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THE INFLUENCE OF CHARGE DENSITY ON THE INTERACTION BETWEEN SODIUM N-DODECYL SULPHATE (SDS) WITH HI

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Abstract

The binding data for the interaction of SDS with HI, in aqueous solution at various ionic strengths have been measured by equlibrium dialysis, and investigated spectroscopically. The effect of charge density on the interaction is shown to be of considerable interest. The presence of NaCl causes conformational changes on the structure of HI; whereas this phenomena seems to become more intensified upon contact with SDS. The existence and the importance of charge density which cause additional forces in the macromolecular structures at identical ionic strengths is reported.

Introduction

All Lysine-rich histones studied thus far possess three - domain structural characterstics consisting of a short basic random - coiled in the N- terminal region (nose), a polar globular central region (head) and a flexible highly basic c-terminal region [1]. The globular part of HI occupies a position close to the entry and exit points of the DNA in the nucleosome [2], while the C-terminus extends over the linker DNA. It is the C-tail of the molecule that is responsible for the folding of the fiber into higher - order structure [3].

A number of studies on the interaction between sodium n - dodecyl sulphate (SDS) and proteins were reported [4-9]. These reports indicated that the binding of detergents to globular proteins in buffered solutions were often pH dependent [10-11]. We have previously reported a number of studies on the interaction of SDS with histones at various pH and temperature [12-14].

We would like to show, our studies on the interaction of SDS and histone HI which were carried out using the technique of equilibrium dialysis to obtain the Gibbs free energy.

Hill and Scatchard plots as well as spectroscopy analysis at various ionic strengths in phosphate buffer, solution pH 6.4 and 27°C, show that the magnitude of interaction between SDS and HI is greatly dependent on the charge density which is produced at the presence of sodium chloride.

Key words: Histone HI; Sodium n-dodecyl sulphate; Sodium Chloride; Charge density; Free energy

Experimental Section

Histone preparation

Histone HI was extracted from calf thymus glands prepared from Tehran (Ziaran) slaughter - house by the method of Johns [15].

Materials

The buffer (phosphate) was prepared in double distilled water in various ionic strengths 3.75 mM ($I = 8.82 \times 10^{-3}$), 5 mM ($I = 10.73 \times 10^{-3}$), 6.25 mM ($I = 12.64 \times 10^{-3}$), 7.5 mM ($I = 14.55 \times 10^{-3}$) and 10 mM ($I = 18.38 \times 10^{-3}$) at pH 6.4. The second type of buffer (1:1 concentration of phosphate and sodium chloride) was prepared as above with identical ionic strengths. Each of the buffer solutions contained 0.02% ($-\frac{W}{V}-$) sodium azide contributing 0.0031 to the ionic strength. Sodium n-dodecyl sulphate (especially pure grade) was from

the Merck company. Rosaniline hydrochloride dye was used as supplied by B. D. H. Visking dialysis tubing (MW cut off 10000 - 14000) was from SIC (East Leigh) Hampshire, U. K. All other chemicals used in this study were reagent grade.

Methods

Equilibrium dialysis was carried out at 27°C as previously described [13-14] using a Hl concentration of 0.01% W/V. The free surfactant concentrations in equlibrium with the complexes were assayed by the Rosaniline hydrochloride method [16].

U. V. spectroscopy measurements were made at the maximum wavelength of 225 nm with a Shimadzu instrument model 260 double - beam recording spectrophotometer. The instrument reading was adjusted to zero with buffer solution in both cuvettes, and difference spectra were obtained for various Hl concentrations (0.005% to 0.05%) in absence of SDS. Difference spectra of the interaction of SDS and Hl were also obtained by adding portions of SDS solutions in the range of 0.1 mM to 1 mM to the sample cuvette. In all measurements of the interaction of SDS and Hl, the Hl concentration was taken 0.01% (W/V). Corrections for inequalities arising from Donnan effects are negligible at the ionic strength used.

In all calculations the molecular weight of Hl was taken as 21,000 [17].

The CMC value obtained 7.5 upto 5 mM for ionic strengths of 8.82×10^{-3} to 18.38×10^{-3} respectively. It is important to note that the concentrations of SDS which were used were below the CMC.

Results and Discussion

Figure 1 indicates the effects of ionic strength caused by phosphate ions on the absorption spectra (λ_{max} = 225 nm) of different concentrations of Hl at pH 6.4, 27°C. A distinct variation was observed in Hl at 0.025% concentration in 5 mM phosphate buffer at the ionic strength of 10.73×10^{-3}. Progressive variations occurred with increasing Hl concentrations especially at an ionic strength of 10.73×10^{-3}.

Figure 2 shows the effects of sodium chloride on the absorption spectra for different concentrations of Hl which are intensified at the variation point ($I = 10.73 \times 10^{-3}$). This probably suggests that a charge effect, due to the influence of sodium chloride changes the conformational stability of Hl molecule. The absorbance changes in Figures 4and 3 arise upon the interaction of SDS and Hl with and without the presence of NaCl at identical ionic strengths respectively.

