THE EFFECT OF IONIC STRENGTH ON THE BINDING OF SODIUM N-DODECYL SULPHATE TO ASPERGILLUS NIGER CATALASE

A.A. Moosavi-Movahedi* and S. Ghobadi

Institute of Biochemistry & Biophysics, University of Tehran, Tehran, Islamic Republic of Iran

Abstract

The binding of sodium n-dodecyl sulphate (SDS) to Aspergillus niger catalase in various ionic strengths at pH 6.4 and a temperature of 27° and 37°C has been studied over a range of SDS concentrations using equilibrium dialysis and fluorescence spectroscopy techniques. The binding data were used and interpreted in terms of theoretical models (Scatchard Equation and Wyman Binding Potential). The cationic ionizable residues were estimated and have been compared with data from amino acid analysis.

Introduction

The study of the binding of large amounts of detergent to proteins should furnish important insights into the more complicated and less easily understood problems of lipid-protein interactions. The lipoproteins of serum have been available in large amounts for years, yet the location of the various types of lipids in relation to the protein side chains and to each other is simply not known. Membrane structure is likewise not understood in detail.

It would seem simpler to approach the understanding of these complex structures by studying the interaction of two componets, the amphipathic negatively charged dodecyl sulphate (the lipid) and various proteins [1-4].

The interaction between detergents and catalase has been studied by several physical methods [5-7]. Whereas, the effect of sodium n-dodecyl sulphate (SDS) on the structure of Aspergillus niger catalase shows much more resistance and less activity than Bovine liver catalase [1].

The aim of this work, raising the ionic strength in the presence of higher concentration of SDS on the interaction with Aspergillus niger catalase, might dissociate these effects more efficiently.

Keywords: Aspergillus Niger Catalase, Sodium n-Dodecyl Sulphate (SDS), Thermodynamic Parameters, Fluorescence Spectroscopy, Ionic Strength

Experimental Section

Materials

Aspergillus niger catalase was obtained from Sigma Chemical Co. Sodium n-dodecyl sulphate (especially pure grade) was from Merck Co. Rosaniline hydrochloride dye was used as supplied by B.D.H. (Poole, U.K.) Visking dialysis tubing (MW cut-off 10000-14000) was from SIC (East Leigh) Hampshire, U.K. All other chemicals used in this study were reagent grade.

The buffer (Phosphate) was prepared in double distilled water in various concentrations, 2.5 mM ($I=6.9\times10^{-3}$), 5mM ($I=10.73\times10^{-3}$) and 10mM ($I=18.38\times10^{-3}$) at pH 6.4. Each of the buffer solutions contained 0.02% ($\frac{W}{V}$) sodium azide contributing 0.0031 to the ionic strength.

Methods

Equilibrium dialysis was carried out at

27° and 37°C as previously described [8]. Fluorescent measurements were carried out using a Hitachi Spectrofluorimeter MPF-4. Fluorescence was measured at right angles. The entrance slit of the excitation monochromator was 8 nm. The samples were excited at 265 nm and in a method similar to that proposed by Jordano, et al. [9].

The critical micelle concentration (cmc) of SDS in the buffer solutions were measured at 27° and 37°C by conductance, or from surface tension measurements made with a Du Nouy tensiometer [10]. Correction for inequalities arising from Donnan effects are negligible at the ionic strengths used.

In all calculations, the molecular weight of Aspergillus niger catalase was taken to be 354000 [11].

Results and Discussion

The binding isotherms (the number, $\bar{\upsilon}$, o surfactant ions bound per protein molecule as a functio of the logarithm of the free surfactant concentration, $[S_f]$)for SDS on Aspergillus niger catalase as a functio of ionic strength are shown in Figure 1 (a,b). The binding isotherm shows an initial gradual rise, which has been attributed to weak binding to ionic sites and a further steep rise attributable to hydrophobic binding. Ionic strength affects both the ionic and hydrophobic binding regions; increase in ionic strength shifts the binding region to lower free SDS concentrations, effectively promoting binding. Also, here the extent of binding at the critical micelle concentration (cmc)

