Thermochimica Acta, 219 (1993) 151–158
Elsevier Science Publishers B.V., Amsterdam

The thermal denaturation of histones (H_2A, H_3, H_2B) in the presence of surfacants

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(First received 7 July 1992; accepted 10 October 1992)

Abstract

The thermal denaturation of the histones H_2A and H_3, and their sodium *n*-dodecyl sulphate (SDS) complexes was studied spectroscopically over the range 30–90°C at pH 6.4. The Pace theoretical model was used to determine the free energy and heat capacity of the denaturations.

The effect of SDS on the stabilization of the histones H_2A and H_3 was estimated from their thermodynamic parameters as well as from their melting temperatures, which indicated a compact structure for the histone–SDS complexes. In contrast, these values are inconsistent for the effect of dodecyl trimethyl ammonium bromide (DTAB), suggesting that DTAB induces the unfolding of the histones, H_2A, H_3, and H_2B.

INTRODUCTION

Histones are highly cationic proteins which are involved in the binding and compaction of DNA in the cell nucleus chromatin [1, 2]. In mammals, there are five sub-classes of histones: H_1, H_2A, H_2B, H_3 and H_4 [3, 4]. Four histones form an octameric core protein aggregate, H_2A, H_2B, H_3 and H_4, around which a 145-bp DNA molecule is wrapped, forming the core particle. A number of studies on the secondary structure of histones have been reported [5–8].

The effects of sodium dodecyl sulphate (SDS) on the secondary structure of proteins have been reported [9, 10] and it is known that the detergent alters the helicity and the β-form content of many proteins. However, the exact nature of the changes induced by SDS in the tertiary structure are not yet completely understood.

In our previous studies, we investigated the interaction between SDS and proteins, especially histones [11–19]. There is currently considerable interest in measuring the conformational stability of globular proteins, i.e.

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the extent to which the folded conformation is more stable than the unfolded conformation. Thermodynamic parameters of the denaturation behavior of proteins provide quantitative estimates of their conformational stability. The aim of the present work is to study the structural features of the histone complexes with surfactants by thermal denaturation.

MATERIALS AND METHODS

The histones were purchased from Sigma Chemical Co. All materials were of analytical grade and the solutions were made using doubly distilled water. Thermal curves were obtained from a UV–temperature scan using a Gilford model 2400-2 instrument with a temperature programmer and digital absorbance meter. The instrument was calibrated with a buffer solution. The optical density at 280 nm was measured in a 1 cm optical path-length, 500 μl in volume. In all calculations, the molecular weights of H_2A, H_2B and H_3 were taken to be 14 000, 13 250 and 15 300, respectively [20]. Histone solutions (0.01%) were prepared in 2.5 mM buffer (sodium phosphate to pH 6.4). Sodium azide was added to each sample to a concentration of 0.02%.

RESULTS AND DISCUSSION

Thermally induced protein unfolding can be detected as a function of temperature from changes in the UV spectra at 280 nm between the native and unfolded states of the protein. Figure 1(a) and (b) show the changes in molar absorption coefficient which occur on unfolding H_2A and H_3. An evaluation of the equilibrium constants in the transition region requires extensions of the pre- and post-unfolding baselines into the transition region. These extensions are considered to be linear and are represented by

$$\Delta\varepsilon_f = \Delta\varepsilon_f^{30} + m_f(T)$$
$$\Delta\varepsilon_u = \Delta\varepsilon_u^{30} + m_u(T) \quad (1)$$

where $\Delta\varepsilon_f$ and $\Delta\varepsilon_u$ are the differences in the extinction coefficients for folded and unfolded protein, respectively, at specified temperatures in the transition region and are functions of temperature; $\Delta\varepsilon_f^{30}$ and $\Delta\varepsilon_u^{30}$ are the intercepts at 30°C, and m_f and m_u are the slopes of the pre- and post-unfolding regions, respectively.

