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Thermodynamic studies on the interaction between sodium *n*-dodecyl sulphate and histone H_2A

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Abstract

The thermodynamic parameters of interaction between histone H_2A and sodium *n*-dodecyl sulphate (SDS) in aqueous solutions of pH 3.2, 6.4 and 10, measured over a wide range of SDS concentration by equilibrium dialysis at 27 °C and 37 °C, are discussed. The data are used to determine the free energy from the Wyman binding potential theoretical model, and the enthalpy of interaction from the temperature dependence of the equilibrium constants using the van't Hoff relation. The data obtained for H_2A are also compared with corresponding data for histone H_2B.

INTRODUCTION

Histone fraction H_2A is the most hydrophobic of the histone fractions which is completely free of the extended antiparallel β-conformation. The H_2A fraction also gave the highest histone/DNA ratio during recombination experiments and it has been suggested that it may play an important part in maintaining the condensation or supercoiling of the DNA molecule [1].

H_2A is located in the nucleosome core in the form of strongly bound dimers (H_2A, H_2B) and (H_2B, H_4) and weakly bound dimers (H_2A, H_4) and (H_2B, H_3) [2].

The interaction of detergents with globular proteins frequently leads to the disruption of their tertiary structure and the formation of protein–detergent complexes. The mechanism of detergent denaturation involves the binding of detergent ions to sites on the protein molecule, which results in denaturing and further binding, often in a cooperative fashion.

We have previously reported several studies involving the interaction of sodium *n*-dodecyl sulphate (SDS) as a potent biological detergent with histones such as H_1, H_2B and H_3 [3–7] and catalase [8–10]. The major aims

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of these investigations were the application of equilibrium dialysis and microcalorimetery in the determination of the thermal effects caused by the interaction of a detergent with histones and catalase, respectively.

The thermochemical study of interaction between H_2A and SDS is reported in this paper. A comparison of the thermodynamic parameters of interaction of SDS with histones H_1, H_3 and H_2B are also reported.

EXPERIMENTAL

Materials

Histone H_2A was obtained from Sigma. 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 10000–14000) was obtained from SIC (Eastleigh, Hampshire, UK) Rosaniline hydrochloride dye was used as supplied by B.D.H. and SDS (especially pure grade) was from Merck.

All the salts used in the preparation of the buffers were of analytical grade, and were solubilized in doubly distilled water.

METHODS

The equilibrium dialysis for measuring bound SDS was carried out according to previously described methods [3,4]. The critical micelle concentration (c.m.c.) of SDS was measured in the cited buffers, using conductivity and surface tension techniques. The c.m.c. value obtained was 7.5 mM at 27°C and 37°C, which is in reasonable accord with the literature [11]. Corrections for inequalities arising from Donnan effects are negligible at the ionic strengths used.

In all calculations, the molecular weight of H_2A was taken to be 14000 [12]. In all cases, the concentration of histones was 0.01% (w/v).

RESULTS AND DISCUSSION

Figure 1(abc) shows the number of moles of SDS bound per mole of histone H_2A ($\bar{\nu}$) measured by equilibrium dialysis as a function of the logarithm of the free SDS concentration at 27°C and 37°C and at pH values of 3.2, 6.4 and 10, respectively. Figure 1(ac) shows an initial plateau region which has been attributed to binding on ionic sites and a further steep rise attributable to hydrophobic binding [13]. Figure 1(b) shows an initial steep rise in $\bar{\nu}$ at a free surfactant concentration which is typical of

