ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

BIOPOLYMER-SURFACTANT INTERACTIONS: BINDING STUDIES OF CETYLTRIMETHYL AMMONIUM BROMIDE, CETYL PYRIDINIUM BROMIDE AND DODECYL TRIMETHYL AMMONIUM BROMIDE WITH B-LACTOGLOBULIN

[1]G Geetha [2]C Suresh Kumar and [3]N Devanna

[*1]Department of Biochemistry, Oil Technological Research Institute, Jawaharlal Nehru Technological University Anantapur, Anantapur 515001 India.

[2]Department of Biochemistry, Sri Krishnadevaraya University, Anantapur 515003, India.

[3]Department of Chemistry, Oil Technological Research Institute, Jawaharlal Nehru Technological University Anantapur, Anantapur 515001 India.

Email: ggeetha552@gmail.com

Received on 24-04-2012 *Accepted on 10-05-2012*

Abstract

Binding studies of cationic detergents such as cetyltrimethyl ammonium bromide, Cetyl pyridinium bromide and dodecyl trimethyl ammonium bromide with β - Lactoglobulin were carried out by equilibrium dialysis, ultraviolet difference and circular dichroism techniques at 25 C. Binding isotherms at pH 5·0, 7·0 and 9·0 show cooperative binding at all concentrations of detergents and the number of available binding sites in β - Lactoglobulin increases with pH. Gibbs free energy of binding calculated on the basis of Wymans' binding poten tial concept increases with pH indicating increased bind in strength at higher pH. The ultraviolet difference spectra of the detergent complexes with β - Lactoglobulin at pH 7·0 and 9·0 in the region of 250-300 nm indicate the involvement of aromatic amino acid residues as probable binding sites and also the carboxylate groups since the binding is cooperative. The circular dichroism spectra also indicate the involvement of aromatic amino acid residues in the binding of these detergents. This is substantiated by the decrease in the intensity of the aromatic positive bands in the near ultraviolet region. The increase in the magnitude of [θ] 222 nm values in the far ultraviolet region with the increase in the concentration of the detergent in the complex indicates conformational changes resulting in an increase of α-helical content producing a more ordered structure of β - Lactoglobulin . These binding studies show that at pH 7·0 and 9·0, hydrophobic interactions play a major role, while at pH 5·0 only electrostatic interactions play prominent

role in the binding of these detergents.

Keywords: β - Lactoglobulin; cationic detergents; cooperative binding; helical content.

Introduction: The interactions of proteins with ions and neutral molecules or a variety of ligands in general have been widely investigated and have been reviewed by Steinhardt and Reynolds (1969). Of them, the interaction studies of ionic detergents with proteins have received much attention (Jones, 1975) and it is generally accepted that the binding of such detergents to proteins occurs by a combination of electrostatic and hydrophobic interactions. The relative importance of these types of interactions can be assessed by a study of the binding of a typical globular protein like β - Lactoglobulin with a series or detergents having hydrocarbon moieties of varying lengths and different polar head groups. Such a study will serve as a model since β - Lactoglobulin has been well characterized physic chemically (Maulik *et al*. 1998) and it s 3-dimensional structure well established (Blake *et al.* 1965, 1967).

The binding studies of β - Lactoglobulin with various detergents have been carried out by several workers using different techniques (Iloukhani *et al.* 2000; Coma *et al.* 2002) under a variety of conditions, in order to determine the thermodynamics of binding, the nature and number of available binding sites, the types of interactions involved and the conformational changes accompanying such interactions. In the present investigation, we describe the interaction of β-Lactoglobulin with cationic detergents such as cetyl trimethylammonium bromide (CTAB), dodecyl trimethyl- ammonium bromide (DTAB) and cetyl pyridinium bromide (CPB) at various concentrations and pH using equilibrium dialysis technique. The thermodynamic parameters of their binding are evaluated. The nature of binding sites and the types of interactions involved are determined by ultraviolet (UV) difference spectra. The conformational changs in the enzy me accompanying the binding of these detergents are followed by the circular dichroism (CD) technique. However, the enzymatic activity of β - Lactoglobulin due to the binding of these detergents has not been undertaken in this study.

Materials and Methods

The concentrations of detergents are determined by extracting their bromophenol blue complex in chloroform as described earlier (Felse and Panda 1999). Jasco J-20 spectropolarimeter, calibrated

