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Thermodynamic studies of the interaction of glucose oxidase with anionic and cationic surfactants

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Abstract

The interaction between glucose oxidase and sodium *n*-dodecyl sulphate (SDS), sodium *n*-hexadecyl sulphate (SHS) and dodecyltrimethylammonium bromide (DTAB) in aqueous solutions by equilibrium dialysis and spectrophotometry at various temperatures is investigated. The binding data are used to obtain thermodynamic parameters, which are interpreted in terms of a theoretical model based on the Wyman binding potential and the van't Hoff relation.

UV absorption spectra show that the cationic detergent DTAB unfolds glucose oxidase immediately, that SDS has no instantaneous effect, and that SHS has a small immediate effect on the structure of glucose oxidase.

The binding of SDS, SHS and DTAB to glucose oxidase differs with time. DTAB plays a distinct role in the immediate interaction with glucose oxidase, which is in marked contrast to other denaturants.

INTRODUCTION

Glucose oxidase (β-D-glucose: oxygen oxidoreductase, EC 1.1.3.4) is a flavoprotein containing glycoprotein which catalyzes the oxidation of β-D-glucose by molecular oxygen to give D-glucono-δ-lactone and hydrogen peroxide [1]. The enzymes isolated from *Aspergillus niger* contain approximately 16% carbohydrate and these sugars are thought to be present as oligomeric polysaccharides covalently attached to the polypeptide chain via serine, glutamic acid and aspartic acid residues [2]. The native enzyme is also known to contain two molecules of flavin–adenine dinucleotide (FAD) [3]. These flavin cofactors are responsible for the oxidation–reduction properties of the enzyme and the available evidence suggests they are firmly bound, but not covalently linked, to the polypeptide portion of the enzyme.

Many measurements of the molecular weight of the native enzyme have been made, and although the reported values range from 150 000 [3] to

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186 000 [4], most of the data fall in the range 155 000 ± 5000 [5]. Although there is general agreement that the holoenzyme has two subunits, O'Malley and Weaver [5] argue that they are covalently linked by disulphide bonds.

The properties of the native enzyme have been well studied but relatively little is known about the denatured enzyme. In this study we examine the thermodynamic and structural properties of the denatured glucose oxidase, using amphipathic molecules such as surfactants like sodium *n*-dodecyl-sulphate (SDS), sodium *n*-hexadecyl sulphate (SHS) and dodecyltrimethyl-ammonium bromide (DTAB) as denaturants which disrupt the native structure of most globular proteins at very low concentrations (approx. mM), previously reported for *Aspergillus niger* catalase [6–9] and histones [10, 11].

EXPERIMENTAL

Materials

Asperigillus niger glucose oxidase, SDS, SHS and DTAB were obtained from Sigma Chemical Co.

Visking membrane dialysis tubing (MW cut-off 10 000–14 000) was obtained from SIC, Eastleigh, Hampshire. Rosaniline hydrochloride dye and orange II were obtained from Sigma Chemical Co. All other materials and reagents were of analytical grade, and solutions were made in double-distilled water. The composition of the buffers used (pH 3.2, pH 6.4) and concentration measurements were as previously described [11].

Methods

Equilibrium dialysis to measure bound *n*-alkyl sulphase (SDS, SHS) and DTAB were carried out by previously described methods [7, 10, 12]. A UV–VIS Shimadzu spectrophotometer Model 160 equipped with temperature control was used. In all calculations the molecular weight and concentration of glucose oxidase were taken to be 160 000 [13] and 0.05% (W/V), respectively.

