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FOLDING OF THE INTERACTION OF HISTONE H_1 WITH SODIUM N-DODECYL SULPHATE

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Abstract

The effects of sodium n-dodecyl sulphate (SDS) on the structure of histone H_1 has been studied by a combination of equilibrium dialysis, U.V. spectroscopy; polyacrylamide gel electrophoresis, protein titration and viscometery techniques using, 2.5 mM phosphate buffer, pH 6.4. The interaction of H_1 and SDS in contrast to many other protein-SDS interactions is organized between $\bar{v}$ 40 to 70. Above $\bar{v}$=40 there is an exothermic contribution from H_1 folding characterized by minima in the enthalpy curve at about 65 Kj $mol.^{-1}$ This subject has been confirmed by spectroscopy, electrophoresis, titrametery and viscometery techniques.

Introduction

Histone H_1 consists of three structural domains. The N-terminal region, of 40 residues, is highly variable in sequence [1]. The next region, of 80 residues, is more conserved and contains most of the hydrophobic residues of the histone molecule [2]. And finally, the C-terminal region contains about 90 residues, which are nearly 90% lysin, alanine and proline, and it has been shown to be the most important DNA-binding domain in the H_1 molecule [3]. The structural role of the H_1 is not yet known. Taking into account structure-function relationship, It is clear that a better knowledge of H_1 structure may lead to a better understanding of its function. The main purpose of denaturation studies has always been to provide additional information on the structure, properties, and function of proteins.

Detergents such as sodium n-dodecyl sulphate (SDS) as a potent biological denaturant reacts readily with proteins, frequently causing dissociation of proteins into subunits and denatruation of the individual polypeptide chains. In addition, SDS is effective at very low concentrations and shows a high degree of strong binding to the protein. The nature of the binding between surfactants and proteins has been investigated and it has been found that certain surfactants can initiate the unfolding of the tertiary and secondary protein structures [4-10]. We report here a study of the folding of H_1 with SDS which has been investigated by a number of different physical techniques, to obtain more evidence for structural analysis of H_1.

Experimental Section

Preparation:

Histone H_1 extracted from calf thymus gland by the method of Johns (11) was obtained from Tehran (Ziaran) slaughterhouse.

Materials:

Sodium phosphate buffer (2.5 m M), pH 6.4 and I = 0.0069 contains 0.02% W/V sodium azide which contributes 0.0031 to the ionic strength (I) Sodium n-

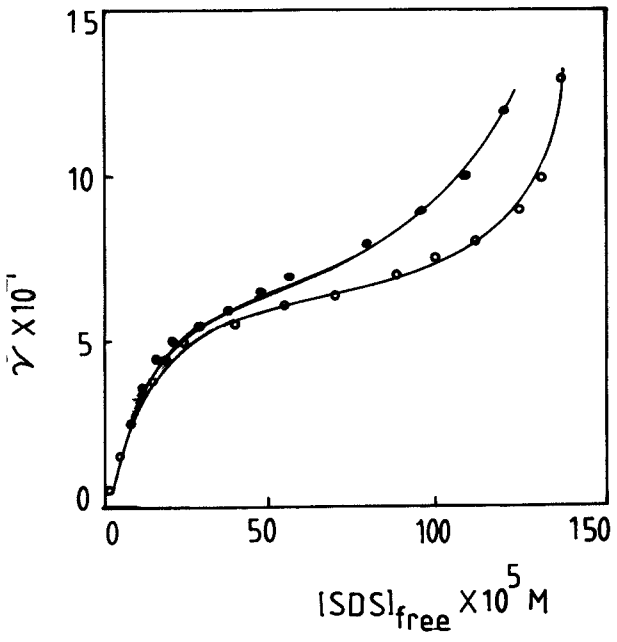

Fig.1. Binding of sodium n-dodecyl sulphate (SDS) to H_1 as a function of free SDS concentration at, pH 6.4 phosphate buffer. 0, 27°C and ●, 37°C

dodecyl sulphate (special pure grade) was obtained from the Merck Company. Acrilamide, bisacrilamide and N,N,N,N´,N´ -Tetramethyl ethylenediamine was obtained from Sigma Ltd. Rosaniline hydrochloride dye was used as supplied by B.D.H. Visking, the membrane dialysis tubing (molecular weight cut off 1000-14000) was from SIC (East Leigh) Hampshire, U.K. All the salts used in the preparation of the buffer were analytical grade and they were made up in doubly distilled water.

