THE UNEXPECTED EFFECT OF SODIUM ARSENATE ON THE INTERACTION BETWEEN HISTONE H_1 AND SODIUM N-DODECYL SULPHATE

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ABSTRACT

A study was made on the interaction between histone H_1 and sodium n-dodecyl sulphate (SDS) in the presence of sodium arsenate inside a phosphate buffer of pH 6.4, using spectroscopy and equilibrium dialysis at 27°C. The binding data has been used to obtain the Gibbs free energy in terms of a theoretical model based on the Wyman binding potential. The binding data has been analysed in terms of Hill equation, to obtain Hill coefficient. The data show that sodium arsenate exhibits an unusually greater degree of denaturation for the structure of H_1.

INTRODUCTION

Histone H_1 has been shown to be involved in the salt-dependent folding of polynucleosome chain giving it a higher-order structure [1-3]. At a functional level, selective H_1 - mediated chromatin condensation plays an important role in the regulation of gene expression [4].

We have previously reported a number of studies on the interaction between sodium n-dodecyl sulphate (SDS) and histones [5-10] and other proteins [11-12]. We have also previous-

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ly noted the effect of sodium chloride on the interaction of SDS, with histone H_1 [13] and catalase [14].

The purpose of the present paper is to report the effect of sodium arsenate on the interaction between histone H_1 and sodium n-dodecyl sulphate.

EXPERIMENTAL

Histone preparation:

Histone H_1 was extracted from calf thymus glands following the method developed by Johns [15].

Materials:

The buffer (phosphate) was prepared in double distilled water with ionic strengths of 2.5 mM ($I=6.69\times10^{-3}$), 3.75 mM ($I=8.82\times10^{-3}$), 5 mM ($I=10.73\times10^{-3}$), 6.25 mM ($I=12.64\times10^{-3}$), and 7.5 mM ($I=14.55\times10^{-3}$) at pH 6.4 . The second type of buffer (1:1 concentration of phosphate and sodium arsenate) was prepared as above with identical ionic strengths. Each of the buffer solutions contained 0.02% (w/v) sodium azide contributing 0.003I to the ionic strength. Sodium n-dodecyl sulphate (especially pure grade) was obtained from the Merck Company, W.Germany. Rosaniline hydrochloride dye was used as supplied by B.D.H. (U.K.). Visking dialysis tubing (Molecular weight cut off 10000-14000) was from SIC (East Leigh) Hampshire, U.K. . All other chemicals used in this study were reagent grade.

Methods:

Equilibrium dialysis was carried out at 27 °C as previously described [16], using a H_1 concentration of 0.01% (w/v). The free surfactant concentrations in equilibrium with the protein surfactant complexes were assayed by rosaniline hydrochloride method [17].

U.V. spectroscopy measurements were made at λ_{max} = 225 nm with a Shimadzu double-beam recording model 160 spectrophotometer.

The instrument adjustments and techniques of work was same as described before [13]. In all calculations the molecular weight of H_1 was taken as 21,000 [18].

RESULTS AND DISCUSSION

The effect of varying phosphate ion concentrations on the absorption spectra (λ_{max} =225 nm) of Histone H_1 at different concentrations was previously studied where a dome like peak was obtained at pH 6.4 at 27°C. Increasing the concentration of H_1 caused the absorption peak to flatten up to 0.025% (w/v) and thereafter it caused an splitting of the peak [13].

Figure 1 shows the effect of varying concentration of sodium (arsenate/phosphate) on the absorption spectra of Histone H_1 at different concentrations. When compared with the effect of phosphate ion alone, under identical conditions , the