The Figures (3 and 4) show the large effect of SDS on Hl structure especially in the presence of NaCl. It is important to note, that at a similar ionic strength ($I = 10.73 \times 10^{-3}$) the spectral changes are highly intensified again.

The binding isotherms (The number, $\bar{\upsilon}$, of SDS ions bound per protein molecule as a function of the logarithm of the free SDS concentration), $[S_f]$ for SDS on histone Hl as a function of ionic strengths in phosphate buffer, pH 6.4 and 27°C are shown in figure 5. These binding isotherms show a dependence on ionic strengths suggesting the initial interaction of SDS is ionic, subsequent binding being hydrophobic and non-specific except for 5 mM concentration of phosphate buffer ($I = 10.73 \times 10^{-3}$) which does not show a hydrophobic binding region (f ig. 5a) [18].

The calculation of the Gibbs energies of binding which can be applied to the entire binding isotherm is based on the Wyman binding potential concept [19]. The binding potential is calculated from the area under the binding isotherm according to the equation:

$$\pi = RT \int_{\bar{\upsilon}_i = O}^{\bar{\upsilon}_i} \bar{\upsilon}_i \, d \, Ln \, [S_f] \qquad (1)$$

and it is related to an apparent binding constant K_{app} as follows:

$$\pi = RT \, Ln \, (1 + K_{app} \, [S_f]^{\bar{\upsilon}}) \qquad (2)$$

The values of K_{app} were determined by application of equations (1) and (2) are used to determine the value of $\Delta G_{\bar{\upsilon}}$ [20]

$$\Delta G_{\bar{\upsilon}} = \frac{\Delta G_{app}}{\bar{\upsilon}} = \frac{RT}{\bar{\upsilon}} \, Ln \, K_{app} \qquad (3)$$

Figures 6 and 7 show ΔG and $\Delta G_{\bar{\upsilon}}$ as a function of $[SDS]_{total}$ and $\bar{\upsilon}$ at various ionic strengths in phosphate buffer pH 6.4 and 27°C. These suggest the interaction of SDS and Hl at an ionic strength equal to 10.73×10^{-3}, being the more ionic interaction than the other ionic strengths. The values of $-\Delta G$ at $\bar{\upsilon} = 10$ are equal to 240, 230, 215, 195 Kj mol^{-1} for 5 mM ($I = 10.73 \times 10^{-3}$), 3.75 mM ($I = 8.82 \times 10^{-3}$) 6.25 mM ($I = 12.64 \times 10^{-3}$) and 7.5 mM ($I = 14.55 \times 10^{-3}$) respectively.

Fig.8shows the cooperative binding isotherms at ionic strength of 10.73×10^{-3} with and without the presence of NaCl in phosphate buffer pH 6.4, 27°C. Binding

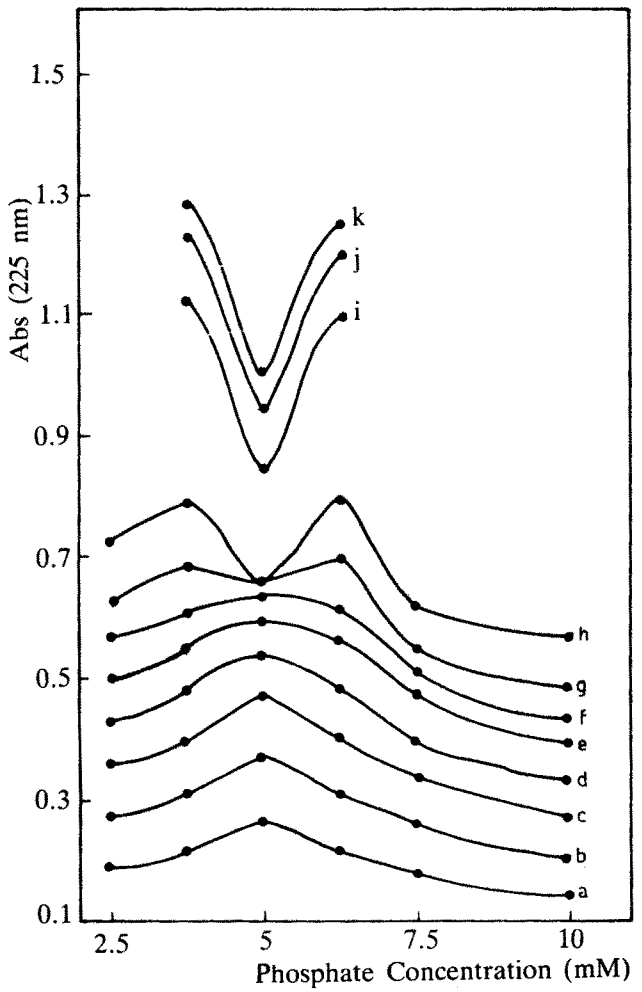


Figure 1- The effect of ionic strength caused by phosphate ions on the absorption spectra (O D max = 225 nm) of different concentrations (W/V %) of Hl at pH 6.4.
a) 0.005% b) 0.0075% c) 0.01% d)0.0125% e) 0.015% f) 0.0175% g) 0.02% h) 0.025% i) 0.03% j) 0.035% k) 0.05%