RESULTS AND DISCUSSION

Glucose oxidase has been shown to be a resistant enzyme against denaturants e.g. temperature, sodium *n*-dodecyl sulphate (SDS) as a potent biological detergent, guanidine hydrochloride and urea. The reasons for

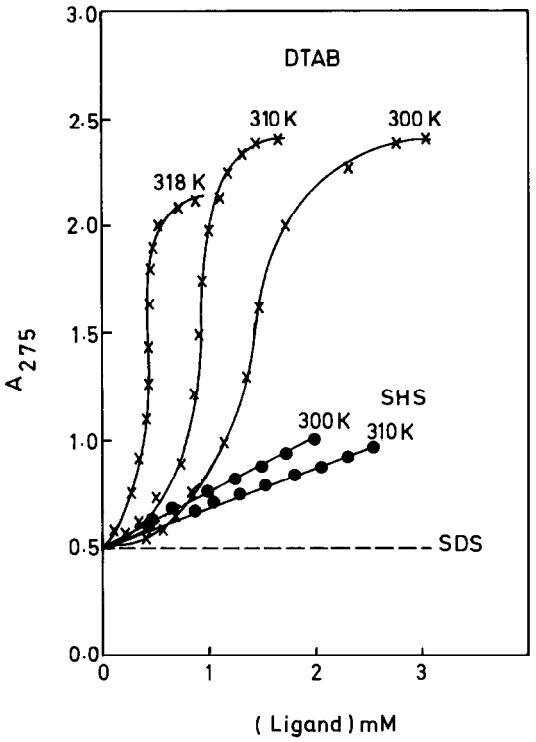

Fig. 1. UV absorption at 275 nm for instantaneous interaction of glucose oxidase with detergents at pH 6.4 at various temperatures: broken line, SDS; ●, SHS; ×, DTAB.

glucose oxidase resistance are the presence of high negative charge at the surface (isoelectric point = 4.2), high hydrophobicity of the internal region (111 Gly, 108 Ala, 79 Val, 96 Leu), and evidence of carbohydrate moieties and of disulphide-bonded inter-enzyme and intra-enzyme molecules [2, 14]. Here, we have tried to interact the anionic detergents SDS and SHS (with the same head groups but different tails) and the cationic detergent DTAB (with the same tail and different head compared to SDS), which are shown in Fig. 1. Figure 1 shows the different effects of detergents on the structure of glucose oxidase. Unlike most globular proteins that bind and are denatured by SDS, glucose oxidase shows resistance to immediate binding by SDS. The binding of SDS to glucose oxidase takes a long time; this will be discussed in terms of the binding isotherms produced by equilibrium dialysis. Some proteins, e.g. histones, bind much of the SDS immediately [15]. Accordingly, the multichain proteins, having subunits which take several days to bind their maximum amount of SDS, show a time-dependent behaviour [16]. *Aspergillus niger* catalase is also equally slow in binding SDS and forming subunits [17, 18]. The effect of a large temperature range on absorption for glucose oxidase, both with and without the presence of SDS, does not cause a significant change in the

denaturation. It was also reported by Nelson [16] that glucose oxidase lost no activity at all in 24 h, even in 0.1 M mercaptoethanol plus SDS at 37°C, but did lose activity quickly at 60°C. It was also reported by Nakamura and Koga [19], that the thermal stability of glucose oxidase was dependent on its redox state. The oxidized form showed an apparent denaturation temperature at 76°C; for the reduced form of the enzyme the denaturation temperature increased by about 10°C, using differential scanning calorimetry (DSC). This provides confirmation by a totally different method of the stability of glucose oxidase. Figure 1 shows the instantaneous absorption measurement of complexes between detergents with glucose oxidase, indicating no complexes for SDS, some complexes for SHS and complete complexes for DTAB. SHS binds to glucose oxidase better than SDS because of the increasing SHS chain length, which demonstrates the relative roles of the ionic and the hydrophobic interactions. For specific interactions to occur it is clearly necessary to have both an ionic head group and a hydrocarbon chain long enough to make hydrophobic contacts with the protein. The high negative charge of glucose oxidase (the ratio of acidic to basic amino acid content is 3.4) must assist in repelling the SDS; for SHS, the hydrocarbon tail plays an important role in the instantaneous interaction with glucose oxidase, causing hyperchromic phenomena, which are indicative of small conformational changes. In spite of the shielding effect and the steric hindrance of the methyl groups of the DTAB head group, immediate strong ionic links between the trimethylammonium bromide and negative sites on glucose oxidase are formed, as has been shown by the complete unfolding curve in the two-transition region. Accordingly, it is important to note that the distinct role of DTAB in the immediate denaturation concomitant with the precipitation of glucose oxidase is stronger than for any denaturant previously used. DTAB interacts with glucose oxidase at lower temperatures. This is shown in Fig. 1.

