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Thermochemical analysis of *Aspergillus niger* catalase and sodium *n*-dodecyl sulphate interaction

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Abstract

The binding of sodium *n*-dodecyl sulphate (SDS) to *Aspergillus niger* catalase was studied in the pH range 3.2–10 by equilibrium dialysis at 25 and 37 °C. The binding data have been used to obtain the thermodynamic parameters of interaction using a theoretical model of the Wyman binding potential and van't Hoff relation.

The theoretical enthalpy (ΔH_{VH}) of interaction between *Aspergillus niger* catalase and SDS was compared with the experimental enthalpy (ΔH_{Cal}) at various pH values between 3.2 and 10. A large value of $\Delta H_{VH}/\Delta H_{Cal}$ is obtained which is consistent with activation of *Aspergillus niger* catalase and SDS complexes at pH 6.4.

INTRODUCTION

The enzyme catalase (EC 1.11.1.6) is widely distributed in nature, being found in almost all aerobic organisms, and was one of the first enzymes to be isolated with a high degree of purity. Catalase protects the cell from the oxidizing action of hydrogen peroxide (H_2O_2) produced by superoxide dismutase and flavoprotein enzymes: catalytically, by carrying out the decomposition of two H_2O_2 molecules into molecular oxygen and water [1], and peroxidatively, oxidizing formate, nitrate or alcohols with H_2O_2 [2].

Catalase from a wide range of sources contains four subunits and, like bovine liver catalase, generally has a molecular weight of approximately 250 000 [3]. In contrast, catalase from *Aspergillus niger* has been found to differ significantly from other catalases in several respects [4,5]. In particular, the molecular weight of *A. niger* catalase has been found to be considerably greater than that of other catalases, and previously has been reported to be 354 000 [6]. The enzyme contains carbohydrate [6], which contributes in a small degree to its thermostability [7]. The catalytic activity of *A. niger* catalase is lost in sodium *n*-dodecyl sulphate (SDS), as a potent biological

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detergent, in acid (pH 3.2) or alkaline (pH 10) solutions but, surprisingly, is activated by SDS up to 180% at pH 6.4 [8].

The thermodynamic investigation of the interaction of SDS with *A. niger* catalase at low and high ionic strength by microcalorimetry at 25 °C was previously reported [9,10].

In this paper we compare the thermodynamic data obtained by the experimental method of microcalorimetry and by the theoretical method from the Wyman binding potential and van't Hoff relation at 25 and 37 °C over a range of pH and SDS concentration using a equilibrium dialysis technique [11,12].

EXPERIMENTAL

Materials

Aspergillus niger catalase was obtained from Sigma Chemical Co. Ltd. Sodium *n*-dodecyl sulphate was purchased from Merck. Visking membrane dialysis tubing (MW cut-off 10000–14000) was obtained from SIC (Eastleigh, Hampshire). Rosaniline hydrochloride dye was received from B.D.H. All other materials and reagents were of analytical grade, and solutions were made in double-distilled water. The composition of the buffer used (pH 3.2, pH 6.4 and pH 10) and concentration measurements were as described previously [6,13].

Methods

Equilibrium dialysis to measure bound SDS was carried out by a previously described method [13,14]. In all calculations the molecular weight and concentration of *A. niger* catalase were taken to be 354000 and 0.05% (w/v) respectively.

RESULTS AND DISCUSSION

Figure 1 shows the number of moles of SDS bound per mole of *A. niger* catalase ($\bar{\nu}$) measured by equilibrium dialysis as a function of the logarithm of the free SDS concentration at 25 and 37 °C and at pH 3.2, 6.4 and 10. The increase in temperature shifts the binding isotherm to a higher free concentration of SDS.

