Thermochimica Acta, 235 (1994) 189–196
Elsevier Science B.V., Amsterdam
SSDI 0040-6031(93)01568-N

Thermodynamic denaturation of glucose oxidase in aqueous dodecyl trimethyl ammonium bromide solution between 25 and 65°C

M.R. Housaindokht [a] and A.A. Moosavi-Movahedi [b,*]

[a] *Department of Chemistry, University of Shiraz, Shiraz (Iran)*
[b] *Institute of Biochemistry and Biophysics, University of Tehran, P.O. Box 13145-1384, Tehran (Iran)*

(Received 3 August 1993; accepted 2 September 1993)

Abstract

The denaturation of glucose oxidase has been studied as a function of dodecyl trimethyl ammonium bromide (DTAB) concentration at temperatures between 25 and 65°C, with 2.5 mM phosphate buffer and pH 6.4. DTAB was found to be very effective in denaturing glucose oxidase, normally resistent to other denaturing agents. At 55°C, the enzyme is expected to have minimum stability in the hydrocarbon environment provided by the detergent. In an otherwise identical situation, when water surrounds the protein, maximum stability is suggested.

These predictions are made using the Gibbs free energies of the transition in water $\Delta G_D(H_2O)$, and in a hydrophobic environment $\Delta G_D(hc)$. The values of $\Delta G_D(H_2O)$ and $\Delta G_D(hc)$ were 26.84 and -180.2 kJ mol^{-1}, respectively.

INTRODUCTION

Glucose oxidase (β-D-glucose:Oxygen Oxidoreductase-EC.1.1.3.4) from *Aspergillus niger*, a flavoprotein, catalyses the oxidation of β-D-glucose by molecular oxygen to give D-glucono-δ-lactone and hydrogen peroxide [1]. Glucose oxidase is used in food processing, in the production of gluconic acid [2] and for the quantitative determination of D-glucose in samples such as blood, food and fermentation products [3].

The enzyme isolated from *Aspergillus niger* contains about 24% of its molecular weight as carbohydrate [4] 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 [5].

Glucose oxidase is a dimer (MW 160 000) composed of two monomers. The dimer contains two disulphide bonds and two free sulphydryl groups [6], and two flavin-adenine dinucleotides (FAD) which are bound tightly to

* Corresponding author.

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the enzyme [7]. The linkage between two monomers of a glucose oxidase dimer has been reported to be by non-covalent interaction [5, 8] or by disulphide bonds [6]. The native enzyme was found to be quite resistant to sodium *n*-dodecyl sulphate (SDS) [9, 10] and does not bind measurable amount of SDS, retaining full activity in its presence [11]. The enzyme is also fairly stable to urea and heating [12].

A microcalorimetric study of glucose oxidase–DTAB and other surfactants has been reported [13]. We report here a study of the interaction of glucose oxidase with dodecyl trimethyl ammonium bromide (DTAB), as a good denaturant for the enzyme, and the stability of glucose oxidase in the presence of DTAB is investigated.