with (+)-10 Camphor sulphonic acid (De Tar, 1969) was used to record the CD spectra. Beckmann spectrophotometer Model-25 was used to record the UV difference spectra.In the equilibrium dialysis experiments, 5 ml of 0·2% β - Lactoglobulin (1·4×10-4 M; molecular weight 14,400) was dialysed against 10 ml of detergent solution at various concentrations in the same buffer as described earlier (Xie *et al.* 2002). The time required to attain equilibrium was found to be 24 h at 25°C. The binding ratio 'r', the number of mol of the detergent bound per mol of the protein, at various free detergent concentrations [D] was calculated in a manner similar to Rosenberg and Klotz (1961). The binding studies were carried out in 3 buffer systems: 0·01 M sodium acetate buffer, pH 5·0, 0·01 M phosphate buffer, pH 7·0 and 0·01 M glycine buffer, pH 9·0 and at varying detergent concentrations with a fixed protein concentration of 1.4× 10-4 M. The critical micelle concentrations (CMCs) of the detergents are 9·2× 10-4 M for CTAB, 1·56 × 10-2 M for DTAB and 5·8 × 10-4 M for CPB (Mukerjee and Mysels, 1971). The free detergent concentrations used were well below and above their CMCs. The concentrations of the buffers used in these studies are such that no unequal distribution of free detergent molecules due to the Donnan effect occur during the equilibrium dialysis. Binding studies were carried out at pH 5·0, 7·0 and 9·0 in the case of CTAB and CPB, while with DTAB the studies were carried out only at pH 9·0. In order to investigate the effect of ionic strength on the binding of detergents to β - Lactoglobulin , experiments were carried out only at pH 9·0 with 0·05 M glycine buffer. The CD spectra of β - Lactoglobulin and its complex with the detergents were measured at a protein concentration of 2·3 × 10-5 M in a 0·5 mm cell from 250-200 nm and 3·5 × 10-5 M in a 10 mm cell from 320–250 nm at 25°C. The results are represented as molar residue ellipticity, $[\theta]\lambda$ in deg cm2 dmol-1, calculated in a manner similar to that of Ohta *et al.* 2001. Taking a value of 111 as mean residue weight for β - Lactoglobulin (Warren and Gordon, 1970). The UV difference spectra of β - Lactoglobulin -detergent complex were recorded at a fixed protein concentration of 7·0×l0-5 M in all cases and varying the detergent concentration. Both UV difference and CD spectra of the complex were recorded 3 h after mixing.

Results and Discussions

Binding isotherm

The binding isotherms obtained for the interaction of β - Lactoglobulin with CTAB and CPB at pH 5·0, 7·0 and 9·0 and with DTAB at pH 9·0 are shownin figure 1 as a plot of r vs log [D] and they follow cooperative binding at all pH. The binding isotherms have been interpreted in terms of the Wyman' s binding potential concept (Muzzarelli *et al.* 2001) as adopted by (Zhulina *et al.* 2000) and subsequently by others in the binding studies (Dedinaite *et al.* 2000). From the plots of r vs log [D], the apparent free energy of binding, Δ Gr is calculated in a manner similar to that of Jones and (Babak *et al.* 2000) from which the apparent Gibbs free energy of binding per bound detergent ion is computed from the relation, Δ Gr =ΔGa /r. The results of these interaction studies with CTAB and CPB at various pH and with DTAB at pH 9·0 only are shown in the plot of ΔGr vs r (figure 2), from which the maximum values of ΔGr are calculated. It has been observed that the binding ratios in each case increase with pH. The increase in the number of available binding sites with pH is in accordance with the fact that there is a decrease in the net positive charge on β - Lactoglobulin with the increase of pH. The number of binding sites, Gibbs' free energy of binding per bound detergent ion, ΔGr, and the binding constant K, at various pH and ionic strengths are summarized in table 1. As the free detergent concentration approaches CMC, the binding isotherms show a marked increase in the average number of detergent ions bound per mol of the protein over a relatively small range of detergent concentration and reaches a limiting value. Thus cooperative binding reaches a limit when β - Lactoglobulin becomes saturated with the detergent. This is in accordance with the observation by earlier workers on the interaction of β - Lactoglobulin with sodium dodecyl sulphate (SDS) (Jones et al., 1981).

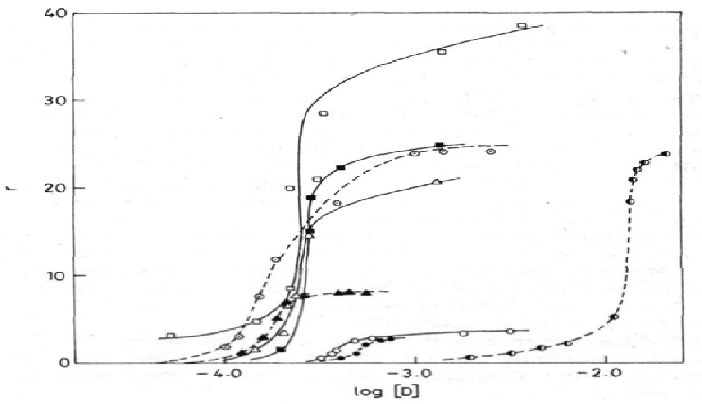

Figure-1: Binding isotherms for the interaction of β - Lactoglobulin with detergents at 25° C: r vs log[D] .
(i) Binding of CTAB: (□), 0·01 M Glycine buffer, pH 9·0; (■), 0·05 M Glycine buffer, pH 9·0; (Δ), 0·01 M Phosphate buffer, pH 7·0; (O), 0·01 M Acetate buffer, pH 5·0
(ii)Binding of CPB: ( ), 0·01 M Glycine buffer, pH 9·0; (▲), 0·01 M Phosphate buffer, pH 7· 0; (Q), 0·01 M Acetate buffer, pH 5·0.
(iii).Binding of DTAB :(◐), 0· 01 M Glycine buffer, pH 9·0.