Figure 2 shows the number of moles of SDS, SHS and DTAB bound per mole of glucose oxidase $\bar{\nu}$, measured by equilibrium dialysis as a function of the logarithm of the free surfactants concentration at 27° and 37°C. Figure 2(a) at 27°C can be divided into three regions: the initial binding of SDS ($\bar{\nu} \approx 200$) to specific binding sites which are independent binding sites; the much larger number of SDS ions in a non-cooperative fashion; cooperative binding [20]. The binding isotherms for SDS (Fig. 2(a) and Fig. 2(b)) and for DTAB (Fig. 2(c)) can each be divided in two regions: initial parts attributable to binding on ionic sites; the further steep rise attributable to hydrophobic binding. The binding isotherms for SHS (Figs. 2(d) and 2(e)) show hydrophobic binding only.

Calculation of the binding constants which are applicable to the entire binding isotherms are based on the Wyman binding potential concept [21]. The binding potential is calculated from the area under the binding

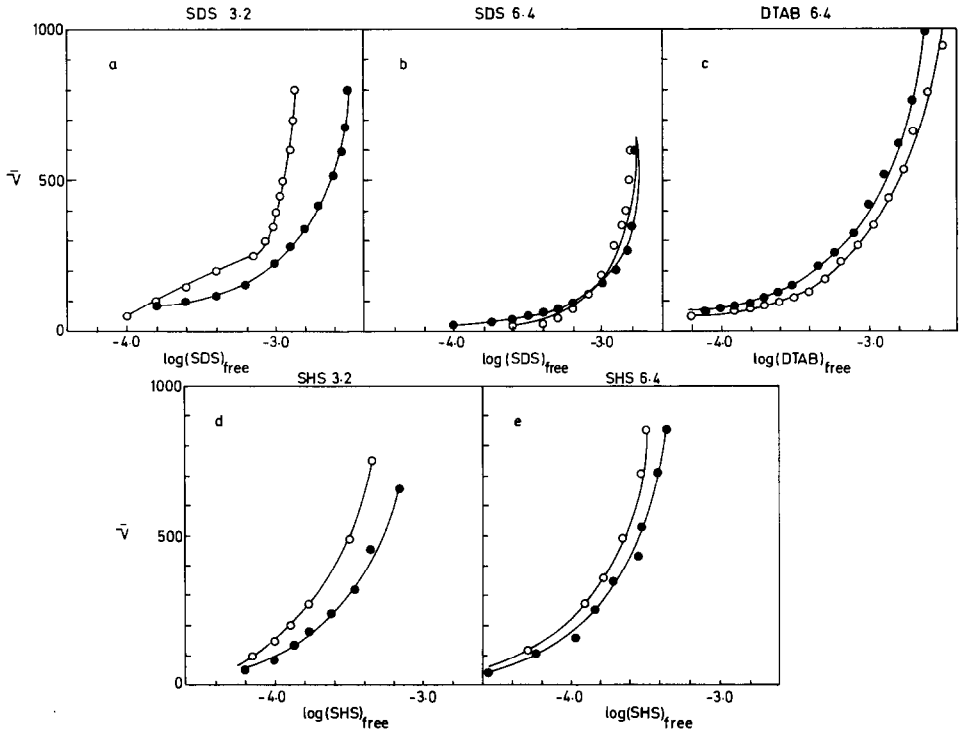

Fig. 2. Binding isotherms for interaction of glucose oxidase with detergents: (a) SDS, pH 3.2; (b) SDS, pH 6.4; (c) DTAB, pH 6.4; (d) SHS, pH 3.2; (e) SHS, pH 6.4; ○, 27°C; ●, 37°C.