Methods

Equilibrium Dialysis:

Equilibrium dialysis experiments were carried out to determine the concentration of free sodium n-dodecyl sulphate (SDS) in equilibrium with the protein-SDS complexes at 27°C and 37°C and hence the amount of SDS bound to the proteins. The experiments were made either with dialysis bags made from Visking tubing (18/32 inch) as semipermeable membrane volume of 2 ml. aliquots of histone H_1 solution in the concentration of 0.01% (W/V) against 2ml aliquots of SDS solutions in the concentration range $1x10^{-4}$-$3x10^{-2}$ mol Lit^{-3}. Equilibration time was 96 hours. The free SDS concentrations in equilibrium with the complexes were assayed by the Rosaniline hydrochloride method [12].

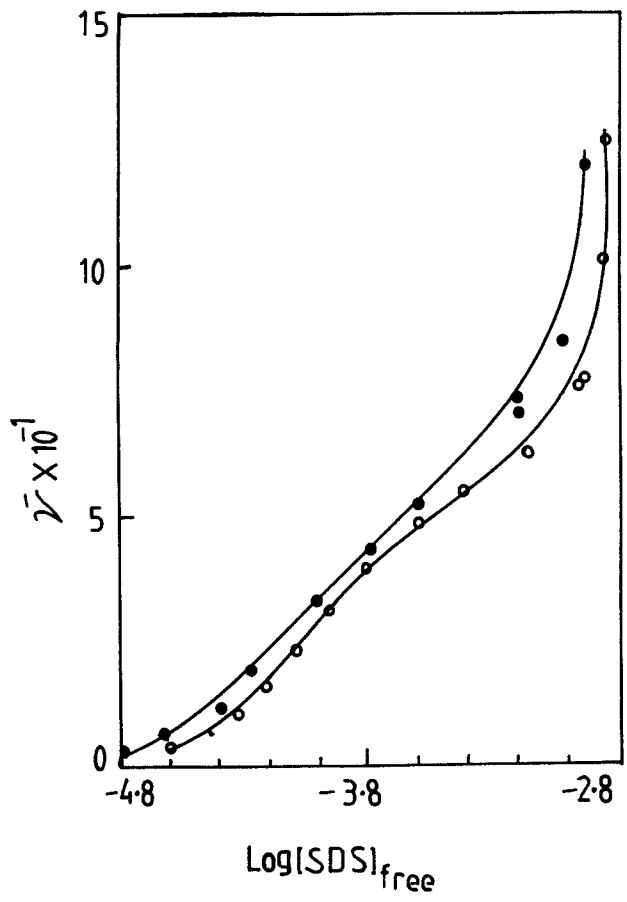

Fig.2. Binding isotherms for sodium n-dodecyl sulphate (SDS) on the interaction with H_1 at pH 6.4 phosphate buffer 0, 27°C and ●, 37°C.

Uv-Vis Spectroscopy

Measurements were made in the range 210-250 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 by adding portions of SDS solutions to the sample cuvette. The SDS concentrations were in the range $1x10^{-4}$-$3x10^{-2}$ mol Lit^{-3}.

Protein Titration:

Protein titrations were made with a Corning pH meter model 130 in conjuction with a nitrogen gas cylinder. Measurements were carried out to determine the numbers of protons dissociated from native H_1 and H_1 in the presence of SDS solutions in the concentration range $1x10^{-4}$-$3x10^{-2}$ mol Lit^{-3} [13].

Viscometery:

Viscosity measurements were made using an Ostwald Viscometer (75-100 sec). The average of five

