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THERMODYNAMIC STUDY OF THE INTERACTION OF SODIUM *n*-DODECYL SULPHATE WITH HISTONE HI

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ABSTRACT

A study has been made of the interaction between histone HI and sodium *n*-dodecyl sulphate (SDS) in aqueous solutions of pH 3.2, 6.4 and 10 by equilibrium dialysis at 27 and 37 °C. The binding data were used to obtain thermodynamic parameters, specifically the Gibbs free energy of interaction, which is interpreted in terms of a theoretical model based on the Wyman binding potential. The data were then used to obtain the enthalpy of interaction from the temperature dependence of the equilibrium constants by the van't Hoff relation, in order to form a more complete picture of the thermodynamics of the interaction.

The enthalpy of interaction between histone HI and SDS is endothermic, in marked contrast to many other protein–SDS interactions. The results show that the interactions of histone HI and SDS are entropy-driven.

INTRODUCTION

A useful key to understanding the stability of proteins in solution is to observe the manner in which these molecules denature. Protein denaturation is any process which results in a change in the three-dimensional structure from the native conformation to some other conformation. Protein denaturation occurs in a number of ways. On the molar scale, sodium *n*-dodecyl sulphate (SDS) is probably one of the most effective denaturants [1–3]. The observation by Anson [4] that SDS is a potent protein denaturant directed the attention of protein chemists to detergent interactions with proteins. The interaction of SDS with proteins has been studied in some detail [5–11]. Anionic surfactants are believed to bind initially to specific cationic sites on the protein; subsequent binding is largely hydrophobic and non-specific [9].

Histones are a family of basic proteins that are fundamental in the structural organization of chromatin [12]. Recently, much progress has been

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made in understanding the general organization of chromatin structure. Histone Hl is particularly important in the stabilization of this structure [13].

The present work reports on a study of the interaction of SDS with histone Hl in which the technique of equilibrium dialysis was used to obtain the enthalpy, Gibbs free energy and entropy of binding between histone Hl and SDS over a range of pH and SDS concentration values, at temperatures of 27 and 37 °C.

EXPERIMENTAL

Histone preparation

Histone Hl was extracted from calf thymus glands prepared by the Tehran (Ziaran) Slaughter-house according to the method of Johns [14]. It was purified by passage through a column of BioGel p-60 (length, 40 cm; diameter, 2.5 cm) using a 1000 ml salt gradient of 0.75–1.25 M with 0.05 M phosphate, pH 6.8. The flow rate was 60 ml h^{-1}. This procedure was found to completely remove other histones. The appropriate fractions from the column effluent were pooled and desalted by dialysis, and the histone was precipitated by addition of four volumes of acetone. The precipitate was then washed with dry acetone and dried under vacuum.

Materials

A number of buffers were used, each of which contained 0.02% w/v sodium azide contributing 0.0031 to the ionic strength I. The buffers were: (i) glycine (50 mM) plus hydrochloric acid, pH = 3.2, $I = 0.0119$; (ii) sodium phosphate (2.5 mM), pH = 6.4, $I = 0.0069$; and (iii) glycine (50 mM) plus sodium hydroxide, pH = 10.0, $I = 0.0318$.

Visking membrane dialysis tubing (molecular weight cut-off 1000–14000) was from SIC (Eastleigh), Hampshire, U.K. Rosaniline hydrochloride dye was used as supplied by B.D.H. SDS (especially pure grade) was from Merck. All the salts used in the preparation of the buffers were of analytical grade, and were made up in doubly distilled water.