The calculation of the apparent binding constant K can be applied to the entire binding isotherm. This is based on the Wyman binding potential concept [11]. The binding potential Π is calculated from the area under the binding isotherm according to the equation

$$\Pi = RT\int_{\bar{\nu}_i=0}^{\bar{\nu}_i} \bar{\nu}_i \, \mathrm{d} \ln [\mathrm{SDS}] \qquad (1)$$

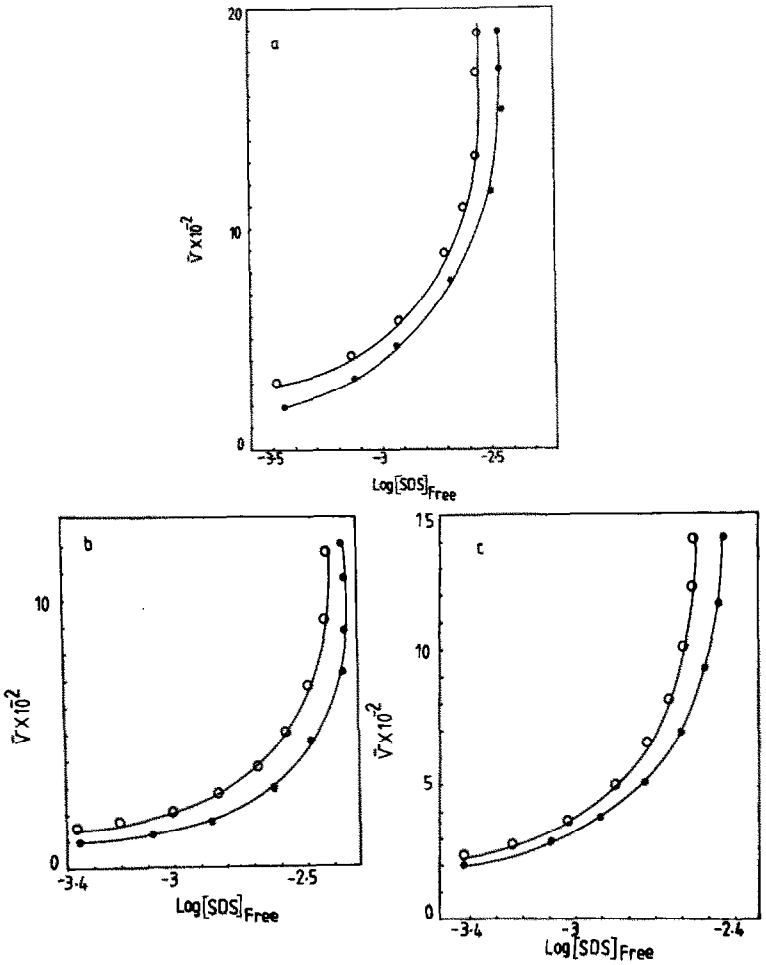

Fig. 1. Binding isotherms for SDS on interaction with *Aspergillus niger* catalase at 25 and 37 °C: (a) pH 3.2; (b) pH 6.4; (c) pH 10. ○, 25 °C; ●, 37 °C.

and is related to an apparent binding constant K_{app} as follows

$$\Pi = RT \ln\left(1 + K_{app}[SDS]^{\bar{\nu}_i}\right) \tag{2}$$

Values of K_{app} were determined by application of eqns. (1) and (2) and were used to determine values of Gibbs energy (ΔG) and the Gibbs energy of binding per surfactant ion ($\Delta G_{\bar{\nu}}$)

$$\Delta G_{\bar{\nu}} = \frac{\Delta G_{app}}{\bar{\nu}} = \frac{RT}{\bar{\nu}} \ln K_{app} \tag{3}$$

The enthalpy of interaction of *A. niger* catalase and SDS is shown in Fig. 2. This was obtained from the temperature dependence of the binding