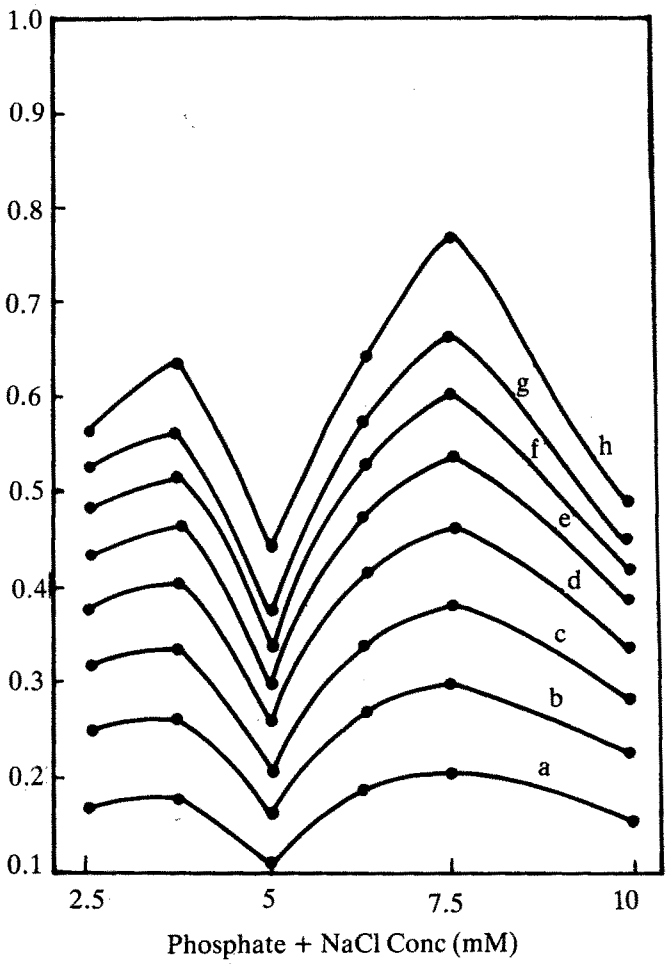


Figure 2- The effect of identical ionic strength caused by phosphate and NaCl ions on the absorption spectra (OD max = 225 nm) of different concentrations (W/V %) of Hl at pH 6.4
a) 0.005% b) 0.0075% c) 0.01% d) 0.0125% e) 0.0125% f) 0.015% g) 0.0175% h) 0.02%

isotherms in the presence of NaCl are shifted to lower concentrations of free SDS and have a higher binding affinity including some hydrophobic interaction.

Fig 7b shows the comparison of free energy change of interaction of SDS and Hl with and without the presence of NaCl.

Figures 9, 10 and 11 show the analysis of binding data which were used in terms of the Scatchard and Hill equations [21-22].

$$\frac{\bar{\upsilon}}{[S_f]} = K(n - \bar{\upsilon}) \qquad (4)$$

Where $[S_f]$ is the free SDS concentration, K is the binding association constant and n, the number of independent binding site in the Scatchard equation. If this equation is followed by a plot of $\frac{\bar{\upsilon}}{[S_f]}$ Vs. $\bar{\upsilon}$ should be linear with a slope of K and an intercept when $\frac{\bar{\upsilon}}{[S_f]} = 0$ of n.

Fig. 9b shows the Scatchard plots are linear at lower values of $\bar{\upsilon}$ (i.e. the specific binding region and the intercepts of the x-axis (n) are 20 and 30 for interaction with and without the presence of NaCl respectively. Inspite of normal binding isotherm in the presence of NaCl which are given in Fig. 8, the Scatchard plot has an unusual shape. In fact there is no theoretical model which would give Scatchard plots with minima as shown in Fig. 9b, except perhaps a combination of negatively cooperative binding sites and a set of positively cooperative binding sites with binding constants differing by perhaps several orders of magnitude. Fig. 9a shows the Scatchard plots in the highly cooperative. Fig. 10 and 11 show an analysis of the binding data in terms of the Hill equation:

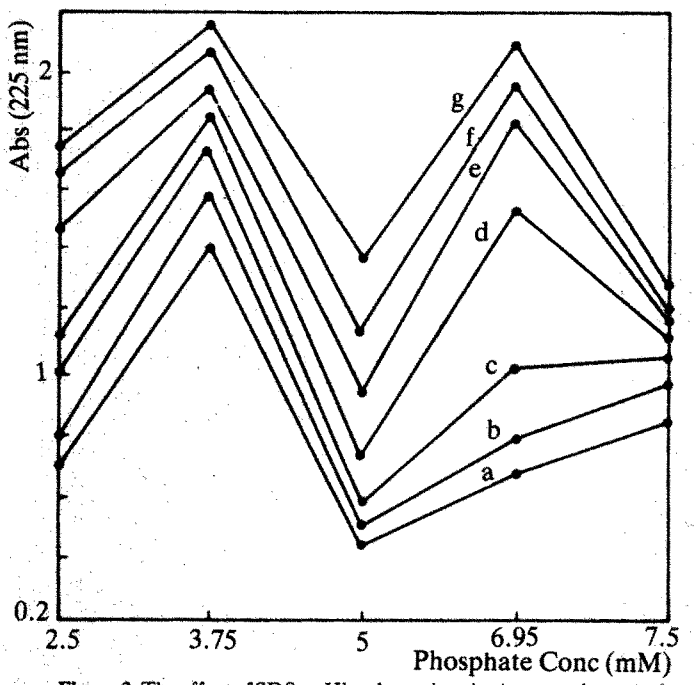
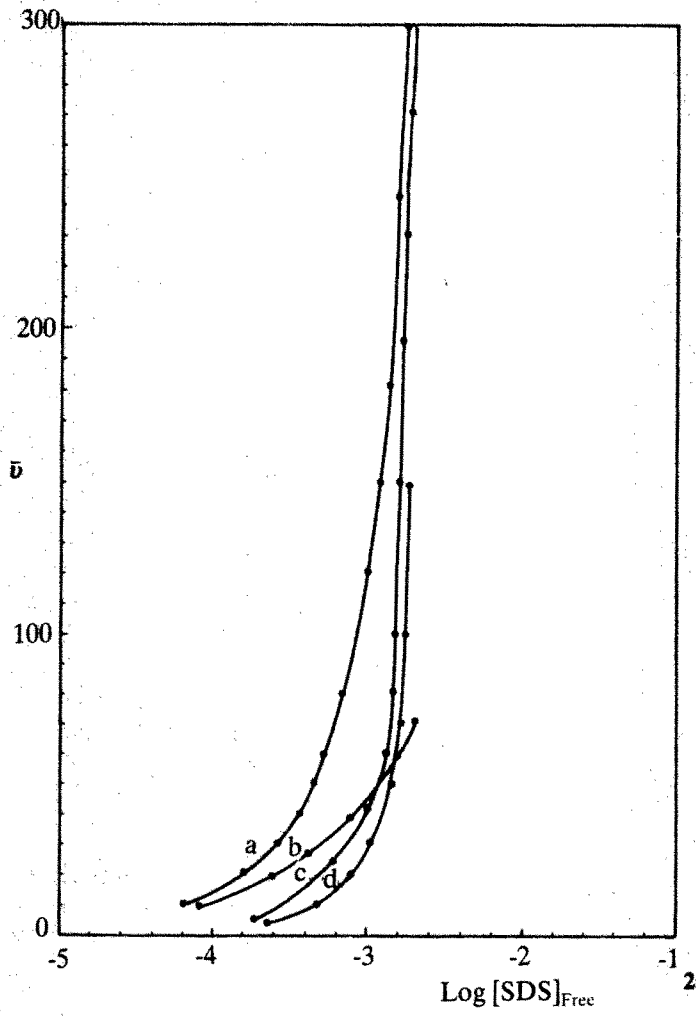


Figure 3- The effect of SDS on Hl at the various ionic strengths caused by phosphate ions at constant concentraction of Hl (0.01% W/V)
a) 1.0 mM, SDS b) 0.9 mM, SDS c) 0.8 mM, SDS d) 0.7 mM, SDS e) 0.6 mM, SDS f) 0.5 mM, SDS g) 0.4 mM, SDS