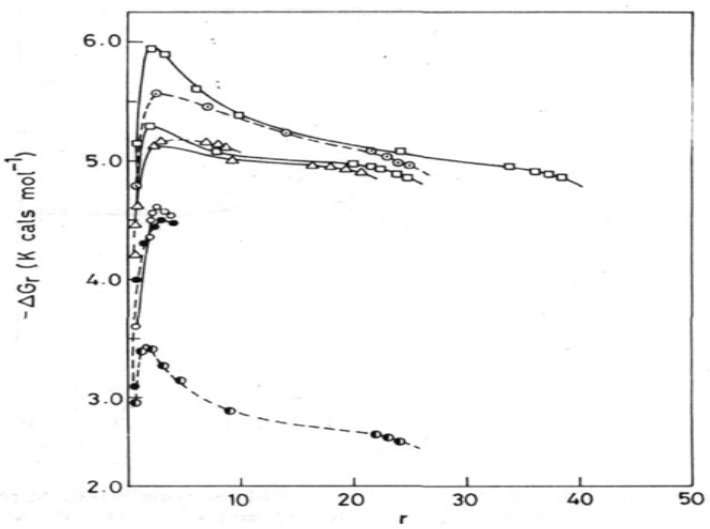

Figure-2: Plot of Δ Gr (kcals mo l -1) vs r for the interaction of β - Lactoglobulin with the detergents at 25°C. Same notations as in figure 1.

Table 1. Thermodynamic parameters for the binding of detergents with β - Lactoglobulin at 25 ° C .

Detergent	Buffer	pH	Total number of binding sites 'n'	ΔG_r max[a] (k cals mol⁻
	Acetate buffer (0·01 M)	5·0	4	–4·62
	Phosphate buffer (0·01 M)	7·0	20	–5·15
CTAB	Glycine buffer:			
	(i) 0·01 M	9·0	38	–5·95
	(ii) 0·05 M	9·0	24	–5·29
DTAB	Glycine buffer (0·01 M)	9·0	24	–3·43

a) Δ G r max is the maximum value of G, computed from the plot of r vs log D
b) Binding constant K values are calculate d from Δ Gr, ma x .

UV difference spectra

The UV difference spectra of β - Lactoglobulin CTAB complex at pH 5·0 (0·01 M) showed no characteristic peaks in the aromatic region while a distinct peak was observed for the complex at pH

7·0 (0·01 M) around 295–297 nm. The intensity of the peak increased with the increase in the concentration of CTAB in the complex (figure 3). The UV difference spectra of the complex at pH 9·0 (0·01 M) showed two peaks one around 295-297 nm and the other at 250 nm (figure 4). Here again, the intensity of both the peaks increased with the increase in the concentration of CTAB in the complex upto a certain limit and then showed a decrease. The UV difference spectra of L-Trp-CTAB complex at pH 9·0 (0·01 M) showed a strong peak at 292 nm and a weak one around 282–283 nm (figure 5). The UV difference spectra of L-Tyr-CTAB complex at pH 9·0 (0·01 M) showed a peak around 245-246 nm in addition to the one observed at 285-286 nm (figure 6). No significant UV difference spectra were observed for these complexes at pH 7·0 and 5·0. The UV difference spectra of β - Lactoglobulin -CPB complexes were recorded in the region of 270–350 nm only, as it was not possible to record them below 270 nm since the detergent itself showed a very high UV absorbance in this region. At pH 5·0, no characteristic peak was observed in the aromatic region, while at pH 7·0 and 9·0, a distinct peak was observed around 295–297 nm, as in the case of β - Lactoglobulin -CTAB complexes but with lesser intensity. The UV difference spectra of L-Trp-CPB complex at pH 9·0 showed a strong peak around 292–293 nm, while no peaks were observed in the case of L-Tyr-CPB complex at this pH.