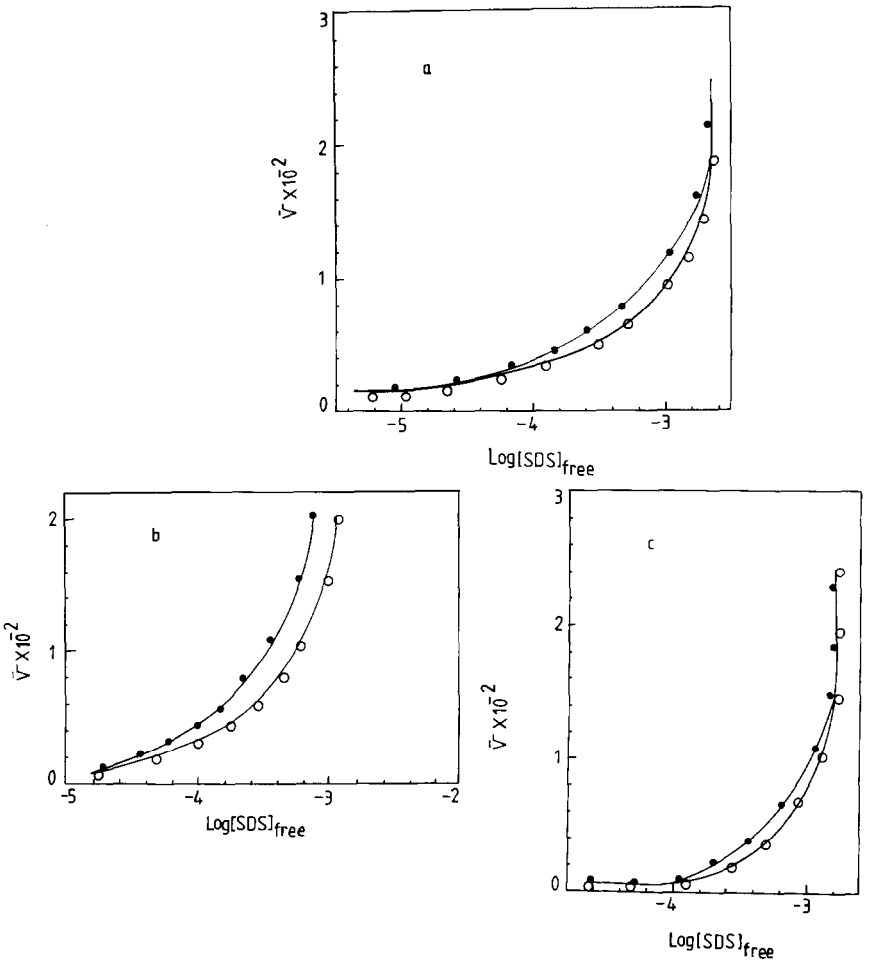

Fig. 1. Binding isotherms for SDS on interaction with histone H_2A: ○, 27 °C; ●, 37 °C. (a) pH 3.2, (b) pH 6.4, (c) pH 10.

cooperative binding. In all cases, the binding isotherms were shifted towards lower free SDS concentration with increasing temperature. It should be noted that the c.m.c. of SDS is approximately the same at 27 °C and 37 °C.

Calculation of the binding constants which are applicable to the entire binding isotherms are based on the Wyman binding potential concept [14]. The binding potential is calculated from the area under the binding isotherm, according to the equation

$$\Pi = RT\int_{\bar{\nu}_i}^{\bar{\nu}_i} \bar{\nu}_i \, \mathrm{d} \ln[\mathrm{SDS}]_{\mathrm{free}} \tag{1}$$

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

$$\Pi = RT \ln\left(1 + K_{\mathrm{app}}[\mathrm{SDS}]_{\mathrm{free}}\right) \tag{2}$$

