THE UNEXPECTED EFFECT OF SODIUM ARSENATE ON THE INTERACTION BETWEEN HISTONE H\textsubscript{1} AND SODIUM N-DODECYL SULPHATE

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Keywords: Histone H\textsubscript{1}; Sodium arsenate; Sodium n-dodecyl sulphate; Free energy; Hill coefficient

(Received 16th Apr. 1990)
(Approved 15th Oct. 1990)

ABSTRACT

A study was made on the interaction between histone H\textsubscript{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\textsubscript{1}.

INTRODUCTION

Histone H\textsubscript{1} has been shown to be involved in the salt-dependent folding of nucleosome chain giving it a higher-order structure[1-3]. At a functional level, selective H\textsubscript{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 ($I=6.69\times 10^{-3}$), $3.75$ mM ($I=8.72 \times 10^{-3}$), $5$ mM ($I=10.73 \times 10^{-3}$), $6.25$ mM ($I=12.64 \times 10^{-3}$), and $7.5$ mM ($I=14.55 \times 10^{-3}$) 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 $0.0031$ 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 $27^\circ C$ 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 rosiniline hydrochloride method[17].

U.V. spectroscopy measurements were made at $\lambda_{max}=225$ 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[10].

RESULTS AND DISCUSSION

The effect of varying phosphate ion concentrations on the absorption spectra ($\lambda_{max}=225$ nm) of Histone $H_1$ at different concentrations was previously studied where a dome like peak was obtained at pH 6.4 at $27^\circ C$. 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$_{\text{max}}$=225 mM) at different concentrations (w/v%) of H$_2$ at pH 6.4.

a) 0.0025%  b) 0.005%  c) 0.0075%

d) 0.01%

absorption peak is seen highly intensified.

Figure 2 shows the effect of SDS on the absorption spectrum of H$_2$ 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$_2$ in the presence of Na$_3$AsO$_4$.

Fig.2: The effect of SDS on the H$_2$ at various ionic strengths of (1:1) mixtures of sodium salts of phosphate and arsenate at constant concentration of H$_2$ (0.0025%) a) 0.1 mM, SDS  b) 0.2 mM, SDS  c) 0.4 to 0.7 mM, SDS

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$_3$ASO$_4$ 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: Δe$^{225}$ = 15 (no SDS), 100 (no salt) and 140 (SDS+salt)[13]. However, in the case of Na$_3$ASO$_4$ Δe$^{225}$ = 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$_3$ASO$_4$ plays the same function as NaCl on the structure of H$_1$ but in much more powerful manner.

The binding isotherms (the number, ν, 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 ν equal to 100, the amount of -ΔG 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 KJ mol$^{-1}$; d) 2300, 1200 KJ mol$^{-1}$; e) 2000, 700 KJ mol$^{-1}$ respectively. Fig. 4 also shows the amount of [SDS] under the same conditions for ν=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 ν 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 cooperativity 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 phosphate buffer occurs only at 5 mM ($I=10.73 \times 10^{-3}$) 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 ($I=6.62 \times 10^{-3}$)  

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⁺ 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 coefficient is plotted against the concentration of buffer.

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

We thank Dr. M. Pooladi for critical reading of manuscript. The financial support from the research council of the university of Tehran is gratefully appreciated.

REFERENCES