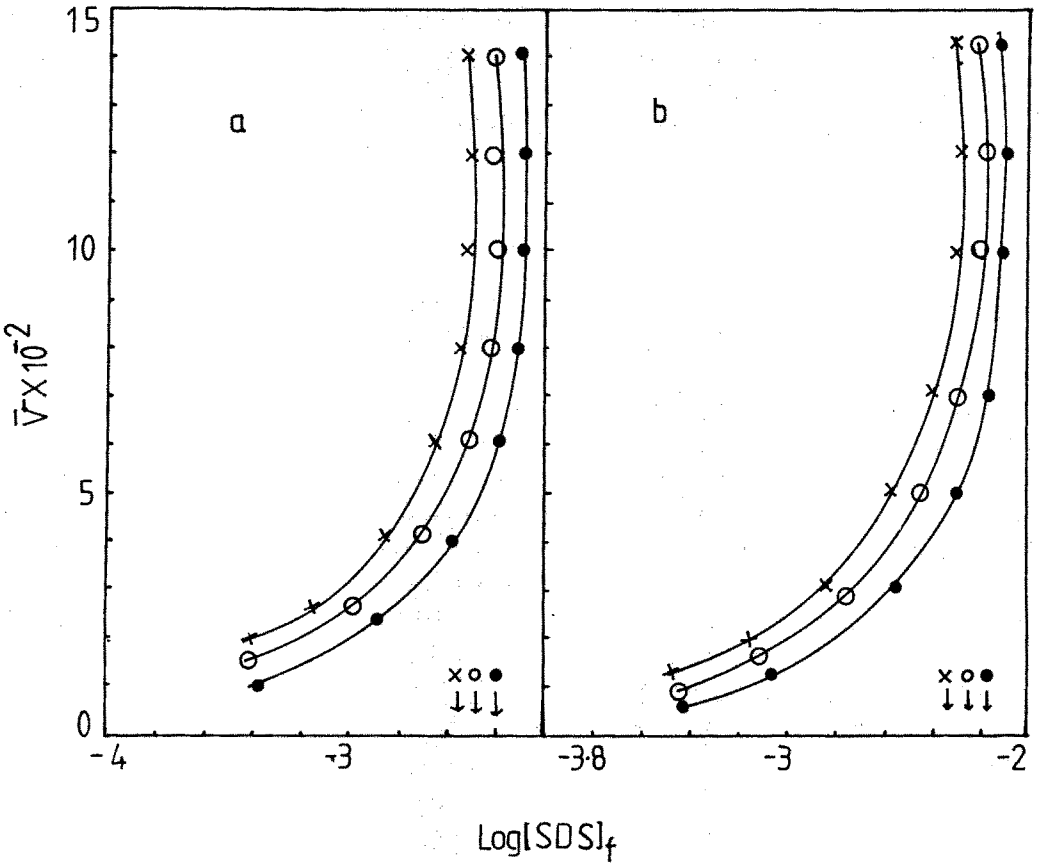

Figure 1- Binding isotherms for SDS on interaction with Aspergillus niger catalase at pH 6.4. ●; 2.5 mM ($I=6.9\times10^{-3}$), O; 5mM ($I=10.73\times10^{-3}$), X; 10 mM ($I=18.38\times10^{-3}$).
a) 27°C b) 37°C

ascends a little as the ionic strength increases: at I=6.9x 10^{-3}, $\bar{\upsilon}$ (cmc)=600; at I=10.73x10^{-3}, $\bar{\upsilon}$ (cmc)=680; at I=18. 38x10^{-3}, $\bar{\upsilon}$ (cmc)=800. Raising the temperature from 27° and 37°C has a negligible effect on the cmc.

The binding isotherms were shifted towards a higher free SDS concentration with increasing temperature from 27° to 37°C, resulting in an exothermic enthalpy of the system.

Calculation of the binding constant and Gibbs energy which can be applied to the entire binding isotherm is based on the Wyman Binding Potential concept which was described previously [12]. The enthalpies of interaction of Aspergillus niger catalase-SDS were obtained from the temperature dependence of K_{app} using the van't Hoff relation which has been described previously [13].

The enthalpies of interaction between Aspergillus niger catalase and SDS at various ionic strengths are shown in Figure 2 (a,b,c). The top axes in the figures show the number of surfactant molecules which would be bound per catalase molecule $\bar{\upsilon}$.