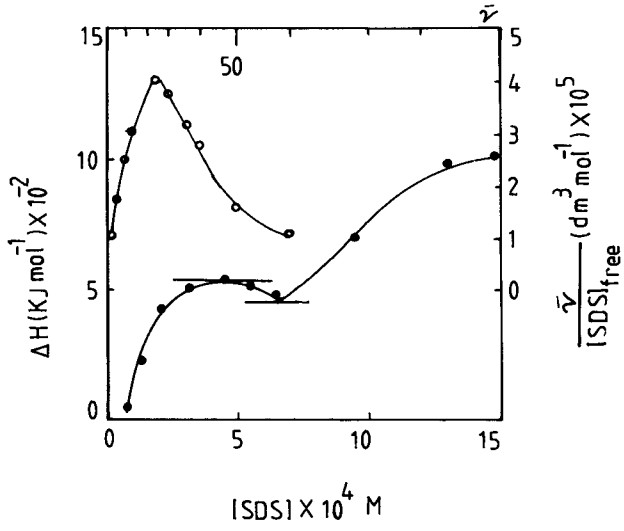

Fig.3. Enthalpy of binding of sodium n-dodecyl sulphate (SDS) to H_1 as a function of SDS concentration, and Scatchard Plot for binding at pH 6.4 phosphate buffer. The upper axis shows the number of SDS molecules bound per H_1 at equilibrium($\bar{\nu}$). ●, Left-hand axis, enthalpy curve. Right-hand axis, O, Scatchard Plot of binding data of Fig.1 at 27°C.

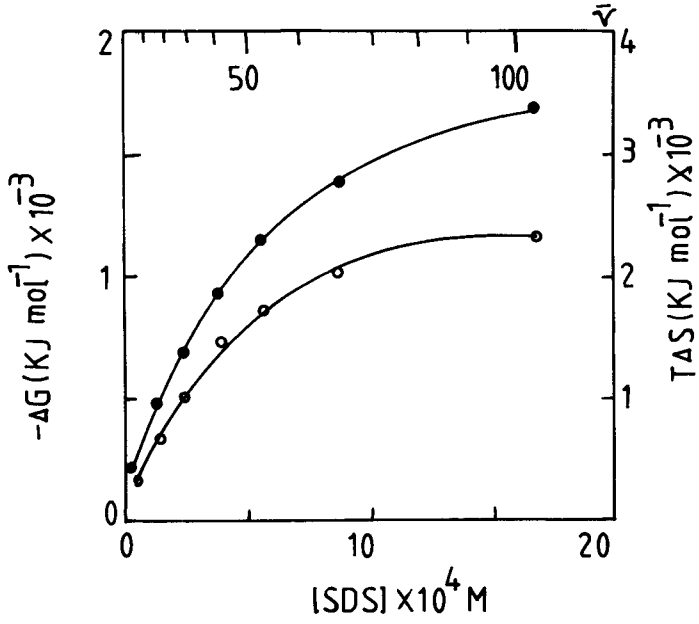

Fig.4. Left-hand axis, apparent Gibbs energy change as a function of SDS concentration at 27°C. Right hand axis O, $T\Delta S$ as a function of SDS concentration at 27°C. The uper axis shows the number of SDS molecules bound per H_1 at equilibrium ($\bar{\nu}$).

flow time (t) of a series of solutions of fixed protein concentration 0.01% and variable SDS concentrations in the range $1x10^{-4}$-$3x10^{-2}$ mol Lit^{-3} were measured in the 2.5 mM phosphate buffer, pH 6.4.

Gel Electrophoresis:

20% Polyacrylamide gel of 10 cm length was run for 3.5 hours at 180 volts. Acetic acid (0.9N) was used as running buffer. Gels were stained with porcion dye, and destained with acetic acid and methanol mixture. Gel electrophoresis was carried out with H_1 and H_1 in the presence of SDS concentraton in the range of $3x10^{-4}$-$1x10^{-2}$ mol Lit^{-3} [15].

All the measurements reported refer to SDS concentrations below the critical micelle concentration. In all calculations the molecular weight ot the H_1 was taken as 21000 [14], and the concentration of H_1 was taken 0.01% (W/V). Corrections for inequalities arising from Donnan effects are negligible at the ionic strength used.

Results and Discussion

Figure 1 shows the number of Sodium n-dodecyl sulphate (SDS) ions bound per molecule of H_1 ($\bar{\nu}$) as a function of the free SDS concentration in solution, measured by equilibrium dialysis. The curves initially rise very steeply, indicating that there are probably between 5 to 40 strongly binding sites which indicates specific binding, and suggests that the protein charge group interacts with the surfacant's head group[16]. At the higher values of $\bar{\nu}$ = 40, the nearly flat region there may be non-cooperative binding. The plateau region $\bar{\nu}$ =40 and 70 indicates that weak binding occurs. At values greater than $\bar{\nu}$ = 70 cooperative binding occurs. Fig. 1 shows increasing temperature affects on the hydrophobic parts much more than hydrophilic region. To clarify the presentation of the subsequent results the binding curve of Fig. 1 has been used to calculate $\bar{\nu}$ in solutions of known total sodium n-dodecyl sulphate concentration and H_1 concentration. The material balance equation requires that:

$$\bar{\nu}=\frac{(SDS)_{total}-(SDS)_{free}}{(H_1)} \qquad (1)$$

Fig. 2 shows the binding isotherms expressed $\bar{\nu}$ as a function of the logarithm of the free SDS concentration for the H_1 + SDS system at pH 6.4 in 27°C and 37°C. The curve shifted to the left by increasing the temperature. The calculation of the Gibbs energy and binding constant which can be applied from the area

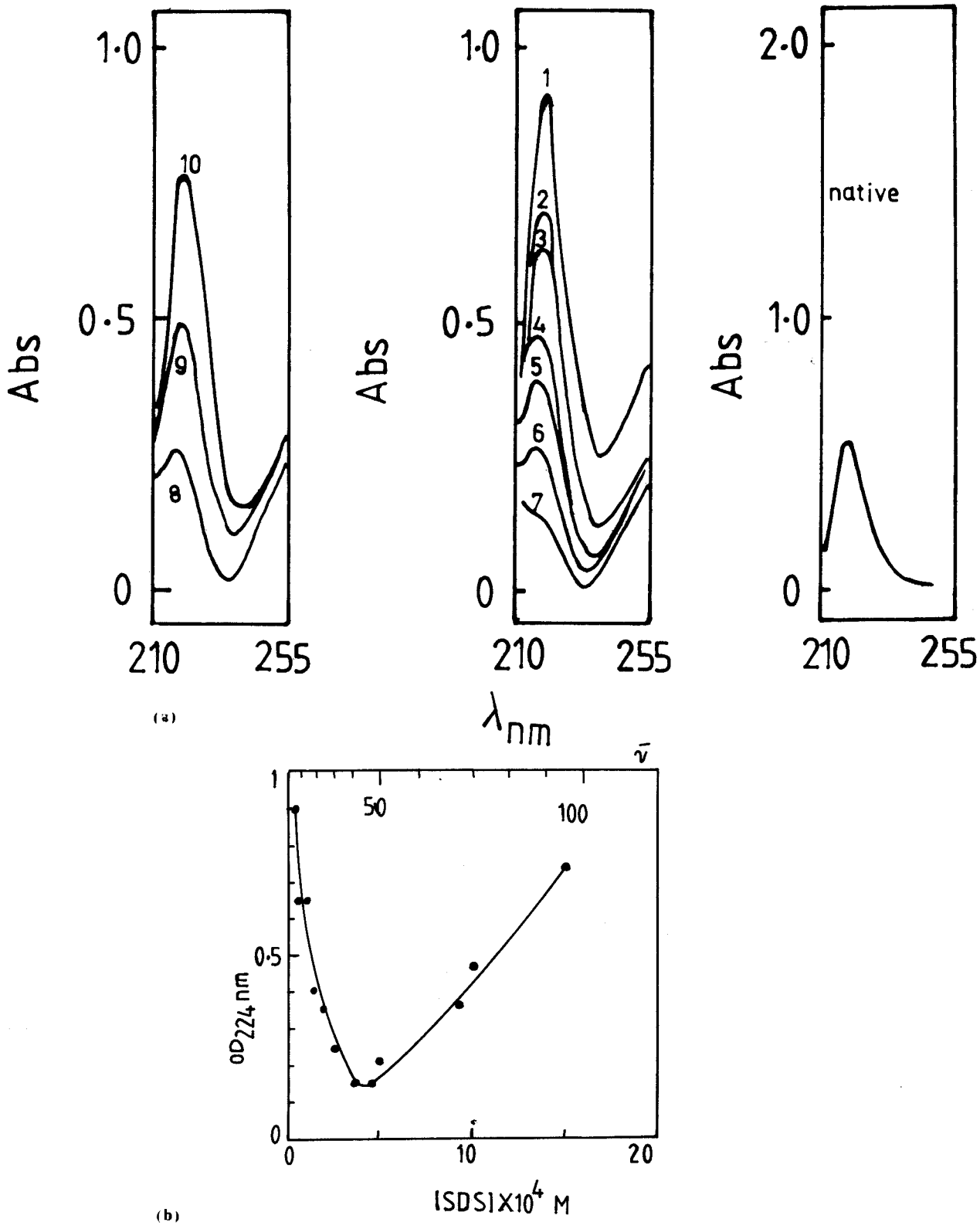

Fig.5.(a) Effect of SDS on the U.V. absorption spectra of histone H_1 at equilibrium in 27°C. (1) 0.0025 mM SDS (2) 0.05 mM SDS, (3) 0.1 mM SDS, (4) 0.15 mM SDS, (5) 0.2 mM SDS, (6) 0.25 mM SDS, (7) 0.35 mM SDS, (8) 0.5 mM SDS, (9) 1.0 mM SDS, (10) 1.5 mM SDS