The fraction of unfolded protein F_d may be calculated using $F_d = (y_f - y_{obs})/(y_f - y_u)$ where y_{obs} is the observed variable, i.e. the optical density or fluorescence intensity, and y_f, y_u are the values of y characteristic of the folded and unfolded conformations. K is defined as [21]

$$K_{obs} = (\Delta\varepsilon_f - \Delta\varepsilon)/(\Delta\varepsilon - \Delta\varepsilon_u) \quad (2)$$

The baselines serve as points of reference in the transition zone in the

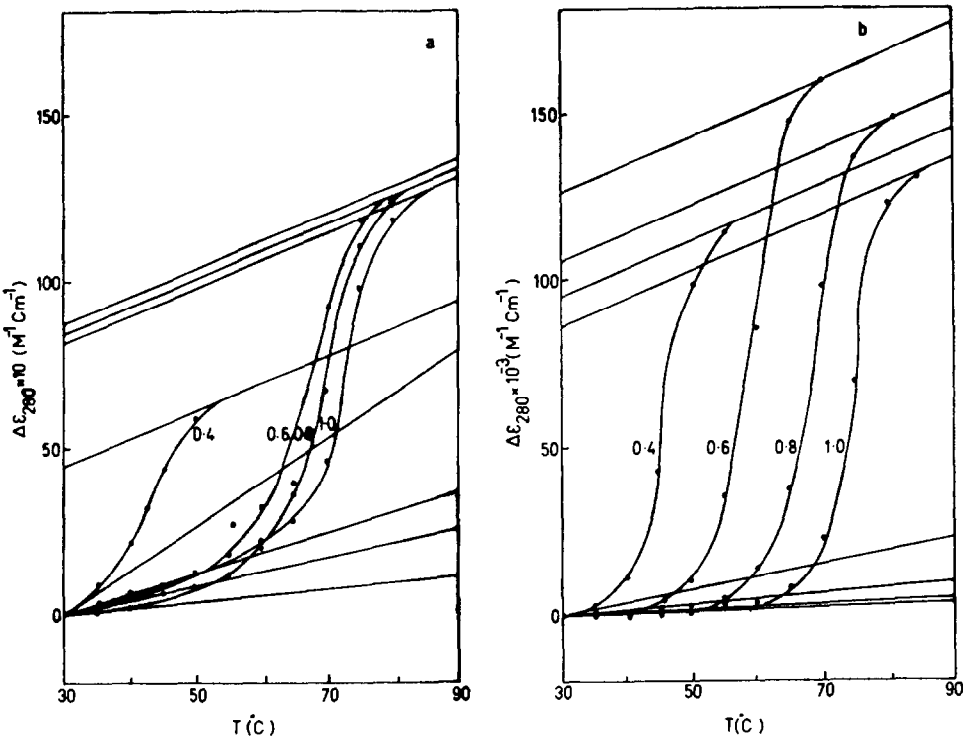

Fig. 1. Difference in molar absorption coefficient at 280 nm as a function of temperature for (a) H_2A; (b) H_3. The full lines are pre- and post-unfolding baselines in the presence of various concentrations (mM) of SDS.

determination of the equilibrium constants for unfolding at any given temperature, and the corresponding values of K_{obs} represent the equilibrium ratios of unfolded/folded protein species at any temperature. Two requirements for this analysis are that the system must be reversible and that only two states of the protein, folded and unfolded, exist in significant populations in the transition region. The extrapolation method is commonly used for evaluating the free energy change for conversion of folded to unfolded protein in the low temperature region (30°C). The method consists of converting the observed equilibrium constants evaluated in the transition region to free energy data, plotting these data as a function of temperature as in Fig. 2(a) and (b), and extrapolating the free energy to 30°C.

