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THERMODYNAMIC STUDIES OF THE INTERACTION OF SODIUM N-DODECYL, SULPHATE WITH CALF - THYMUS HISTONE H3

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Abstract

The binding of Sodium n-dodecyl sulphate (SDS) to histone H3 was studied in the pH range 3.2-10 by equilibrium dialysis at 27° and 37°C. The binding data have been used to obtain the Gibbs free energy of interaction using a theoretical model of the Wyman binding potential; and the enthalpy of interaction from the temperature dependence of the equilibrium constants from the Van't Hoff relation. The enthalpy of interaction of H3 and SDS is exothermic in some buffer solutions which is in marked contrast to other histone - SDS complexes. The entropy of initial interaction of H3 and SDS is negative in some other buffer solutions, with increasing binding of SDS, the entropy becomes positive.

Introduction

Histone H3 is one of the five main fractions of the basic proteins found in association with DNA in somatic cells of eukaryotes [1]. The primary structure of calf thymus histone H3 (135 residues) is known, and it indicates the presence of two cysteine residues at positions 96 and 110 of the molecule [2].

The presence of crevices containing cysteine in the tertiary structure of calf thymus histone H3 is detected by EPR spectroscopy [3].

With a view to obtaining more information on the structure of proteins, we have applied the denaturation methodology.

A number of studies have been previously reported on the interaction between detergents and proteins [4-8], including a number of studies on the interaction between sodium n-dodecyl sulphate and histones [9-12].

In the present work, we have tried to obtain a better insight into the tertiary structure of histone H3 by the thermodynamic studies on the interaction between H3 and SDS as a denaturant.

Key words: Histone H3, Sodium n-dodecyl sulphate equilibrium dialysis; Exothermic enthalpy, Free energy

Experimental Section

Materials

Calf thymus histone H3 was obtained from Sigma Chemical.

A number of buffers were used, each of which contained 0.02% W/V sodium azide contributing 0.0031 to ionic strength (I). The buffers were:

(i) glycine (50 mM) plus HCl pH 3.2, I = 0.0119,

(ii) sodium phosphate (2.5 mM), pH 6.4, I = 0.0069,

(iii) glycine (50 mM) plus NaOH pH 10.0, I = 0.0318 and

(iv) sodium bicarbonate + sodium carbonate (1 mm) plus NaOH pH 10.0, I = 0.00508

Visking membrane dialysis tubing (MW cut - off 10,000 - 14,000) was obtained from SIC (East Leigh) Hampshire, U. K., Rosaniline hydrochloride dye was received from B. D. H., Sodium n - dodecyl sulphate (especially pure grade) was purchased from Merck. All the salts used in the preparation of the buffers were of analytical grade and they were made up in doubly distilled water.

Methods

Equilibrium dialysis to measure bound SDS was

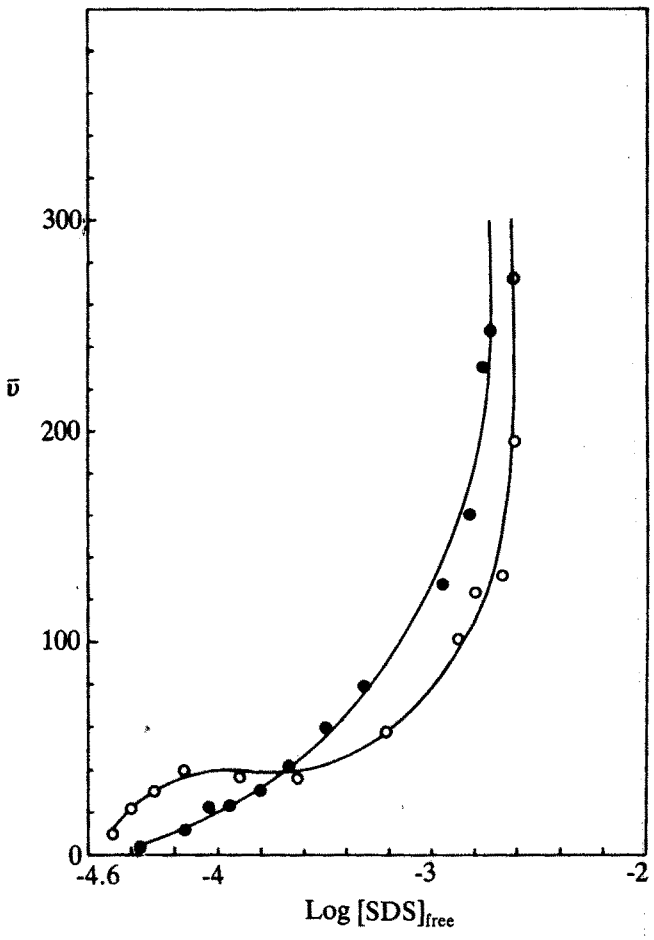

Fig 1. Binding isotherms for (SDS) on the interaction with H3 at pH 3.2, 50 mM glycine.
●, 37°C; 0,27°C

