INTERACTION OF DNA WITH THE FOLDED AND UNFOLDED HISTONE H_1 IN THE PRESENCE OF SURFACE ACTIVE AGENTS

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Abstract

Interaction between DNA and histone H_1 was investigated in the presence and absence of sodium-n-dodecyl sulphate (SDS) and dodecyl trimethylammonium bromide (DTAB) at temperatures of 27 and 37°C, in 2.5 mM phosphate buffer, pH 6.4 by UV spectrophotometry, equilibrium dialysis and titration. The presence of 1.33 mM SDS caused histone H_1 to fold and to further contact DNA. Binding data were used in the Wyman theoretical model to calculate the free energy and enthalpy of the interaction and were compared with equilibrium state. All data confirm the higher compaction for DNA in the presence of folded histone H_1 (H_1 in the presence of 1.33 mM SDS) relative to native histone H_1 (H_1 in the absence of SDS).

Introduction

Histones are found complexed with DNA in higher eukaryotic cells, except for some sperm cells, and in well-defined lower eukaryotes, and their chromosomal proteins have been biochemically characterized. In most somatic tissues, histones form a group of five classes of proteins, H_1, H_2A, H_2B, H_3 and H_4 [1]. It seems that the binding of H_3 and H_4 to DNA causes its folding and results in the formation of a binding site for H_2A and H_2B [2]. Chromatin in the interphase nucleus exists mainly in the form of 30 nm fiber loops [3]. Chromatin fragments can be isolated from nuclei by limited digestion with micrococcal-nuclease followed by lysis [4]. Nucleosomes consist of two parts, the core particle and the linker, and the former contains two copies of histones: H_2A, H_2B, H_3 and H_4 plus a 146 base pair stretch of DNA wrapped around the histones. The DNA is wrapped around the core histones as a 1.8-turns forming left-hand solenoid. The histone H_1 class of histones is associated with the linker region [5].

The consequence of wrapping the DNA around the protein core is that the formation of a nucleosome is favoured by pre-existing negative supercoils or by preformed DNA bends in the same direction; negative supercoiling does indeed allow nucleosome core particle formation in topologically stringent conditions [6]. Histone H_1 clamps the folds of the DNA molecule, once it has been coiled around the core [7], therefore, it seems that, although histone H_1 is not required for folding the DNA around the histone core, its role may be to stabilize such folding [8]. Recently, much progress has been made in understanding the general organization of chromatin structure [9-10]. Histone H_1 is particularly important in the stabilization of this structure. It has been shown that histone H_1 is involved in chromatin condensation and packaging of the polynucleosome chain into higher order structures [11].

The interactions of anionic and cationic surfactants like, sodium-n-dodecyl suphate (SDS) and dodecyl trimethylammonium bromide (DTAB) with histone H_1 were previously studied [12, 13, 14]. The aim of such studies was to gain an insight into the structure of histones especially histone H_1 from the pathway of denaturation. The effect of SDS has indicated folding for H_1 at a specific concentration of SDS [14], whereas DTAB causes unfolding structure [15].

Keywords: DNA; Folded histone H_1; Interaction; Surface active agents

In the present work, the thermodynamic study on the interaction of folded and unfolded histone H_1 with DNA is reported.

Materials and Methods

Materials

0.15 M NaCl, 10 mM EDTA, 10 m M Tris with pH = 8.0 is defined as NET buffer, and 10 mM Tris, 1 mM EDTA pH 7.5-8.0 is defined as TE buffer, 2.5 mM sodium phosphate buffer, pH 6.4, I = 0.0069 which contained 0.02% (w/v) sodium azide contributing 0.0031 to the ionic strength was also prepared.

DTAB, SDS and rosaniline dye were from Sigma. Visking membrane dialysis tubing (molecular weight cut-off 10,000-14,000) was from SIC (East Leigh, Hampshire, U.K.), proteinase-K and RNase-A from GIBCO BRL (Scotland, U.K.), CM C-50 Sephadex from Pharmacia (Sweden). All other chemicals and solvents used in the preparation of buffers or extraction of DNA and histone H_1 were analytical grade.

