A circular dichroism study of the pH-dependent activation/inactivation equilibrium in the glutamate dehydrogenase of *Clostridium symbiosum*

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Glutamate dehydrogenase from *Clostridium symbiosum* has been studied by circular dichroism spectroscopy. The far UV spectrum shows negative bands at 205 nm and 220 nm typical of α-helical structure. Change in the pH from 7 to 8.8 has little effect on this spectrum, suggesting no major change in secondary structure. The near UV spectrum is strongly negative with a maximum at 208 nm, shoulders at 293, 287, 274 and 266 nm and a trough at 258 nm. This spectrum is intensified by about 50% on-going from pH 7 to 8.8 indicating a perturbation of the aromatic chromophores in keeping with the conformational change suggested by the concomitant time-dependent inactivation of the enzyme. The dichroic absorption at 280 nm was used to follow the time course of change from pH 8.8 (inactive) to pH 7 (active). This transition at 30°C was virtually complete in 15 min. The pH dependence of the CD changes suggests a pK of 7.8 for the conformational change.

Glutamate dehydrogenase; Circular dichroism; Conformational change; pH-dependence; (*C. symbiosum*)

1. INTRODUCTION

Glutamate dehydrogenase (GDH) catalyses the reversible oxidative deamination of L-glutamate with NAD(P)$^+$ as coenzyme. The enzyme's metabolic and regulatory interest have made it the subject of many investigations. The NAD$^+$-dependent GDH (EC 1.4.1.2) of *Clostridium symbiosum* has been studied from a kinetic standpoint in our group [1,2] in conjunction with crystallographic work which has provided the first detailed three-dimensional structure for GDH from any source [3,4]. We find that, on transferring the enzyme from pH 7.0 to pH 8.8, more than 95% of the activity is lost in a time-dependent and reversible manner [1,2]. Previous studies of the NADP$^+$-dependent GDH (EC 1.4.1.4.) from *Neurospora crassa* and *Candida utilis* [5,6] have identified a similar pH-dependent transition but in the opposite sense, activity being associated with the high pH state. The inactivation of *C. symbiosum* GDH at high pH is not accompanied by any alteration in quaternary structure, and it seems that the enzyme may undergo a slow conformational change in response to the change in pH [1,2]. We now provide direct evidence of such a change obtained by circular dichroism spectroscopy.

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2. MATERIALS AND METHODS

GDH from *Clostridium symbiosum* was purified by dye-ligand chromatography [1] and stored at 4°C as an ammonium sulphate precipitate. Stock solutions were prepared by dialysis against 50 mM Tris/HCl, pH 7 or 8.8, at 4°C and then clarified by centrifugation. The concentration of GDH, based on A_{280} [1], for circular dichroism measurements in the near UV (250–340 nm) and far UV (200–260 nm) ranges was 5–6.55 mg/ml and 0.125 mg/ml, respectively. The time-dependence of the conversion of the high pH to the low pH form of GDH was determined by following changes in the CD signal at 280 nm after diluting the pH 8.8 stock 20-fold into 50 mM Tris-HCl, pH 7.

The pK_a of the transition of the low pH form to the high pH form of the enzyme was determined by a 20-fold dilution of GDH from 20 mM Tris-HCl, pH 7, into 0.1 M Tris-HCl buffer at various pHs from 6.75 to 9.0 (final GDH concentration was 1.11 mg/ml). To ensure complete equilibration, each dilution was incubated at 30°C for 30 min before recording near UV CD spectra [1]. Spectra were recorded digitally from 340–250 or 260–200 nm using a Jasco J41-C spectropolarimeter with a model J-DPY data processor at a sensitivity of 50×10^{-2} m°/cm with an instrumental time constant of 16 s. Fused silica cells of path length 0.1–4 cm were used as required for near and far UV. The reported spectra are the average of 5 scans and are expressed as molar circular dichroism, $\Delta\epsilon$, based upon a mean residue weight of 110.