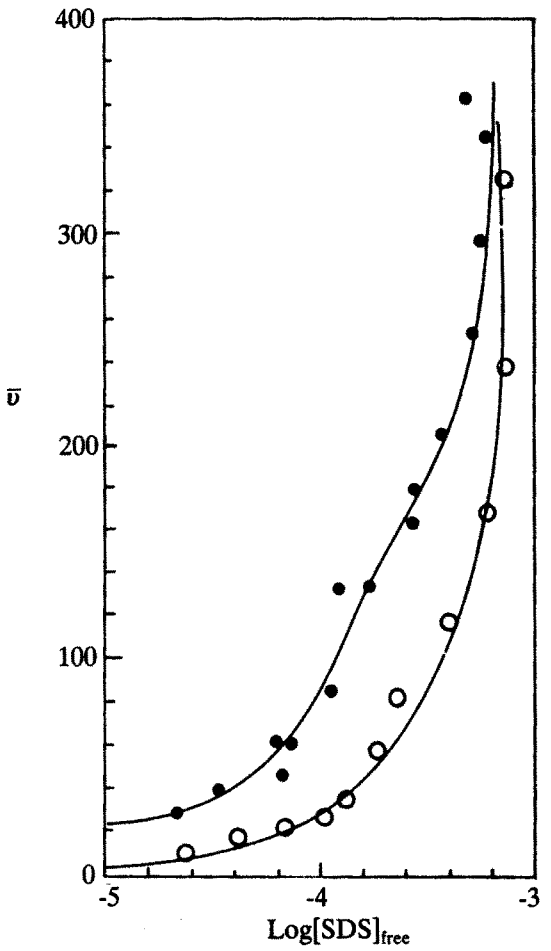

Fig 2. Binding isotherms for SDS on the interaction with H3 at pH 6.4, 2.5 mM Phosphate. ●, 37°C; 0, 27°C

made as described previously [9, 11]. Spectrophotometer Shimadzu model 160 was used for the measurement of optical density.

In all calculations the molecular weight of H3 was taken as 15,300 [13] and concentration of H3 was 0.01% (W/V).

Results and Discussion

The binding isotherms, the number, $\bar{\upsilon}$, of SDS ions bound per H3 as a function of the logarithm of the free SDS concentration, $[SDS]_f$ on interaction of H3 with SDS at various pH and temperatures are shown in figures 1, 2, 3 and 4. The type of buffer solutions and pH change the state of charges on histone H3 markedly; Whereas the same effect is shown to be much less for histones H_1 and H_2B [10, 17].

Increasing the temperature from 27° to 37°C produced a shift to a higher and lower free SDS concentrations in glycine buffer. Results at pH10 (carbonate buffer) and pH 6.4 (phosphate buffer) are shown in figures 2 and 3 respectively. It is generally accepted that binding of surfactant molecules to proteins occurs by a combination of ionic and hydrophobic interactions [14].

Increasing the temperatures from 27° to 37°C produced the shift for ionic part to higher free concentrations of SDS and subsequent variations occurs in hydrophobic part to lower SDS free concentration for glycine buffer solution, pH3.2 and carbonate buffer solution, pH 10 are shown in figure 1 and 4 respectively, and also the shift has happened to lower and higher free concentration from the temperature dependence of K_{app} using Van't Hoff relation: [16]

$$\mathrm{Ln}\frac{K_2}{K_1} = -\frac{\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

The enthalpy of interaction between H3 and SDS in

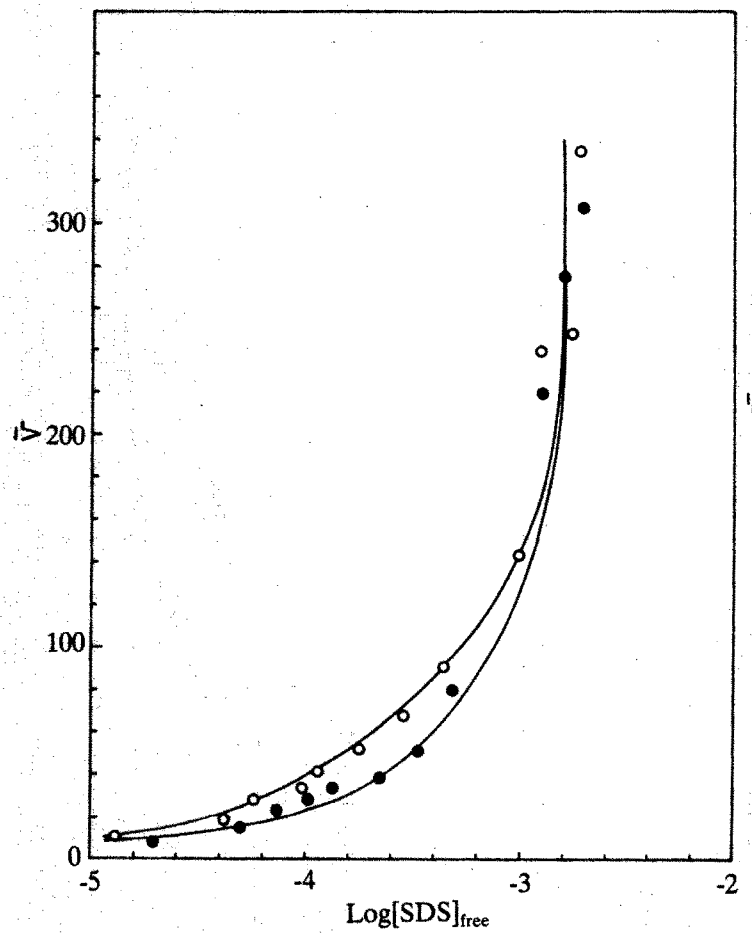

Fig 3. Binding isotherms for SDS on the interaction with H3 at pH10 50mM glycine ●, 37°C; 0,27°C.