The enthalpy curves show that there is an exothermic contribution to Aspergillus niger catalase and SDS complexes. Addition of SDS to catalase initially rise until $\bar{\upsilon} \simeq 600$. The minima in the curves suggest the presence of an endothermic contribution that is attributable to unfolding of catalase. By raising the ionic strength, the enthalpy curves shift to a higher initial concentration of SDS whereas the value of $\bar{\upsilon}$ at which endothermic effect occurs does not change ($\bar{\upsilon}$ =600), but, the steep rise after the minima shows the effect of higher ionic strength after unfolding.

Figure 3 (a,b,c) shows the relation of the denatured

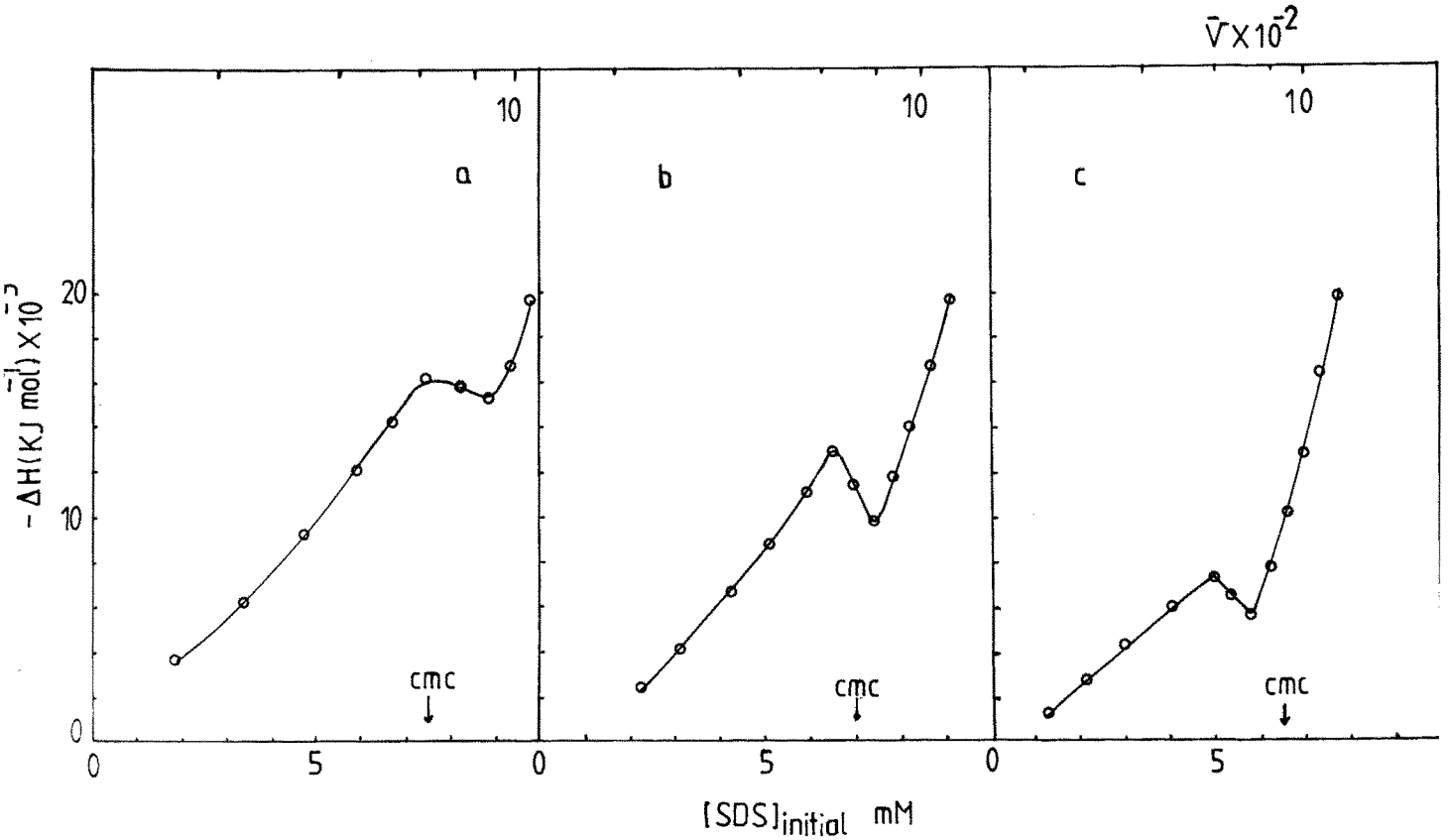

Figure 2- Enthalpy of interaction between Aspergillus niger catalase with SDS at various ionic strengths. The upper axis shows the number of SDS molecules bound per catalase molecule.
a) I=6.9x10^{-3} b) I=10.73x10^{-3} c) I=18.38x10^{-3}