3. RESULTS AND DISCUSSION

The far UV CD spectra of GDH at pH 7 and pH 8.8 in Tris-HCl (fig. 1) show two negative bands at 205 nm and 220 nm, typical of α-helical structure [7,8], and assignable to the parallel-polarized component of the $\pi^{\circ}-\pi$ transition and the peptide $n_1-\pi^-$ transition, respectively [7]. The CD spectrum at pH 7 is not markedly different from that at pH 8.8, indicating that

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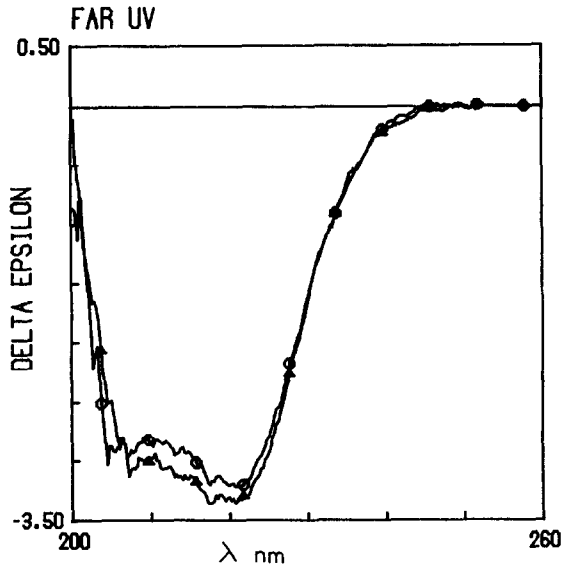

Fig.1. Far UV circular dichroism spectra of GDH (0.125 mg/ml) at pH 7 (▲) and 8.8 (○) in 50 mM Tris-HCl.

the alteration in pH does not significantly affect secondary structure.

Fig.2 shows CD spectra of GDH at pH 7 and 8.8 in Tris-HCl in the near UV region (250–340 nm). These are strongly negative, showing a maximum centred around 280 nm with a trough at 258 nm and distinct shoulders at 266, 274, 287 and 293 nm. The unresolved fine-structure details represent the overlapping effects of all the aromatic chromophores and may be compared with the relatively well-resolved positive CD spectrum of ox-liver GDH at pH 7 [9] with clearly defined features at 259, 265, 280 and 288 nm. The positive maximum of this spectrum was only +8.8 litre·mol^{-1}·cm^{-1} ($\Delta\epsilon$) based on subunit molarity, as compared to a value of -30 litre·mol^{-1}·cm^{-1} for clostridial GDH (pH 8.8).

The dichroic absorption at 293 nm may tentatively be assigned to the red-shifted L_a electronic transitions of tryptophan, presumably corresponding to buried residues. The shoulder at 287 nm and the maximum at 280 nm could derive from the typical L_b electronic transitions of tryptophan, as judged from the spectra of model compounds [10]. The unresolved shoulder at 266 nm and the trough at 258 nm may be correlated with the resolved fine structure of phenylalanine derivatives [11]. The trough at 258 nm suggests that the phenylalanines may be producing a net positive contribution at this wavelength. The unresolved features at 247 nm could be attributed to tyrosine, as also found for model compounds [10].

Fig.2. demonstrates that in going from pH 7 to 8.8, there is intensification of the dichroic absorption of all the aromatic amino acids and in particular of the tryptophan contribution at 280 nm ($\Delta\epsilon$ of −30 litre·mol^{-1}·cm^{-1} at pH 8.8 as compared to -20 litre·mol^{-1}·cm^{-1} at pH 7). The extent of this change strongly indicates perturbation of the chromophoric environments and suggests that the enzyme undergoes a pH-dependent conformational change, thereby supporting the conclusions drawn from kinetic studies [1,2].

In contrast to the clostridial GDH, the active and inactive states of the *N. crassa* enzyme showed identical CD spectra in the near UV range [5] implying no major difference in the orientation or environment of exposure to solvent of aromatic chromophores. Instead, fluorescence spectral measurements were used to monitor the conformational changes in this enzyme [5].

The increase in the dichroic absorption at 280 nm was used to monitor the rate of conversion of the pH 8.8 (inactive) form to pH 7 (active) form of clostridial GDH. Fig.3 is a plot of the CD signal vs time. The tran-

