THERMAL ANALYSIS OF ADENOSINE DEAMINASE IN THE PRESENCE OF SODIUM N-DODECYL SULPHATE

*Moosavi-Movahedi, Ali Akbar**
Moghaddamnia, Hassan
Institute of Biochemistry and Biophysics, University of Tehran, P.O.Box: 13145-1384 Tehran, Iran.
Hakimelahi, Gholam Hossein
Institute of Chemistry, Academia Sinica, Taipei, Taiwan 11529, Republic of China

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ABSTRACT : *The thermal denaturation of adenosine deaminase (ADA) has been investigated in the presence of sodium n- dodecyl sulphate (SDS) over the temperature range of (293-363K) in 2.5mM phosphate buffer, pH 6.4 by temperature scanning spectroscopy. The interaction of SDS caused the folding of adenosine deaminase resulting in a decrease of T_H (temperature of minimum solubility), T_S(temperature of maximum stability), $\frac{\Delta H_{VH}}{\Delta H_{298}}$ (intermolecular force between hydrophobic parts of adenosine deaminase with water) and other corresponding thermodynamic parameters. The folding of adenosine deaminase by SDS, induced minimum solubility at lower temperatures indicating enhanced apolar interactions in the interior phase resulting in a lower value for T_H. In contrast the interaction of ADA with dodecyl trimethylammonium bromide (DTAB) led to the unfolding of the enzyme and a higher value of T_H.*

KEY WORDS : *Adenosine deaminase, Sodium n- dodecyl sulphate, Thermal denaturation.*

* Corresponding author

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INTRODUCTION :

Macromolecules of biological importance are generally characterized by a degree of rigidness of three- dimensional structure which far exceeds that usually observed with synthetic polymers. This arises from the existence of strong intramolecular forces exhibiting much cooperation, one manifestation of which is the relatively sharp thermally induced conformational transitions which have been observed with many biopolymers. The upper limit of cooperativity for small globule of one subunit protein in a transition is exhibited in a simple two state transition of folded $\rightleftharpoons$ unfolded, where the cooperative unit coincides with the entire molecule [1,2].

The conformational stability of a globular protein is generally defined as the difference in free energy between the folded and unfolded conformational states at 25°C or in the absence of a denaturant for the reaction; folded $\rightleftharpoons$ unfolded.

A commonly used method for estimating the conformational stability of a protein is the analysis of urea or guanidine hydrochloride denaturation curves [4-8]. The urea and guanidine hydrochloride are solution components which affect the water structure surrounding a protein [9], whereas the surfactants usually affect the protein system. There is a lack of information on thermal and chemical denaturation of ADA especially in the presence of surface active agents, while the denaturation of ADA in the presence of dodecyl trimethylammonium bromide (DTAB) has been done by us [11]. In the presence of sodium n- dodecyl sulphate as denaturant which affects the protein system directly, the free energy (ΔG), enthalpy (ΔH), entropy (ΔS), and heat capacity (ΔC_p) has been obtained.

EXPERIMENTAL :

Materials :

Adenosine deaminase from calf intestinal mucosa and sodium n- dodecyl sulphate (SDS) were obtained from Sigma (St. Louis, Missouri, USA). Chemicals used in the preparation of the buffer were analytical grades and they were dissolved in doubly distilled water. Sodium phosphate buffer (2.5mM), pH 6.4 and I= 0.0069 were used.

Methods :

A Gilford model 2400-2 (Ciba, Corning, Ohio, USA) multiple sample absorbance spectrophotometer equipped with recorder was used for melting at a constant heating rate of 2/3°C per min. The decreased hypochromicity refered to A280 started at 20°C was recorded at 5 degree intervals.

All measurement reported refer to SDS concentrations below the critical micelle concentration (cmc). In all measurements the concentration of adenosine deaminase was 0.02% (W/V).

RESULTS AND DISCUSSIONS :

Thermal denaturation curves for adenosine deaminase (ADA) in the presence of sodium n-dodecyl sulphate (SDS) are shown in Fig.1. ADA, like many other small globular proteins closely approaches a two state mechanism. Assuming a two state denaturation mechanism, the fraction of denatured protein, F_d, may be calculated using [12]:

$$F_d = (y_n - y_{obs}) / (y_n - y_d) \quad (1)$$

where y_{obs} is the observed variable parameter (e.g. absorbance intensity, Fig. 1-a), and y_n and y_d are the values of y characteristic of the native and denatured conformations(obtained as shown in Fig. 1-a). The difference in free energy between the native and denatured conformations, ΔG, can then be calculated using:

$$\Delta G = -RT\ln[F_d/(1- F_d)] = -RT \ln[(y_n - y_{obs}) / (y_{obs} - y_d)] \quad (2)$$