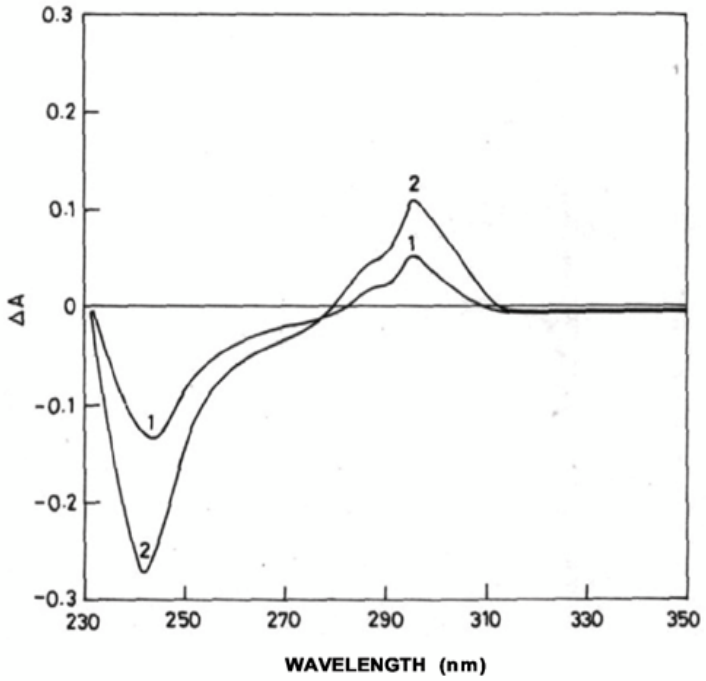

Figure-3: UV difference spectra of 7× 10-5 M β - Lactoglobulin in 0·01 M phosphate buffer, pH 7·0 produced b y C T AB. (1), l· 37×10 -3 M and (2), 2·74×l0 -3 M.

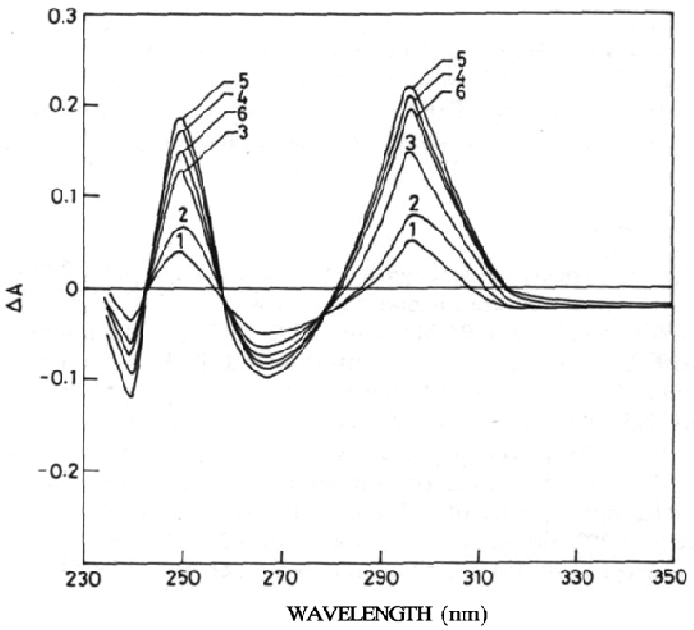

Figure-4: UV difference spectra of 7× 10-5 M β - Lactoglobulin in 0·01 M glycine buffer, pH 9·0, produced by CTAB. (1), 0·685×10-3 M; (2), 0·96×10-3 M; (3), 1·371 ×10-3 M; (4), 2·0 55×10 -3 M ; (5) ; 2·74×l0 -3 M and (6) , 4·11×1 0 -3M .

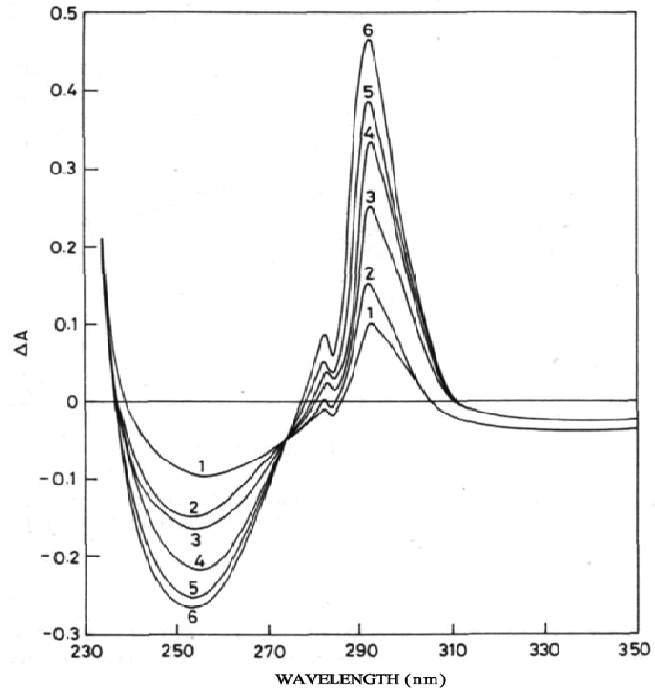

Figure-5: UV difference spectra of 5 ×10-4 M L-Trp in 0·01 M glycine buffer, pH 9·0 p rod u ced b y C TAB. (1), 0·6 8 5×1 0 -3 M; (2), 0·96×l 0 -3 M; (3), 1·371×1 0 -3 M ; (4), 2·055×1 0 - 3 M; (5), 2· 74 ×1 0 - 3 M and (6), 4·11×1 0 -3 M.

