

# THERMODYNAMIC STUDIES ON THE INTERACTION BETWEEN DODECYL TRIMETHYL AMMONIUM BROMIDE AND HISTONE H<sub>2</sub>A

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## Abstract

The thermodynamic parameters of interaction between calf thymus histone H<sub>2</sub>A and dodecyl trimethyl ammonium bromide (DTAB), a cationic surfactant, in aqueous solutions of pH 3.2, 6.4 and 10.0 were measured over a wide range of DTAB concentrations by equilibrium dialysis at 27°C and 37°C and 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<sub>2</sub>A-DTAB are compared with the corresponding data for H<sub>2</sub>A and sodium n-dodecyl sulphate (SDS) complexes. The difference between the cationic and anionic surfactants interaction in regard to histone H<sub>2</sub>A is discussed.

## Introduction

The interaction of surfactants at very low concentrations (approximately millimolar) with globular proteins frequently leads to the disruption of their tertiary structure and the formation of protein-surfactant complexes. The mechanism of surfactant denaturation involves the binding of the surfactant ions to the sites onto the protein molecule, which results in unfolding and further binding, often in a cooperative fashion.

The interaction between anionic surfactants especially sodium n-dodecyl sulphate (SDS) and globular proteins has received much attention [1, 2, 3] and it is generally accepted that binding of surfactant molecules to proteins occurs through a combination of

ionic and hydrophobic interactions [4]. The interaction between proteins and cationic surfactants has received less attention but several studies have been carried out [5, 6, 7].

We have previously reported the interaction of calf thymus histone H<sub>2</sub>A with SDS [8]; the aim of this investigation was to study the nature of the interaction between histone H<sub>2</sub>A and dodecyl trimethyl ammonium bromide (DTAB), a cationic surfactant the hydrophobic tail of which is similar to SDS.

## Experimental Section

### Materials

Histone H<sub>2</sub>A 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

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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 cutoff 10,000-14,000) was obtained from SIC (Eastleigh, Hampshire, UK). Orange II dye and DTAB were used as supplied by Sigma. All the salts used in the preparation of the buffers were of analytical grade, and were solubilized in doubly distilled water.

### Methods

Equilibrium dialysis was carried out at 27° and 37°C. The histone H<sub>2</sub>A solutions (2 Cm<sup>3</sup>) were placed in dialysis bags which were then equilibrated with equal volumes of DTAB solutions covering a range of concentrations up to approximately 5 mM in closed glass tubes. The tubes were placed in a gently shaking incubator and the systems allowed to come to equilibrium (>96 hours).

At equilibrium, the concentration of unbound DTAB outside the dialysis bags was assayed by the Orange II method of Few and Ottewill [9] with reference to a previously determined calibration curve. A uv-vis Shimadzu spectrophotometer, model 160, was used for the measurement of optical density at 485 nm.

In all the calculations, a molecular weight of 14,000 was taken for histone H<sub>2</sub>A [10]. In all cases, the concentration of histones was 0.01% (W/V).

### Results and Discussion

Figure 1 (a,b,c) shows the number of moles of DTAB bound per mole of histone H<sub>2</sub>A ( $\bar{v}$ ) measured by equilibrium dialysis as a function of the logarithm of the free DTAB concentration at 27° and 37°C and at pH values of 3.2, 6.4 and 10.0, respectively. Figure 1b shows the most initial plateau region of Figures (1a, c) which is attributed to the binding, mostly anionic sites, and a further steep rise is also attributable to the hydrophobic binding [11]. The increasing pH was induced to the right shift (higher free concentration of DTAB) for binding isotherms which are shown in Figure 1 (a,b,c).

It should be noted that the critical micelle concentration (cmc) of DTAB is approximately the same at 27° and 37°C and is equal to 14 mM. The areas of all the peaks under cmc point indicate that the monomer concentration of DTAB is interacted to histone H<sub>2</sub>A.