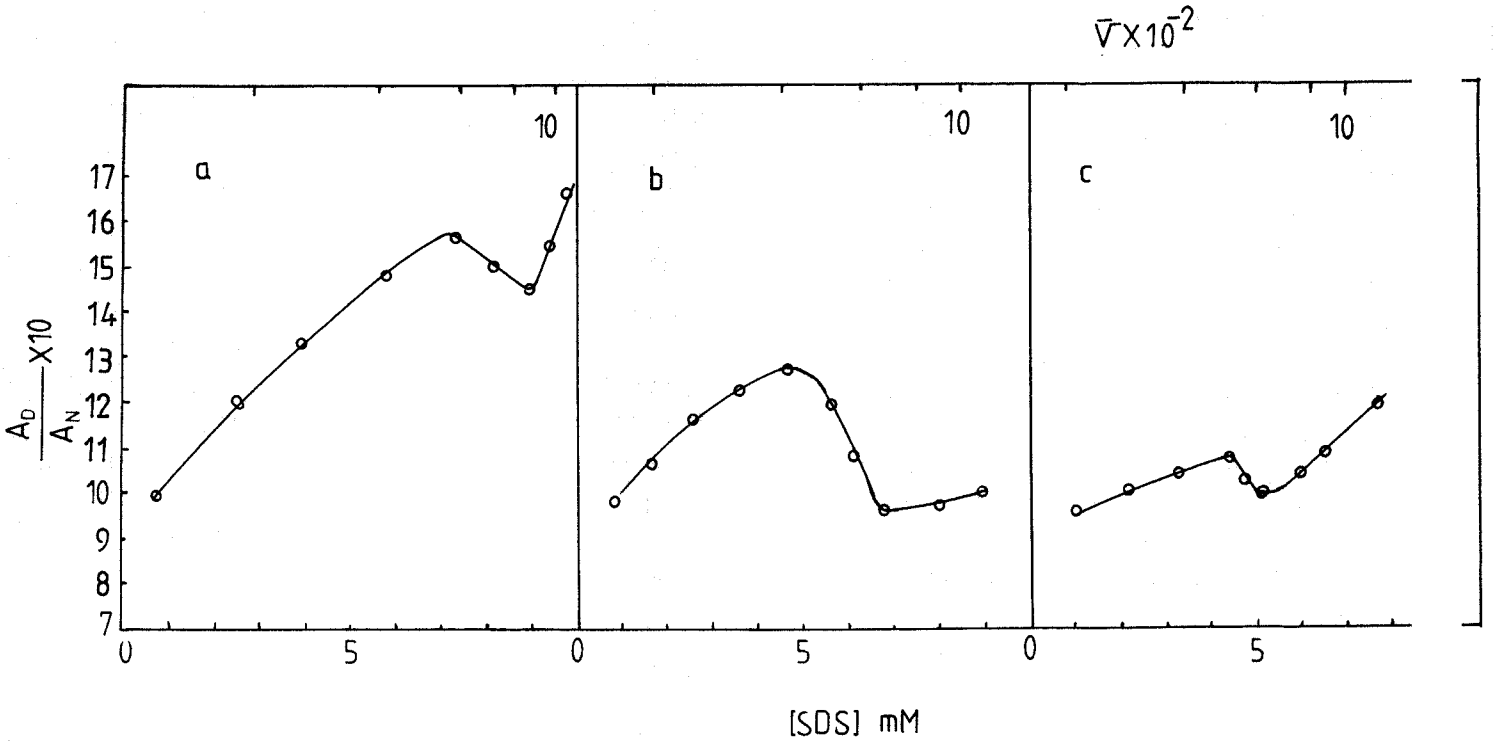

Figure 3- The relative area under the fluorescence emission spectrum between 250 and 400 nm for denatured and native states for the interaction between Aspergillus niger catalase-SDS complexes.
a) $I=6.9x10^{-3}$ b) $I=10.73x10^{-3}$ c) $I=18.38x10^{-3}$