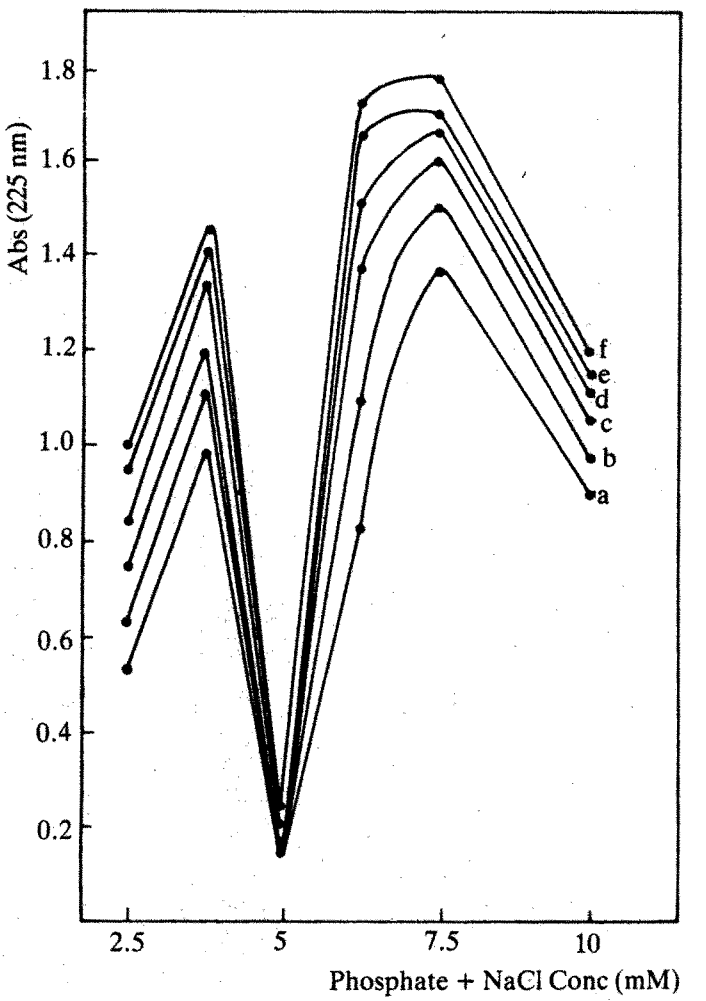


Figure 4- The effect of SDS on Hl at various ionic strength caused by phosphate and NaCl ions at constant concentration of Hl (0.01% W/V)
a) 1.0 mM, SDS b) 0.9 mM, SDS c) 0.8 mM, SDS d) 0.7 mM, SDS e) 0.6 mM, SDS f) 0.5 mM, SDS g) 0.4 mM, SDS

Figure 5- Binding isotherms for SDS on the interaction with Hl at phosphate buffer, pH 6.4 and 27°C
a) 3.75 mM phosphate ($I = 8.82 \times 10^{-3}$)
b) 5 mM phosphate ($I = 10.73 \times 10^{-3}$)
c) 6.25 mM phosphate ($I = 12.64 \times 10^{-3}$)
d) 7.5 mM phosphate ($I = 14.55 \times 10^{-3}$)

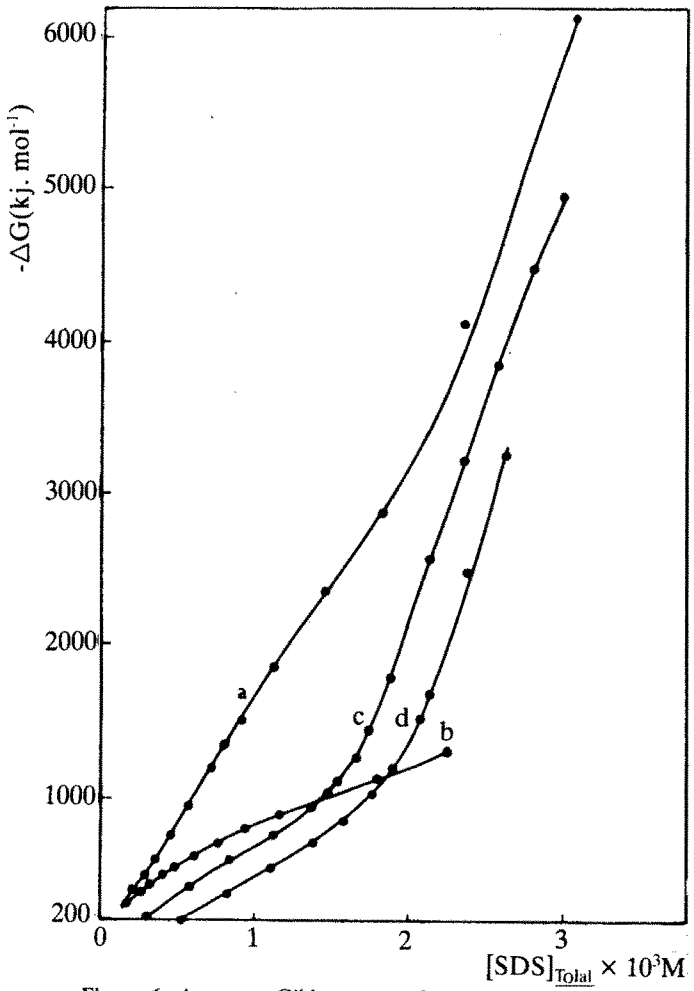


Figure 6- Apparent Gibbs energy change as a function of final concentration of SDS
a) 3.75 mM phosphate b) 5 mM phosphate
c) 6.25 mM phosphate d) 7.5 mM phosphate