isotherm, according to the equation

$$\pi = RT \int_{\bar{v}_{i=0}}^{\bar{v}_i} \bar{v}_i \, \mathrm{d} \ln[\text{surfactant}] \tag{1}$$

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

$$\pi = RT \ln(1 + K_{app}[\text{surfactant}]^{\bar{v}_i}) \tag{2}$$

The values of K_{app} were determined from eqns. (1) and (2) and were used to determine the value of $\Delta G_{\bar{v}}$ by

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

The values of the enthalpy of interaction ΔH were obtained from the temperature dependence of K_{app} using the van't Hoff relation [22]

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

The enthalpies of interaction of glucose oxidase with SDS, SHS and

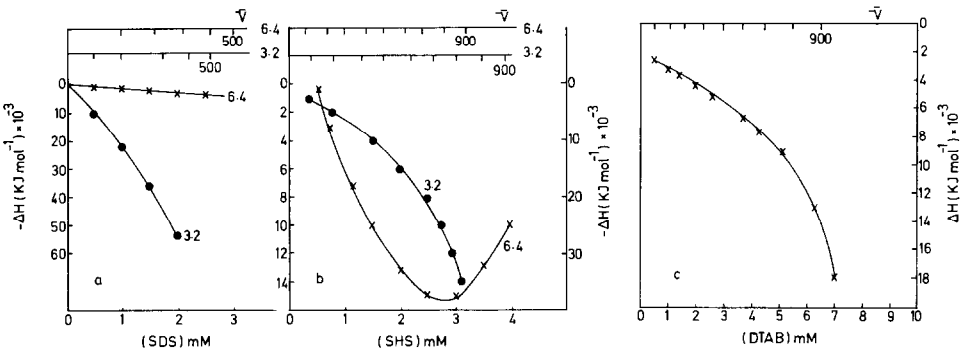

Fig. 3. Enthalpy of interaction between glucose oxidase and detergents: (a) SDS; (b) SHS: left axis, pH 6.4; right axis, pH 3.2; (c) DTAB, pH 6.4. the upper axis shows the number of surfactant molecules bound per glucose oxidase molecule at equilibrium.

DTAB are shown in Fig. 3, as obtained from the Wyman and van't Hoff theoretical relations. The top axes in Fig. 3 show the number of detergent molecules $\bar{\nu}$ which would be bound per glucose oxidase molecule ($\bar{\nu}$) at equilibrium. Figure 3(a) shows the enthalpy of interaction of glucose oxidase with SDS at pH 3.2 and pH 6.4. At pH 6.4, the enthalpy change was insignificant, whereas at pH 3.2 a greater energy change occurs, which probably originates from specific ionic interactions between the SDS anion and cationic amino acid residues at a certain period of time (>96 h). The time dependency of the binding of SDS to glucose oxidase is very important, because no binding occurs immediately for SDS to glucose oxidase. Jones et al. have also previously reported [23] the enthalpies of interaction of glucose oxidase with SDS from microcalorimetry, measured over a 20 min period after mixing. They report no enthalpy change detected at pH 6.0, but a greater enthalpy change at pH 3.2. Figure 3(b) shows the enthalpy of interaction of glucose oxidase with SHS at pH 3.2 and pH 6.4. the enthalpies for SDS and SHS at pH 3.2 are of almost the same magnitude, whereas there is a marked difference at pH 6.4. Figure 3(c) shows the enthalpy of interaction of glucose oxidase with DTAB at pH 6.4, which is higher in magnitude than for SHS and SDS at pH 6.4, but lower in magnitude than for SHS and SDS at pH 3.2. This probably confirms that the anionic parts of SDS and SHS bind readily with the cationic part of glucose oxidase, causing the higher evolution of heat, whereas the initial interaction by DTAB is difficult because of the shielding effect and the steric hindrance caused by the methyl groups. In SDS and SHS the negative charge is spread over the exposed sulphate oxygen atoms amd much stronger ionic links can be envisaged for a period of time at pH 3.2. The enthalpy change of interaction between glucose oxidase with DTAB is endothermic, in contrast with the exothermic enthalpy change observed on interaction with SDS and SHS.