Empirically, it is observed that the pre-unfolding intercepts $\Delta\varepsilon_N^{30}$ fall around zero for each denaturant concentration, while the post-unfolding intercepts $\Delta\varepsilon_u^{30}$ cluster around 60 000 M^{-1} cm^{-1} for 0.6, 0.8 and 1.0 mM SDS concentration, around 20 000 M^{-1} cm^{-1} for 0.4 mM SDS for H_2A, and around 70 000, 100 000, 80 000 and 60 000 M^{-1} cm^{-1} for 0.4, 0.6, 0.8 and 1.0 mM SDS, respectively, for H_3 (Fig. 1). For protein unfolding, ΔH is

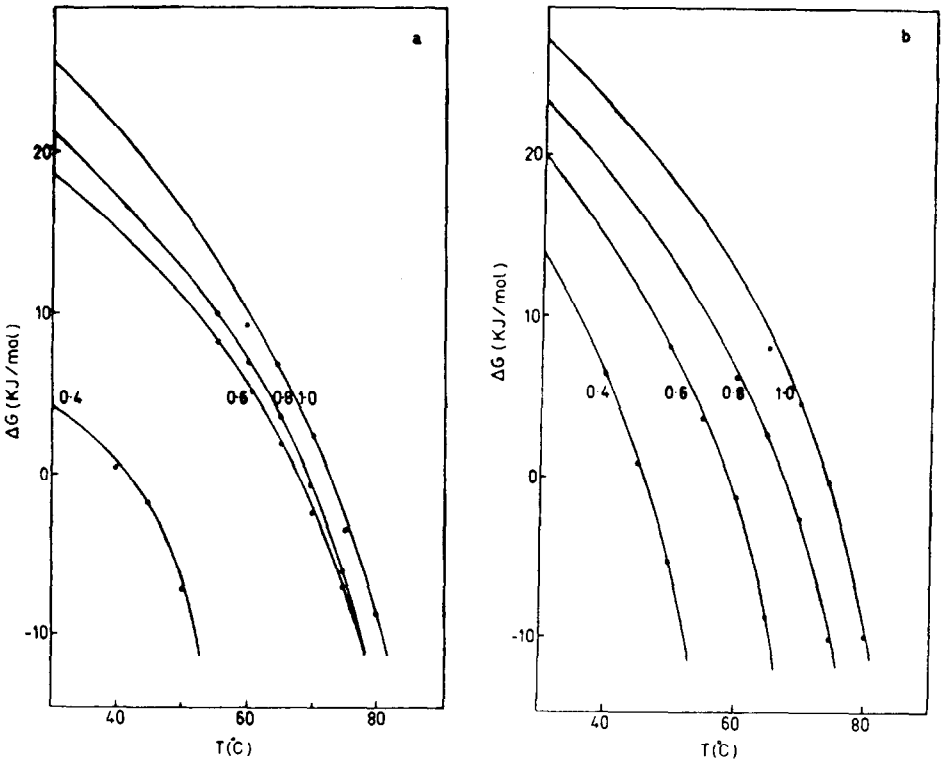

Fig. 2. The free energy change for denaturation ΔG as a function of temperature for (a) H_2A; (b) H_3.

generally found to increase markedly with temperature because the heat capacity of the unfolded protein $C_p(u)$ is greater than that of the folded protein, $C_p(f)$. There is a large difference in the heat capacity ΔC_p for the reaction

fold $\rightarrow$ unfold

$$\Delta C_p = C_p(u) - C_p(f) \tag{3}$$

ΔH can be measured as a function of temperature and ΔC_p can be evaluated using the Kirchhoff equation

$$d(\Delta H)/dT = \Delta C_p \tag{4}$$

For protein folding, ΔG can be measured more accurately than ΔH. The determination of the unfolding curves as a function of temperature yields $\Delta G(T)$ values that are used with ΔH_m and T_m in the Gibbs–Helmholtz equation (eqn. (5)) to calculate ΔC_p [21]

$$\Delta G(T) = \Delta H_m(1 - T/T_m) - \Delta C_p[T_m - T + T \ln(T/T_m)] \tag{5}$$

where $\Delta G(T)$ is ΔG at temperature T, T_m is the midpoint of the thermal

TABLE 1

Thermodynamic parameters of characterizing the thermal denaturation of H_2A and H_3 complexes with SDS