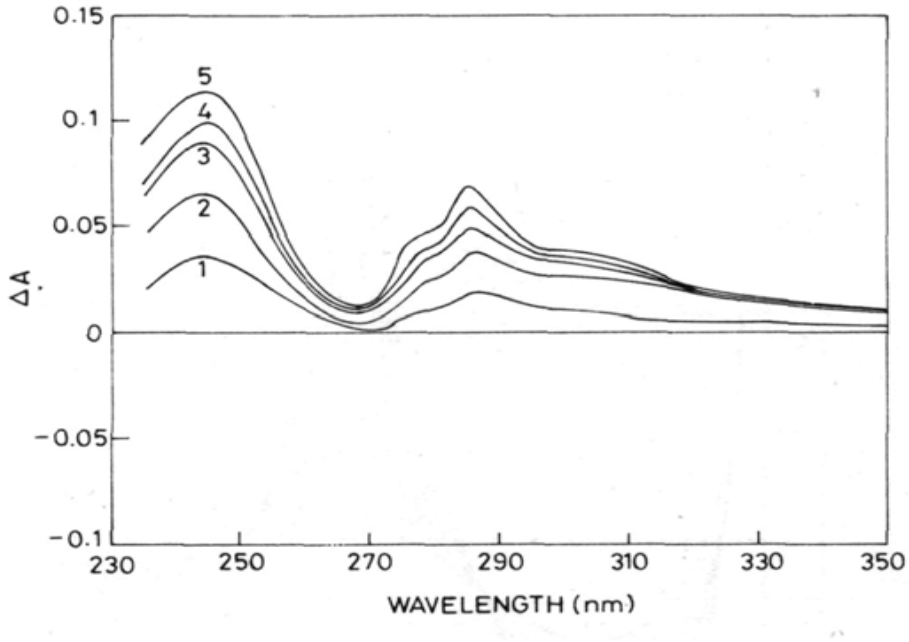

Figure-6: UV difference spectra of 5× 10-4 M L-Tyr HCl in 0·01 M glycine buffer, pH 9·0, produced by CTAB. (1), 0·685× 10-3 M; (2), 0·96×10-3 M; (3), 1·37× 10-3 M: (4). 2·055 × 10^{-3} M and (5), 2·76× 10^{-3} M.

The absence of peaks in the UV difference spectra in the aromatic region for the complexes of β - Lactoglobulin either with CTAB or CPB at pH 5·0 indicates the non-involvement of aromatic amino acid residues in the interaction at this pH. However, the binding observed at this pH may be only due to the interaction of cationic head groups of the detergents with carboxylate anions on the β - Lactoglobulin molecule involving mainly electrostatic interactions. With the increase in pH, more detergent ions bind at additional sites available on account of the ionization of side chain carboxyl groups of the enzyme, probably involving the rupture of some hydrogen bonds. The appearance of 295–297 nm peak in the UV difference spectra of the complexes of these detergents with β - Lactoglobulin at pH 7·0 and pH 9·0 indicates the involvement of indole rings of tryptophan residues in addition to the carboxyl sites. This is in accordance with the earlier observation of (Dedinaite and Claesson 2000) for the interaction of β - Lactoglobulin with dimethyl benzyl myristyl ammonium bromide (DBMA), where they have shown that DBMA which acts as a competitive inhibitor for β - Lactoglobulin binds at all the tryptophan residues lying on the surface and with one or two in the cleft by hydrophobic interactions.

The appearance of two peaks in the case of β - Lactoglobulin -CTAB complex at pH 9·0 (0·01 M) as

well as in the UV difference spectra of the complexes of CTAB with L-Trp and L-Tyr · HCl at the same pH indicates the involvement of both tryptophan and tyrosine residues along with the carboxyl groups in the binding of CTAB with β - Lactoglobulin . The red shift of 4–5 nm in the 297 nm band of β - Lactoglobulin -CTAB complex from that of L-Trp-CTAB complex at the same pH may be due to the binding of the CTAB at or near the tryptophan residues which are buried in the non-polar environment and also probably due to the partial ionization of a tyrosine residue in β - Lactoglobulin at this pH. The UV difference spectrum for the ionization of tyrosine residues in β - Lactoglobulin (Togo et al., 1966) at alkaline pH showed two maxima, one at 295 nm and the other at 245 nm, their difference in molar extinction coefficients being 2650 and 13,200, respectively. So the appearance of an intense peak around 250 nm in the β - Lactoglobulin CTAB complex at pH 9·0 (0·01 M) may be due to the involvement of partially ionized tyrosine residues as the binding sites, while the appearance of only one peak in the UV difference spectra of β – Lactoglobulin- CTAB complexes at pH 7·0 (0·01 M) is probably due to the binding of the detergent molecules only at tryptophan residues as tyrosines do not ionize at this pH. The appearance of a strong peak around 292-293 nm in the UV difference spectra of L-Trp-CPB complex at pH 9·0 also indicates the involvement of tryptophan residues in the binding of CPB with β - Lactoglobulin, and absence of any peak in the UV difference spectra of L-Tyr-CPB complexes shows the non-involvement of tyrosine residues as binding sites at this pH.

