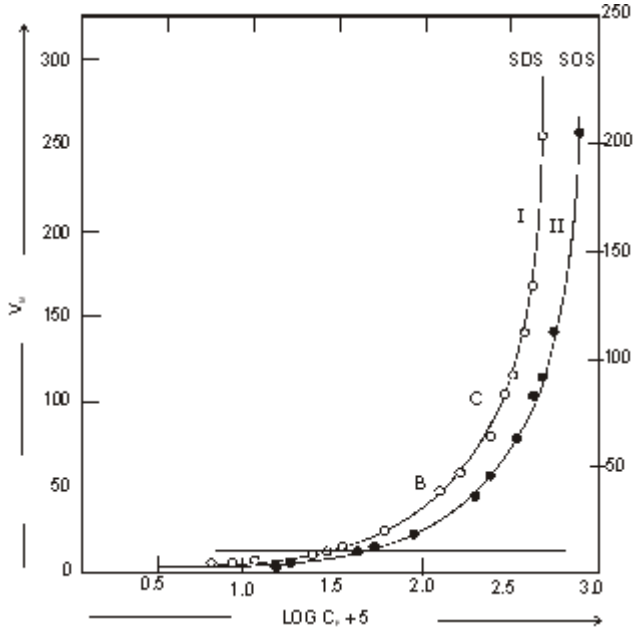

Fig. 1: Plots of V_M VS log C_F for surfactant gliadin systems

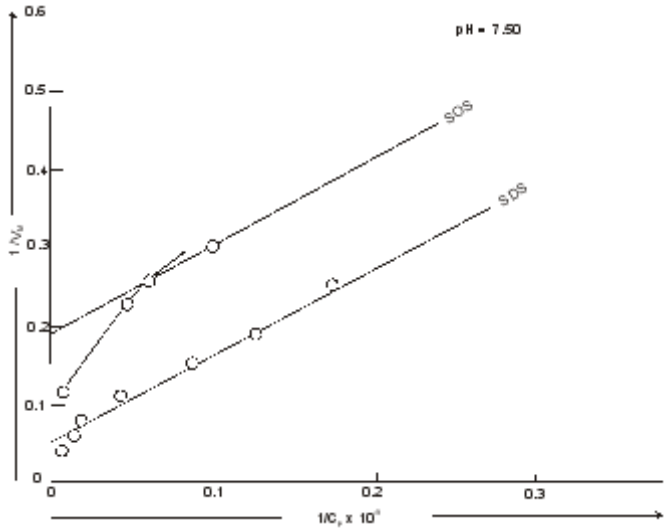

Fig. 2: Plots of $1/V_M$ VS $1/C_F$ for surfactant gliadin systems

reciprocal of the intercepts on the ordinate obtained after extrapolation of the straight lines of SDS and SOS and gliadin are 16 and 23, respectively. These values represent the maximum number of binding sites (n) available in the region A or the maximum value of V_M in this region of statistical binding.

Beyond the region A, the way of binding is found to change and the binding appears to be no more statistical in nature. It may be observed that towards the end of the region A, the curves in Fig. 2 deviate from linearity in the direction of higher values of V_M. After reacting with the number of moles of surfactants i.e. 16 and 23, as a maximum for statistical binding, there is a significant change in the interaction process and a large number of ions enter into combination presumably as a unit. It is possible that after a certain number of sites have been occupied, the surfactant ions disrupt the once tightly folded protein molecule and enter into combination with the less accessible sites. With the loosening of protein structure, potential barrier to the entrance of the anions is reduced resulting in the all or none reaction. Lundgren *et al.*[38] has first reporte 'all or none' characterstic of the binding of surfactant ions with ovalbumin which was confirmed later by Yang and Foster[35]. These workers on the basis of electrophoretic moboility studies further concluded that bovine serum albumin is not fully unfolded even in the second stage.