Methods

Histone H_1 Extraction

Histone H_1 was extracted from calf thymus glands according to the previously reported methods [16,17]. Briefly, fresh calf thymus glands were collected from the Ziaran slaughter house of the city of Tehran, then stored frozen at -70°C until use. A hundred grams of the frozen glands were thawed, cut into small pieces, cleaned, washed and homogenized completely in the same saline at 4°C. The homogenate was centrifuged at 1100 g for 30 minutes at 4°C. The supernatant was discarded and the pellet was re-suspended in the same saline, and centrifuged under the same conditions. The sediment was re-suspended in three volumes of 5% (v/v) perchloric acid and homogenized completely using a PT 10 polytron homogenizer (Brinkmann Instrument, INC, Westbury, USA). The homogenate was centrifuged at 2000 g for 30 minutes at 4°C. The supernatant was collected and the pellet was re-extracted twice in the same way with 5% perchloric acid. The three supernatants were pooled and filtered through a Buchner glass funnel. Histone H_1 was precipitated following higher acidification of the filtrate with 5 N HCl (0.3 N final concentration), and the addition of three volumes of acetone. The precipitate was collected, then centrifuged at 2000 g for 20 minutes. The sediment was washed three times with acidified acetone (2000: 1, acetone; concentrated HCl) by centrifugation at 2000 g for 20 minutes. The precipitate was dried under vacuum and prepared for further purification.

Histone H_1 Purification

For further purification of histone H_1, the extracted H_1 was dissolved in 20 ml of 10 mM phosphate buffer, pH 9 containing 7 mM boric acid and 3 mM NaOH. The dissolved H_1 was loaded on the top of CM C-50 Sephadex column (25 × 4 cm) pre-equilibrated with the 10 mM sodium phosphate buffer pH 9.0. The application of this type of ion exchange chromatography showed that peak three of the pooled fractions contains proteins that lack histidine, and it has only a low amount of aspartic and glutamic acids compared to the other two peaks of the column. Such characteristics among the histone proteins are typical only of histone H_1. Histone H_1 was eluted with 1000 ml of a linear gradient between 0 and the buffer containing 1 M sodium chloride in the same buffer. The fraction size was 5 ml collected with a flow rate of 0.5 ml/minute. Appropriate fractions were pooled, desalted by dialysis and protein precipitation was followed by adding four volumes of acetone. The precipitate was washed with acetone three times, dried under vacuum and stored at 4°C until use.

Core Histones Extraction

The sediment remaining after the third extraction of histone H_1 with 5% perchloric acid was re-suspended and mixed with 0.25 N HCl and left overnight at 4°C [16]. The mixture was centrifuged at 2000 g for 20 minutes, the supernatant was collected and the pellet was washed and centrifuged with 0.25 N HCl under the same conditions. The supernatants were pooled and the core histones were precipitated following the addition of six volumes of acetone. The precipitate was washed with acetone three times, dried under vacuum, then stored at 4°C until use.

DNA Extraction

High-molecular weight DNA was isolated from calf thymus as follows [18]: an appropriate amount of fresh calf thymus was cut into small pieces at 4°C then suspended in NET buffer at a ratio of 1:5 (w/v). The suspension was homogenized using a PT 10 Polytron homogenizer, then SDS at 0.2% final concentration was added and the lysate was incubated at 60°C overnight. DNA was extracted with the addition of an equal volume of phenol-choloroform-isoamylalcohol at a 25:24:1 ratio, respectively. The DNA was precipitated with sodium acetate (0.3 M final concentration) and two volumes of ice-cold absolute ethanol. The DNA was pooled and washed twice with ice-cold 70% ethanol following centrifugation at 3000 rpm for 10 minutes each time, then re-suspended and dissolved in sterilized bidistilled water.

RNase-A was added to the DNA solution at 2.5 µg/ml (final concentration), and incubated for an overnight period at 37°C. Proteinase-K at 100 µg/ml (final concentration) was later added to the mixture and incubated at 60°C for 12 hours. The DNA was re-extracted with an equal volume of

phenol-chloroform-isoamylalcohol, then precipitated and and the dried DNA was dissolved in TE buffer then quantified and qualitified spectrophotometrically at 260 and 280 nm and stored at 4°C until use.