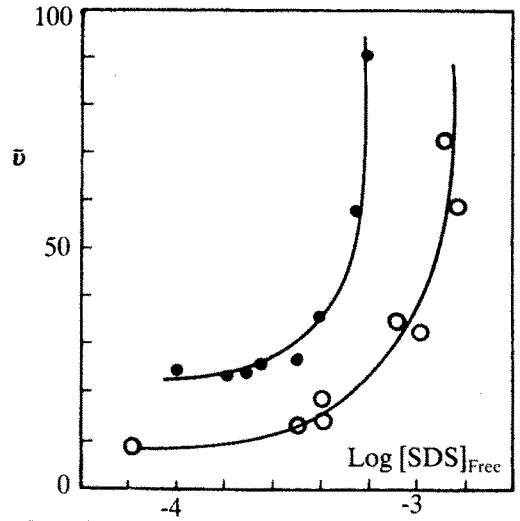


Figure 8- Binding isotherms for SDS on the interaction with Hl at I = 10.73×10^{-3}
0, phosphate ions ●, phosphate and NaCl ions at identical ionic strength

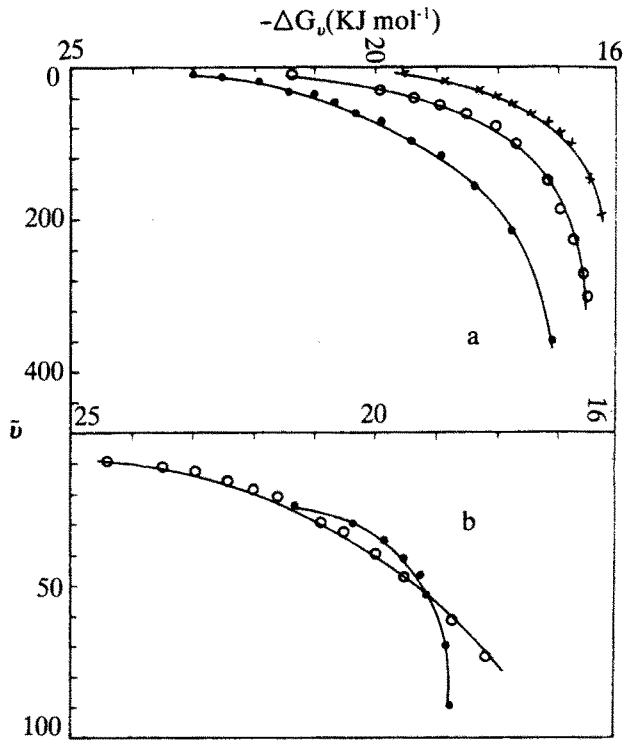


Figure 7- Apparent Gibbs energy change per SDS bound ($\Delta G\upsilon$) as a function of $\bar{\upsilon}$.
a) ●, 3.75 mM phosphate; 0, 6.25 mM phosphate; x, 7.5 mM phosphate
b) 0, 5 mM phosphate ($I = 10.73 \times 10^{-3}$); ●, $I = 10\ 73 \times 10^{-3}$ caused by phosphate and NaCl ions

$$\bar{\upsilon} = g \frac{(K[S_f])^{n_H}}{1 + (K[S_f])^{n_H}} \qquad (5)$$

where g is the maximum value of $\bar{\upsilon}$, n_H the Hill coefficient (a measure of the cooperativity of the interaction) and K the mean binding constant.

In order to fit the data to the Hill equation, a value of g is equal to 100 which are based on the binding of 1.4 g^{SDS}/g of protein(23).The determination of K and n_H from Hill plots based on the linear form of equation (5):

$$\text{Log}\left(\frac{\bar{\upsilon}}{g - \bar{\upsilon}}\right) = n_H \text{ Log } [S_f] + n_H \text{ Log } K \qquad (6)$$

Fig. 12 shows the n_H Hill cooperativity coeffients as a function of phosphte buffer concentrations without the presence of NaCl, indicating negative cooperativity ($n_H < 1$) at 5 mM phosphate buffer ($I = 10.75 \times 10^{-3}$), nevertheless, other ionic strengths are positively cooperative ($n_H > 1$).

Fig. 11 shows biphasic Hill plots for the interaction of SDS and Hl at the presence of NaCl, indicating a two step interaction, specific and cooperative binding. At $\bar{\upsilon}$