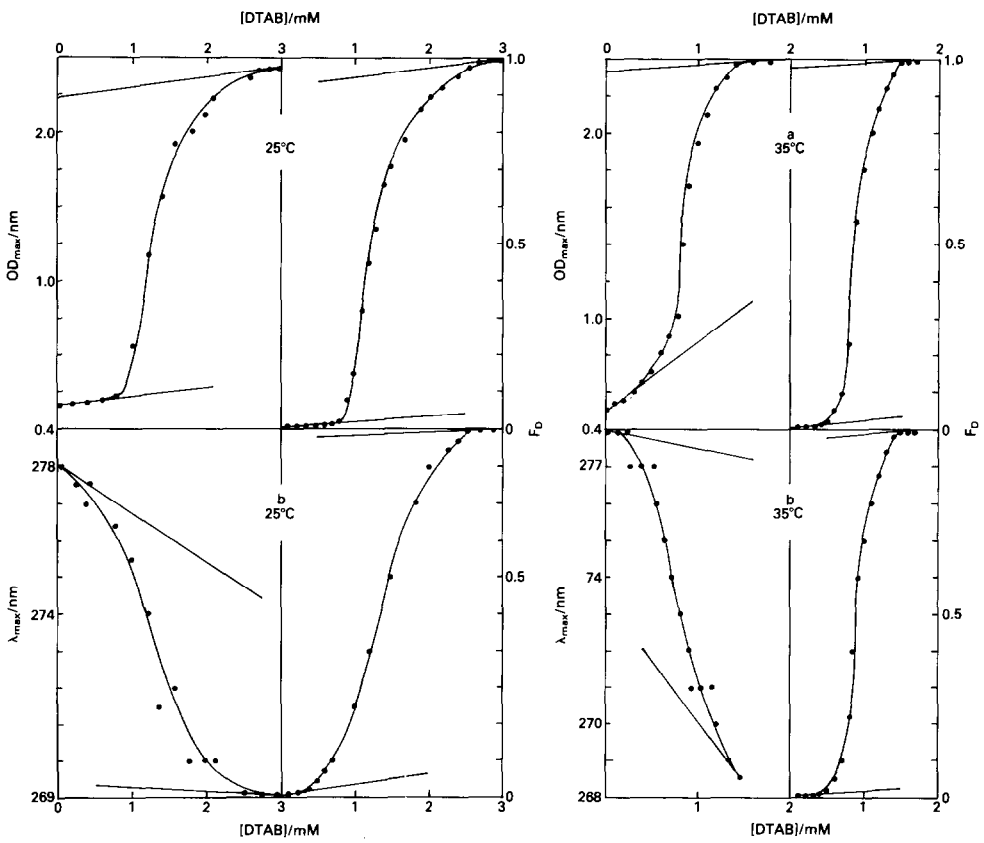

Fig. 1. DTAB denaturation curve for glucose oxidase in 2.5 mM sodium phosphate buffer, pH 6.4 at 25, 35, 45, 55 and 65°C.

a. Left side: optical density at maximum wavelength (273 ± 5 nm) OD_{max} vs. DTAB concentration. Right side: the fraction of denaturation F_D obtained from eqn. (1).

b. Left side: blue-shifted λ_{max} vs. DTAB concentration. Right side: the fraction of denaturation F_D obtained from eqn. (1).