The calculation of the apparent binding constant K

may be applied to the entire binding isotherms (Fig. 1). This is based on the Wyman binding potential concept [12]. The binding potential  $\pi$  is calculated from the area under the binding isotherm according to the equation:

$$\pi = RT \int_{\bar{v}_1=0}^{\bar{v}_1} \bar{v}_1 d \ln [\text{DTAB}]_{\text{free}} \quad (1)$$

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

$$\pi = RT \ln (1 + K_{\text{app}} [\text{DTAB}]_{\text{free}}^{\bar{v}_1}) \quad (2)$$

Values of  $K_{\text{app}}$  were determined by the application of equations 1 and 2 and they were used to determine the values of Gibbs energy ( $\Delta G$ ) and the Gibbs energy of binding per surfactant ion ( $\Delta G_{\bar{v}}$ )

$$\Delta G_{\bar{v}} = \frac{\Delta G_{\text{app}}}{\bar{v}} = \frac{-RT}{\bar{v}} \ln K_{\text{app}} \quad (3)$$

The enthalpy of interaction of histone H<sub>2</sub>A and DTAB is shown in Figure 2. This was obtained from the temperature dependence of the binding constant ( $K_{\text{app}}$ ) using the van't Hoff relation [13].

$$\Delta H = \frac{-R d(\ln K_{\text{app}})}{d(1/T)} \quad (4)$$

Figure 2 shows the exothermic changes in enthalpy on the formation of the histone H<sub>2</sub>A-DTAB complexes, which is markedly inconsistent with histone-SDS interaction which is plotted for comparison in Figure 2. The amount of enthalpy of interaction of H<sub>2</sub>A-SDS complexes is much higher than H<sub>2</sub>A-DTAB interaction, because the ratio of cationic to anionic amino acid of H<sub>2</sub>A is equal to 4.33, therefore SDS, an anionic detergent, can act better than DTAB, as a cationic detergent. Of course, the head of DTAB may interfere with the interaction with protein. The difference in the enthalpy sign probably resulted from a difference in charge groups of both surfactants. The top axes in the figures show the number of detergent molecules which would be bound per H<sub>2</sub>A molecule ( $\bar{v}$ ) at equilibrium. These values were taken from binding isotherms (Fig. 1).

Figure 3 shows the thermodynamic parameters of H<sub>2</sub>A-DTAB interaction at pH 3.2, 6.4 and 10.0 whilst the entropy variations are evident at pH 10.0 (Fig. 3c). The change in  $\Delta G_{\bar{v}}$  with increasing  $\bar{v}$  implies that, after the initial binding to the higher energy sites, the subsequent binding is weaker. The change in  $\Delta H_{\bar{v}}$  with increasing  $\bar{v}$  shows less exothermic heat which is related to the unfolding of H<sub>2</sub>A-DTAB complexes.

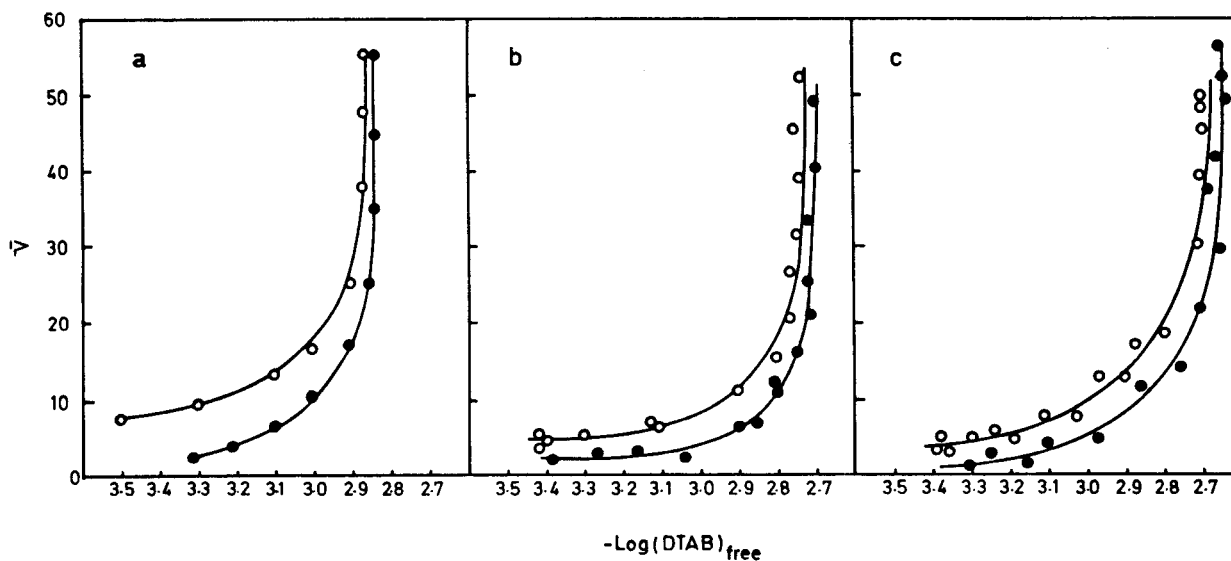


Figure 1. Binding isotherms for DTAB on interaction with, histone H<sub>2</sub>A at 27° and 37°C: (a) pH 3.2; (b) pH 6.4; (c) pH 10.0 O, 27°C; ●, 37°C.