The UV difference spectra of β - Lactoglobulin -CTAB complex recorded at pH 9·0 at a higher ionic strength of 0·05 also showed two distinct peaks (figure 7) in the region 295–297 nm and 250 nm, however, with lesser intensities, the 250 nm peak decreasing to a larger extent than the one observed at a lower ionic strength of 0·01 at the same pH. This effect of ionic strength in decreasing the interaction of CTAB with β - Lactoglobulin is supported by the fact that the binding isotherms also showed decreased binding at this ionic strength and pH. Accordingly there has been a decrease in the number of available binding sites and ΔGr. A comparison of the results of the binding of CPB with those of CTAB to β - Lactoglobulin indicates that only a lower number of binding sites are available to CPB at

any pH. Accordingly, the magnitude of ΔG r values shows a decrease in the case of CPB. The small difference in ΔGr values at any given pH, for the binding of CTAB and CPB with l β - Lactoglobulin (table 1) indicates that electrostatic interactions play only a very minor role even though they possess different cationic head groups. The small difference may be attributed to the bulkiness of the polar head group in the case of CPB, since both the detergents have the same hydrocarbon chain length. The decreased binding in the case of CPB may also be due to the non-involvement of tyrosine residue in binding.

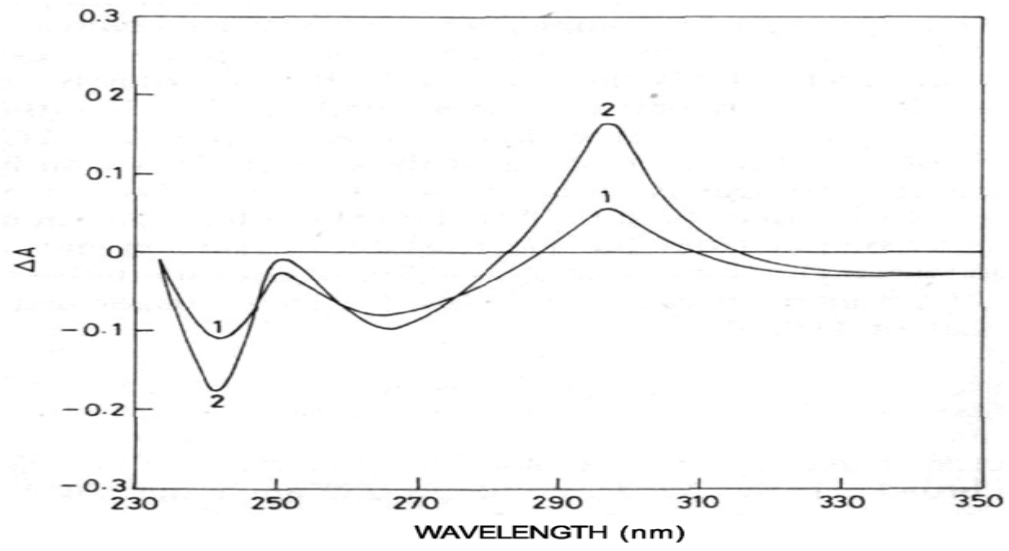

Figure-7: UV difference spectra of 7 × 10-5 M β – Lacto globulin in 0·05 M glycine buffer, pH 9·0 produced by CTAB. (1), 1· 37×1 0 -3 M and (2), 2· 74 ×10 -3 M.

Although the extent of binding in the case of CPB is lower than that of CTAB, the observed high binding ratios at pH 9·0 in both the cases cannot be explained with all the available carboxyl and aromatic amino acid residues in β - Lactoglobulin . Moreover, the absence of peaks in the 240–300 nm region of the UV difference spectra of L-Phe-detergent complexesindicate the non-participation of any phenylalanine residues. So the observed high binding ratios can be explained as follows: the electrostatic interaction of the detergent with the carboxylate anions of β - Lactoglobulin and the resultant binding are stabilized probably by the mutual affinity of the hydrocarbon chain of the adjacently bound detergent ion (Claesson *et al.* 2000) by non-polar forces which are presumed to be the same as those that bind the detergents to form micelles. The UV difference spectra of DTAB with β -

Lactoglobulin and with model compounds L-Trp and L-Tyr· HCl at pH 9·0 follow a pattern similar to those of CTAB complex with β - Lactoglobulin at pH 9·0 (0·01 M). The intensities of both the peaks at 250 and 297 nm of the β - Lactoglobulin-DTAB complex were lower when compared with those of β - Lactoglobulin e-CTAB complex at the same pH. This indicates a lesser interaction between β - Lactoglobulin and DTAB than with CTAB. Accordingly, the number of binding sites are lower for β - Lactoglobulin -DTAB complex at pH 9·0 (0·01 M) than for β - Lactoglobulin -CTAB complex at the same pH. A large difference in ΔGr and K values have also been observed in the binding of these detergents with β - Lactoglobulin . Thus these data suggest a stronger interaction for CTAB with β - Lactoglobulin and a weaker interaction for DTAB. Since both CTAB and DTAB have the same cationic head groups, the contribution from the electrostatic interaction must be the same. Therefore, the very high binding energy and binding constant observed in the case of β - Lactoglobulin -CTAB interaction are probably due to its longer hydrocarbon chain length than that of DTAB.