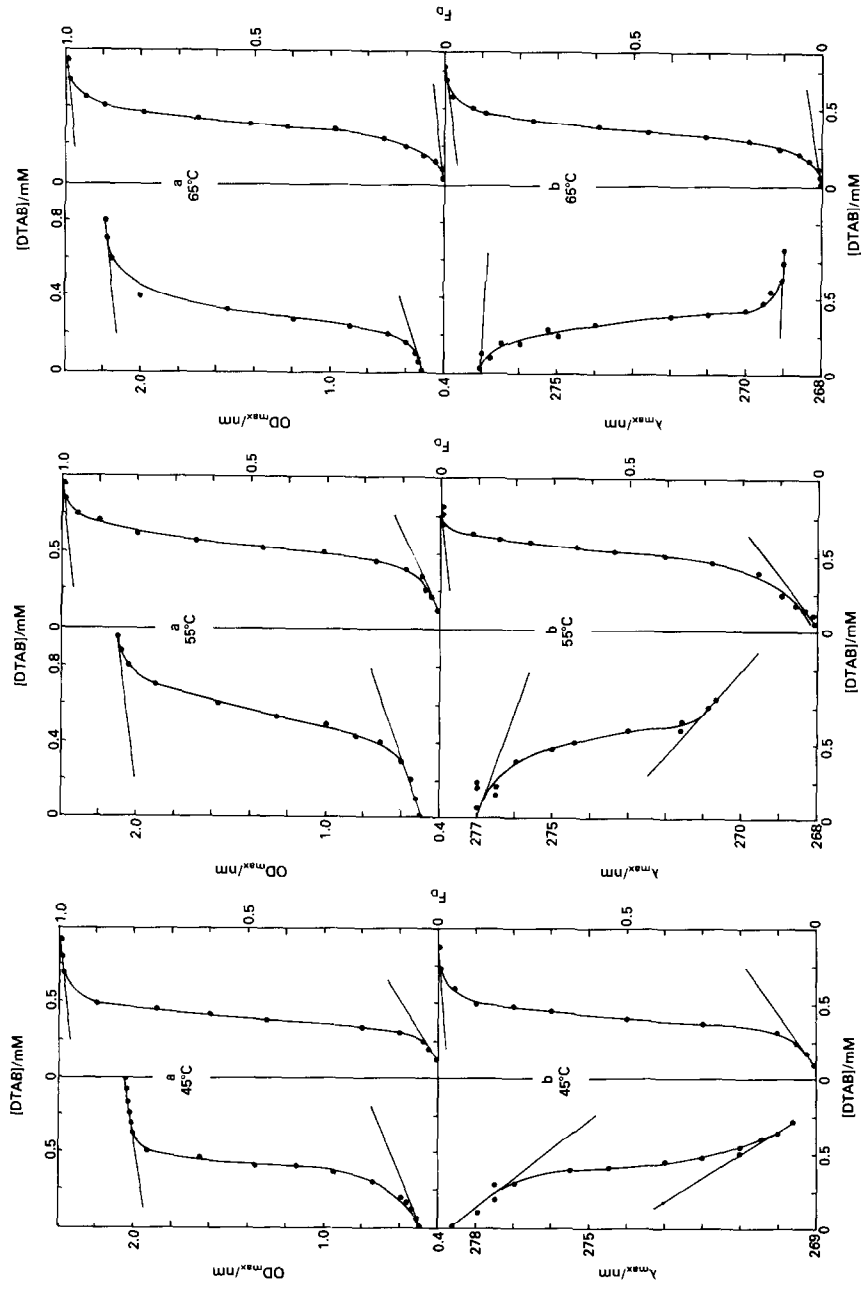

Fig. 1 continued.

EXPERIMENTAL

Material

Glucose oxidase from *Aspergillus niger* and dodecyl trimethyl ammonium bromide (DTAB) were obtained from Sigma. All the salts used in the preparation of the buffer were analytical grade and they were made up in distilled water. Sodium phosphate (2.5 mM), pH 6.4, and $I = 0.0069$, was used.

Method

DTAB denaturation curves were determined by measuring the maximum optical density (273 ± 5 nm) of the solutions containing 3.125 μM glucose oxidase using a Shimadzu model 160 spectrophotometer and 1-cm cuvettes thermostated to maintain the temperature at ± 0.1 K. At lower temperatures, care was taken to assure that the unfolding reaction had reached equilibrium. All measurements were made after glucose oxidase and DTAB had been incubated for over 30 min, after which time the spectral absorbance did not change.

RESULTS AND DISCUSSION

The DTAB denaturation curves for glucose oxidase are shown in Fig. 1. In all cases, denaturation was followed by measuring the maximum optical density at 273 ± 5 nm and λ_{max} which was blue-shifted by interaction with DTAB.

The free energy of unfolding ΔG was calculated as a function of DTAB concentration by assuming a two-state mechanism [14, 15] and using the equations [16]

$$F_D = (y_N - y_{obs})/(y_N - y_D) \tag{1}$$

$$\Delta G_D = -RT \ln K = -RT \ln[F_D/(1 - F_D)]$$

$$= -RT \ln[(y_N - y_{obs})/(y_{obs} - y_D)] \tag{2}$$

where K is the equilibrium constant, y_{obs} is the observed value of the parameter used to follow unfolding in the transition region, and y_N and y_D are the values of y characteristic to the native and denatured conformations of the protein, respectively.