Measurements

The complex of solubility product was determined at a DNA concentration of about 0.004% (w/v) (A_{260}^{1cm}=0.734), when complex of H_1 - surfactant interacted with DNA incubated for one hour in an air-conditioned room at 20°C and later centrifuged for 10 min at 10,000 rpm in an Eppendorf centrifuge model 5415-C. The precipitation percent (P%) was calculated as follows [19]:

$$(1 - \frac{A_{260}\ \text{supernatant}}{A_{260}\ \text{DNA in 0.004\%}}) \times 100 = \text{precipitation \%}.$$

Equilibrium dialysis method was used to determine the number of moles of surfactant bound to the macromolecule and therefrom to calculate the thermodynamic parameters. The procedure involved equilibrating 1.5 cm^3 aliquots of H_1-DNA solution with a function of the H_1 (0.006%) over DNA (0.004%) mass ratio (1.5) as required against 2 cm^3 aliquots of SDS solution in the concentration range of 0.375-4.5 mM for 98 hours. The free SDS concentration in equilibrium with the complexes was assayed spectrophotometrically by the rosaniline hydrochloride method at optical density at 550 nm [20-21].

All absorbance measurements were recorded on a Shimadzu model of 3100 uv/vis spectrophotometer. The molecular weight of histone H_1 was taken 21,000 [22] and that of the DNA was taken 1.3 $\times 10^7$ [23]. Protein-DNA titration was made with a Beckman pH meter model of ϕ-50 in conjunction with nitrogen-gas cylinder. Three millilitres of sample prepared in 10 mM NaCl was titrated by NaOH of concentration 0.01 to 5 M.

Results and Discussion

Physical techniques such as equilibrium dialysis, UV spectroscopy, acrylamide gel electrophoresis, protein titration and viscometry confirms the organized structure (folding) for interaction of H_1 with SDS at a concentration of 1.33 mM which has been reported previously [12,13]. Here, the interaction of DNA and histone H_1 with and without SDS is depicted in Figure 1. The maximal saturation for sigmoidal curves (Fig. 2), occurs at a ratio of H_1/DNA equal to 1.5-1.75 which is consistent with Figure 1, showing the maximum precipitation at this ratio. Figure 2-a shows the left shift for sigmoidal curve at 1.33 mM SDS which means a stronger interaction with DNA.

The folded state of histone H_1 causes more interaction with DNA, whereas the unfolded state of histone H_1 in the presence of DTAB or specific concentrations of SDS causes less interaction with DNA. H_1 in its folded state probably carries more surface charge as a consequence of its interaction with DNA, whereas the unfolded state bears a hydrophobic surface.

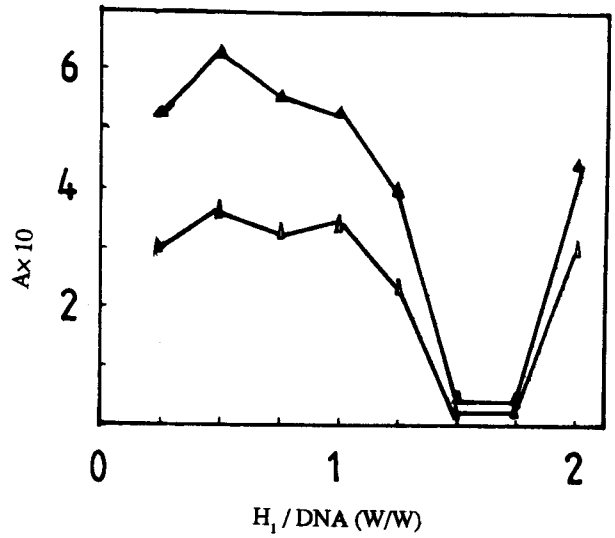

Figure 1. Optical density in two wavelengths 260 and 280 vs. H_1/DNA ratio for supernatant portion. (▲) 260 nm (Δ) 280 nm

The interaction of folded H_1 with DNA represents a sigmoidal curve showing a higher concentration of H_1-DNA complex (Fig. 2-a) whereas the unfolded H_1-DNA complex with DTAB at even higher concentrations of SDS produces a linear curve (Fig. 2b). The concentration of 2 mM DTAB causes the complete unfolding of H_1.