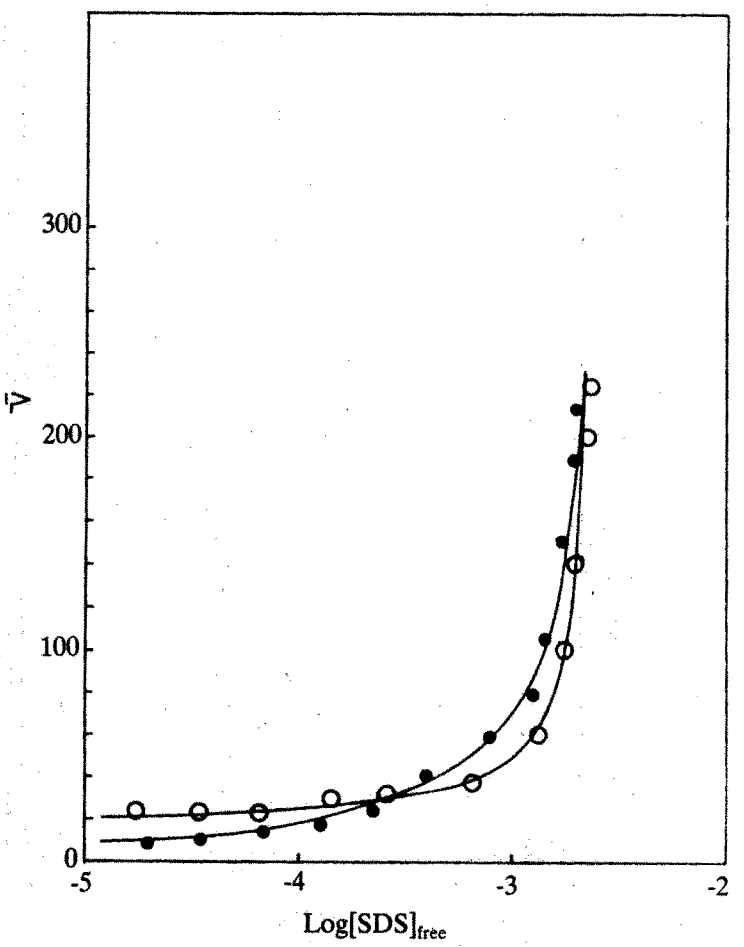

Fig 4. Binding isotherms for SDS on the interaction with H3 at pH 10, 1mM Sodium bicarbonate + Sodium carbonate ●, 37°C; 0,27°C.

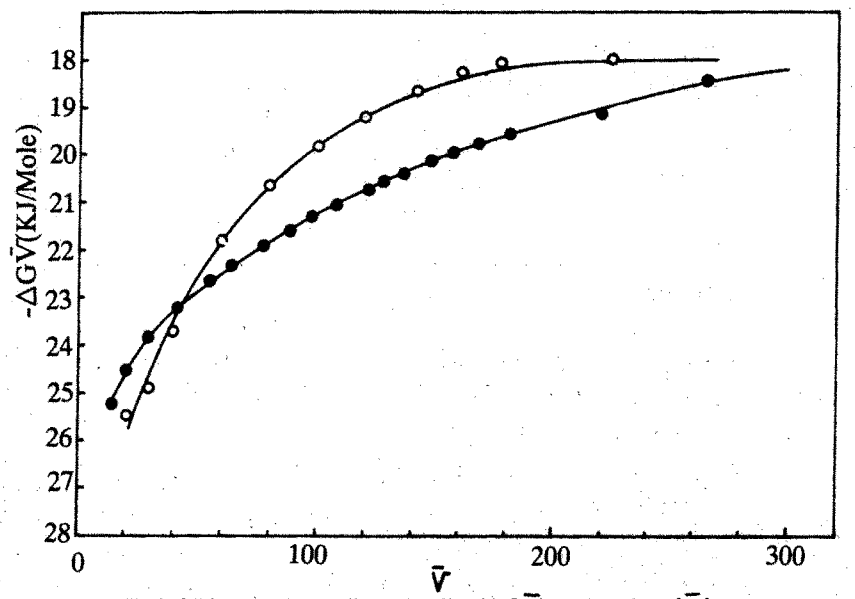

Fig 5. Gibbs energies per ligand binding ($\Delta G\bar{\upsilon}$) as a function of $\bar{\upsilon}$ for SDS on the interaction with H3 at pH 3.2, 50 mM glycine. ●, 37°C; 0,27°C.

