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Interaction of *Aspergillus niger* catalase with sodium *N*-dodecyl sulphate

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Abstract

The interaction of *Aspergillus niger* catalase (*A. niger*) and sodium n- dodecyl sulphate (SDS) has been studied by various physical methods to confirm the compaction of *A. niger* catalase - SDS complexes during the activated state of enzyme. *A. niger* catalase could be activated upto 180% by SDS at pH 6.4, phosphate buffer and pH 8.0 Tris buffer. This is inconsistent with other catalases (bovine and bacterial) which are inactivated by SDS complexes.

The relation of thermal stability of catalase to activity assay was described and suggested as the better method for activity measurments.

INTRODUCTION

Catalase occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxides by catalysing the reaction of $2H_2O_2 -> 2H_2O + O_2$.

Catalase is one of the first enzymes to be isolated in a high state of purity. Catalase are haem enzymes with four iron atoms per molecule attached to protein and chelated to protoporphyrin IX. All catalases are oligomers containing four, tetrahedrally arranged, subunits (ref. 1). Each subunit consists of a single polypeptide chain that associates with a single prosthetic group, ferric protoporphyrin IX (ref. 2). The subunits apparently function independently of one another (ref. 3). Catalases obtained from a wide range of sources (bacterial, fungal, animal) generally have a molecular weight of approximately 250,000 (refs. 1,4). In contrast catalase from *Aspergillus niger* (*A.niger*) has been found to differ significantly from other catalases in several respects. In particular the molecular weight has been found to be considerably greater than other catalases. Gruft et. al, (ref. 5) reported a molecular weight of 323,000 from sedimentation equilibrium measurements while Kikuchi - Tori et al. (ref. 6) and Moosavi-Movahedi et al. (ref. 7) using the same technique found it to be 385,000 and 354,000 respectively. The enzyme contains 12% carbohydrate moiety (ref. 7) which contributes to its higher degree of thermostability (refs. 8,9) and detergent stability (ref. 10).

The catalytic activity of *A. niger* catalase is lost in sodium n-dodecyl sulphate (SDS) as a potent biological denaturant, both in acidic (pH 3.2) or alkaline (pH 10) solutions, however at pH 6.4 (ref.10) and pH 8.0 (ref. 11), a certain concentration of SDS activates the enzyme upto 180%. The use of SDS as an activating agent is particularly interesting because only few enzymes are known to be activated by SDS (refs. 12-14) in contrast to a number of enzymes which are inactivated.

This study is an attempt to interpret the activation of *A. niger* catalase by SDS according to thermodynamic and other physical basis.

MATERIALS AND METHODS

A. niger catalase and SDS were obtained from Sigma Chemical Co. Ltd., and E. Merck respectively. Visking membrane dialysis tubing (MW cut-off 10,000 - 14,000) was obtained from SIC (East Leigh) Hampshire, UK. Rosaniline hydrochloride dye was received from B.D.H. All other materials and reagents were of analytical grade. 50 mM Tris, pH 8.0 and 2.5 mM phosphate, pH 6.4 were used as a buffer. Double distilled water was used where ever required.

Equilibrium dialysis was carried out at 25° and 37°C to measure bound SDS (ref. 15). Absorbance profiles which describe the thermal denaturation of catalase SDS complexes were measured on a Gilford spectrophotometer, model 2400-5, with temperature programmer described previously (ref. 11). The activity of catalase was determined by the rate of decomposition of hydrogen peroxide spectroscopically (refs. 10,11). The calorimetery enthalpy measurement was carried out on LKB 10700 batch microcalorimeter (refs. 9,16). The viscosity was measured using a Haake microviscometer; partial specific volume was also measured using a DMA 60 processing unit and a PAAR digital oscillating densitometer. Electrophoretic mobility was determined by acrylamide gel electrophoresis (refs. 10,11). In all calculations the molecular weight of *A.niger* catalase was assumed as 354,000 (ref.7). In all measurements the concentration of catalase was 0.05% W/V.

RESULTS AND DISCUSSION

The interaction of the *A. niger* catalase with SDS was made at pH 3.2, 6.4, 8.0 and 10. The catalytic activity of *A. niger* catalase is lost in SDS at pH 3.2 and 10.0 whereas it is activated up to 180% at 6.4 and pH 8.0 at low concentration of SDS (Figure 1). The upper axis shows the number of SDS molecule bound per catalase molecule at equilibrium which was measured by equilibrium dialysis method.