Figure 3 shows the enthalpy of interaction for H_1-DNA in the presence and absence of SDS at temperatures of 27 and 37°C. The pace model may be used to calculate the thermodynamic parameters by assuming a two-state mechanism as follows [24]:

$$F_D = (Y_N - Y_{obs})/(Y_N - Y_D) \quad (1)$$

$$\Delta G_D = -RT\ln K = -RT\ln [\frac{F_D}{1 - F_D}]$$

$$= -RT\ln [(Y_N - Y_{obs})/(Y_{obs} - Y_D)] \quad (2)$$

where K is the equilibrium constant, Y_{obs} is the observed value of the parameter used to follow unfolding in the transition region and Y_N and Y_D are the values of Y characteristics of the native and denatured conformations of the protein respectively. Free energy (ΔG) is calculated from equation (2) and inserted in the Gibbs-Helmholtz equation to obtain enthalpy of interaction as follows [26]:

$$\frac{\Delta G_2}{T_2} - \frac{\Delta G_1}{T_1} = \Delta H\ (\frac{1}{T_1} - \frac{1}{T_2}) \quad (3)$$

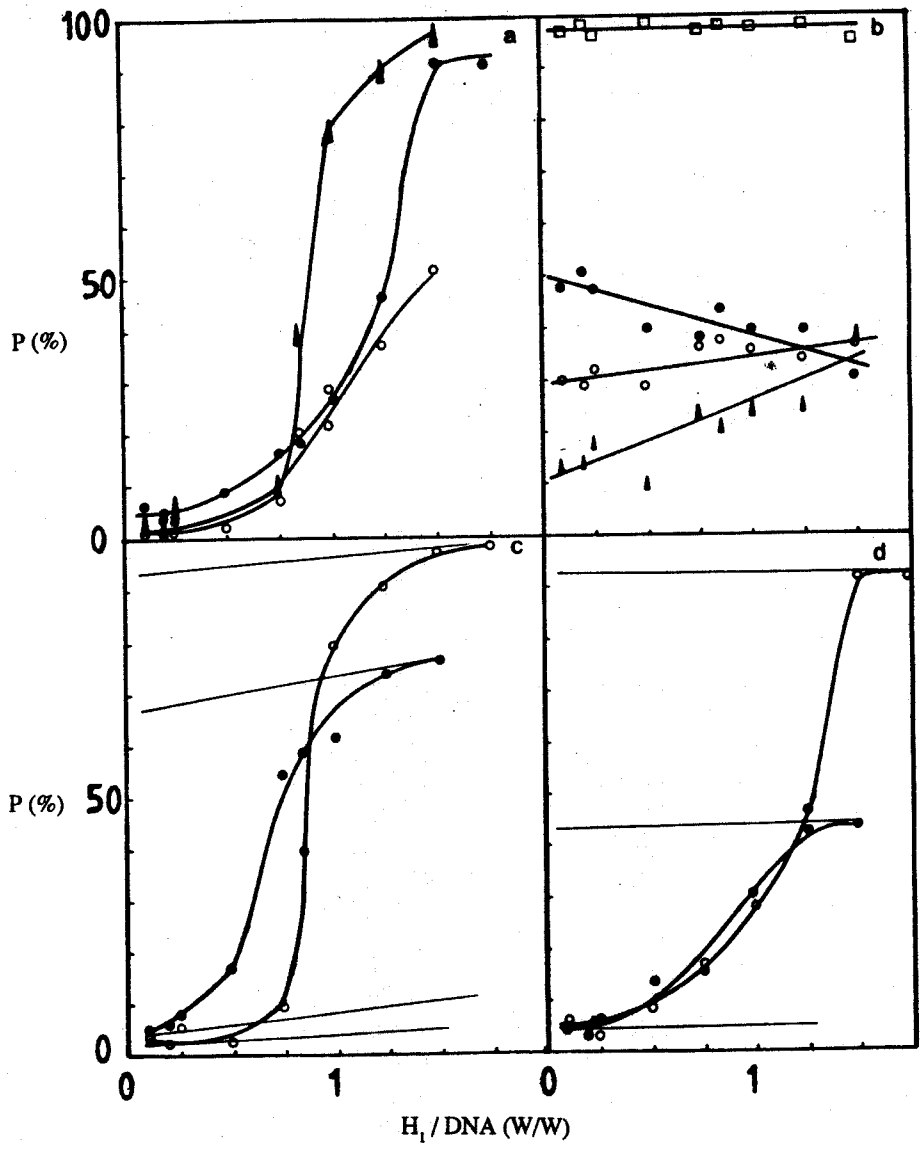

Figure 2. Percent precipitation (P%) of DNA upon histone H_1 in 2.5 mM phosphate buffer pH = 6.4 at a DNA concentration of 40 μg/ml
a) as a function of the H_1/DNA (w/w) ratio with and without the SDS concentration, 0 mM SDS (●); 1.33 mM SDS (▲); 2 mM SDS (○). b) 1 mM SDS (●); 3 mM SDS (○); 4 mM SDS (▲); 2 mM DTAB (□). c) in the presence of 1.33 mM SDS at two temperatures 27°C (○) and 37°C (●). d) without SDS at two temperatures 27°C (○) and 37°C (●).