and native states to the area under the fluorescence emission spectrum between (250 and 400 nm) vs. SDS concentrations. The probability of fluorescence is described by the quantum yield; that is, the ratio of the number of emitted to absorbed photons. Whereas, the quantum yield is related to the area under the fluorescence emission spectrum. On the other hand, the quantum yield predicts the value of the hydrophobicity. Here, the interaction of SDS with Aspergillus niger catalase can affect the fluorescence of aromatic residues and hence provides information on their exposure in Aspergillus niger catalase. Figure 3 shows the changes in the availability of hydrophobic regions, induced by the interaction of Aspergillus niger catalase-SDS complexes. Increasing the concentration of SDS results in the enhancement of the hydrophobicity up to a critical amount of SDS which is dependent on ionic strength which corresponded to the folding, then it was quenched (the minima) which corresponded to the unfolded State. Figure 3 shows the degree of folding decreases with increasing ionic strengths.

Plots based on the Scatchard equation (14):

$$\frac{\bar{\upsilon}}{[SDS]_{free}} = K(n - \bar{\upsilon}) \qquad (1)$$

where K and n are the intrinsic association constant and the number of binding sites respectively are shown in Figure 4. If this equation is obeyed, a plot of $\bar{\upsilon}/[SDS]_{free}$ vs. $\bar{\upsilon}$ should be linear with a slope of K and an intercept when $\frac{\bar{\upsilon}}{[SDS]_{free}} = 0$ of n.

The plots (Figure 4) are clearly linear at lower values of $\bar{\upsilon}$ (i.e. the specific binding region) and the intercepts in higher ionic strength ($I=18.38x10^{-3}$) are very close to 285, the number of cationic amino acid residues in the