Methods

Equilibrium dialysis was carried out at 27 and 37 °C in an orbital incubator, equilibrating 2 cm^3 aliquots of histone Hl solution (0.02% w/v) as required against 2 cm^3 aliquots of SDS solution in the concentration range 50×10^{-6} to 5×10^{-3} mol dm^{-3}. Equilibration times were >96 h. The free SDS concentrations in equilibrium with the complexes were assayed by the rosaniline hydrochloride method [15–17]. A UV–VIS Shimadzu spectropho-

tometer, model 260, was used for the measurement of optical density at 550 nm. A calibration curve of E550 vs. amount of SDS was prepared and used to determine the free SDS concentrations at equilibrium. The critical micelle concentration (cmc) of the SDS was measured in 50 mM, $I = 0.0088$ glycine/HCl and glycine/NaOH, and 2.5 mM, $I = 0.0069$ phosphate buffers of pH values 3.2, 10 and 6.4, respectively, using conductivity and surface tension techniques [18,19]. The cmc value obtained was 7.5 mM at 27 and 37 °C, which is in reasonable accord with the literature [19]. Corrections for inequalities arising from Donnan effects are negligible at the ionic strength used.

In all calculations, the molecular weight of histone Hl was taken to be 21 000 [20].

RESULTS AND DISCUSSION

Figures 1 and 2 show the binding isotherms (the number $\bar{\nu}$ of surfactant ions bound per protein molecule as a function of the logarithm of the free surfactant concentration $[S_f]$) for SDS on histone Hl as a function of pH and temperature. These figures indicate that the binding of SDS to histone Hl in buffer solutions is pH and temperature-dependent. Qualitatively, the pH determines the state of charge of the protein as a consequence of changes in the degree of ionization of the ionic side chains of the amino acid residues. Since the initial interaction of SDS with histone Hl may be ionic at

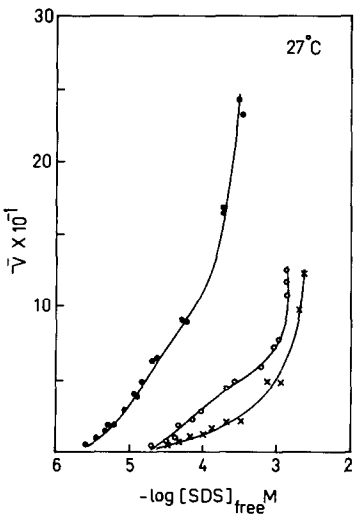

Fig. 1. Binding isotherms for SDS on interaction with histone Hl at 27 °C: ●, pH 3.2; ○, pH 6.4; ×, pH 10.0. The initial histone Hl concentration was 0.02% w/v.

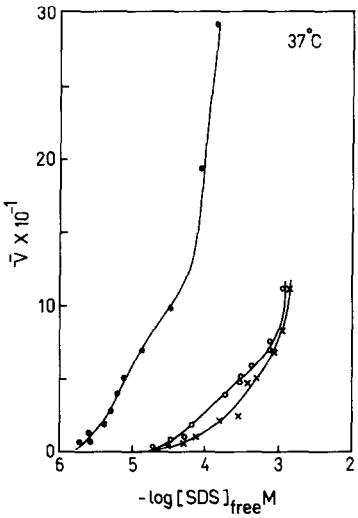

Fig. 2. Binding isotherms for SDS on interaction with histone Hl at 37 ° C: ●, pH 3.2; ○, pH 6.4; ×, pH 10.0. The initial histone Hl concentration was 0.02% w/v.

low concentrations of SDS, the initial pH of the protein solution would be expected to influence surfactant binding.

The ionic binding of SDS ions to the protein initially increases the hydrophobicity of its surface and can lead to aggregation. The increase in negatively charged residues with increasing pH would have an inhibitory effect on binding so that the isotherms were shifted towards lower free SDS concentration with increasing pH and temperature. It should be noted that the cmc of SDS is approximately the same at 27 ° C and 37 ° C.

Calculation of the Gibbs energies of binding which can be applied to the entire binding isotherm is based on the Wyman binding potential concept [21]. The binding potential is calculated from the area under the binding isotherm, according to the equation

$$\pi = RT\int_{v_i=0}^{v_i} v_i \, \mathrm{d} \ln[S_f] \tag{1}$$

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

$$\pi = RT \ln(1 + K_{app}[S_f]) \tag{2}$$

Values of K_{app} were determined by application of eqns. (1) and (2) and used to determine values of

$$\Delta G_{\bar{\nu}} = \frac{\Delta G_{app}}{\bar{\nu}} = \frac{RT}{\bar{\nu}} \ln K_{app} \tag{3}$$

Figures 3 and 4 show $\Delta G_{\bar{\nu}}$ and ΔG_{app}, respectively, as a function of $\bar{\nu}$ and $[SDS]_{final}$, at various pH values and temperatures. The shapes of these curves show that the binding energy gradually decreases as saturation is approached, in contrast to the effect of increasing the temperature.