In region C of the binding isotherms, VM increases apparently without limit. The values of VM far exceed the number of total cationic groups available in the protein molecule. The linking may probably be much weaker in this region than in the region A due to the involvement of the non-specific forces. The present results find support in the work of Yang and Foster[35] on the interaction of sodium dodecylbenzene sulphonate with serum albumin. Such, extrabound surfactant ions were also found by Lundgren *et al.* [38] from electrophoretic analysis in case of ovalbumin. Strauss *et al.*[39] also reported very high value of V_M=165 in case of serum albumin from electrical transference method. He further concluded that the reaction in this region is irreversible.

The excessive binding of surfactant to this protein can be explained by assuming that the binding of one surfactant ion at a site on the protein favours the binding of additional surfactants in its immediate vicinity through the hydrophobic interactions of the apolar (paraffin) chains. It may be concluded from this that surfactant linking sites may act as a nuclei for the formation of a kind of micellar cluster on the protein[40]. Sodium dodecyl sulphate (SDS) well below critical micelle concentration (CMC) is known to form a solubilizing complex with a dye in presence of bovine serum albumin (BSA), a property shown by surfactants above CMC, which goes to show that surfactant on the protein forms a kind of micelle or aggregate[41].

A certain minimum concentration of the surfactant is desired to form these aggregates on the protein which may correspond to the value of V_M at which extensive uncoiling of the protein resulting to denaturation takes place. This is in accordance with the results of present study which indicate extensive unfolding in the upper range of region B. These aggregates or micelles may consist of a single palisade layer of a few molecules clustered about a binding site on the protein.

It may be expected that the hydrocarbon tails of the surfactants bound in monomeric or polymeric form are stabilized through the interaction with hydrophobic residues of amino acids along the polypetide chain. Unfolding of the polypeptide chain through denaturation permits a close approach of these hydrophobic residues to the bound micelles. The interaction of hydrophobic parts of protein and bound aggregate may be the driving force for denaturation of the protein by alkyl sulphonates. From the results of present binding study a probable mechanism for surfactant binding and consequent unfolding can be proposed. The linking presumably involves two possible modes of linkages of gliadin, and the other is non-electrostatic involving forces that normally bind the surfactant ions into micelles. In the initial stages (region A) it is the former which dominates while in region B, the latter type of linkage is more probable. In region C, the phenomenon of uncurling seems to tend towards completion. The data show that either any conformational change or binding of surfactant with gliadin gives the abnormal binding isotherms[42].