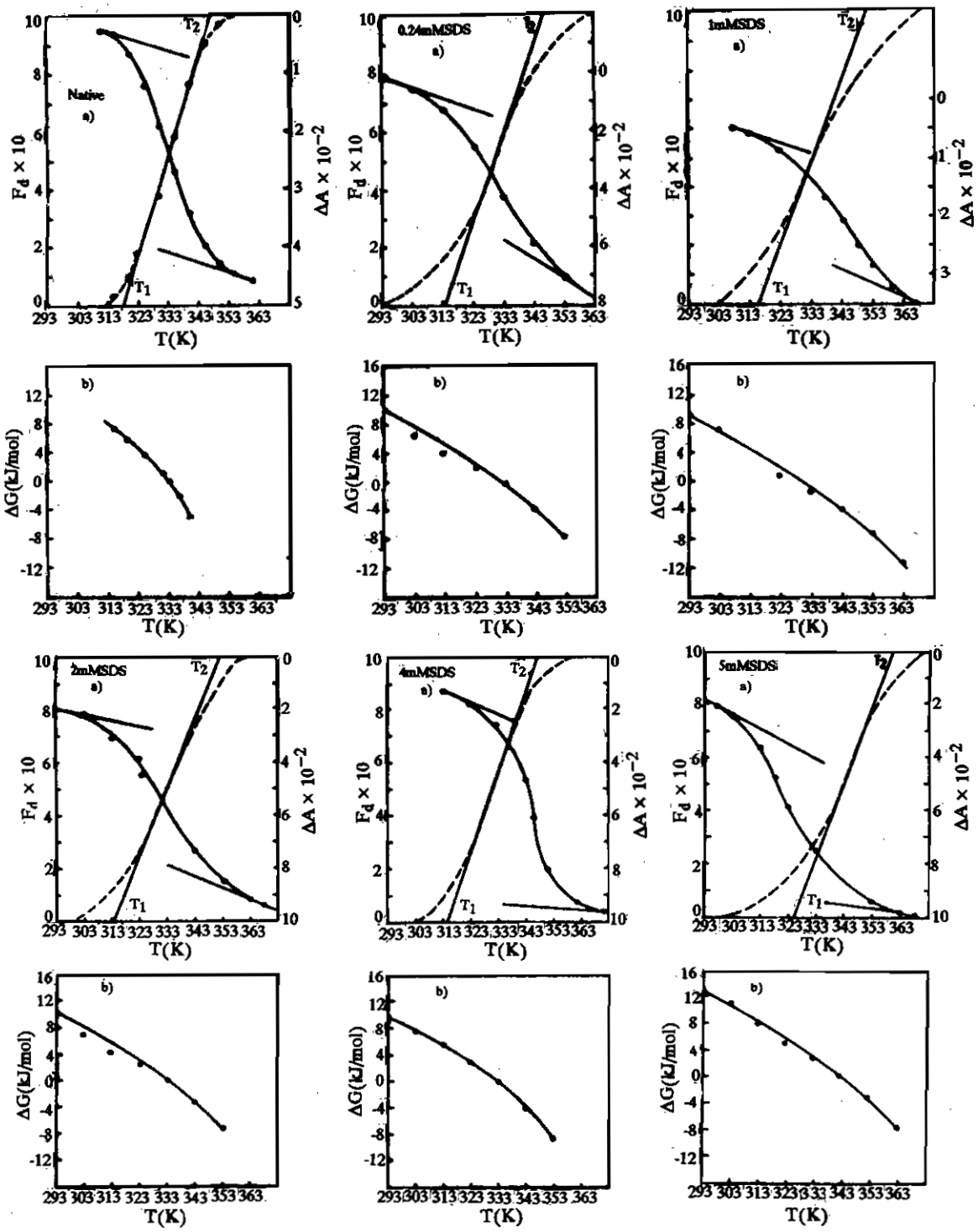

Fig. 1-a) Thermal denaturation curves for adenosine deaminase (ADA) in the presence of sodium n- dodecyl sulphate (SDS). Left hand side shows the denatured fraction (F_d) (dashed curve) and right hand side shows the difference in absorbance (ΔA) at 280nm as a function of the temperature (solid curve). T_1 and T_2 was obtained from van't, Hoff line and used to calculate ΔH_{VH} (see text); b) ΔG as a function of temperature in the presence of SDS as obtained from the data in left hand (F_d) of Fig. 1-a.

where R is the gas constant and T is the absolute temperature. The free energy of denaturation, ΔG, as a function of temperature for ADA in the presence of SDS is shown in Fig. 1. It can be used to determine T_m (melting temperature) at which $\Delta G=0$ and $\Delta H_m = (T_m \text{ in K}) \times[-(\text{Slope at } T_m=\Delta S_m)]$ which is obtained from the plots of ΔG vs. temperature (Fig. 1). The temperature depeudence of ΔG is given by a modified form of the *Gibbs- Helmoltz* equation [13]:

$$\Delta G(T)=\Delta H_m(1-T/T_m)-\Delta C_p[T_m-T+TLn(T/T_m)] \quad (3)$$

where ΔG (T) is ΔG at a temperature T, and $\Delta C_p = C_p(U) - C_p(N)$, is the change in heat capacity upon denaturation. The heat capacity of the unfolded protein $C_p(U)$, always is greater than that of the folded protein, $C_p(N)$, and this gives rise to the positive change in heat capacity.