Conc. SDS (mM)	H_2A			H_3		
	ΔG(30°C) (kJ mol^{-1})	T_m (°C)	ΔC_p (J mol^{-1} K^{-1})	ΔG(30°C) (kJ mol^{-1})	T_m (°C)	ΔC_p (J mol^{-1} K^{-1})
0.4	3.9	41	23	14.5	46	10.5
0.6	18.5	66.5	5	20	58	7.3
0.8	21	68	4.5	23.5	67	8.1
1.0	25.5	71	4.1	27.5	73	7.9

unfolding curve, and ΔH_m is the enthalpy change for unfolding measured at T_m. Equation (5) shows that at temperatures near T_m, ΔG depends on ΔH_m but not significantly on ΔC_p. However, if ΔG could be measured at temperatures considerably below T_m, it should be possible to use the results to estimate ΔC_p.

The values of ΔG, ΔC_p and T_m in the presence of SDS and DTAB, calculated from the thermal denaturation curves (Figs. 2 or 4) are tabulated in Tables 1 and 2 respectively.

The temperature of maximum stability (T_s) occurs at the temperature where $\Delta G(T)$ is maximum and $\Delta S = 0$ and is given by [21]

$$T_s = T_m \exp(-\Delta H_m / T_m \Delta C_p) \quad (6)$$

The unusual temperature dependence of protein denaturation depends on ΔC_p and, in turn, ΔC_p (and the hydrophobic interaction) depends mainly on the ordering of the water molecules around the non-polar groups that are brought into contact with water when a protein unfolds [22, 23]. In previous studies, the effects of temperature and pH on the interaction between SDS and histones were investigated [16, 17, 24–26].

It is interesting to evaluate the structure of proteins from their heat capacity; this should give an estimate of the hydrophobicity of the system. Table 1 shows some thermodynamic parameters which have been determined for the interaction of the histones H_2A and H_2B with SDS. The values of ΔC_p for the H_2A and H_3 complexes with SDS indicate that the structure of H_2A and H_3 compacts with increasing concentration of SDS in the system. The H_2A structure shows more compaction than H_3; this is consistent with our previous work [25, 26].

We also compared the thermal analysis of some histones in the presence of the cationic detergent dodecyl trimethyl ammonium bromide (DTAB), which has the same hydrocarbon tail but a different cationic group from that of SDS. Table 2 shows some thermodymamic parameters for the histones H_2A, H_2B and H_3 obtained from the thermal denaturation curves in the presence of DTAB (Figs. 3 and 4). The heat capacity shows that the

TABLE 2

Thermodynamic parameters characterizing the thermal denaturation of H_2A, H_3 and H_2B complexes with DTAB

Conc. DTAB (mM)	H_2A			H_2B			H_3		
	ΔG(30°C) (kJ mol^{-1})	T_m (°C)	ΔC_p (kJ mol^{-1} K^{-1})	ΔG(30°C) (kJ mol^{-1})	T_m (°C)	ΔC_p (kJ mol^{-1} K^{-1})	ΔG(30°C) (kJ mol^{-1})	T_m (°C)	ΔC_p (kJ mol^{-1} K^{-1}
1	4.0	56	0.91	3.0	55	0.83	–	–	–
2	5.7	62	1.75	4.0	60	1.63	–	–	–
3	–	–	–	–	–	–	14.2	60	2.28
4	6.3	66	2.10	6.0	65	1.90	13.6	56.5	2.67
6	–	–	–	–	–	–	12.0	45	3.95
7	–	–	–	–	–	–	8.8	45	3.95
8	7.0	72	4.52	6.5	70	2.88	–	–	–