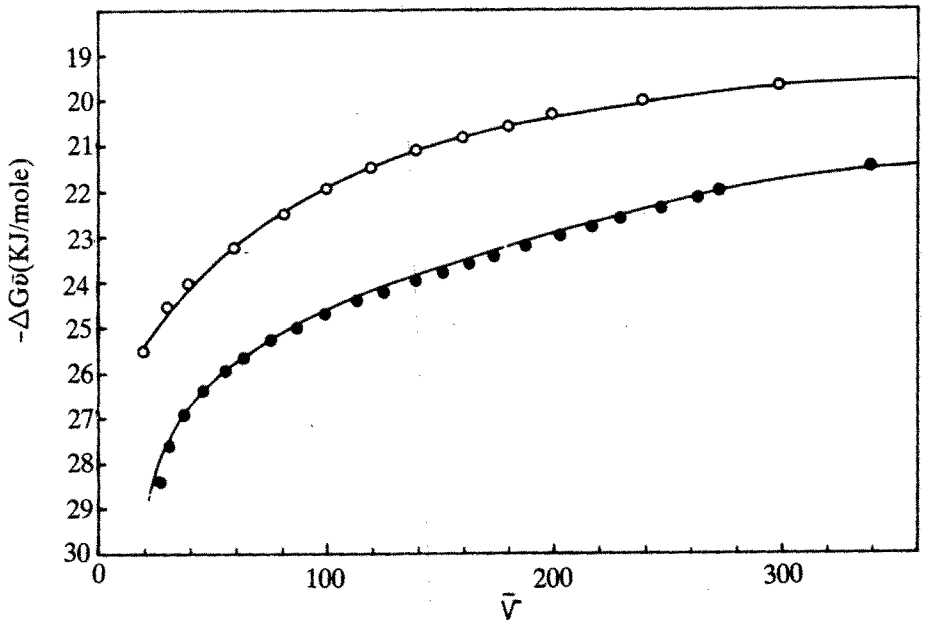

Fig 6. Gibbs energies per ligand binding ($\Delta G\,\bar{\upsilon}$) as a function of $\bar{\upsilon}$ for SDS on the interaction with H3 at pH 6.4, 2.5 mM Phosphate. ●, 37°C; 0.27°C.

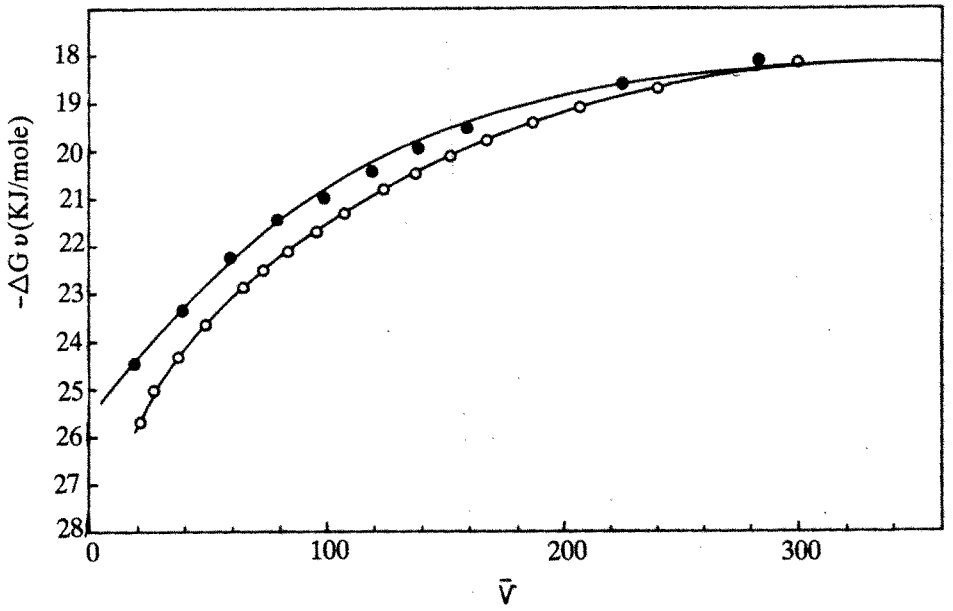

Fig 7. Gibbs energies per ligand bond ($\Delta G\,\bar{\upsilon}$) as a function of $\bar{\upsilon}$ for SDS on the interaction with H3 at pH 10.50 mM glycine. ●, 37°C; 0.27°C.

glycine buffer solution, pH10 (fig. 11) is exothermic which is in contrast to other buffers which is in figures 9,10 and 12 SDS complexes of H1 and H_2B [9, 12]. Figures 9 and 12 show the enthalpy of interaction between H3 and SDS in glycine buffer, pH3.2 and bicarbonate buffer, pH10 respectively. These data illustrate the initial interaction (ionic part) is exothermic and subsequent interaction (hydrophobic part) is endothermic. It is important to note, the transition point form exothermic to endothermic occurs at $\bar{V} = 20$ and $\bar{V} = 40$ in bicarbonate and glycine buffers, pH10 and pH3.2 respectively and the initial interaction released a quantity of heat about 1000 Kj mol^{-1} at the cited $\bar{\upsilon}$. $\Delta G_{\bar{\upsilon}}$ at $\bar{\upsilon} = 20$ and $\bar{\upsilon} = 40$ are equal to -24 and