The data on the right side of Fig. 1 are shown as the fraction of denatured glucose oxidase which is derived from data on the left side of Fig. 1 by application of eqn. (1).

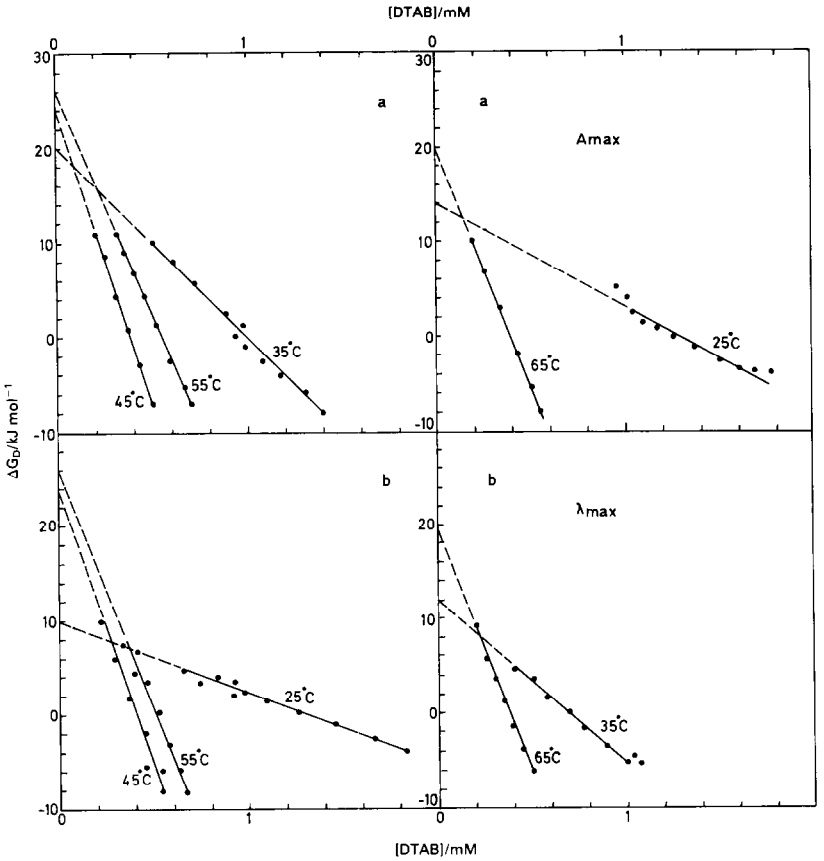

Fig. 2. ΔG_D (calculated from measurement in the transition region using eqn. (2)) vs. DTAB concentration. Broken lines were obtained by a least-squares fit of the data to eqn. (3).

Figure 2 shows the free energy ΔG_D which is calculated from eqn. (2) based on the data on the right side of Fig. 1 which varies linearly with DTAB concentration in the limited region. The simplest method of estimating the conformational stability in the absence of DTAB, $\Delta G_D(H_2O)$, is to assume that this linear dependence continues to zero concentration and to use a least-squares analysis to fit the data to the equation [17]

$$\Delta G_D = \Delta G_D(H_2O) - m[\text{DTAB}] = 0 \tag{3}$$

where m is a measure of the dependence of ΔG on DTAB concentration, obtained from the slope of Fig. 2 and depicting the sharpness of Fig. 2. An increase in the degree of cooperativity with increasing temperatures up to 45°C is indicated. At higher temperatures, no further increase in cooperativity is observed.