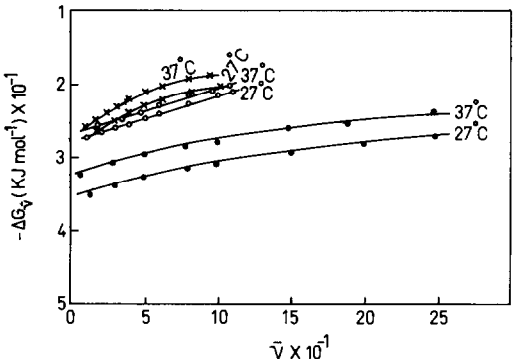

Fig. 3. Apparent Gibbs energy change per SDS molecule bound ($\Delta G_{\bar{\nu}}$) as a function of $\bar{\nu}$, at 27 and 37 °C: ●, pH 3.2; ○, pH 6.4; ×, pH 10.0.

$\Delta G_{\bar{\nu}}$ at $\bar{\nu} = 100$ changes from −27.2 to −18.6 kJ mol^{-1} at 27 °C and from −30.5 to −20.0 kJ mol^{-1} at 37 °C on increasing the pH from 3.2 to 10.0.

The corresponding entropy changes $\Delta S_{\bar{\nu}}$ ($\bar{\nu} = 100$) are 101, 180 and 323 J mol^{-1} K^{-1} at pH values of 6.4, 10 and 3.2, respectively (Fig. 5).

The enthalpies of interaction of histone Hl with SDS are shown in Fig. 6. These values were obtained from the temperature dependence of K_{app} using the van't Hoff relation [2]

$$\Delta H = \frac{R\mathrm{d}(\ln K_{app})}{\mathrm{d}(1/T)} \tag{4}$$

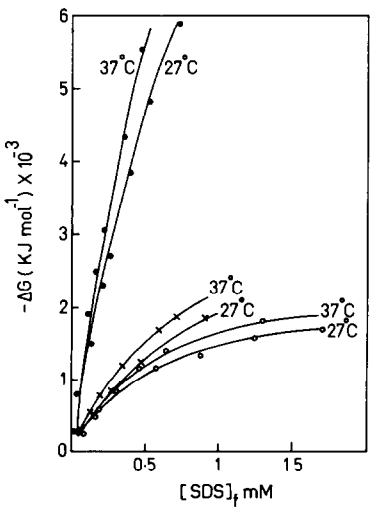

Fig. 4. Apparent Gibbs energy change as a function of final concentration of SDS, at 27 and 37 °C: ●, pH 3.2; ○, pH 6.4; ×, pH 10.0.

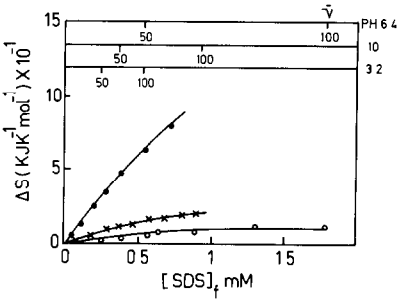

Fig. 5. Entropy change as a function of final concentration of SDS, at 27 and 37° C: ●, pH 3.2; ○, pH 6.4; ×, pH 10.0. The upper axis shows the number of SDS molecules bound per histone Hl molecule at equilibrium.