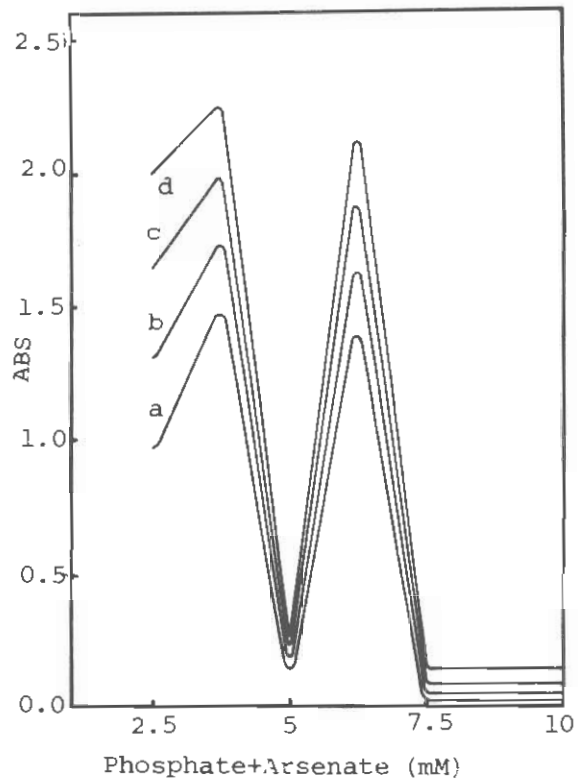


Fig.1:The effect of ionic strength of mixtures of (1:1)sodium salts of phosphate and arsenate on the absorption spectra (OD_{max}=225 nm) at different concentrations (w/v%) of H_1 at pH 6.4.
a)0.0025% b)0.005% c)0.0075%
d)0.01%

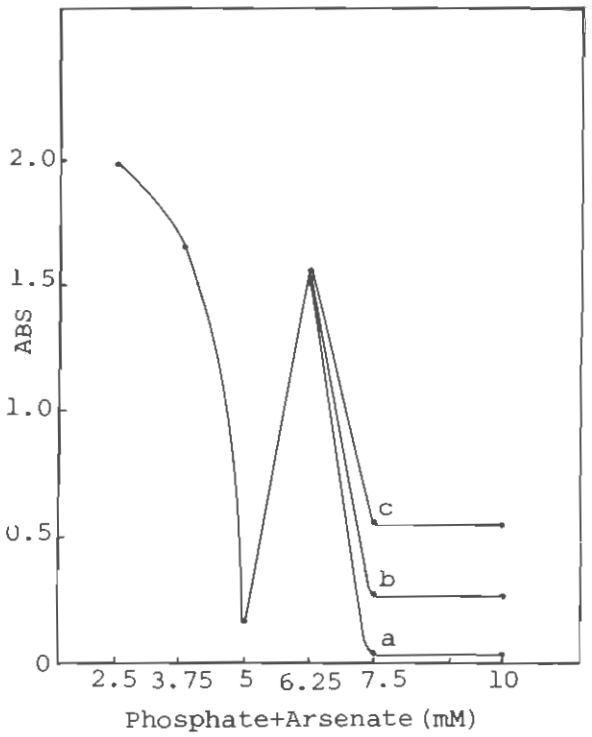


Fig.2 :The effect of SDS on the H_1 at various ionic strengths of (1:1) mix - tures of sodium salts of phosphate and arsenate at constant concentra - tion of H_1 (0.0025%)
a)0.1 mM,SDS b)0.2 mM,SDS
c)0.4 to 0.7 mM,SDS

absorption peak is seen highly intensified.

Figure 2 shows the effect of SDS on the absorption spectrum of H_1 in the presence of sodium(arsenate/phosphate) under conditions identical to those of Fig 1. It is thus , obvious that SDS shows no remarkable interaction with H_1 in the presence of Na_3ASO_4 .

It is important to note,that most salts in comparison to detergents are weak denaturing agents for protein structures. Detergents occupy an entirely unique position among protein denaturants and are able to produce drastic cooperative conformational changes at remarkably low concentrations. Among detergents,SDS is the most potent one for protein denatura -

tion. Thus,we observe here that in this regard Na_3ASO_4 seen as powerful as SDS. The effect of SDS on H_1 structure(0.01% $w/_v$)in the presence and absence of NaCl/ sodium phosphate which has been previously studied indicates that the change in absorption coefficient are as follows: $\Delta\varepsilon^{\%}_{225}$=15(no SDS),100(no salt)and 140 (SDS+salt)[13]. However, in the case of Na_3ASO_4 $\Delta\varepsilon^{\%}_{225}$=215(no SDS) under the same conditions. Therefore it is concluded that sodium arsenate could be a better denaturant for H_1 structure.