(b) Optical density at 224 nm for H_1 as a function of SDS concentration at equilibrium in 27 C. The upper axis shows the number of SDS molecules bound per H_1 at equilibrium in 27°C.

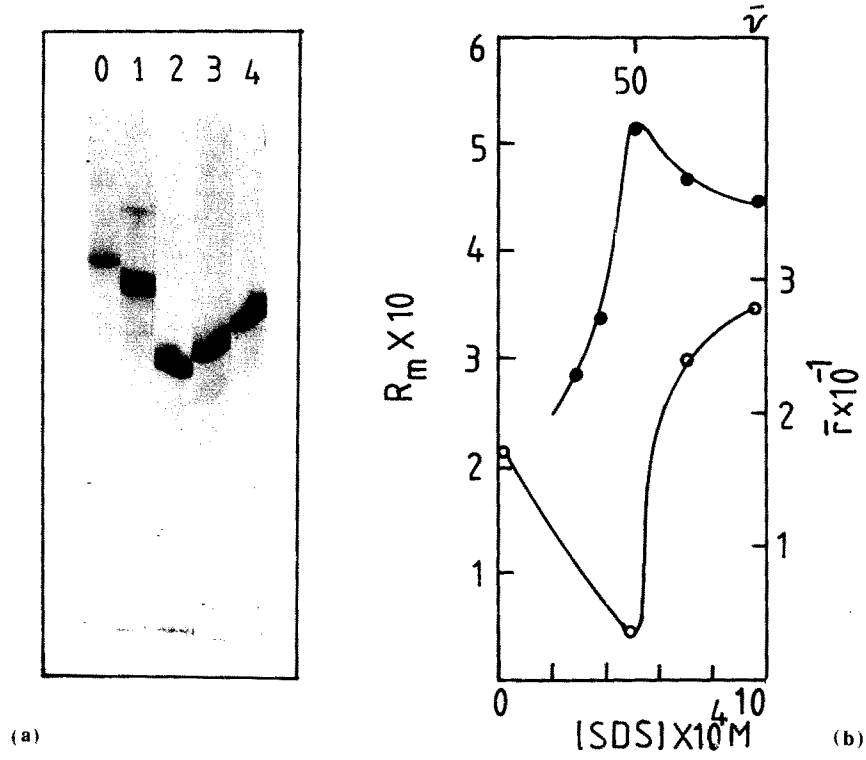

Fig. 6. (a) Polyacrylamide gel electrophoresis of H_1 with presence and absence of SDS concentration. O, Without SDS; (1), 0.4 mM SDS (2), 0.5 mM SDS; (3), 0.7 mM SDS; (4), 1.0 mM SDS. **(b)** left hand axis, ●, R_m electrophoretic mobility as a function of SDS concentration. Right hand axis, O, the number of proton dissociated ($\bar{\gamma}$) in the presence of various concentrations of SDS. The upper axis shows the number of SDS molecules bound per H_1 at equilibrium in 27°C.

under the binding isotherms based on the Wyman binding potential concept [8,17].