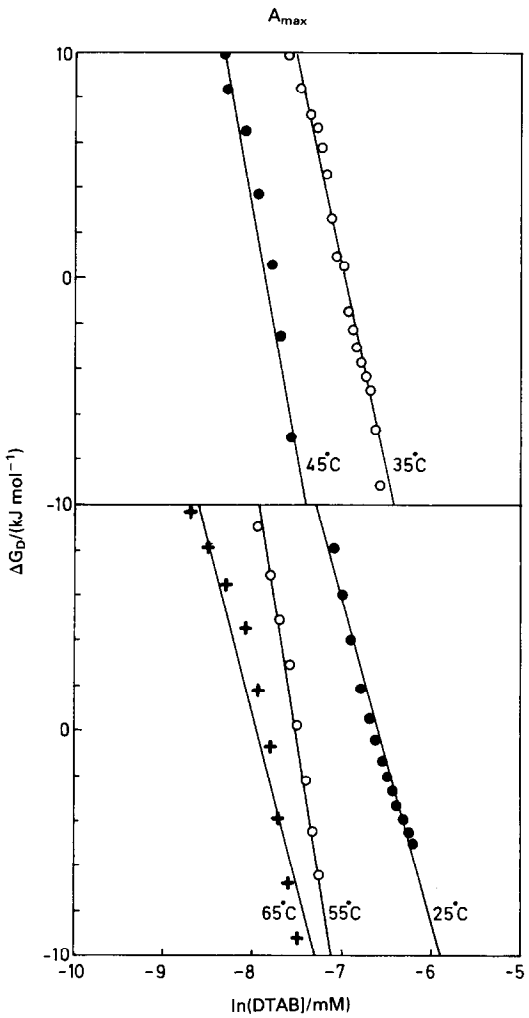

Fig. 3. ΔG_D vs. ln[DTAB] at temperatures between 25 and 65°C, calculated from Fig. 1a.

The results from analysis of the absorbance denaturation curves (Fig. 1(a)) and λ_{max} (Fig. 1(b)) are virtually consistent, which may provide a good test for a two-state mechanism [18].

Figure 3 shows the linearity of the plot of ΔG_D versus ln[DTAB]. Using a least-squares analysis to project the line to [DTAB] = 0 means that a surfactant concentration of 1 M has been reached. From the least-squares analysis of these plots (up to [DTAB] = 0), the free energy in the hydrocarbon ΔG_D(hc) was obtained. A concentration of DTAB of 1 M is in good agreement for the transition of the native state of protein (from water) to the denatured state of protein (to hydrocarbon). The unfolding of a protein by a chemical denaturant at constant temperature and pH can be broken down into thermodynamically defined stages according to the scheme [19]

$$\begin{array}{ccc} & \overset{\Delta G_D(H_2O)}{\rightleftharpoons} & \\ N_{H_2O} & & D_{H_2O} \\ \Delta G_{tr,N} \downarrow\uparrow & & \downarrow\uparrow \Delta G_{tr,D} \\ N_{hc} & \underset{\Delta G_D(hc)}{\rightleftharpoons} & D_{hc} \end{array}$$

The denaturation process can be viewed as a process in which the native protein starts in water (N_{H_2O}) and is treated conceptually in one of two ways. Either it is first transferred to the denaturing solution without unfolding (N_{hc}), after which it is transferred to the denaturing solvent (D_{hc}), or it is first denatured in water and then transferred to solvent. The total free energy changes for N_{H_2O} to D_{hc} will, of course, be the same by either pathway. Thus, it follows that ΔG, for example, for the process N_{H_2O} to D_{hc} is given by the relation

$$\Delta G_{tr,N} + \Delta G_D(hc) = \Delta G_D(H_2O) + \Delta G_{tr,D}$$

or

$$\Delta\Delta G = \Delta G_D(hc) - \Delta G_D(H_2O) = \Delta G_{tr,D} - \Delta G_{tr,N} \quad (4)$$

where $\Delta G_{tr,N}$ and $\Delta G_{tr,D}$ represent the free energies of transfer of the protein, respectively, from water to denaturant solution at a given concentration of DTAB. $\Delta G_D(hc)$ is the free energy change for the process N_{hc} to D_{hc} at 1 molar concentration of DTAB.