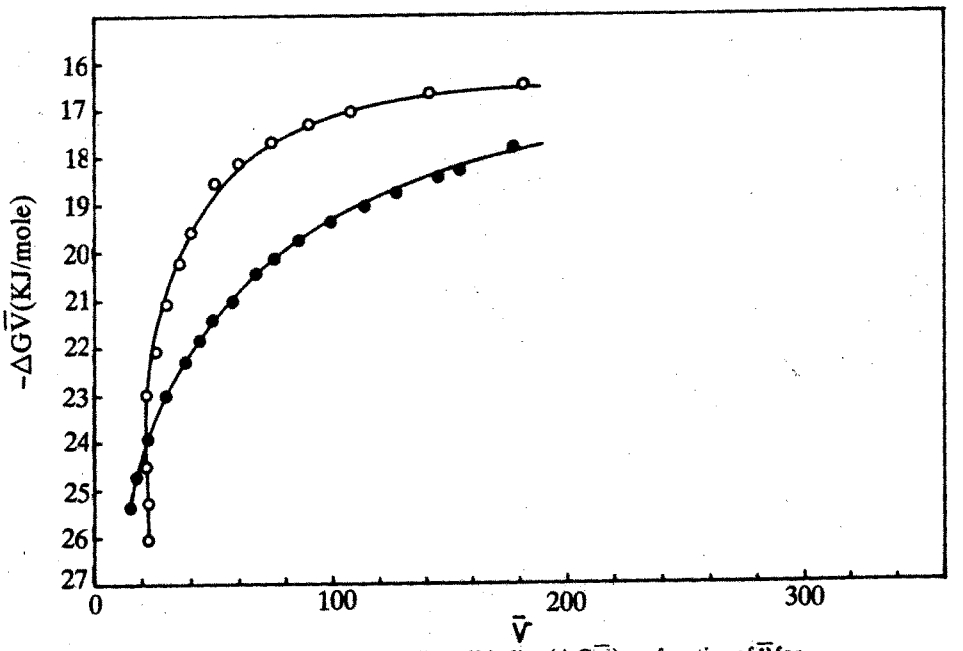

Fig 8. Gibbs energies per ligand binding ($\Delta G\bar{\upsilon}$) as a function of $\bar{\upsilon}$ for SDS on the interaction with H3 at pH 10, 1 mM Sodium bicarbonate + Sodium Carbonate.

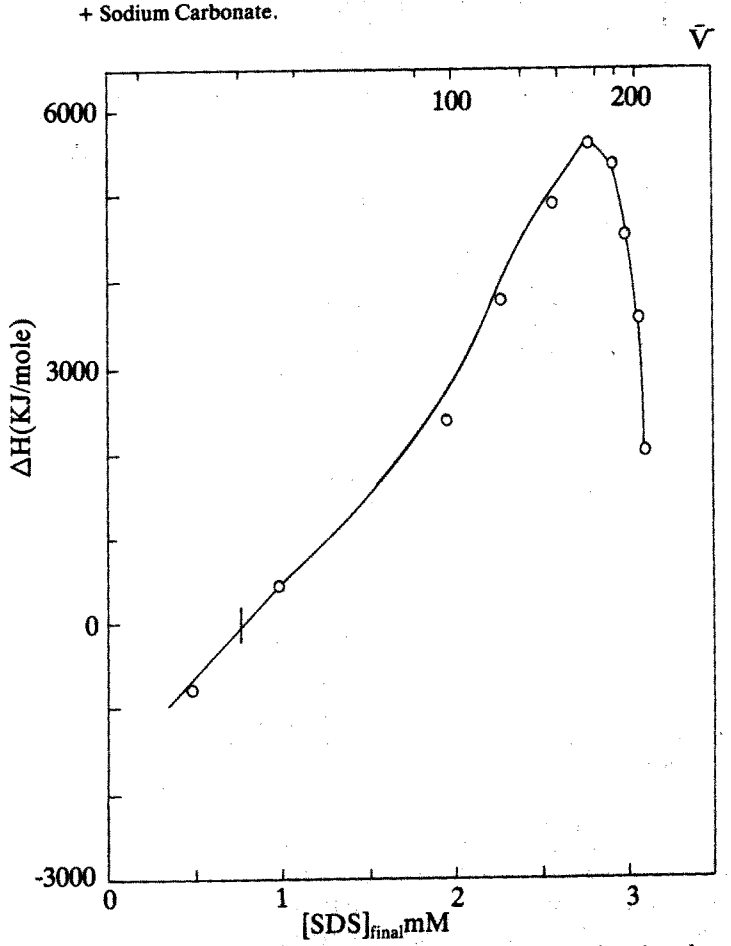

Fig. 9. Enthalpy of interaction between H3 and SDS as a function of final concentration of SDS at pH3.2, 50 mM glycine. The upper axis shows the number of SDS molecules bound per H3 at equilibrium.

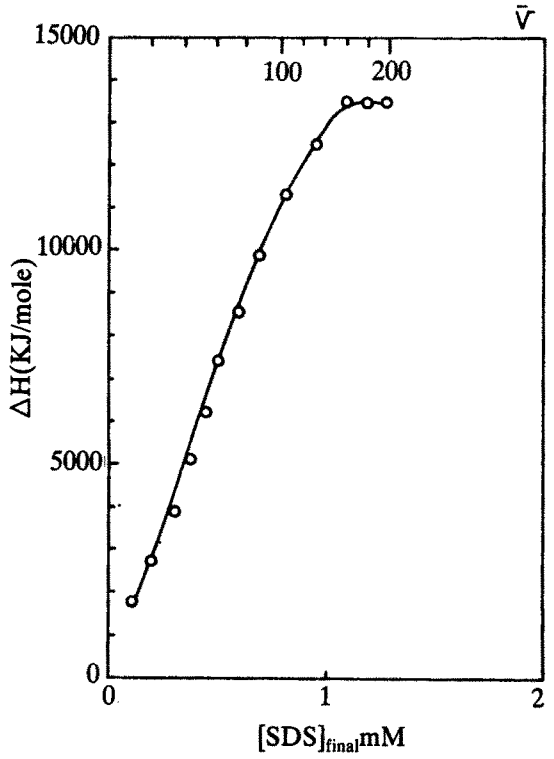

Fig 10. Enthalpy of interaction between H3 and SDS as a function of final concentration of SDS at pH 6.4, 2.5 mM Phosphate. The upper axis shows the number of SDS molecules bound per H3 at equilibrium.