The enthalpy of interaction of H_1 with SDS is shown in Fig. 3 and was obtained from the temperature dependence of binding constant (K_{app}) using Van't Hoff relation [18].

$$\Delta H = \frac{Rd\,(Ln\,K_{app})}{d(\frac{1}{T})} \qquad (2)$$

Fig. 3 shows that the enthalpy change on the formation of the H_1 - SDS complex is positive in marked contrast to many other protein-SDS interactions [4-11]. The magnitude (530 Kj mol^{-1}) is comparable with the enthalpy change found for the binding of 40 n-dodecyl sulphate ions to H_1. Above $\bar{\nu}$ = 40 there is an exothermic contribution from H_1 folding characterised by minima in the enthalpy curve which is shown in Fig. 3. It is important to note the effect of SDS on the secondary structure of proteins are known that the detergent alters the helicity and form content of many proteins. It is also known that SDS disrupts tertiary structure as well [19, 20]. Therefore, SDS-protein interaction is usually disorganized. But on the contrary the interaction of H_1 and SDS, is organized at $\bar{\nu}$ = 40 and $\bar{\nu}$ = 60. Optical Rotatory Dispersion (ORD) confirms the folding of histones by anionic detergents [21].

The inset diagram in Fig. 3 shows the Scatchard Plot which can illustrate the important role of hydrophobic interaction in binding [13]. Above $\bar{\nu}$=40 the Scatchard Plot is consistent to minima in the enthalpy curve as shown in Fig.3. The amount of exothermic enthalpy contribution to folding is 65 Kj mol^{-1} which has been signed in enthalpy curve. The Gibbs energy and entropy of folding region at $\bar{\nu}$=40 and 60 equal to 950 and 1260 Kj mol^{-1} and 4.70 and 6.20 Kj K^{-1} mol^{-1} respectively which is shown in Fig.4.

Fig.5a shows the equilibrium U.V. spectra of H_1 as a function of various concentration of SDS. Fig. 5b

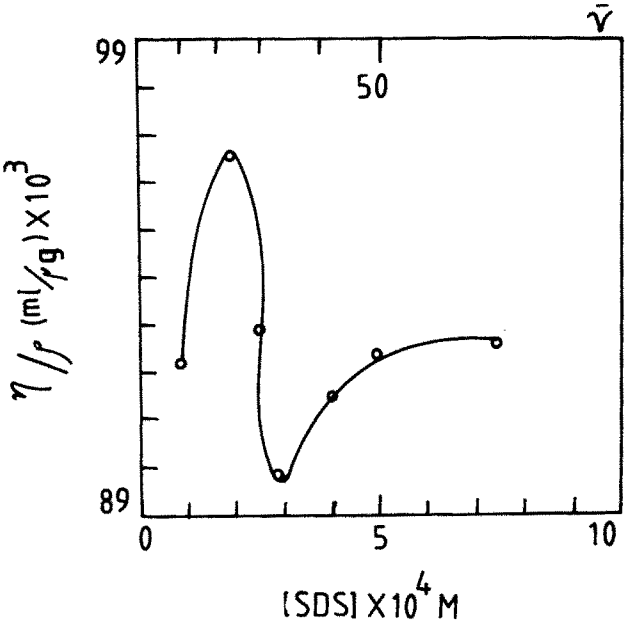

Fig.7. The kinematic viscosity as a function of SDS concentration. The specific density of 0.01% (W/V) H_1 equal to 1.006 (mg.ml^{-1}) at 27°C. The upper axis shows the number of SDS molecules bound per H_1 at equilibrium in 27°C.

shows the optical density for the 224 nm peak as a function of the SDS concentrations (0-15x10^{-4} M) at equilibrium. The upper axis shows the number of SDS molecules bound per H_1 at equilibrium. Addition of SDS to H_1 gives hypochromism to a U.V. spectrum with a maximum absorption at 224 nm until 0.4 mM SDS concentration, then gives rise at $\bar{v}$=50 which has confirmed the minima in the enthalpy curve in Fig.3.

Fig.6 a shows typical electrophoretic patterns of histone H_1 when 20% polyacrylamide gels were used. This pattern shows various concentration of SDS which is added to H_1, reaching to equilibrium, then runs were carried out. The maximum electrophoretic mobility occurs for 0.5 mM SDS concentration at $\bar{v}$=50 which has completely clarified the association of H_1 and is shown in Fig. 6b. The proton dissociation curve in Fig. 6b illustrates the folding phenomena in 0.5 mM SDS concentration [13].

Fig.7 shows the kinematic viscosity (η/p) with the extent of binding which appears in the minima at $\bar{v}$=40. This suggests that the maximum fluidity happened in the minima region of the enthalpy curve which is shown in Fig. 3. Therefore, Rheogram firmly settled the folding phenomena of H_1 in the presence of 0.5 mM SDS concentration.

Acknowledgements

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