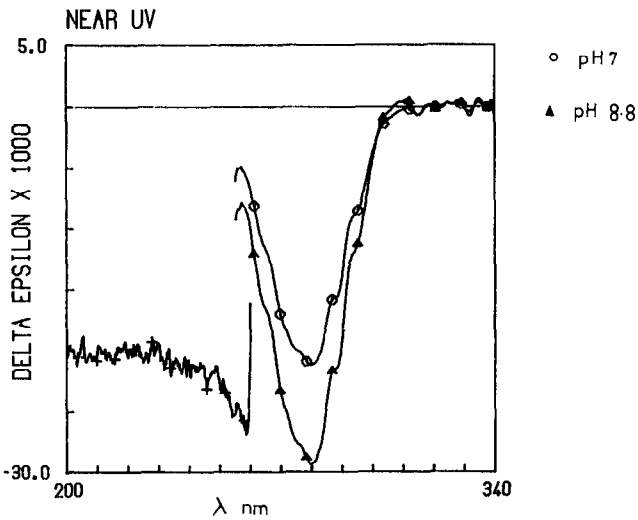

Fig.2. Near UV circular dichroism spectra of GDH (5 mg/ml) at pH 7 (○) and 8.8 (▲) in 50 mM Tris-HCl.

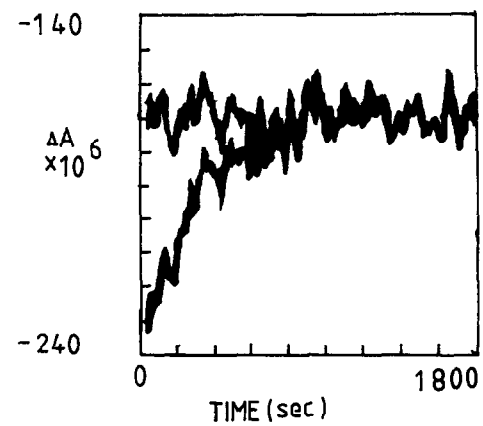

Fig.3. Transition of high pH form of GDH to the active low pH form on dilution of pH 8.8 stock into 50 mM Tris-HCl, pH 7, at 31°C. The final GDH concentration was 0.25 mg/ml. The decrease in the CD signal at 280 nm was followed with time.

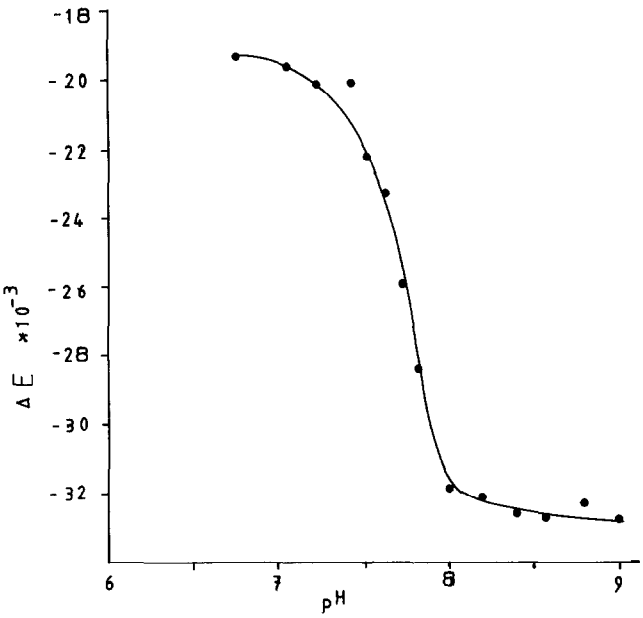

Fig.4. The pK_a of the transition of the high pH form to the low pH form of the enzyme. GDH in 20 mM Tris-HCl, pH 8.8, was diluted 20-fold into 0.1 M Tris-HCl buffer adjusted to different pHs. The final GDH concentration was 1.11 mg/ml. CD spectra were recorded in the near UV range. Changes in the signal at 280 nm are shown.

sition to the active form is virtually complete after 15 min of incubation at pH 7, in good agreement with the activity measurements [1,2].

The shape and position of the CD spectra did not change significantly with pH (spectra not shown). Fig.4 shows a plot of $\Delta\epsilon$ values (litre·mol^{-1}·cm^{-1}) at 280 nm vs pH, yielding an apparent pK_a value of about 7.8. In *N. crassa* GDH, the pK of the conformational transition was 7.2 [5] and similar results were obtained for the enzyme from *Candida utilis* [6].

Gore et al. [12] have reported that, in *N. crassa* GDH, diethylpyrocarbonate modifies a histidine residue, inferred from the primary structure and supporting data to be His-142. Brett et al. [13] have shown this histidine to be important in maintaining the active conformation of the enzyme. Studies [14] involving the photo-oxidation of histidines confirm these findings. Similar chemical modification studies may be useful in investigating the role of the group(s) with pK_a at 7.8 in the conformational changes described above for clostridial GDH.

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