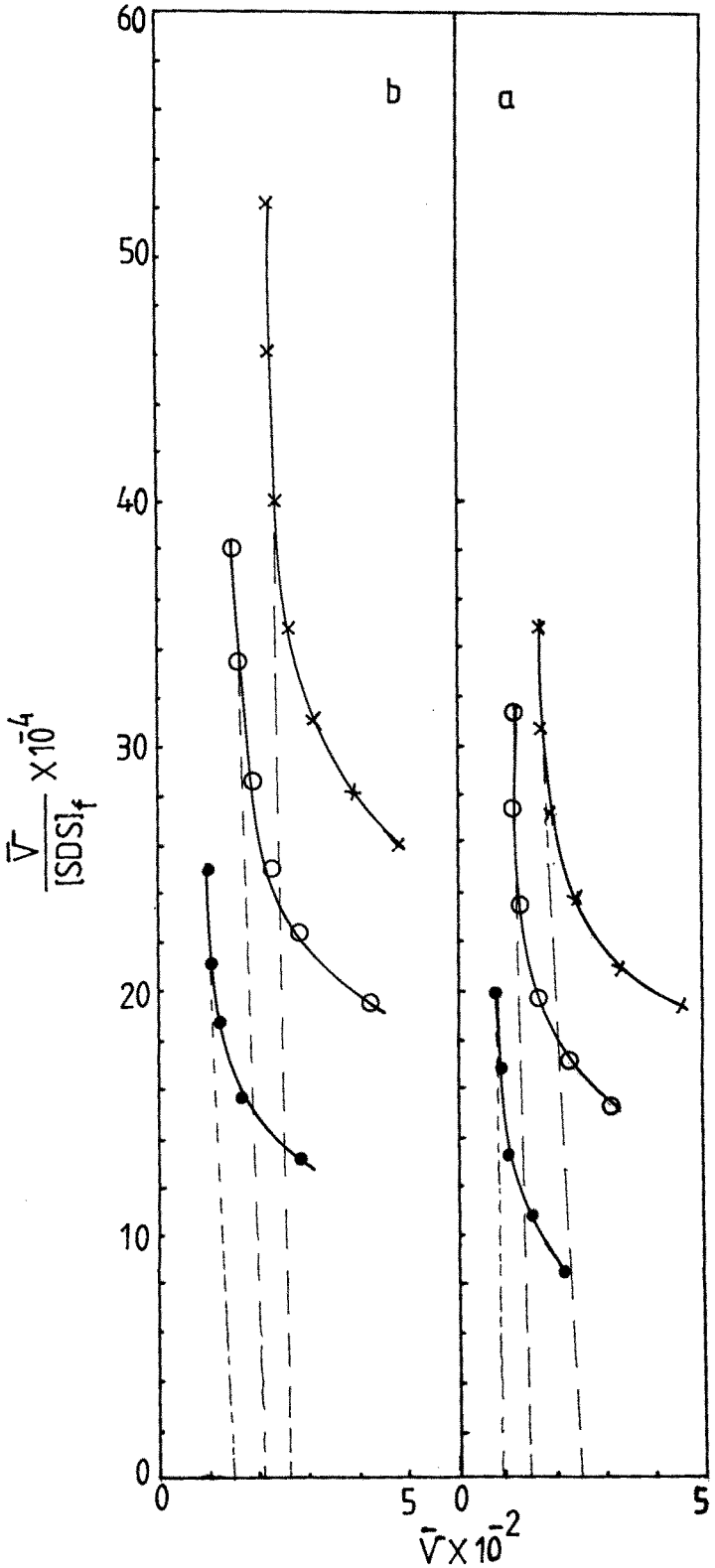

Figure 4- Scatchard plots of the interaction between Aspergillus niger catalase and SDS. ●; 2.5 mM ($I=6.9\times10^{-3}$), O;5 mM ($I=10.73\times10^{-3}$)X; 10 mM ($I=18.38\times10^{-3}$).
a) 27^0C b) 37^0C