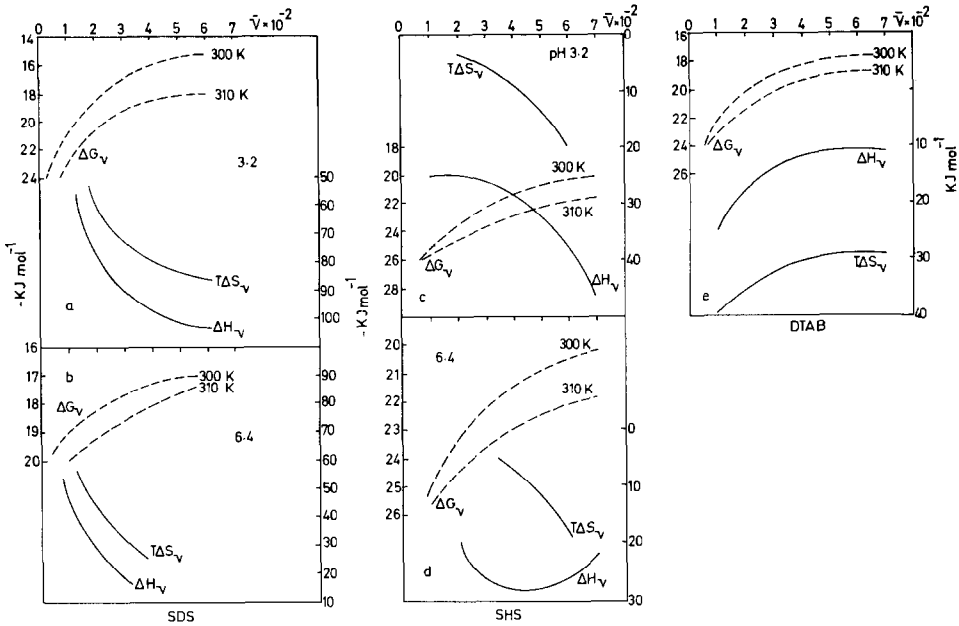

Fig. 4. Thermodynamic parameters of interaction between glucose oxidase and detergents: (a) SDS, pH 3.2; (b) SDS, pH 6.4; (c) SHS, pH 3.2; (d) SHS, pH 6.4; (e) DTAB, pH 6.4. Left axis, $\Delta G_{\bar{v}}$; right axis $\Delta H_{\bar{v}}$ and $T\Delta S_{\bar{v}}$.

Figure 4 shows thermodynamic parameters of interaction of glucose oxidase with SDS, SHS and DTAB. Figure 4 gives $\Delta G_{\bar{v}}$ as a function of $\bar{v}$; the former becomes less negative with increasing $\bar{v}$. The change in $\Delta G_{\bar{v}}$ with increasing $\bar{v}$ implies that, after the initial binding to the "higher energy" sites, subsequent binding is weaker [24]. $\Delta H_{\bar{v}}$ and $T\Delta S_{\bar{v}}$ become less negative with increasing $\bar{v}$ for SDS and SHS (Figs. 4(a)–4(d)), whereas $\Delta H_{\bar{v}}$ and $T\Delta S_{\bar{v}}$ become less positive with increasing $\bar{v}$ for DTAB.

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