The enthalpies of interaction of SDS with histone Hl become more endothermic with decreasing pH. Studies have been made of the enthalpies of interaction of several globular proteins and enzymes (including bovine serum albumin [22,23], ovalbumin [22], *b*-lactoglobulin [24], trypsin [25], lysozyme [26,27] and catalase [5–7,28]) with SDS, and all have been found to be exothermic. Histone Hl, like ribonuclease [29], is thus exceptional, as the enthalpies of interaction with SDS over a range of pH values in phosphate and glycine buffers are endothermic. The upper axes in the enthalpy curves show the number of SDS molecules bound per histone Hl molecule at equilibrium. The SDS concentrations at which binding to histone Hl saturates are approximately 1.9, 0.95 and 1.5 mM for pH values of 6.4, 10 and 3.2, respectively. This is assuming that saturation binding corresponds to ~1.4 g SDS per gram of proteins [7] at pH values of 6.4 and

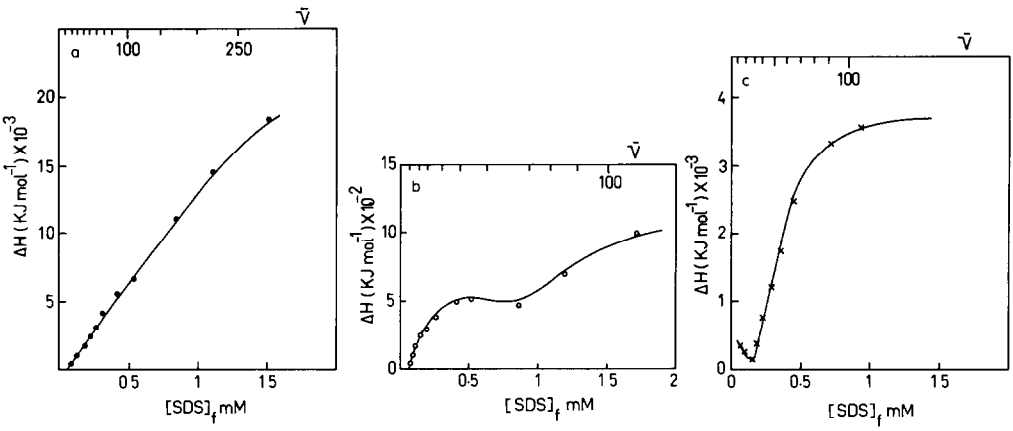

Fig. 6. Enthalpy of interaction between histone Hl and SDS in aqueous solution between 27 and 37° C: (a) pH 3.2; (b) pH 6.4; (c) pH 10. The initial histone Hl concentration was 0.02% w/v and the lower horizontal axis gives the SDS concentration after equilibrium.

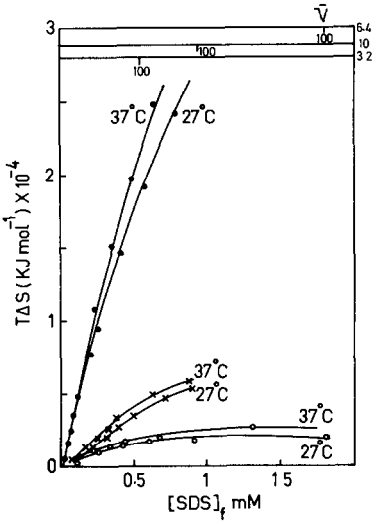

Fig. 7. $T\Delta S$ parameter for the interaction between histone Hl and SDS, at 27 and 37 °C: ●, pH 3.2; ○, pH 6.4; ×, pH 10.0. The upper axis shows the number of SDS molecules bound per histone Hl molecule at equilibrium at 27 °C.

10. Saturation binding at pH = 3.2 is > 3.4 g SDS per gram of protein [30]. At these SDS concentrations, the enthalpies of interaction are 3600, 1000 and > 18000 kJ mol^{-1} for pH values of 10.0, 6.4 and 3.2, respectively.

Figure 7 shows another thermodynamic parameter, $T\Delta S$, as a function of SDS concentration. It can be seen that $T\Delta S > \Delta H$.

At SDS concentrations above the cmc thermal effects can arise from the dissociation of micelles, but in the present work all measurements were made below the cmc.

ACKNOWLEDGEMENTS

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