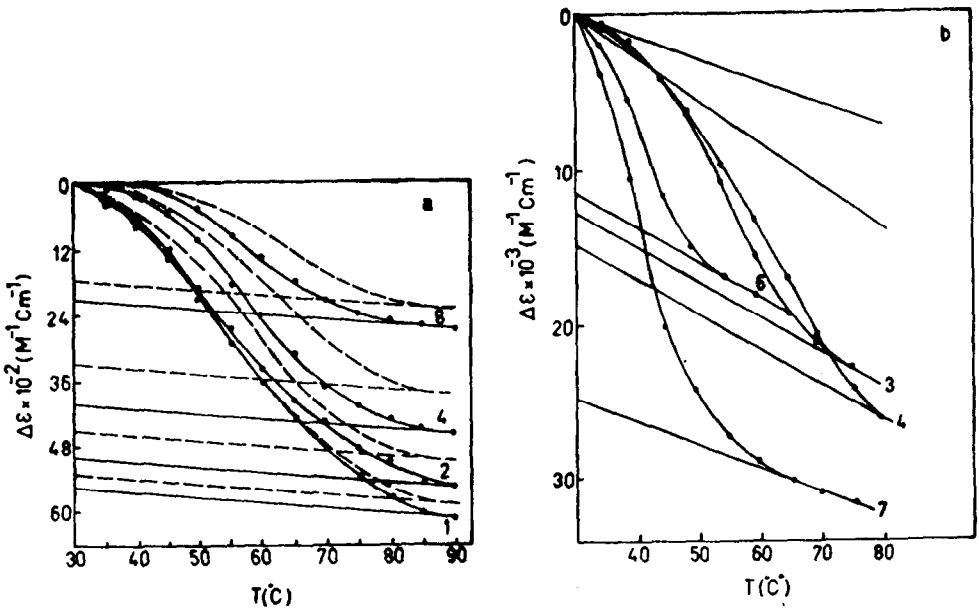

Fig. 3. Difference in molar absorption coefficient at 280 nm as a function of temperature for (a) H_2B (solid curves) and H_2A (broken curves); (b) H_3 in the presence of various concentrations (mM) of DTAB.

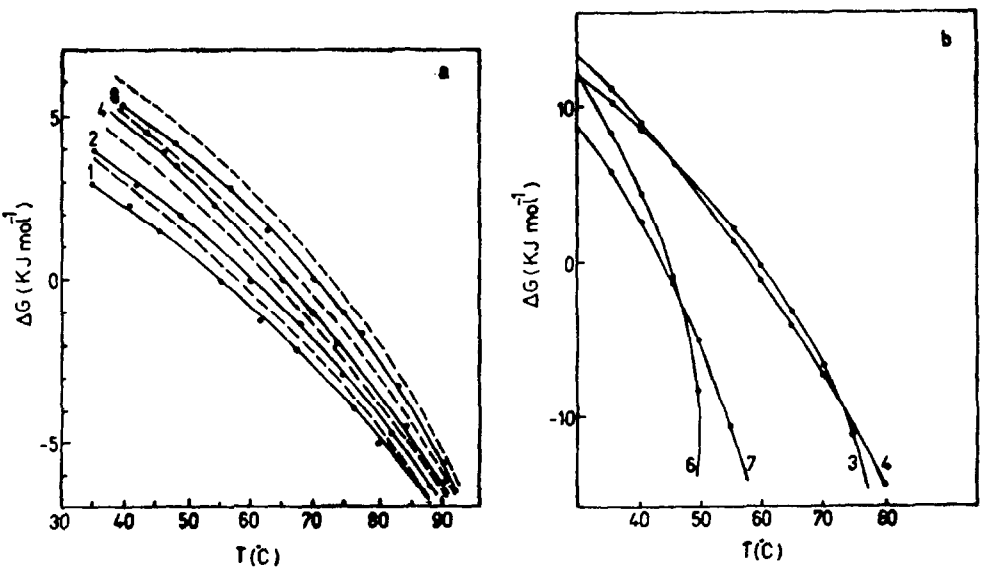

Fig. 4. Free energy change for denaturation ΔG as a function of temperature for (a) H_2B (solid curves) and H_2A (broken curves); (b) H_3 in the presence of various concentrations (mM) of DTAB.

unfolded state for histone–DTAB complexes is consistent with that of the SDS complexes. This study provides details of the nature of the interaction between proteins and surfactants which in turn gives some insight into the interaction between the binding of surfactant ion to oppositely charged sites on the surface of the native histone molecules although the ionic interaction is modulated by hydrophobic effects.

ACKNOWLEDGEMENT

Financial support from the Research Council of the University of Tehran is gratefully acknowledged.

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