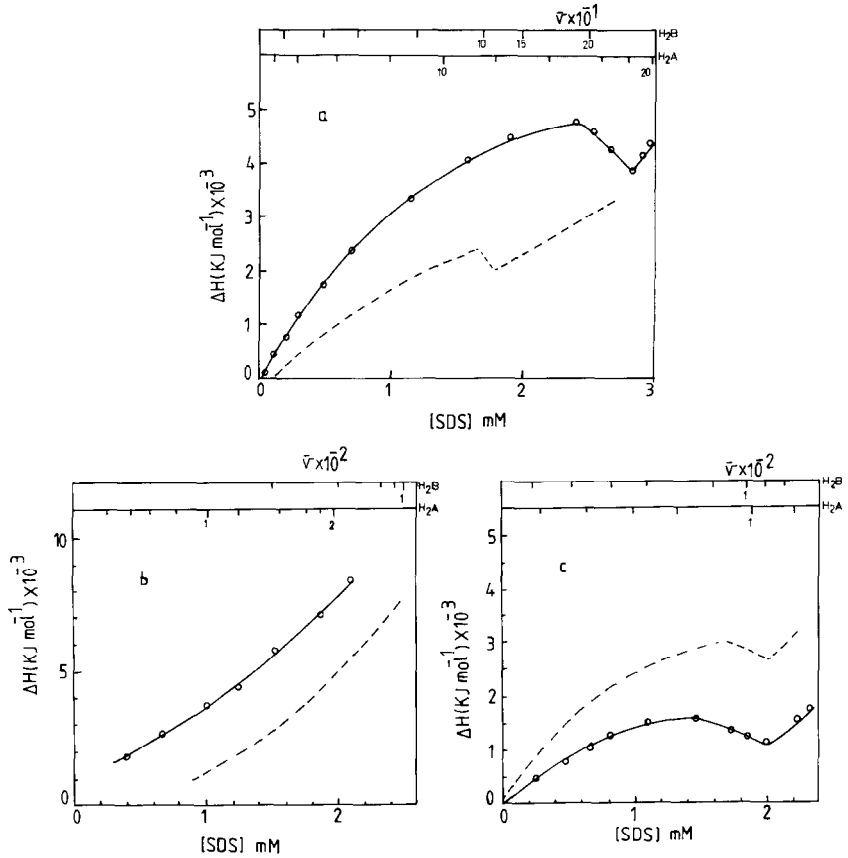

Fig. 2. Enthalpy of interaction between histone H_2A with SDS in aqueous solution. The upper axis shows the number of SDS molecules bound per histone molecule at equilibrium. The broken line is for histone H_2B, taken from ref. 4. (a) pH 3.2, (b) pH 6.14, (c) pH 10.

The values of K_{app} were determined from equations (1) and (2) and were used to determine the value of $\Delta G_{\bar{\nu}}$ as follows

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

The values of the enthalpy of interaction ΔH were obtained from the temperature dependence of K_{app} using the van't Hoff relation [15]

$$\frac{\mathrm{d} \ln K_{app}}{\mathrm{d}T} = \frac{-\Delta H}{RT^2} \tag{4}$$

The enthalpies of interaction of histone H_2A with SDS are shown in Fig. 2(abc), in which they are compared with the corresponding data obtained previously for histone H_2B [4]. At all pH values (Fig. 2) the curves for both types of histones are of similar shape, even though the enthalpy is more endothermic for histone H_2A than it is for histone H_2B except at pH 10

which is less endothermic for histone H_2A than it is for histone H_2B. This difference could probably be due to the cationic:anionic ratio of H_2A (2.2) being higher than that of H_2B (1.7) [16].

Figure 2(ac) shows minima which suggest the presence of an exothermic contribution from corresponding interactions. The exothermicities of H_2A and H_2B are similar at pH 10 (Fig. 2c) whereas at pH 3.2 they are different, that of H_2A being about 900 kJ mol^{-1} and that of H_2B about 400 kJ mol^{-1}. Unlike the enthalpies obtained in acid and alkaline solution (pH 3.2 and 10) the curve in Fig. 2(b) (pH 6.4) appears to differ markedly in shape from the others and also does not show minima. These curves seem to become more endothermic as the pH approaches neutrality.

The binding affinity of H_2B–SDS complexes is much higher than that of H_2A–SDS at pH 6.4. For example, at $\bar{\nu} = 70$ (assuming saturation binding corresponds to about 1.4 g SDS per gram of protein [17]), $\Delta H_{\bar{\nu}}$ is equal to 40