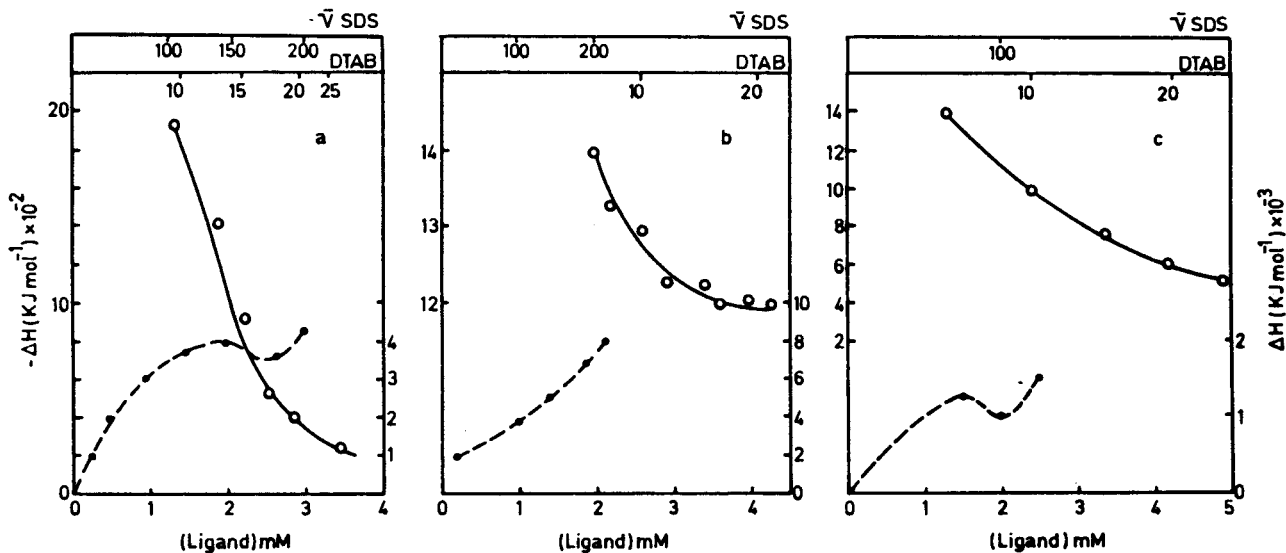


Figure 2. Enthalpy of interaction between histone H<sub>2</sub>A and DTAB, (solid line). Right hand, the broken line is for histone H<sub>2</sub>A-SDS complexes, taken from Ref. 8. The upper axis shows the number of surfactant molecules bound per histone H<sub>2</sub>A at equilibrium. Right hand shows the enthalpy of H<sub>2</sub>A-SDS complexes. (a) pH 3.2, (b) pH 6.4, (c) pH 10.0