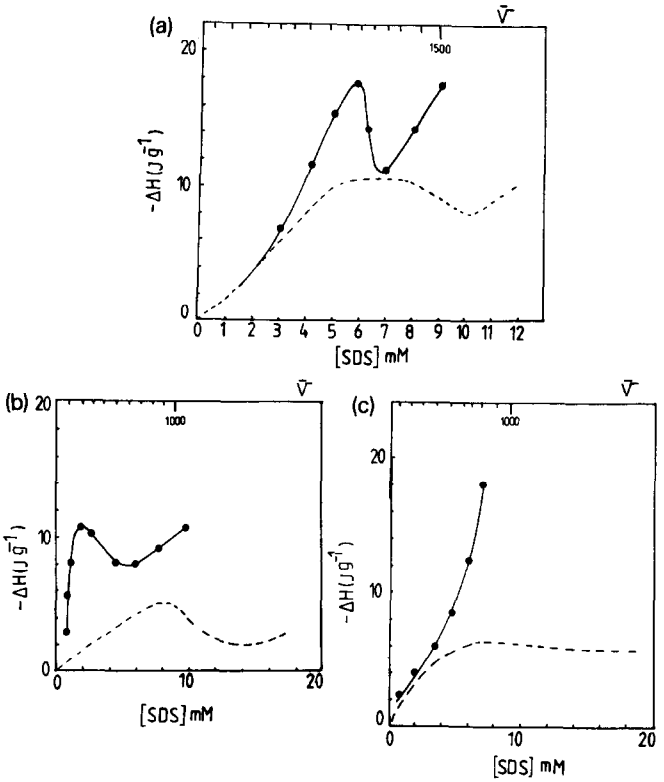

Fig. 2. Enthalpy of interaction between *Aspergillus niger* catalase and SDS. ΔH_{VH} (solid line); ΔH_{Cal} (broken line) was taken from Ref. 10. The lower horizontal axis gives the final SDS concentration. The upper axis shows the number of SDS molecules bound per catalase molecule at equilibrium at 25 °C: (a) pH 3.2; (b) pH 6.4; (c) pH 10.

constant (K_{app}) using the van't Hoff relation [15]

$$\Delta H = \frac{R\, \mathrm{d}(\ln K_{app})}{\mathrm{d}(1/T)} \tag{4}$$

Figure 2 shows the exothermic changes in enthalpy on the formation of the *A. niger* catalase–SDS complexes which were obtained from the Wyman and van't Hoff theoretical equations, ΔH_{VH} (solid line), and by the experimental method of microcalorimetry, ΔH_{Cal} (broken line), which were taken from a previous report [9].

At pH 3.2 and 6.4 (Fig. 2a and b) the enthalpy curves of ΔH_{VH} and ΔH_{Cal} are of similar shape, although the ΔH_{VH} is more exothermic and shifted to the left side (lower concentration of SDS), but at pH 10 a difference appeared for values of $\bar{\nu} > 500$.

By comparing ΔH_{Cal} with ΔH_{VH} it can be established whether the transition of the system is a two-state mechanism or not. When ΔH_{VH} is smaller than ΔH_{Cal}, this indicates that the transition is a multi-state one,

TABLE 1

The relation of ΔH_{VH} and ΔH_{Cal} at various concentrations of SDS at pH 3.2, 6.4 and 10. The minima are shown in italics

SDS, mM	$\frac{\Delta H_{VH}}{\Delta H_{Cal}}$		
	pH 3.2	pH 6.4	pH 10
1.0	–	–	*1.05*
2.0	–	7.33	1.1
3.0	1.17	4.54	1.2
4.0	1.31	2.66	1.4
5.0	1.51	2.22	1.57
6.0	1.69	1.9	2
6.5	1.14	*1.79*	–
7.0	*1.05*	1.84	2.8
8.0	1.4	2.08	–
9.0	1.88	2.75	–

whereas, if ΔH_{VH} is greater than ΔH_{Cal}, this proves that there exist some intermolecular interactions [16].

The relation of $\Delta H_{VH}/\Delta H_{Cal}$ for interaction between *A. niger* catalase and various concentrations of SDS at pH 3.2, 6.4 and 10 is shown in Table 1.

The value of $\Delta H_{VH}/\Delta H_{Cal}$ at low SDS concentrations, around $\bar{\nu} = 150$ at pH 6.4, is very high, although it is normal at other pH values.