CD spectra

The CD spectra of native β - Lactoglobulin show positive maxima at 293, 288, 282 and 278 nm and a broad negative band around 258–259 nm in the 320–250 nm region at all pH. The aromatic positive bands for the complexes of β - Lactoglobulin with CTAB and CPB at pH 9·0 (0·01 M) decrease gradually and are replaced by an overall negative spectra with the increase in the concentration of the detergents in the complexes (figure 8a). The magnitude of molar ellipticity values around 257–259 nm decreases with the increase in the concentration of the detergents in the complex and also shows a gradual blue shift of 4–5 nm. The CD spectra of the complexes of β - Lactoglobulin with these detergents recorded at pH 7·0 and 5·0 show similar patterns to those at pH 9·0, but with a lesser intensity of the aromatic bands. In the far UV region of 250–200 nm the CD spectrum of β - Lactoglobulin has an extremum at 208 nm and a shoulder at 222 nm. However, the magnitude of molar ellipticity [θ] 222 nm values for the complexes of β - Lactoglobulin with CTAB and CPB at pH 9·0 (0·01 M) increases upto certain concentrations of the detergents indicating an increase in the -helical content (figure 8b).

The α-helical content calculated by the method of (Wei et al. 1993) increased from a fH value of 0·24 for the native β - Lactoglobulin to a maximum value of 0·32 in the case of CTAB and 0·286 for CPB indicating more ordered structures. In this region, however, at pH 7·0 and 5.0, there was no detectable difference between CD sp spectra of native β - Lactoglobulin and its complexes with these detergents.

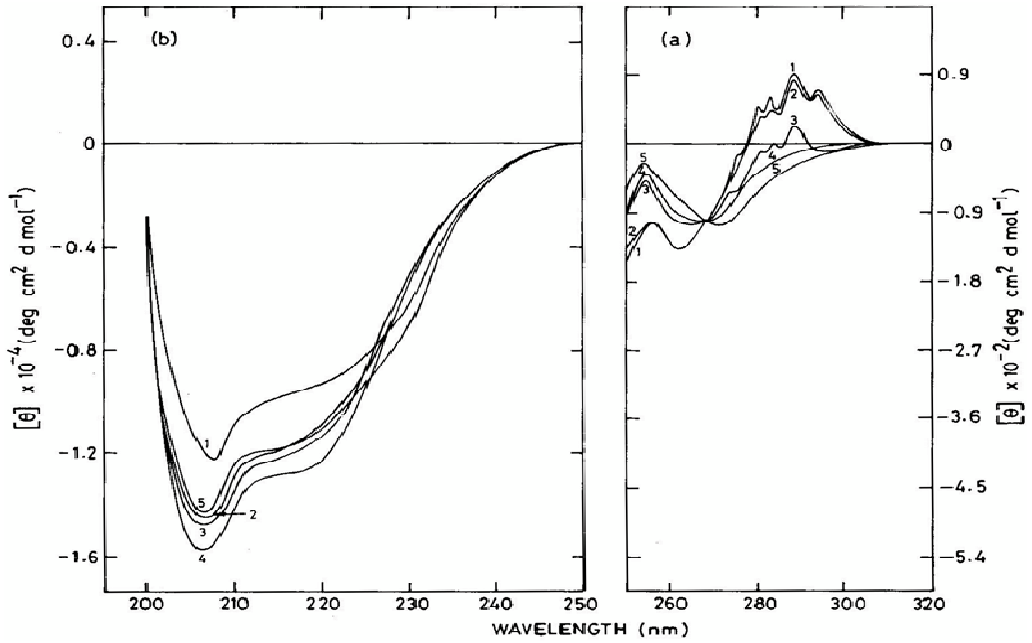

Figure-8: (a), Effect of CTAB on the CD spectrum of β - Lactoglobulin in 0·01 M Glycine buffer, pH 9·0 (320–250 nm region). (1), 0; (2), 0·274× 10-3 M; (3), 0·683× 10-3 M; (4), 0·891× 10-3 M and (5), 1·095 × 10-3 M. Protein concentration: 3·5 × 10-5 M.
(b), Effect of CTAB on the CD spectrum of β - Lactoglobulin in 0·01 M glycine buffer, PH 9·0 (250–200 nm region). (1), 0; (2), 0·274×l0-3M; (3), 0·639×10-3 M; (4), 0·91 × 10-3 M and (5), 1·09× 10-3 M. Protein concentration: 2·3× 10-5 M.