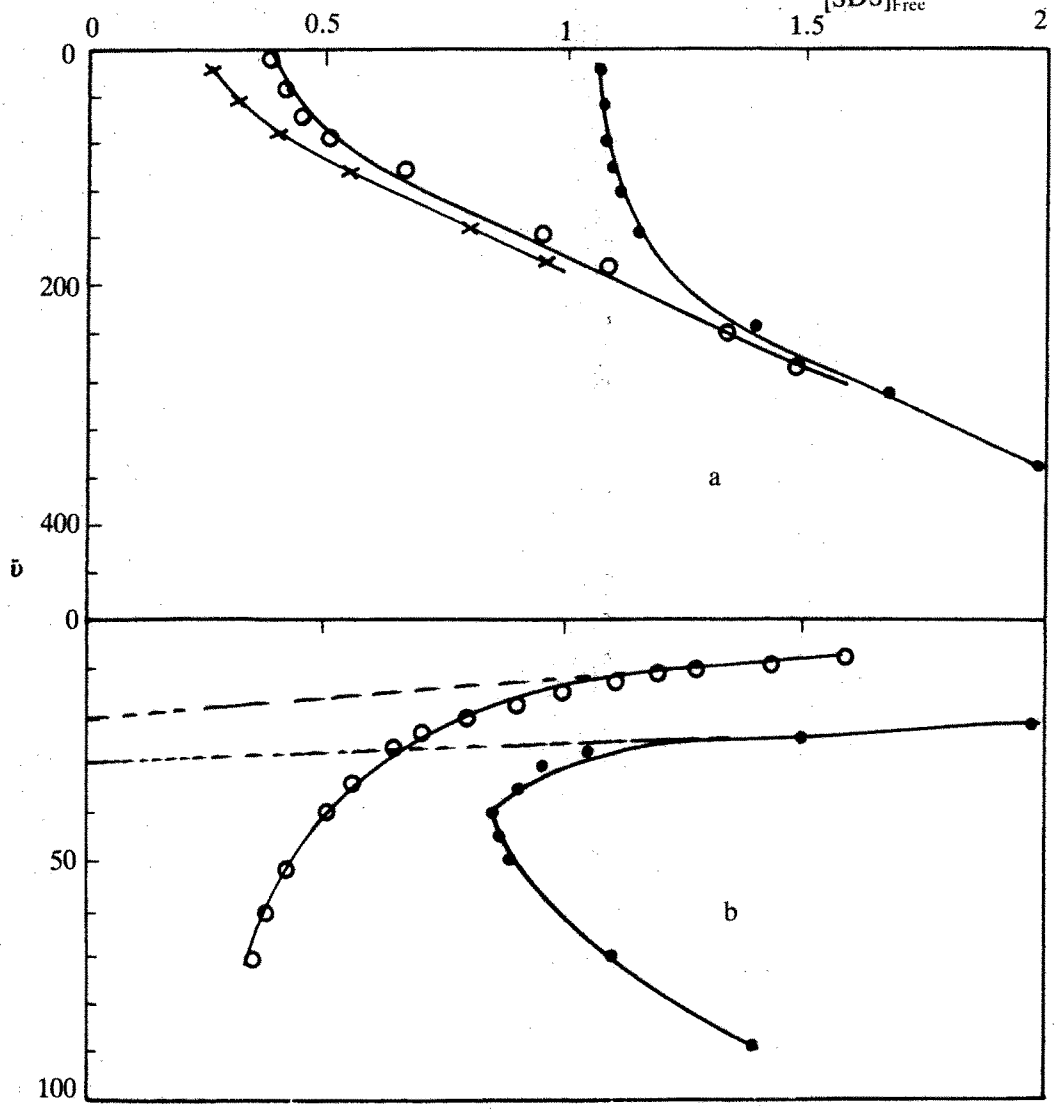


Figure 9- Scatchard plots for SDS on interaction with Hl at 27°C and pH 6.4.
a) ●, 3.75 mM phosphate ($I = 8.82 \times 10^{-3}$); ○, 6.25 mM phosphate ($I = 12.64 \times 10^{-3}$); x, 7.5 mM phosphate ($I = 14.55 \times 10^{-3}$)
b) ○, 5 mM phosphate ($I = 10.73 \times 10^{-3}$); ●, $I = 10.73 \times 10^{-3}$ caused by phosphate and NaCl ions

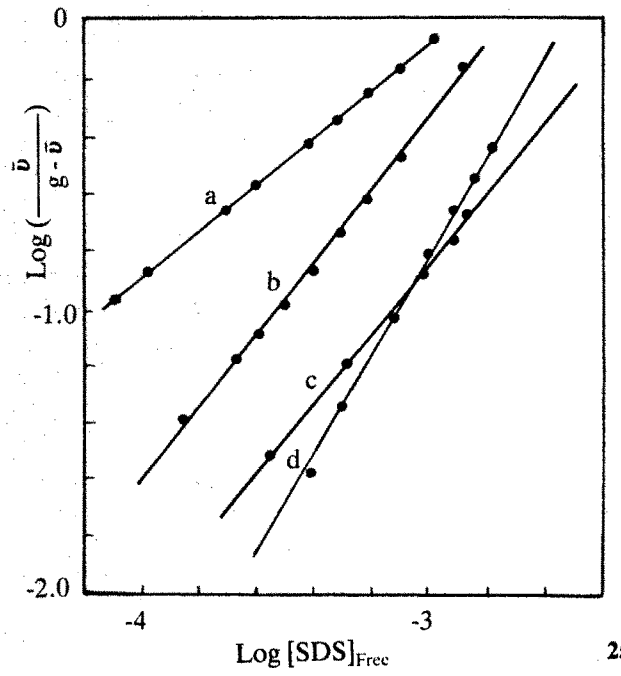


Figure 10- Hill plots for SDS on interaction with Hl at phosphate buffer, pH 6.4 and 27°C. g = 100
a) 5 mM b) 3.75 mM c) 6.25 mM d) 7.5 mM