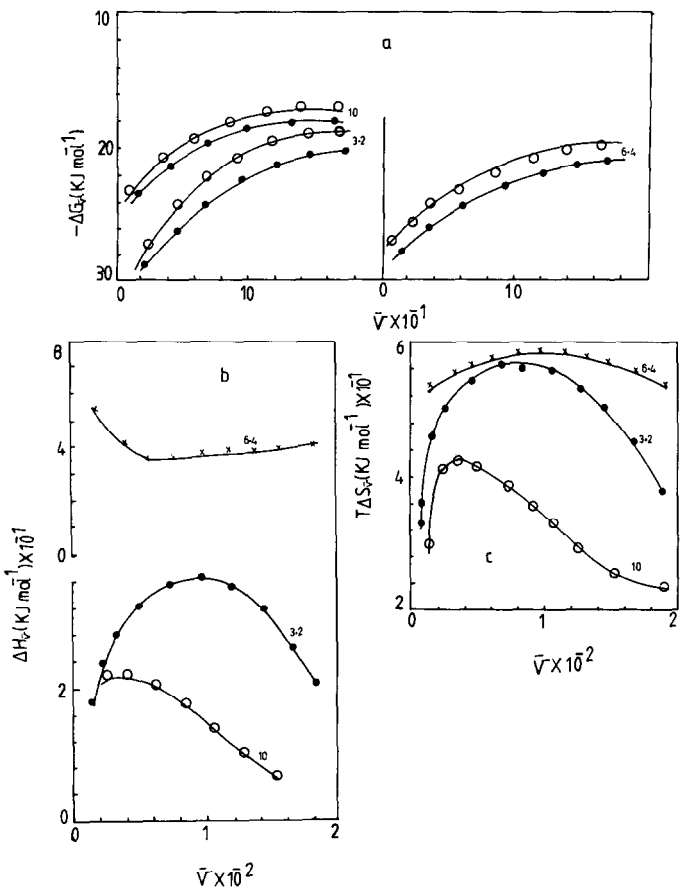

Fig. 3. Thermodynamic parameters for interaction between histone H_2A and SDS: ○, 27 °C; ●, 37 °C. (a) $\Delta G_{\bar{\nu}}$, (b) $\Delta H_{\bar{\nu}}$, (c) $T\Delta S_{\bar{\nu}}$.

TABLE 1

Thermodynamic parameters for the formation of SDS–histone (H_2A, H_2B, H_3 and H_1) complexes at various pH values and 27 °C

Thermodynamic parameters	H_2A ($\bar{\nu} = 70$)			H_2B ($\bar{\nu} = 70$)			H_3 ($\bar{\nu} = 75$)			H_1 ($\bar{\nu} = 100$)		
	3.2 [a]	6.4	10	3.2	6.4	10	3.2	6.4	10	3.2	6.4	10
$\Delta H_{\bar{\nu}}$ (kJ mol^{-1})	36.0	38.0	20.0	26.5	85.7	37.85	22.7	53.33	−46.7	90.0	10.0	36.0
$-\Delta G_{\bar{\nu}}$ (kJ mol^{-1})	22.0	22.0	19.0	21.4	22.9	22.85	21.0	22.7	21.9	50.0	16.5	18.0
$\Delta S_{\bar{\nu}}$ (J mol^{-1} K^{-1})	190	200	133	157	356.6	171.7	131.7	253.3	84.43	500	86.7	183.3

[a] pH value.

and 100 kJ mol^{-1} for H_2A and H_2B respectively. Figure 2(ac) shows the difference of binding affinity for histones H_2A and H_2B with SDS at pH values of 3.2 and 10, whilst variations are evident at pH 6.4 (Fig. 2b).

Figure 3 shows thermodynamic parameters of H_2A–SDS interaction at pH 3.2, 6.4 and 10. Figure 3(a) shows $\Delta G_{\bar{\nu}}$ as a function of $\bar{\nu}$, which becomes less negative with increasing $\bar{\nu}$. The change in $\Delta G_{\bar{\nu}}$ with increasing $\bar{\nu}$ implies that, after the initial binding to the "higher energy" sites, subsequent binding is weaker [18]. Figure 3(abc) shows the effect of charge (pH dependency) on the interaction of H_2A–SDS complexes. Figure 3(bc) shows $\Delta H_{\bar{\nu}}$ and $T\Delta S_{\bar{\nu}}$ ($T = 300$ Kk) as functions of $\bar{\nu}$; the curves are convex, except for $\Delta H_{\bar{\nu}}$ at pH 6.4 (Fig. 3b). The positive entropy of binding is the dominant contribution to $\Delta G_{\bar{\nu}}$ which is consistent with a decrease in the structure of water on SDS binding; decrease in entropy is also probably consistent with an increase in the structure of water.

Finally the thermodynamic parameters of the interaction of histones (H_1, H_2B, H_2A, H_3) with SDS were compared at pH 3.2, 6.4 and 10; the results, shown in Table 1. The saturated $\bar{\nu}$ values assume saturation binding of SDS to histones H_2A, H_2B, H_3 and H_1 is equal to 70, 70, 75 and 100 moles SDS per mole of histone, if respectively, saturation binding corresponds to about 1.4 g SDS per gram of protein).

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