REFERENCES

1. Arora J.P.S., Singh R.P., Soam D. and Singh S.P., *Bulletin De La Societe Chimique De France,* **1**(2): 19 (1984).
2. Arora J.P.S., Singhal V.K., Singh S.P. and Kumar R., *Tenside Detergents*, **21**: 152 (1984).
3. Arora J.P.S. and Malik S.P., Studia Biophysica, **100**(3): 181 (1984).
4. Arora J.P.S., Singh R.P., Malik S.P. and Jain S., *Tenside Detergents*, **23**: 186 (1986).
5. Arora J.P.S., Singh S.P. and Malik S.P., Bulletin De La Societe Chimique De France, **2**, 167 (1985).
6. Arora J.P.S., Singh R.P. and Singhal V.K., *Tenside Detergents*, **21**: 197 (1984).
7. Arora J.P.S., Singhal V.K., Singh S.P. and Kumar R., *Tenside Detergents*, **21**: 22 (1984).
8. Arora J.P.S., Singh R.P., Singhal V.K. and Kumar R., *Tenside Detergents,* **21**, 87 (1984).
9. Arora J.P.S., Singh R.P., Singhal V.K. and Singh S.P., *Tenside Detergents*, **22**(3): 123 (1985).
10. Arora J.P.S., Pal C. and Jain P.B., *Studia Biophysica*, **126**: 61 (1988).
11. Arora J.P.S., Pal C. and Dutt D., *Tenside Surfact. Detergents*, **28**(3): 215 (1991).
12. Arora J.P.S., Verma Km. V. and Balyan Km. K.L., *Tenside Surfact. Detergents,* **29**(2): 124 (1992).
13. Arora J.P.S. and Balyan Km. K. L., *Tenside Surfact. Detergents*, **29**(1): 48 (1992).
14. Arora J.P.S., Vineeta and Singh R.P., Tenside Surfact. Det. **29**(6): 418 (1992).
15. Arora J.P.S., Vineeta Km. and Chand M., *Tenside Surfact. Detergents,* **30**(2): 136 (1993).
16. Arora J.P.S., Arora S.K. and Duggal A.K., *Tenside. Surfact. Det.* **35**(1): 24 (1998).
17. Karpenko V., Skrabana R. and Chand M., *Tenside. Surfact. Det.*, **35**(1): 8 (1998).
18. Kumar A., Pattarkine M., Bhadhade M. and Sastry M., Adv. Mater., **13**: 341 (2001).
19. Li M., Wong K. and Mann S., *Chem. Mater,* **11**: 23 (1997).
20. Storhoff J.J., Lazarides A.A., Mucie R.C., Mirkin C.A., Letsinger R.L. and Schatz S.C., *J.Am. Chem. Soc.,* **122**: 4640 (2000).
21. Kumar S., Phadtare S., Pastricha R., Guga P., Ganesh K.N. and Sastry M., *Current Science.,* **84**(1): 71 (2003).
22. Dubey R.K. and Tripathi D.N., *Ind. J. Bochemistry and Biophysics*, **49**: 301 (2005).
23. Chatterjee A. and Moulik S.P., *Ind. J. Biochem. Biophys.*, **42**(4): 205 (2005).
24. Pozharski E. and MacDonald R.C., *J. of Biophysics*, **83**: 556 (2002).
25. Sobate R. and Estelrich J., *International Journal of Biological Macromolecules* **28**(1-2): 151 (2001).
26. Buron C., Filiatre C., Membrey F., Foissy A. and Argillier J.F., Colloid and Polymer Science, **28**(5): 446-453 (2004).
27. Bordbar A.K., Omidiyan K. and Hosseinzadeh H., Colloids and Surface B, **40**: 67 (2005).
28. Lu R.C., Cao A.N., Lai L.H. and Xiao J. X, *Journal of colloid and Interface Science* **293**: 61-68 (2006).
29. Blanco E., Ruso J.M., Sabin J., Prieto G. and Sarmiento F., *J. Thermal and colorimetry,* **87**(1): 211-215 (2007).
30. Krejci J., *International Journal of Cosmetic Science*, **29**(2): 121 (2007).
31. Azzam E.M.S., *Journal of Surfactant and Detergent*, **10**(1): 13-17 (2007).
32. Singh R.P., Tomer A.S. and Chaudhary Romy, *Asian Journal of Chemistry*, **20**(6): 4503-10 (2008).
33. Singh R.P., Arya S.V. and Chaudhary Romy, *Journal of Acta Ciencia Indica*, (Accepted 2007).
34. Singh R.P., Arora P., Arora S.K. and Chaudhary R., *Asian Journal of Chemistry,* **20**(6): 4647-58 (2008).
35. Yang J.T. and Foster J.F., *J. Am. Chem. Soc.,* **75**: 5560 (1953).

36. Karush F. and Sonenberg M., *Anal. Chem.,* **22**: 175 (1950).
37. Koltz I.M., Walker F.M. and Pivan R.B., *J.Am. Chem. Soc.,* **68**: 1486 (1946).
38. Lundgren H.P., Elam D.N. and Connel R.A.O., *J. Biol. Chem.*, **149**: 183(1943).
39. Strauss G. and Strauss U.P., *J. Phys. Chem.,* **62**: 1321 (1958).
40. Karush F. and Sonenberg M., *J. Am. Chem. Soc.*, **71**: 1369 (1949).
41. Blei I., *J. Coll. Sci.*, **15**: 370 (1960).
42. Ray A., Reynolds J.A., Polet H. and Steinhardt J., *Biochemistry,* **5**: 2606 (1966).