The temperature of maximum stability (T_s) occurs at the temperature where $\Delta S=0$ and is given by equation [14]:

$$T_s = T_m \exp [-\Delta H_m / (\Delta C_p \cdot T_m)] \quad (4)$$

The temperature at which the enthalpy becomes zero T_H can be written [7]:

$$T_H = T_m - \Delta H_m / \Delta C_p \quad (5)$$

T_H is defined as the temperature of minimum solubility by *Baldwin*[15]. At T_H ,the enthalpies of interactions of the apolar surfaces with water, including the enthalpy of water restructuring and the solute/ solvent van der Waals interactions, are equal and opposite to the enthalpic interactions that the apolar surface experiences in the respective initial phases [15]. As the apolar interaction with water is independent of the initial phase, greater apolar interactions in this phase result in a lower value for T_H [16]. In approximation, $\Delta H(T)$, it can be written:

$$\Delta H(T) = \Delta H(T_m) - (T_m - T) \Delta C_p \quad (6)$$

The van't Hoff enthalpy can be also expressed as [17]:

$$\Delta H_{VH} = 4RT_m^2 / (T_2 - T_1) \quad (7)$$

Where T_1 and T_2 are indicated in Fig.1. The line which is crossed at melting point (T_m) is the van't Hoff line. The projection of van't Hoff line onto the x-axis shows T_1 and its extension on the opposite direction (where $F_d=1$) is indicated by T_2. Thermodynamic data of T_m , ΔH_m , ΔS_m , ΔH_{VH} , ΔH_{298} , ΔC_p , T_s , T_H and $\Delta H_{VH}/\Delta H_{298}$ at various concentrations of SDS interacted with ADA , are tabulated in Table 1.

With increasing the concentration of SDS ΔS_m , ΔH_m , ΔC_p , T_s , T_H and $\Delta H_{VH}/\Delta H_{298}$, decreases whereas the ΔH_{VH} does not change so much, and ΔH_{298} increases. T_m was changed at 5mM concentration of SDS.

The decrease in T_m , ΔS_m , ΔH_m may be attributed to the folding of the structure of ADA.

Table 1 : Thermodynamic parameters characterizing the interaction of adenosine deaminase with SDS.

[SDS] mM	T_m (K)	ΔS_m $Jmol^{-1}K^{-1}$	ΔH_m $kJmol^{-1}$	ΔH_{VH} $kJmol^{-1}$	ΔH_{298} $kJmol^{-1}$	$\frac{\Delta H_{VH}}{\Delta H_{298}}$	ΔC_p $kJmolK^{-1}$	T_s (K)	T_H (K)
0	333.5	468	152	129	30.4	4.22	3.43	292	289
0.24	333.5	300	100	109	52.1	2.08	1.35	267	259
1	333.5	320	107	106	66.0	1.60	1.15	252	240
2	333.5	330	110	109	76.6	1.42	0.944	235	216
4	333.5	340	113	109	100.5	1.08	0.739	211	180
5	342	290	102	109	76.7	1.40	0.582	199	166

ΔC_p was calculated from equation (3) which is a good estimation of ΔC_p if $T < T_m$.

The positive values of ΔC_p is commonly attributed to the hydrophobic interactions [15] and the reducing value of ΔC_p in Table 1 shows the exposure of polar groups to water [18] which means the folding for ADA - SDS complexes.

The reducing value of T_H , due to interaction of ADA - SDS complexes decreasing the T_H (temperature of minimum solubility) which means the folding of ADA induced the minimum solubility at lower temperature. T_s is the temperature of maximum stability which is also decreased by ADA - SDS complexes.

ΔH_{298} is calculated from equation (6) which is assumed a good agreement to enthalpy of calorimetery, ΔH_{cal} , for a two state mechanism [3]. By comparing ΔH_{cal} with ΔH_{VH} it can be established whether the transition of the system; if ΔH_{VH} is greater than ΔH_{cal} , this proves that there exist some intermolecular interaction [19]. The relation of ΔH_{VH} / ΔH_{298} , can show the transition of the ADA in the presence of SDS. This shows the interaction of protein with water, with decreasing amount of ΔH_{VH} / ΔH_{298} predict the less interaction of ADA with water, which means the minimum solubility of ADA is brought at lower temperature. All these parameters indicate the folding for ADA when interacted with SDS. It is markedly inconsistent with interaction of ADA with dodecyl trimethylammonium bromide (DTAB) as a cationic detergent which has similar tail with SDS and different head [10]. ΔC_p (best criteria for determination of hydrophobic contribution [15]) and T_H increased by interaction of ADA with DTAB which is attributed to unfolding of ADA because of the exposure of apolar groups to water (ΔC_p was increased). The elevation of T_H for ADA - DTAB complexes (unfolding state) induced the minimum solubility at higher temperature. Therefore, it can be concluded that the minimum solubility for ADA - SDS complexes (folding state) shifting to lower temperature, whereas for ADA - DTAB complexes (unfolding state) shifting to higher temperature. This means for increasing the solubility for folding which have to elevate the temperature, whereas for unfolding decreasing the temperature.

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