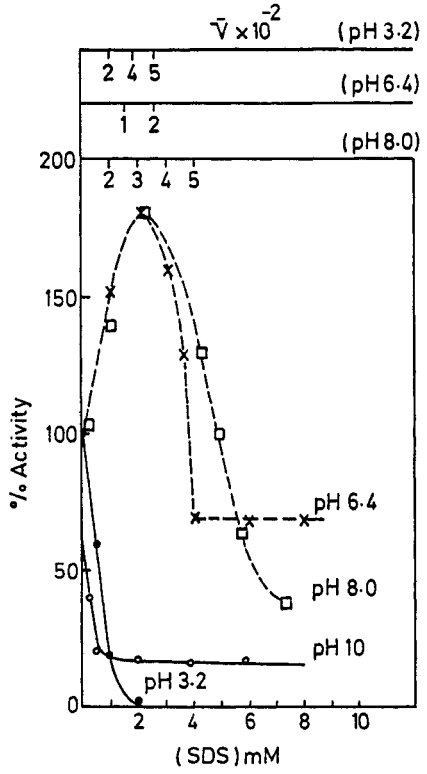

Fig. 1. Relative enzyme activity of *Aspergillus niger catalase* in the presence of SDS. The upper axis show the number of SDS molecules bound per catalase molecule ($\bar{v}$). For the data points at pH 3.2, glycine, •; pH 6.4, phosphate, X; pH 8.0, Tris□ and pH 10.0, glycine, o.

The catalases which have been investigated earlier behave anomalously with SDS, i.e. both bovine and bacterial catalases (*M.Luteus*) are deactivated only slowly, while *A. niger* catalase as demonstrated in present study can be activated. This resistance to SDS deactivation is not simply related to the overall charge balance in the catalases but does relates to the structural environment of the heam active sites. It is noteworthy that at pH 6.4 SDS activates *A. niger* catalase but denatures bovine catalase (ref. 17), the SDS concentration at which binding to *A. niger* catalase saturates is approximately 10 mM assuming saturation binding corresponds to 1.4g SDS g^{-1} of protein (ref. 18). At this concentration of SDS the enthalpies of interaction for bovine liver (ref. 19) and *Aspergillus niger* (ref. 9) are $-4Jg^{-1}$ and $-18Jg^{-1}$ respectively.

It is also noteworthy that at pH 6.4 the enthalpy of interaction of the bacterial catalase from *Micrococcus Luteus* (ref. 17) is zero up to an SDS concentration of 10mM. Thus there are very distinct species variations between the enthalpies of SDS-catalases interactions at pH 6.4.

The reason for the activation of the *A. niger* catalase and its SDS complexes can be interpreted thermodynamically and using other physical methods.

From a comparison of calorimetery enthalpy (ΔH_{cal}) measured directly using LKB microcalorimetry (ref. 9) with van't Hoff enthalpy (ref. 20) (ΔH_{VH}) which is calculated from binding constants at two different temperatures (25°C and 37°C), the area under binding isotherm based on the Wyman binding potential (ref. 21) can be established irrespective of whether the transition of the system follows a two-state mechanism (ref. 22) or not. When ΔH_{VH} is smaller than ΔH_{cal}, this indicates that the transition is a multi-state one, whereas, if ΔH_{VH} is greater than ΔH_{cal}, this proves that there exist some intermolecular interactions (ref. 23). The relation of $\Delta H_{VH}/\Delta H_{cal}$ for interaction between *A. niger* catalase at various concentrations of SDS and pH are shown in Table 1.

Table 1. The relation of $\Delta H_{VH}/\Delta H_{Cal}$ and ΔS_V at various concentrations of SDS at pH 3.2, 6.4 and 10.

[SDS]mM	$\Delta H_{VH}/\Delta H_{Cal}$	$\Delta S_V JK^{-1}mol^{-1}$	$\Delta H_{VH}/\Delta H_{Cal}$	$\Delta S_V JK^{-1}mol^{-1}$	$\Delta H_{VH}/\Delta H_{Cal}$	$\Delta S_V JK^{-1}mol^{-1}$
	pH 3.2		pH 6.4		pH 10.0	
1.0	-	-	-	-	1.05	-
2.0	-	40	7.33	-10	1.1	45
3.0	1.17	35	4.54	5	1.2	42
4.0	1.31	33	2.66	10	1.4	40
5.0	1.51	32	2.22	15	1.57	38
6.0	1.69	31	1.9	30	2	35
6.5	1.14	30	1.79	35	-	-
7.0	1.05	30	1.84	40	2.8	30
8.0	1.4	32	2.08	-	-	-
9.0	1.88	-	2.75	-	-	-