Allan,J;et al(1980)has reported that the H_1 structure is almost fully compacted into a higher-order structure in 80 mM NaCl[19],whereas we have shown the same event at 5 mM(phosphate/NaCl)in presence of SDS[13]. Here,Na_3ASO_4 plays the same function as NaCl on the structure of H_1 but in much more powerful manner.

The binding isotherms (the number, $\bar{\nu}$,of SDS ions bound per H_1 molecule)as a function of the logarithm of the free SDS concentration, at various ionic strengths for phosphate ions and combined phosphate and arsenate ions(1:1),pH 6.4 and 27°C are shown in Fig.3. When compared with the phosphate binding,these isotherms indicate a shift towards left due to presence of arsenate. The leftward shift of isotherm leads to a lower concentration of SDS indicating a decrease in the SDS affinity. The calculation of Gibbs energies of binding,which applies to the entire binding isotherm is based on the Wyman binding potential concept as previously described[6-9]. Fig.4 shows free energy(ΔG)of H_1-SDS interaction as a function of final concentration of SDS at various ionic strenghts in phosphate or in phosphate - arsenate buffer systems. It is clear that the arsenate has a drastic effect on H_1-SDS interaction. For instance , for $\bar{\nu}$ equal to 100,the amount of $-\Delta G$ at mentioned ionic strengths (in presence and absence of arsenate are equal to a)3000,1400 KJ mol^{-1};b)2500 ,1300 KJ mol^{-1};c)2300,1300 KJmol^{-1};d)2300,1200 KJ mol^{-1};e)2000,700 KJ mol^{-1} respectively. Fig.4 also shows the amount of $[SDS]_{final}$ under the same conditions for $\bar{\nu}$=100,is equal to:a)0.2,1.6 mM ; b)0.2,0.8 mM;c)0.2,2.5 mM;d)0.12, 1.6 mM;and e)0.1,1.6 mM,respectively. These data show that arsenate has a larger effect that SDS;therefore the quantity of SDS in interaction with H_1 is reduced.

Fig.5 shows the binding data in terms of the Hill equation [6,13] , where g is the maximum value of $\bar{\nu}$ and n_H is the Hill coefficient which estimates the cooperativity of the interaction.

The Hill plots (Fig.5) in the presence of 1:1 of arsenate and phosphate ions show that with increasing

the amount of arsenate the coopera - tivity is decreased,i.e. n_H is changed from 3.75 to 1.2 when the buffer concentration is increased from 2.5 to 7.5 mM. This subject is markedly different for the phosphate ion alone [13]. The compaction of H_1 in phos - phate buffer occurs only at 5 mM(I= 10.73×10^{-3})whereas in the case of phosphate/arsenate buffer the increase in ionic strengths seem to yield a compact and stable structure.