Figure 3 shows the enthalpy of interaction of H_1-DNA with and without SDS vs. ratio value of H_1/DNA. This figure indicates more interaction causes for H_1-SDS interaction with DNA at a higher ratio of H_1/DNA. The crossing of two curves takes place at a ratio of DNA/H_1 equal to 1.1 [1].

Figure 4 shows the binding isotherms (the number $\bar{\nu}$ of SDS bound per complex of protein and DNA as a function of the logarithm of the free SDS concentration [S_f]) on the complex (H_1-DNA) at 27 and 37°C. Gibbs energies may be calculated from the binding data using the Wyman binding potential [25]. The binding potential is calculated from the area under the binding isotherm according to the equation:

$$\pi = RT\int_{\nu_i=0}^{\nu} \nu_i \, d\ln [S_f] \qquad (4)$$

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

$$\pi = RT\ln (1 - K_{app} [S_f])^{\nu_i} \qquad (5)$$

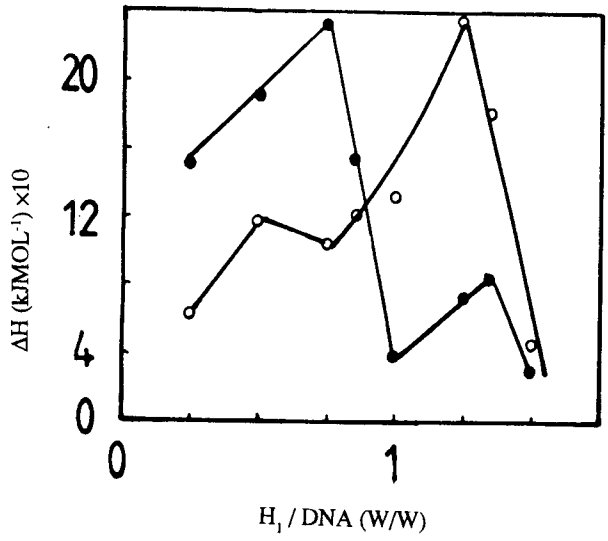

Figure 3. The comparison of enthalpy change for interaction H_1/DNA with and without the presence of SDS which is calculated by Pace model. Data used from Figure 2 c and d. (●) with SDS (○) Native.