The value of $\Delta H_{VH}/\Delta H_{cal}$ at 2 mM concentration of SDS is very high which may indicate the strong intermolecular force between subunits occuring during the interaction of *A. niger* catalase at pH 6.4 A higher value of $\Delta H_{VH}/\Delta H_{cal}$ indicates a higher order structure for *A. niger* catalase which inturn activates *A. niger* catalase-SDS complexes. It is important to note that there is negative entropy ($\Delta \bar{S}_V$) for binding of *A. niger* catalase at 2 mM concentration of SDS at pH 6.4 as shown in Table 1, whereas the said interaction at other pH's (i.e. 3.2 and 10) has positive entropy as reported earlier (ref. 21). This is also indicative of the higher ordered structure of *A. niger* catalase-SDS complexes.

Table 2 shows physical properties of *A. niger* catalase and SDS complexes which includes the compaction of the enzyme during activation by SDS.

Table 2. Physical properties of *A. niger* catalase with and without SDS

[SDS] (mM)	$[\eta] \times 10^{-4}(M^{-1})$	Tm(°C)	k′	$V(CCg^{-1})$	Stokes radius A_0	R_m
0	1.12	70	56.0	0.7696	149.98	1.0
1	1.15		148.0		122.32	-
2	0.85	45	194.0	0.6570	110.60	1.53
3		55				1.48
4		65		0.8225		1.46
7	2.2	80	177.0	0.8236	151.85	1.4
10		80		0.8484		1.5

The intrinsic viscosities, [η] of the *A.niger* catalase-SDS complexes have been determined using the Huggins equation (ref. 24).

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c$$

Where k is the Huggins constant and c is the catalase concentration. The k' decreases as the flexible coil expands and the exponent on the Mark-Houwink equation ([η] = KM^a) increases (ref. 25).

The amount of intrinsic viscosity and the Huggins constant for optimum activity at 2 mM SDS concentration is lowest and highest respectively, indicating the higher ordered structure for catalase. This can also be shown by experimental value of the partial specific volume, electrophoretic mobility (R_m) and calculated value of Stoke radius (ref. 10), indicating the folding of the catalase during the optimum activation by 2 mM concentration of SDS.

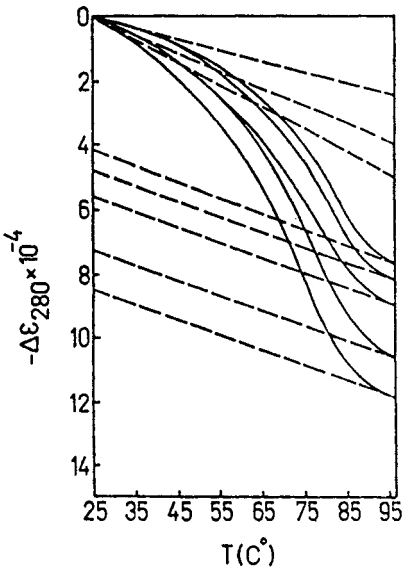

Fig. 2. Thermal profile for *A. niger* catalase in the presence of different concentrations of SDS at pH 6.4.

Table 3. Physical properties of *A. niger* catalase with and without SDS which is calculated from data of Fig. 2 based on ref.26.

[SDS](mM)	ΔG (kJ.mol^{-1})	ΔH_m (kJ.mol^{-1})	ΔC_p (kJ.mol^{-1}K^{-1})
0	10.8	121.1	1.98
2	8.7	113.02	1.70
3	11.0	121.62	1.73
5	11.2	121.8	1.75
8	11.4	122.15	1.80

The melting point (T_m) of catalase indicating the thermal stability of the protein obtained from derivative melting profile of catalase with and without SDS shows sigmoidal thermal denaturation curves (ref. 11). The relation of activity to T_m is one of the important part of this article. The increase in activity decreases T_m. T_m is dependent on the stability of the protein; thus decreasing T_m lowers stability which relates to more activity for the catalases. Here, the less stable state (T_m = 45°C) is optimal for the activity status of catalase-SDS complexes. Table 3 shows the thermodynamic parameters obtained from thermal denaturation profile (Fig. 2) assuming a two-state mechanism (ref. 26), which indicates the minimum free energy and enthalpy of melting temperature and maximum heat capacity for the optimum activation at 2 mM SDS concentration. Ichijo et al. (ref. 28) also reported the relation of thermal stability with activity for invertase (ref. 27) and glucose oxidase and suggested that both methods i.e. activity assay and calorimetery can be employed. Therefore, the activity of the enzyme can be related to its thermal stability which may be a better method for determining enzyme activity.

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