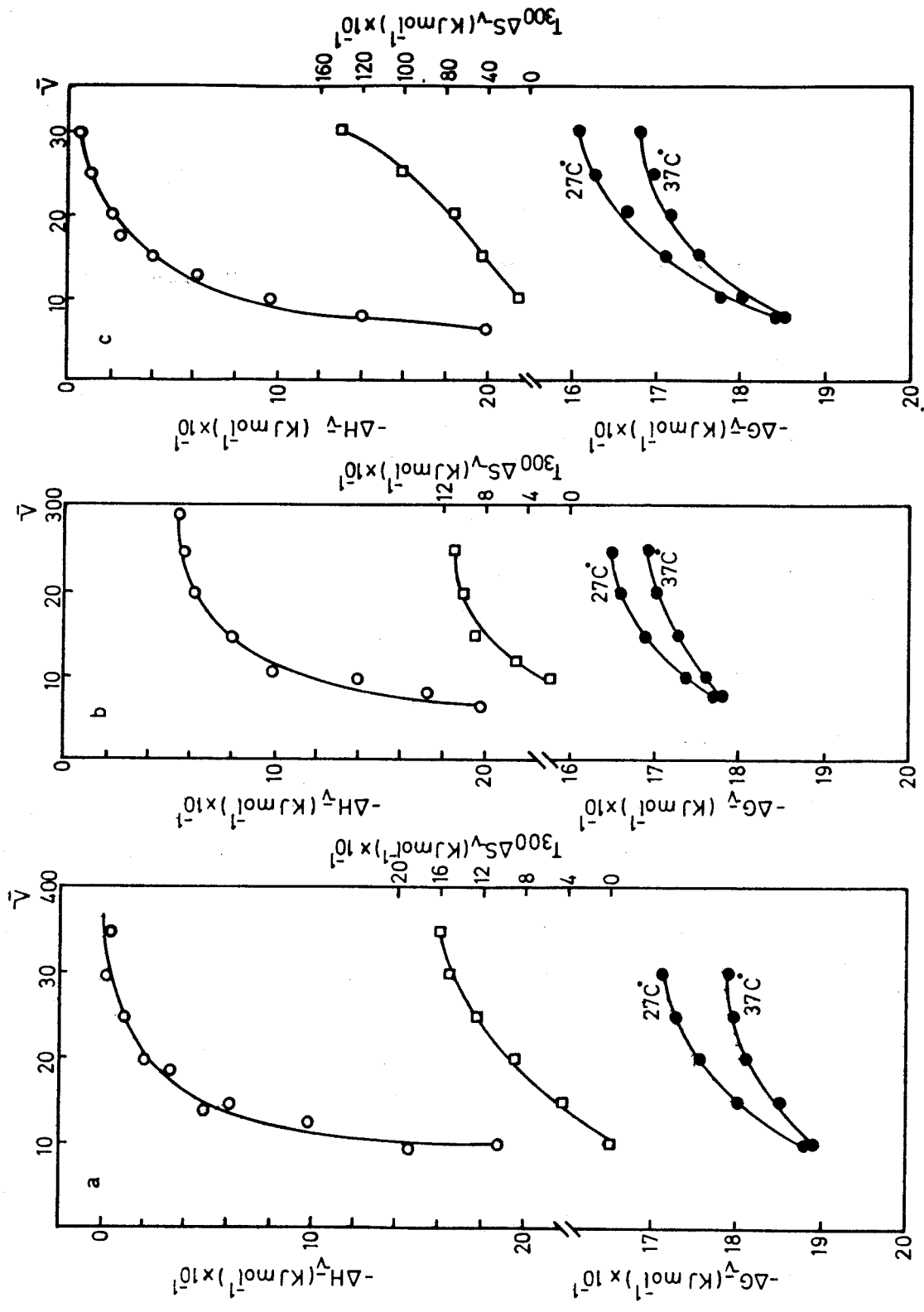


Figure 3. Thermodynamic parameters of the interaction between histone H<sub>2</sub>A and DTAB: (a) pH 3.2; (b) pH 6.4; (c) pH 10.0.  $\Delta H_V$ ;  $\square$ , TAS;  $\bullet$ ,  $\Delta G_V$

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### References

1. Moosavi-Movahedi, A.A., Jones, M.N. and Pilcher, G. *Int. J. Biol. Macromol.*, **10**, 75, (1988).
2. Jones, M.N. and Manley, P. *J. Chem. Soc. Faraday Trans.*, **1**, 77, 827, (1981).
3. Takeda, L. and Moriyama, Y. *J. Protein Chem.*, **9**, 573, (1990).
4. Jones, M.N. and Manley, P. *J. Chem. Soc. Faraday Trans.*, **1**, 75, (1979).
5. Moosavi-Movahedi, A.A., Pilcher, G. and Jones, M.N. *Thermochimica Acta*, **146**, 215, (1989).
6. Moosavi-Movahedi, A.A. and Housaindokht, M.R. *Ibid.*, **219**, 143, (1993).
7. Birdi, K.S. *Biochem. J.*, **135**, 253, (1973).
8. Moosavi-Movahedi, A.A. and Razeghifard, M.R. *Thermochimica Acta*, **188**, 183, (1991).
9. Few, A.V. and Ottewill, R.H. *J. Colloid Sci.*, **11**, 34, (1956).
10. Saenger, W. *Principles of nucleic acid structure*. Springer-Verlag, New York, p. 439, (1984).
11. Jones, M.N. *Biological Interface*. Elsevier, Amsterdam, p. 101, (1975).
12. Wyman, J. *J. Mol. Biol.*, **11**, 63, (1965).
13. Freifelder, D. *Physical chemistry with application to biol. sciences*. Jones Publishers, Inc. Boston, p. 174, (1985).