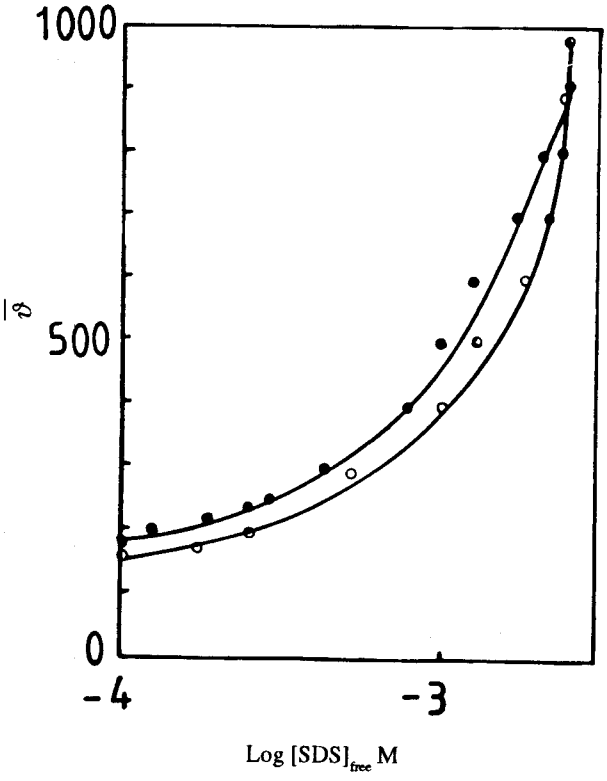

Figure 4. Binding isotherms for SDS on interaction with complex (H_1/DNA) at 27°C (○) and 37°C (●) at pH = 6.4

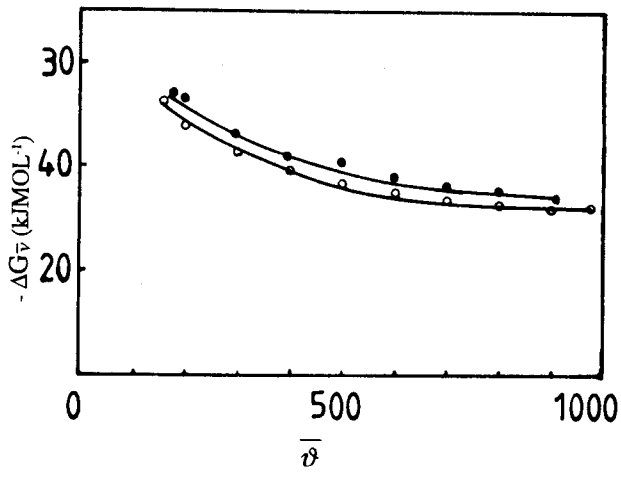

Figure 5. Apparent Gibbs energy change per SDS molecule bound ($\Delta G\ \bar{\nu}$) as a function of $\bar{\nu}$ at 27°C (○) and 37°C (●) calculated from Wyman binding potential

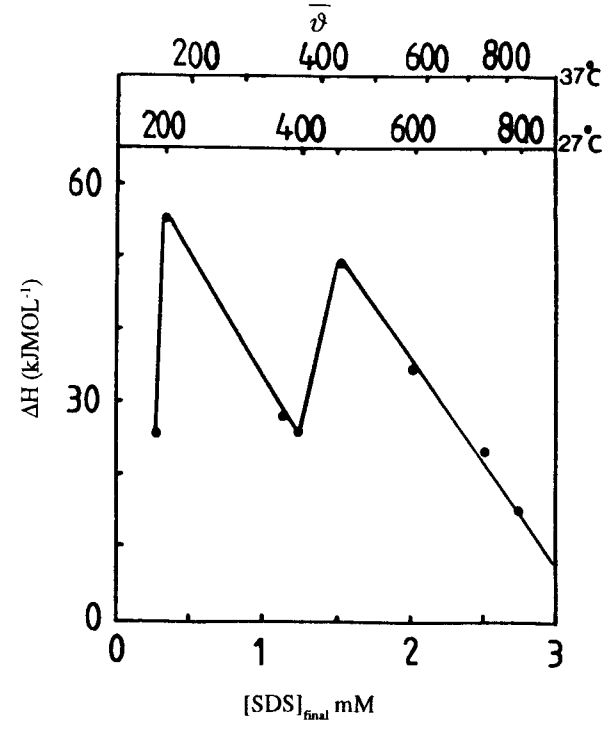

Figure 6. Enthalpy change as a function of final concentration of SDS at 27 and 37°C in pH = 6.4. The upper axis shows the number of SDS molecules bound per complex H_1 -DNA at equilibrium.