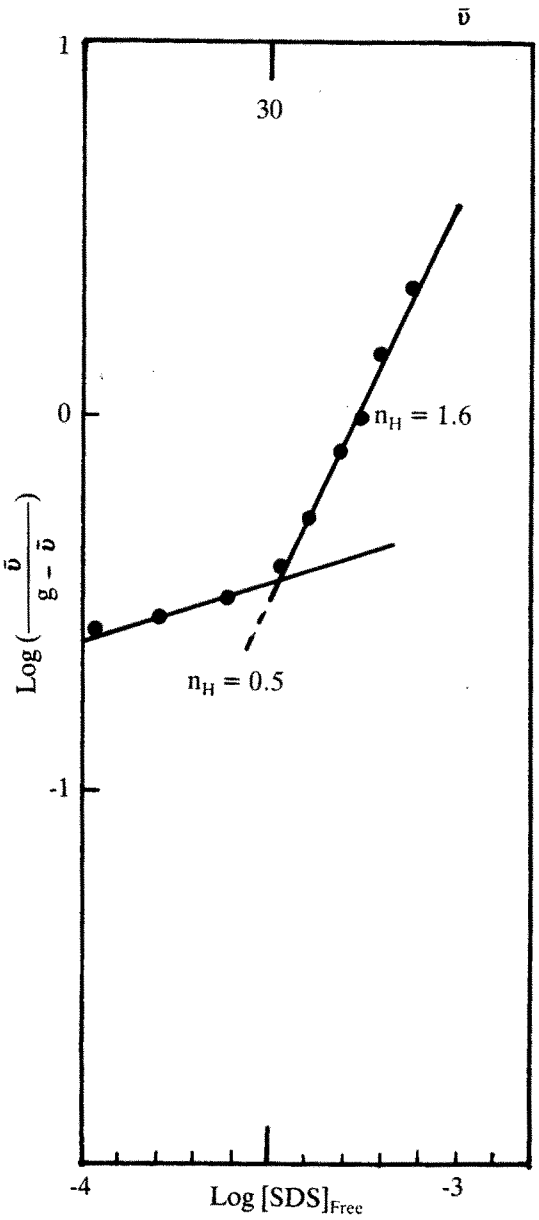


Figure 11- Biphasic Hill plot for SDS on interaction with Hl in the presence of NaCl ($I = 10.73 \times 10^{-3}$) at pH 6.4 and 27°C. g = 100

< 30 and $\bar{\upsilon} > 30$ the interactions are specific and cooperative respectively, which are confirmed by the Scatchard plots (Fig 9b).

The free energy for $\bar{\upsilon} = 30$ is equal to - 610 or $\Delta G_{\bar{\upsilon}:\overline{30}}$ 20.3 KJ mol^{-1} as the boundary between the specific and cooperative region. The numbers of binding sites for interaction of SDS and Hl with and without NaCl are equal to 30 and 20 which are shown in Figure 9b. Fig.11 indicates the cooperativity occurs at $\bar{\upsilon} > 30$ in the presence of NaCl, But cooperativity is not observed without the presence of NaCl (Fig 10).

Finally, we can propose that the smaller ions with a higher charge desity causing difference in electrostatic

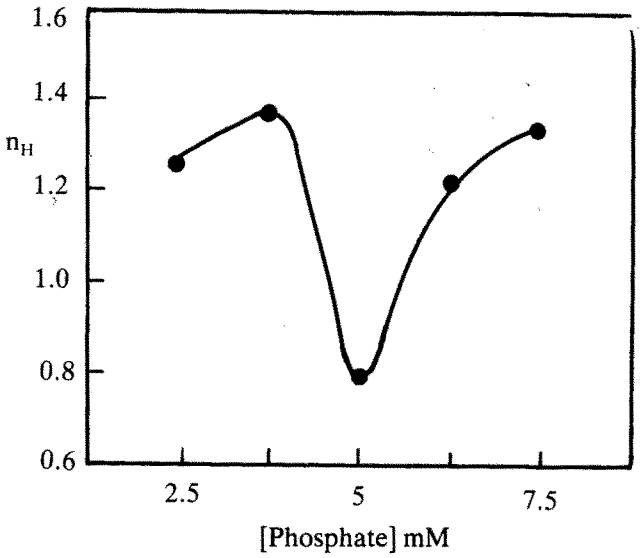


Figure 12- Hill coefficents as a function of various concentration of phosphate buffer.

and hydrophobic interactions. The charge density probably affects the water of solvation and solute stablization. These observations confirm that charge density is a very important factor in the interaction of protein - protein and protein - surfactant complexes. The state of charge at a concentration of 5 mM ($I = 10.75 \times 10^{-3}$) indicates unshielding which compacts the structure of Hl. Hl - Hl interaction may be intensified in this case. The results lead to the belief that the presence of charge density cause additional forces in the macromolecule structure.

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