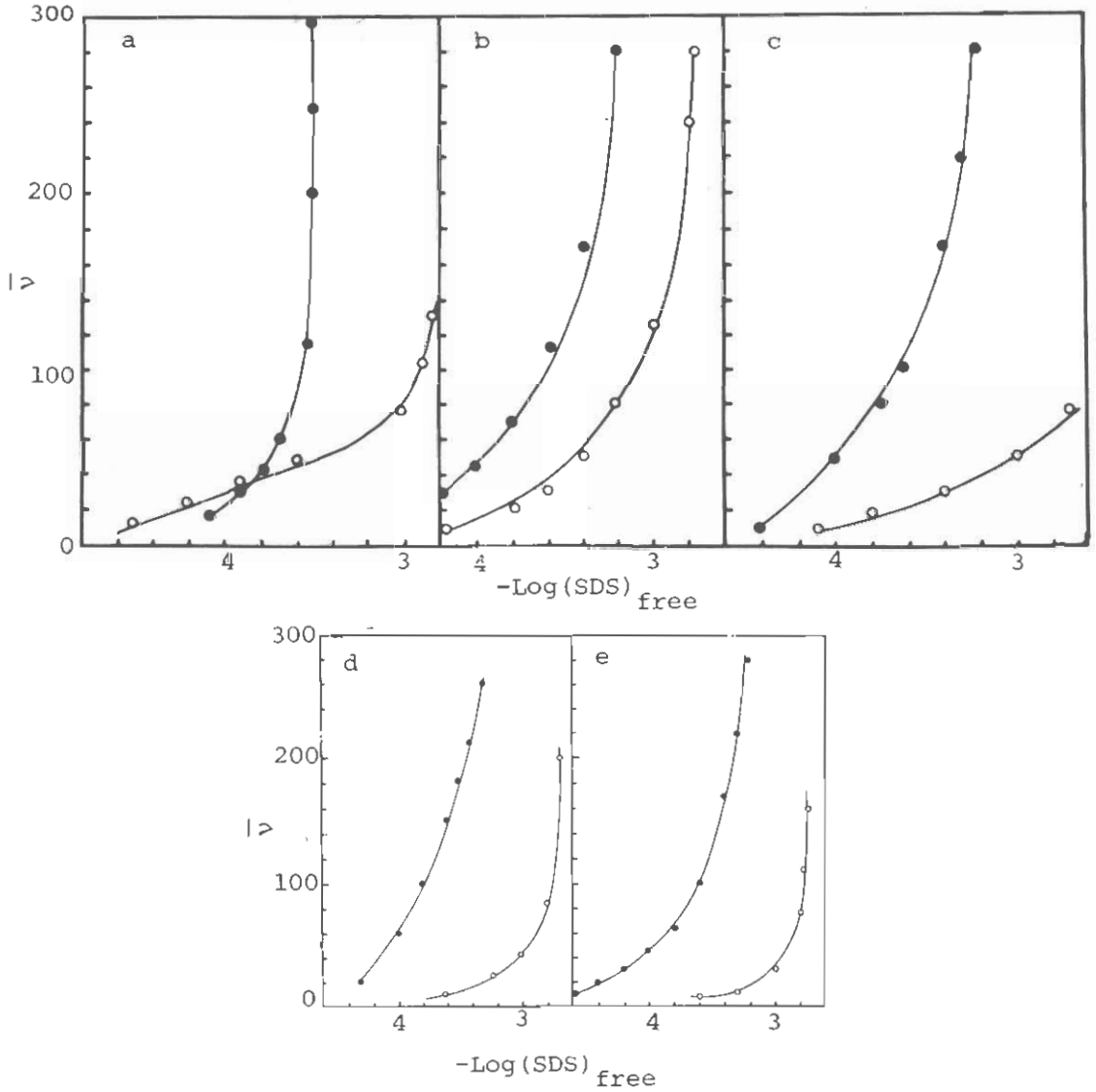


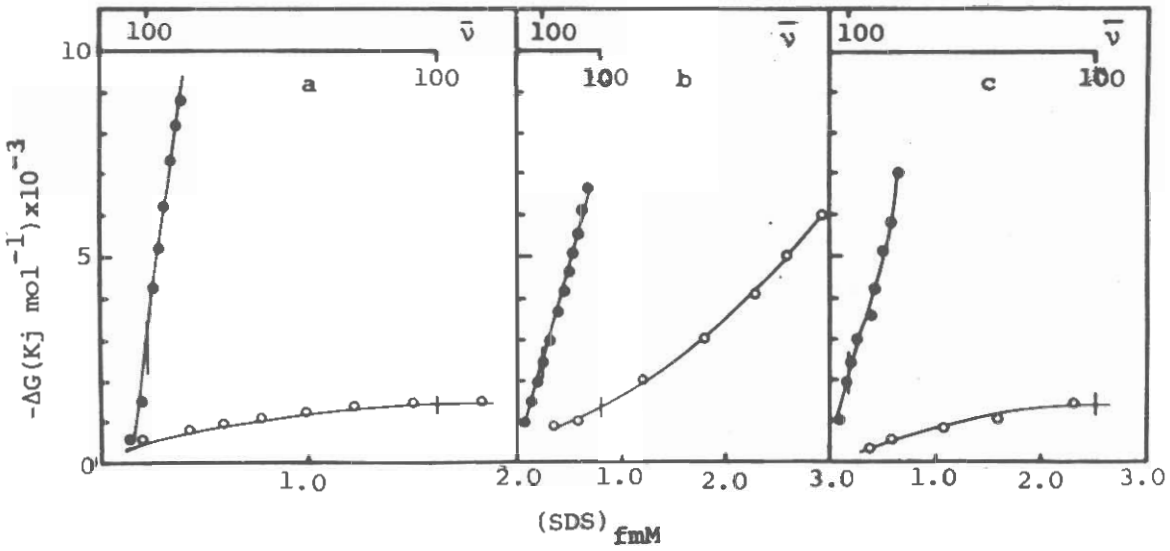
Fig.3 :Binding isotherms for SDS on the interaction with H_1 at various ionic strengths caused by phosphate alone and (1:1) mixture of the sodium salts of phosphate and arsenate at 27°C.