Table 1 shows a positive value for $\Delta G_D(H_2O)$ and negative values for $\Delta G_D(hc)$ and $\Delta\Delta G$. The maximum in $\Delta G_D(H_2O)$ and the minimum in $\Delta G_D(hc)$ occur at 55°C. This means that maximum stability occurs at 55°C (the temperature of maximum stability T_s at which the entropy is zero) [20] without the presence of denaturant (the protein is completely surrounded by water). The minimum stability in the presence of denaturant is also at 55°C (the protein is completely surrounded by denaturant, i.e. the hydrocarbon is exposed to water). $\Delta\Delta G$ is also less negative at this temperature which is the best state for unfolding proteins.

TABLE 1

Parameters characterizing the DTAB-induced glucose oxidase unfolding at temperatures between 25 and 65°C

T/°C	m/(kJ mol^{-1} M^{-1})	$\Delta G_D(H_2O)$/(kJ mol^{-1})	$\Delta G_D(hc)$/(kJ mol^{-1})	$\Delta\Delta G$/(kJ mol^{-1})
25	10.0	13.6	−93.2	−106.8
35	21.0	20.32	−127.0	−147.3
45	60.0	24.72	−167.6	−192.3
55	50.0	26.84	−180.2	−207.0
65	50.0	18.52	−119.7	−138.2

It is important to note that, without additional heating, DTAB is ineffective in causing denaturation of the protein. Therefore, the unfolding of glucose oxidase (the ratio of negative/positive charges of the amino acids is 3.27 [9]) in the presence of DTAB as a cationic denaturant is affected first and, subsequently, the temperature is helpful in the denaturation of glucose oxidase.

ACKNOWLEDGEMENT

Financial assistance from the Research Council of the University of Tehran is gratefully acknowledged.

REFERENCES

1 R. Bentley, Enzymes, 7 (1963) 567.
2 M. Rohr, C.P. Kuvicek and J. Kominek, in H.J. Rehm and G. Reed (Eds.), Biotechnology, Verlag Chemie, Weinheim, 1983.
3 R.D. Schmid and I. Karube, in H.J. Rehm and G. Reed (Eds.), Biotechnology, Verlag Chemie, Weinheim, 1988.
4 H.M. Kalisz, H.J. Hecht, D. Schomburg and R.D. Schmid, Biochim. Biophys. Acta, 1080 (1991) 142.
5 H. Tsuge, O. Natsuakui and K. Ohashi, J. Biochem., 78 (1975) 835.
6 J.J. O'Malley and J.L. Weaver, Biochemistry, 11 (1972) 3527.
7 H. Tsuge and H. Mitsuda, J. Biochem, 73 (1973) 199.
8 M.N. Jones, P. Manley and A. Wilkinson, Biochem. J., 203 (1982) 285.
9 S. Nakamura, S. Hayashi and K. Koga, Biochim. Biophys. Acta, 145 (1976) 249.
10 A.A. Moosavi-Movahedi, M.R. Housaindokht and J. Moghadasi, Thermochim. Acta, 219 (1993) 143.
11 C.A. Nelson, J. Biol. Chem., 246 (1971) 3895.
12 W.N. Ye and D. Combes, Biochim. Biophys. Acta, 999 (1989) 86.
13 M.R. Housaindokht, A.A. Moosavi-Movahedi, J. Moghadasi and M.N. Jones, Int. J. Biol. Macromol., 15 (1993) 337.
14 W. Pfeil and P.L. Privalov, Biophys. Chem., 4 (1976) 33.
15 P.L. Privalov, Adv. Protein Chem., 33 (1979) 167.
16 C.N. Pace, TIBTECH, 8 (1990) 93.
17 R.F. Greene and C.N. Pace, J. Biol. Chem., 249 (1974) 5388.
18 S. Lapanje, Physicochemical Aspect of Protein Denaturation, Wiley, New York, 1978.
19 C. Tanford, Adv. Protein Chem., 24 (1970) 1.
20 A.A. Moosavi-Movahedi, B. Samiee and G.H. Hakimelahi, Int. J. Colloid Interface, 161 (1993) 53.