The CD spectra of β - Lactoglobulin -DTAB complex at pH 9·0 (0·01 M) in the 320–250 nm region showed a decrease in the aromatic bands in a manner similar to that of β - Lactoglobulin - CTAB complex, but with a less pronounced effect. In the 250-200 nm region, the CD spectrum of β - Lactoglobulin -DTAB complex with the detergent concentrations well below its CMC showed no change in the α-helical content. However, the CD spectra of the complex at and above its CMC cannot be recorded as the DTAB showed a high absorbance. The CD spectra of β - Lactoglobulin -detergent complexes in the 320-250 nm and 250-200 nm regions show that the

detergents might bind with the indole rings of the tryptophan residues, 62, 63 or 108, the former two lying on the molecular surface and the latter at the entrance to the cleft of the hydrophobic box in the tertiary structure of β - Lactoglobulin . The cationic head groups of the detergents CTAB and DTAB probably bind at the phenolic group of tyrosine 20 or 23 lying at the entrance to the cleft or at a side chain carboxyl group of Asp 101 or 103 on the molecular surface or Glu 35 which lies close to Trp 108 at the active site of the enzyme. For CPB cationic head group also, all the binding sites are essentially the same as in the case of CTAB and DTAB, except for the tyrosine residue involvement. Since all the sites involved in the interactions lie in the non-helical region of the tertiary structure, a more ordered conformation is induced into the enzyme molecule, with the resultant increase in α-helical content. Generally, when the polypeptide chain(s) of a globular protein folds in 3-dimensions to give a tertiary structure, all the polar groups will be exposed to the surface and the non-polar groups will be buried in the interior of the molecule sc that the molecule is thermodynamically stable. In the case of β - Lactoglobulin , which contains a larger proportion of non-polar amino acid residues, in addition to all the polar groups being on the surface, it is likely that some of the non-polar amino acid residues such as tryptophan, phenylalanine and few other non-polar residues lie on the molecular surface of the enzyme (Bergstro *et al.* 2002). In such a situation, the hydrophobic interactions play a dominant role not only in maintaining the stability of the tertiary structure (Babak *et al.* 2000) but also in the binding of detergents with longer hydrocarbon moieties involving several points of contact. Therefore, our binding studies on β - Lactoglobulin with the cationic detergents reveal that hydrophobic interactions play a major role, while electrostatic interactions play only a minor role.

References

1. Babak VG, Merkovich EA, Galbraikh LS, Shytkova EV, Rinaudo M. (2000) Surfactant polyelectrolyte complexes on the basis of β - Lactoglobulin Mendelee Commun. 94
2. Bergstro M, Kjellin URM, Claesson MP, Pedersen JS, Nielsen MM (2002) Study of Complexes Formed in Mixtures of a Cationic Polyelectrolyte and an Anionic Surfactant J.Phys.Chem.B, 106, 11412- 11419.

3. Blake CCF, Mair GA, North ACT, Philips DC, Sarma VR (1967) Surfactants and polymers in aqueous solution. Proc. R. Soc., **B167,** 365.
4. Blake CCF, Koening DF, Mair GA, North ACT, Philips DC, Sarma VR (1965) Interactions of surfactants with polymers and proteins Nature (London), **206,** 757.
5. Claesson PM, Bergstro ¨m M, Dedinaite A, Kjellin M, Legrand JF, Grillo IJ (2000) Phys. Chem.B 104, 11689.
6. Coma V, Martial-Gros A, Garreau S, Copinet A, Salin F, Deschamps AJ (2002) Food Sci. 67,1162.
7. Dedinaite A, Claesson MP, Bergstro ¨m, M, (2000) Langmuir 16, 5257.
8. Dedinaite A, Claesson PM (2000) Langmuir, 16, 1951.
9. Felse, PA Panda T (1999) Bioprocess Eng. 20,505.
10. Iloukhani H, Molaei M, Gharib, F (2000). J. Phys. Chem. Liq., 38, 607.
11. Jones, MN. (1975) in Biological Interfaces, Chapter 5 (Amsterdam: Elsevier). Jones MN, (1983) Commun. J. Comm. Esp. Deterg., 14th, 117 (Eng).
12. Maulik S, Dutta P, Chattoraj DK, Moulik SP (1998) Biopolymer–surfactant interactions: 5 Equilibrium studies on the binding of cetyltrimethyl ammonium bromide and sodium dodecyl sulfate with bovine serum albumin, b-lactoglobulin, hemoglobin, gelatin, lysozyme and deoxyribonucleic acid. Colloids and Surfaces B: Biointerfaces 11 1–8.
13. Muzzarelli RAA, Muzzarelli C, Tarsi R, Miliani M, Gabbanelli F, Cartolari M (2001) Bio macromolecules 2,165.
14. Ohta A, Takiue T, Ikeda N, Aratono M (2001) Calorimetric study of micelle formation in poly ethylene glycol mono octyl ether solutions. J. of Solution Chem. 30,355 350.
15. Rosenberg RM, Klotz IM (1961) Analytical Methods in Protein Chemistry (eds P. Alexander and P. J. Block) (London: Pergamon Press) Vol. 2, p. 136.
16. Steinhardt J, Reynolds JA (1969) Multiple Equilibria in Proteins (New York: Academic Press) p. 234.
17. Wei YC Hudson SM (1993) Macromolecules 26, 4151.

18. Xie WM, Xu PX, Wang,W, Liu Q (2002) Carbohydr.Polym. 50,35.

19. Zhulina EB, Borisov OV, Birshtein TM (2000) Macromolecules, 33.

Corresponding Author:

G.Geetha*,

Email: *ggeetha552@gmail.com*