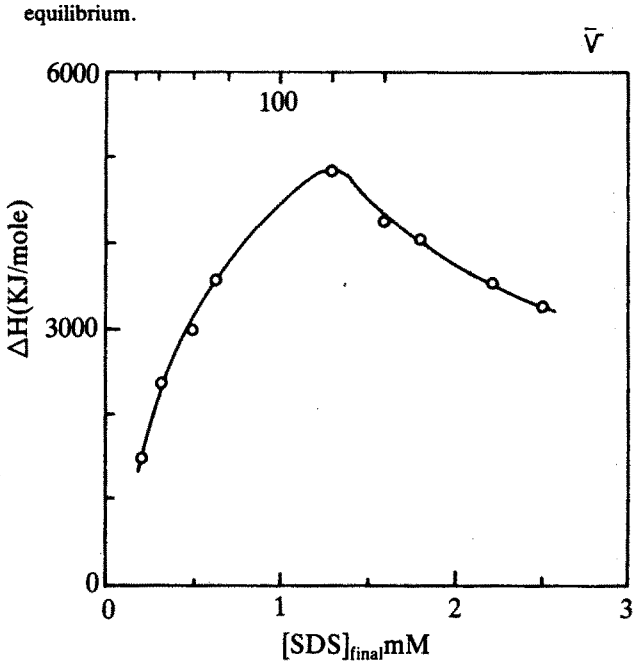

Fig 12. Enthalpy of interaction between H3 and SDS as a function of final concentration of SDS at pH 10, 1 mm Sodium bicarbonate + Sodium Carbonate.

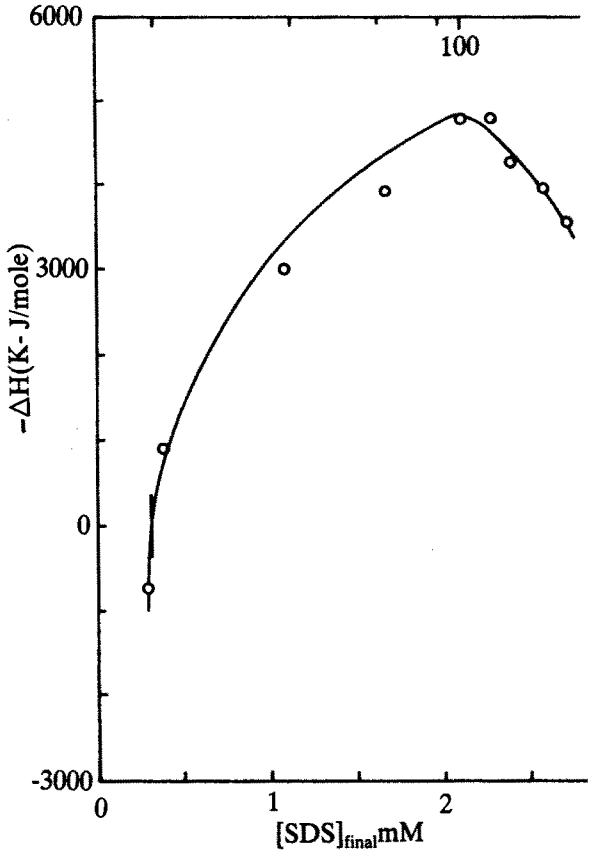

Fig 11. Enthalpy of interaction between H3 and SDS as a functio final concentration of SDS at pH 10, 50 mM glycine. The upper a Shows the number of SDS molecules bound per H3 at equilibri

-23.2 Kjmol^{-1}respectively. The enthalpy of subsequ interaction ($\bar{\upsilon} > 20$ and $\bar{\upsilon} > 40$) are endothermic up maxima at $\bar{\upsilon} = 100$ and $\bar{\upsilon} = 160$ for bicarbonate a glycine buffers at pH's 10 and 3.2 respectively. Thi probably the indication of unfolding of H3 by SDS. The shape of the enthalpy curves after the maxi suggested an exothermic contribution to the SDS - I interaction which are probably indicated by the foldi of H3 by SDS. $\Delta_{H\bar{\upsilon}}$ at $\bar{\upsilon}$ =100 a $\bar{\upsilon}$ =160 are equal to and 35 Kj mol^{-1} in bicarbonate and glycine buffe pH10 and pH3.2 respectively.

The enthalpy curve for glycine at pH10 shows t maxima at $\bar{\upsilon} = 120$ ($\Delta H\,\bar{\upsilon} = -40$ Kjmol^{-1}) and $\bar{\upsilon} > 1$ appears the endothermic contribution which is indication of unfolding of H3.

In contrast to the enthalpies in acid and alkali solution, nearer to the neutrality point (pH6.4, fig 1 the entbalpy of interaction of SDS and H3 diffe markedly from the other pH's. This curve saturates $\bar{\upsilon} = 160$ and $\Delta H\,\bar{\upsilon} = 160 = 84.375$ Kjmol^{-1} which seer to be rather high with respect to other pH's