and values of K_{app} used to determine ΔG_ν;

$$\Delta G_\nu = \frac{\Delta G_{app}}{\bar{\nu}} = \frac{RT}{\bar{\nu}} \ln K_{app} \qquad (6)$$

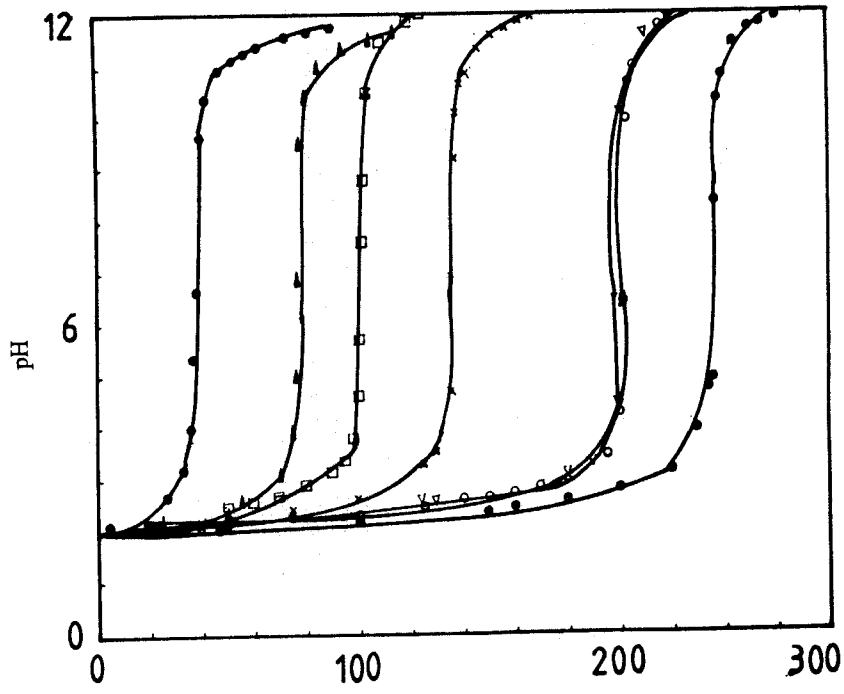

Figure 7. Titration curves for H_1-DNA complex in the presence of SDS at 27°C; (X), H_1; (□), (H_1-SDS) DNA 1mM SDS; (●), (H_1-SDS) DNA 1.33 mM SDS; (▲), (H_1-SDS) DNA 2 mM SDS, (▽) (H_1-SDS) DNA 3 mM SDS; (⊗), (H_1-SDS) DNA 4 mM SDS; and (○), H_1-DNA 0 mM SDS.

Equation 3 was used to calculate the enthalpy of interaction at equilibrium state and the results are shown in Figure 6. This figure shows the enthalpy of interaction of H_1 - SDS (1.33 mM) with DNA. The concavity of the enthalpy curve is consistent to 1.33 mM of SDS. The number of SDS molecules bound ($\bar{v}$) at this concentration of SDS is equal to 400 and 390 for 37°C and 27°C respectively. The concavity of the curve showing minimum endothermicity is in agreement with more precipitation or higher interaction of H_1 (folded) with DNA. The amount of free energy for this binding is depicted in Figure 5. For $\bar{v}$ = 390 at 27°C and 400 at 37°C the free energy ($\Delta G_{\bar{v}}$) is equal to -20.0 and -20.75 kJ/mol respectively.

Another indication for DNA compaction is the titration curves for H_1, H_1-DNA and (H_1- SDS)-DNA that are depicted in Figure 7. The figure shows that the histone H_1 in the presence of 1.33 mM SDS has a better packing for DNA.

It can be concluded that the folded state of H_1 interacts more strongly with DNA and this is confirmed by thermodynamic data.

Acknowledgements

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