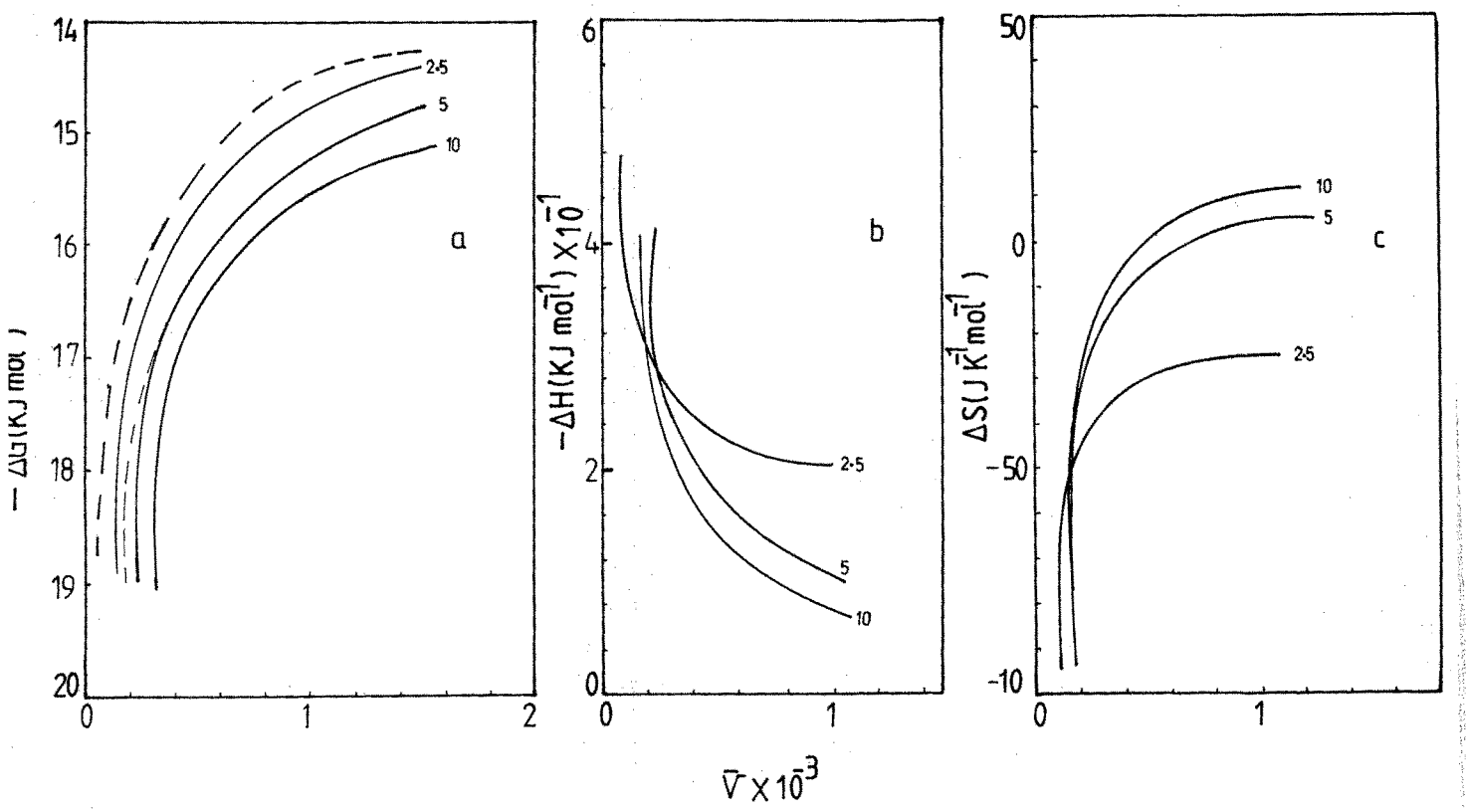

Figure 5-Thermodynamic parameters for the interaction between Aspergillus niger catalase and SDS ●; 2.5 mM ($I=6.9\times10^{-3}$),O; 5 mM ($I=10.73\times10^{-3}$)X; 10 mM ($I=6.9\times10^{-3}$).

a) ΔG_{υ}^{-}, dashed line is for 37 ^{0}C

b) ΔS_{υ}^{-} c)ΔS_{υ}^{-}

Aspergillus niger catalase (i.e. 73 His+83 Lys+129 Arg) [11].

In comparison with data from the amino acid analysis, at ionic strengths; 6.9×10^{-3}; 10.73×10^{-3} and 18.38×10^{-3}, 37%(27^{0}C), 52%(37^{0}C); 52%(27^{0}C), 75%(37^{0}C); 88%(27^{0}C), 91%(37^{0}C) of the cationic ionizable amino acid residues can be titrated with SDS respectively. Scatchard plots in Figure 4 confirm the fluorescence studying which at lower ionic strengths suggests increased folding but on increasing the ionic strength the unfolding of catalase increases.

Figure 5 (a,b,c) shows the thermodynamic parameters (ΔG_{υ}^{-}, ΔH_{υ}^{-}, ΔS_{υ}^{-}) for the interaction of Aspergillus niger catalase with SDS at various ionic strengths. The data show, with increasing $\bar{\upsilon}$ and ionic strength, ΔG_{υ}^{-} and ΔH_{υ}^{-} become less negative and ΔS_{υ}^{-} become more positive.

Acknowledgements

The financial assistance from the Research Council of the University of Tehran is gratefully acknowledged. We also thank Mrs. J.D. Noor-Mohammadi for revising the English.

References

1) A.A.Moosavi-Movahedi, M.N. Jones and G. Pilcher, *Int. J. Biol. Macromol.* **10**,75 (1988).

2) A.A. Moosavi-Movahedi, M.N. Jones and G. Pilcher, *Int. J. Biol. Macromol.* **11**, 26 (1989).

3) M.N. Jones, A. Finn, A.A. Moosavi-Movahedi and B. Waller, *Biochem. Biophys. Acta* **913**, 395 (1988).

4) M. Nogueria, A. Finn and M.N. Jones, *Int. J. Biol. Macromol.* **8**, 270 (1986).

5) A.A. Moosavi-Movahedi, G. Pilcher and M.N. Jones, *Thermochimica. Acta,* **146**, 215 (1989).

6) R. Pitt-Rivers and F.S.A. Impiombato, *Biochem. J.* **109,** 825 (1968).

7) M.N. Jones, A.E. Wilkinson and A. Finn, *Int. J.Biol. Macromol.* **7,** 33 (1985).

8) A.A. Moosavi-Movahedi, M.Goodarzi and M.R. Housaindokht, *J. Sci. I.R. Iran,* **1,** 81 (1990).

9) J. Jordano, J.L. Barbero, F. Montero and L. Franco, *J. Biol. Chem.* **258,** 315 (1983).

10) M.N. Jones, P. Manley and A. Holt, *Int. J. Biol. Macromol. 6,* 65 (1984).

11) A.A. Moosavi-Movahedi, A.E. Wilkinson and M.N. Jones, *Int. J. Biol. Macromol.* **9,** 327 (1987).

12) A.A. Moosavi-Movahedi and M.R. Housaindokht, *Physiol. Chem. Phys.& Med. NMR,* **22,** 19 (1990).

13) A.A. Moosavi-Movahedi and M.R. Razeghifard, *J. Sci. I.R. Iran,* **1,** 244 (1990).

14) G. Scatchard, Ann. N. Y. Acad. Sci, **51**, 660 (1949).