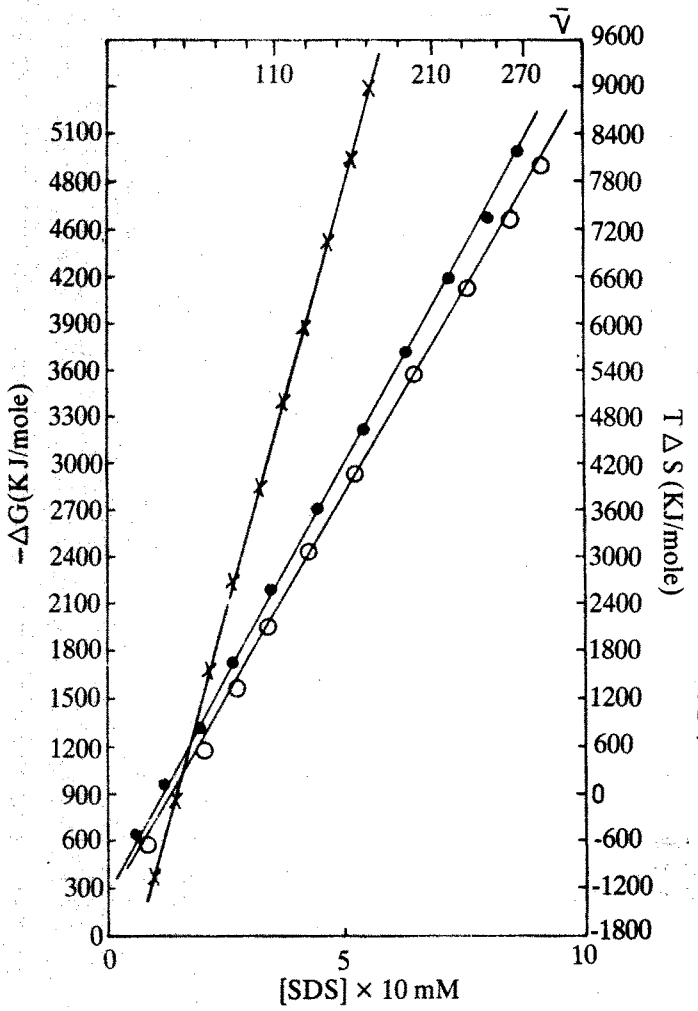

Fig 13. Enthalpy of interaction between H3 and SDS as a function of final concentration of SDS at pH 6.4, 2.5 mM. phosphate. The upper axis shows the number of SDS molecules bound per H3 at equilibrium. Left hand, ΔG, 0,27°C ●, 37°C. Right hand $T\Delta S$: X, 27 and 37°C.

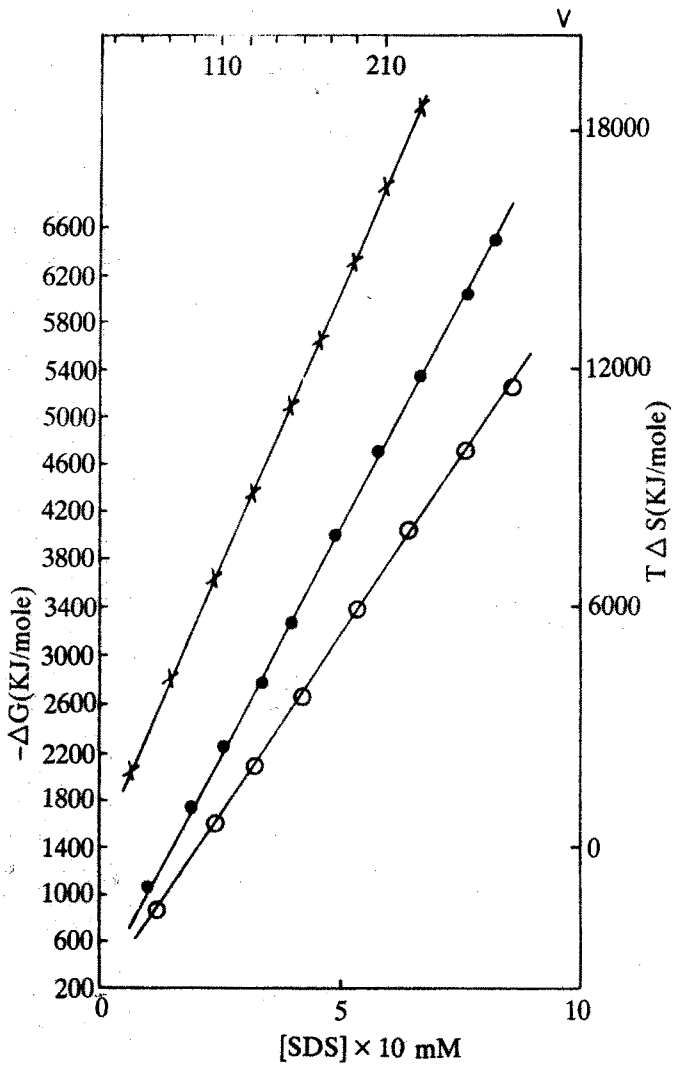

Fig 14. Thermodynamic parameters for interaction between H3 and SDS as a function of intial concentration of SDS at pH 3.2, 50 mM glycine. The upper axis shows the number of SDS molecules bound per H3 at equilibrium. Left hand, ΔG, 0,27°C; ●, 37°C Right hand $T\Delta S$: X, 27° and 37°C.

Figures 13, 14, 15 and 16 show corresponding ΔG and $T\Delta S$ as the functions of concentrations of SDS at various ΔH and temperatures of 27° and 37°C. The figures 13 and 15 show the negative entropy for initial interaction of H3 and SDS for phosphate and glycine buffer at pH's 6.4 and 10 respectively.

The calculation of the apparent Gibbs energies of binding which is applied to the entire binding isotherm is based on the Wyman binding potential concept [15], which was described previously (9). Figures 5, 6, 7 and 8 show $\Delta G_{\bar{v}}$ ($\frac{\Delta G}{\bar{v}}$) as a function of $\bar{v}$ from pH 3.2 to 10 at temperatures of 27° and 37°C. $\Delta G\upsilon$ indicates that SDS binding affinity to H3, is less negative with increasing $\bar{v}$ which this is believed to be due to the hydrophobic forces.