In marked contrast to most enzymes, it is found that at pH 6.4 the activity of the *A. niger* catalase is increased on binding of SDS. Activation of the *A. niger* catalase up to 180% is found under optimum conditions when approximately 150 SDS molecules are bound per catalase molecule at pH 6.4. Activation does not occur under acid (pH 3.2) or alkaline (pH 10) conditions [8]. The data from Table 1 indicate a very strong intermolecular force between subunits occurring during the interaction of *A. niger* catalase and low concentration of SDS at pH 6.4, but with an increase in the concentration of SDS the value of $\Delta H_{VH}/\Delta H_{Cal}$ decreases. A minimum occurs at 6.5 mM SDS, then the value again increases. It is possible that the value of $\Delta H_{VH}/\Delta H_{Cal}$ is related to the interval between subunits. A higher value of $\Delta H_{VH}/\Delta H_{Cal}$ indicates a higher order structure for *A. niger* catalase, and could indicate the activation of *A. niger* catalase–SDS complexes resulting from a higher order structure of the enzyme. The folding and unfolding structure could be estimated from Table 1.

Figure 2 shows the enthalpy curves for *A. niger* catalase–SDS complexes. The broken line was taken from Ref. 10, which involve measurements by microcalorimetry. The solid line was obtained from equilibrium dialysis at temperatures of 25 and 37 °C. The difference between theoretical (ΔH_{VH})

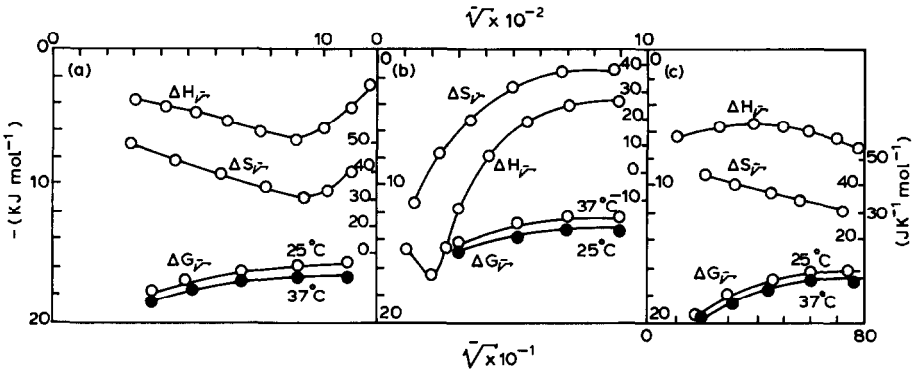

Fig. 3. Thermodynamic parameters for the interaction between *Aspergillus niger* catalase and SDS obtained from the Wyman–van't Hoff relation: (a) pH 3.2; (b) pH 6.4; (c) pH 10. ○, 25 °C; ●, 37 °C.

and experimental (ΔH_{Cal}) enthalpy curves lies in the left shift and the higher value for ΔH_{VH}. The left shift arises from thermal equilibrium and chemical equilibrium (SDS and catalase), in spite of the fact that there is only thermal equilibrium in the microcalorimetry measurement. The time of equilibrium between surfactant and catalase is very important. For example, the activation of *A. niger* catalase on binding of SDS resulted after 7 days' incubation, or the electrophoretic mobility of catalase–SDS complexes changed during 7 days' incubation.

The high value of ΔH_{VH} in relation to ΔH_{Cal} indicates that the whole molecule undergoes a single cooperative transition (two-state system) [17].

The shapes of the ΔH_{VH} and ΔH_{Cal} curves are similar at pH 6.4 and 3.2, but they differ at higher concentrations of SDS for pH 10.

Figure 3 shows the thermodynamic parameters of binding per surfactant ion ($\Delta H_{\bar{\nu}}$, $\Delta G_{\bar{\nu}}$, $\Delta S_{\bar{\nu}}$) which were obtained from the Wyman–van't Hoff relation. It is important to note that there is negative entropy ($\Delta S_{\bar{\nu}}$) for binding of *Aspergillus niger* catalase at low concentrations of SDS ($\bar{\nu} \approx 150$) at pH 6.4, as is shown in Fig. 3b. This is also indicative of the higher order structure of *A. niger* catalase–SDS complexes. This factor is probably the root of the activation of binding between *A. niger* catalase and SDS.

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