a)2.5 mM($I=6.69 \times 10^{-3}$) b)3.75 mM($I=8.82 \times 10^{-3}$)

c)5 mM($I=10.73 \times 10^{-3}$) d)6.25 mM($I=12.64 \times 10^{-3}$)

e)7.5 mM($I=14.55 \times 10^{-3}$)

O, Phosphate buffer; ●, Phosphate/Arsenate buffer



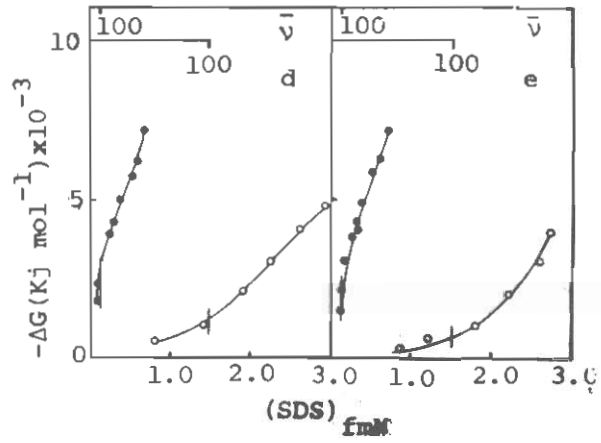


Fig.4 :Changes in apparent Gibbs energy as a function of final concentration of SDS at various ionic strengths of buffer made by phosphate alone or (1:1)mixtures of the sodium salts of phosphate and arsenate at 27 °C.

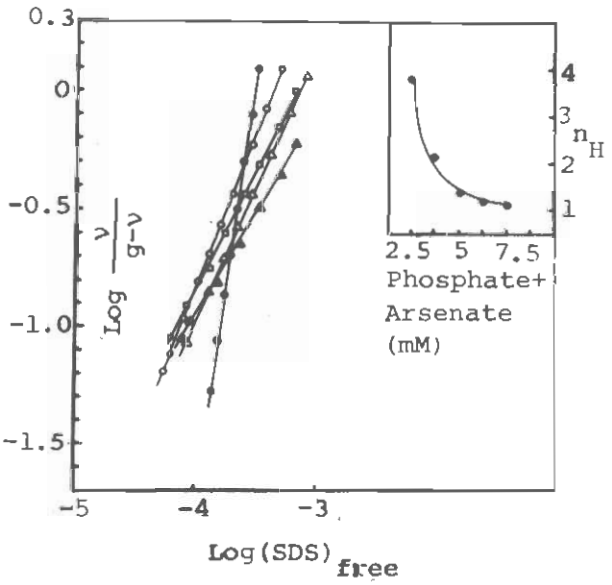
○,Phosphate buffer; ●, Phosphate / Arsenate buffer

a)2.5 mM b)3.75 mM c)5 mM

d)6.25 mM e)7.5 mM

Fig.5 :Hill plots for SDS on the interaction with H_1 at various ionic strength of the buffer caused by(1:1) mixture of the sodium salts of phosphate and arsenate at 27°C. In the insert,each values of Hill coeffi - cient is plotted against the concentration of buffer.

● 2.5 mM,g=440; ○ 3.75 mM,g=350;△5mM, g=370;□6.25 mM,g=400; ▲7.5 mM,g=370



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