Among several pH's the increasing temperature from 27° to 37°C showed a larger varaition at pH 6.4 which this is an indication of larger binding SDS - H3 complexes (fig. 6); whereas, the smaller binding was observed at glycine buffer pH10. The increasing temperature reduced binding affinity of SDS - H3 interaction.

The enthalpies of interaction of H3 with SDS are shown in figures 9, 10, 11 and 12, these were obtained at pH's 3.2 and 10, the subsequent interaction approaches to positive entropy; whereas, the figures 14 and 16 show the positive entropy. The figures show the

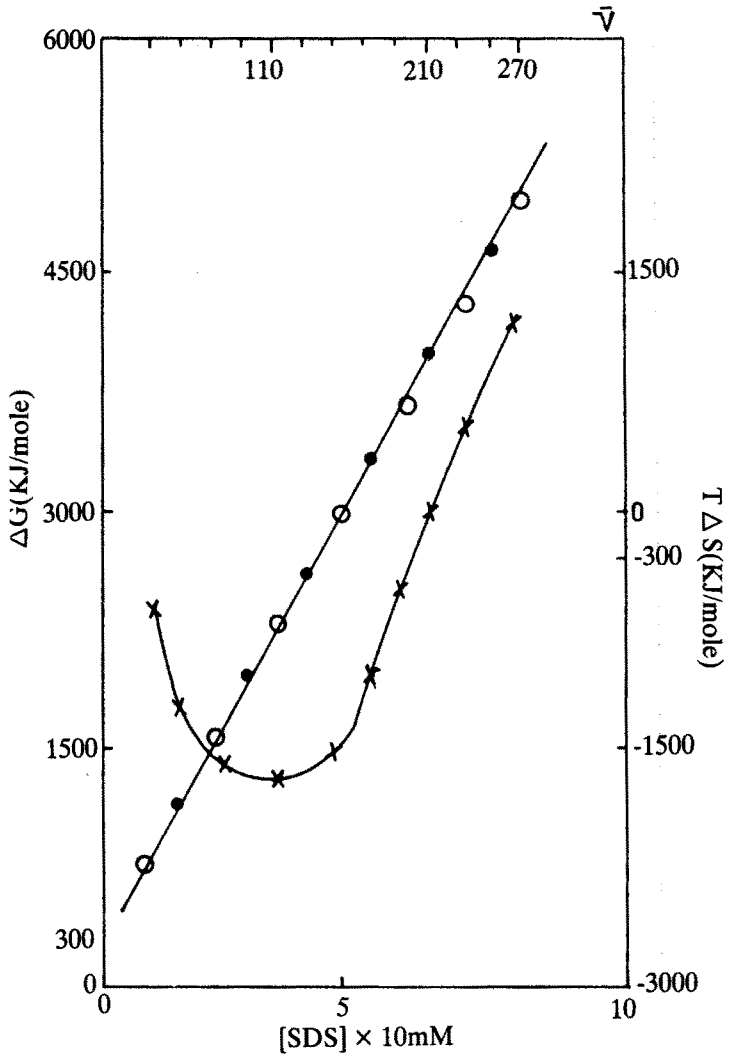

Fig 15. Thermodynamic parameters for interaction between H3 and SDS as a function of initial concentration of SDS at pH 10,50 mM glycine.
The upper axis shows the number of SDS molecules bound per H3 at equilibrium. Left hand, Δ G, 0,27°C; ●, 37°C.Right hand, T Δ S; X, 27°C and 37°C.

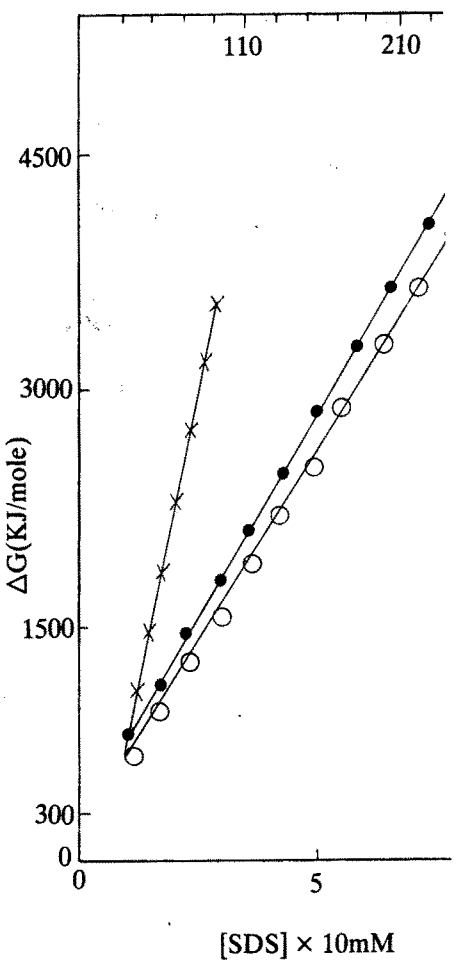

Fig 16. Thermodynamic parameters for interaction betw SDS as a function of initial concentration of SDS at p Sodium bicarbonate + Sodium carbonate. The upper ax number of SDS molecules bound per H3 at equilibrium ΔG, 0,27°C; ●, 37°C. Right hand, TΔS; X, 27°C and 3

quantitative amount of ΔG and TΔS which are indicating the interaction forces of a three dimensional structure of H3. The amount of thermodynamic parameters for H1 and H_2B were reported previously (9, 10, 12, 17). A comparison of the thermodynamic data of H1, H_2B and H3 would estimate the stability of their structures which are constructed by noncovalent forces.

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