CIRCULAR DICHROISM OF L-GLUTAMIC ACID DECARBOXYLASE*

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Shukuya and Schwert (1960a) have shown that L-glutamic acid decarboxylase (GAD) from E. coli exhibits a sharply pH-dependent reversible spectral change centered at pH 5.6. At pH's less than 5, the enzyme has an absorbance peak at 415 mμ, while at pH's greater than 6, this peak is replaced by one at 340 mμ. The range of enzymatic activity lies on the acid side of this transition. The 415 mμ absorbance peak is presumably due to a Schiff's base between pyridoxal-5-phosphate (PLP) and an ε-amino group of a lysyl residue. Anderson and Chang (1965) have shown that the species absorbing at 340 mμ cannot be reduced by sodium borohydride ($NaBH_4$), whereas that absorbing at 415 mμ reacts rapidly with this reagent to give an enzymatically inactive species which has an absorbance inflection at 330 mμ and has the PLP bound to an ε-amino group of a lysyl residue. Shukuya and Schwert (1960a) demonstrated that hydroxylamine

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(NH_2OH) reacts with GAD at pH 6.3 to give a product with absorbance peaks at 380 mμ and 330 mμ.

Optical rotatory dispersion (ORD) measurements have been used to investigate the manner in which PLP is bound to aspartate-glutamate transaminase (Torchinsky and Koreneva, 1963; Fasella and Hammes, 1964) and to aspartate β-decarboxylase (Wilson and Meister, 1966). Measurements of circular dichroism (CD) have been used to study aspartate-glutamate transaminase (Breusov, et al., 1963) and phosphorylase (Torchinsky, et al., 1965; Johnson and Graves, 1966). Data reported here extend the use of CD measurements to L-glutamic acid decarboxylase.[1]

CD measurements were made with a modified Jouan dichrograph as described by Johnson and Graves (1966). Sensitivity under the conditions used in these experiments was approximately 2×10^{-5} absorbance units. All CD curves shown in this paper were obtained by drawing smooth curves through the experimental traces.

L-glutamic acid decarboxylase was purified from E. coli by a modification of the method used by Shukuya and Schwert (1960b):

1.) Growth conditions were the same as those used by the preceding authors except for the addition of 1% L-glutamic acid to the growth media and an increase in the concentration of yeast extract to 0.6%.
2.) The cells were harvested by centrifugation and suspended in distilled water (20% w/v). The suspension was sonicated, and the solid material was separated by centrifugation and discarded.

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[1] Sukhareva and Torchinsky (1966) in a recent abstract (Second Symposium on Chemical and Biological Aspects of Pyridoxal Catalysis, September, 1966) reported experiments somewhat overlapping those in this paper.

3.) A 2% solution of protamine sulfate was added to the supernatant until precipitation ceased.
4.) The published procedure was then followed exactly as described, except that only one DEAE cellulose chromatography was used, after which the protein was precipitated with ammonium sulfate and was collected by centrifugation. The yellow solid was dissolved in a small volume of distilled water, and the protein concentration was determined by the method of Warburg and Christian (1941). From 250 gm of E. coli cells (wet weight), 650 mg of protein were obtained with a specific activity approximately the same as that found by Shukuya and Schwert after the use of their first DEAE cellulose column.

Aliquots of the purified enzyme solution were dialyzed against 0.1M potassium phosphate (pH 6.5) or 0.1M sodium acetate (pH 4.6) and clarified by centrifugation prior to study. The form absorbing at 415 mμ (pH 4.6) showed strong CD, while the 340 mμ absorbing form (pH 6.5) had very little CD. (Fig. 1 and Table 1)

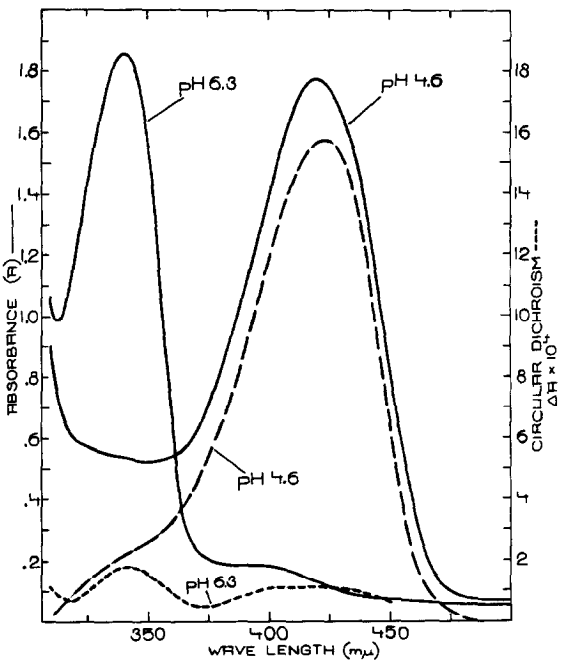

Figure 1 Absorbance and CD of GAD. The solid lines and the dashed lines represent absorbance and CD respectively. The pH 4.6 solution was obtained by dialysis against a 0.1M sodium acetate buffer at that pH. The pH 6.5 solution was obtained by dialysis against a 0.1M potassium phosphate buffer at that pH.

Table 1

		λ max	$\left(\frac{\Delta A}{A}\right)$ λ max
Glutamic Acid Decarboxylase	pH 4.6	420 mμ	+ 0.89 x
	pH 6.5	340 mμ	+ 0.09 x
Reduced Glutamic Acid Decarboxylase	pH 4.6	335 mμ	+ 0.34 x
	pH 6.5	335 mμ	+ 0.38 x
	8M Urea	335 mμ	0
Glutamic Acid Decarboxylase + NH_2OH	pH 4.6	385 mμ	- 0.30 x
		327 mμ[a]	+ 0.64 x
	pH 6.3	385 mμ	+ 0.31 x
		327 mμ	+ 0.20 x

[a] CD maximum occurs at 316 mμ. The absorbance maximum occurs at 327 mμ. $\left(\frac{\Delta A}{A}\right)$ was calculated by using the values at these two different wavelengths (see text).

The enzyme in the pH 4.6 buffer was reduced with $NaBH_4$ (Anderson and Chang, 1965) and was then dialyzed against 0.1M potassium phosphate (pH 6.5) or 0.1M sodium acetate (pH 4.6) and clarified by centrifugation. The absorbance and CD spectra of the reduced enzyme at these two pH's were almost identical (Table 1). The spectra for pH 6.5 are shown in Fig. 2. A solution of the reduced enzyme, to which was added solid urea to a concentration of 8M, showed no CD in its 335 mμ absorption band (Table 1).

Aliquots of the purified enzyme solution were added

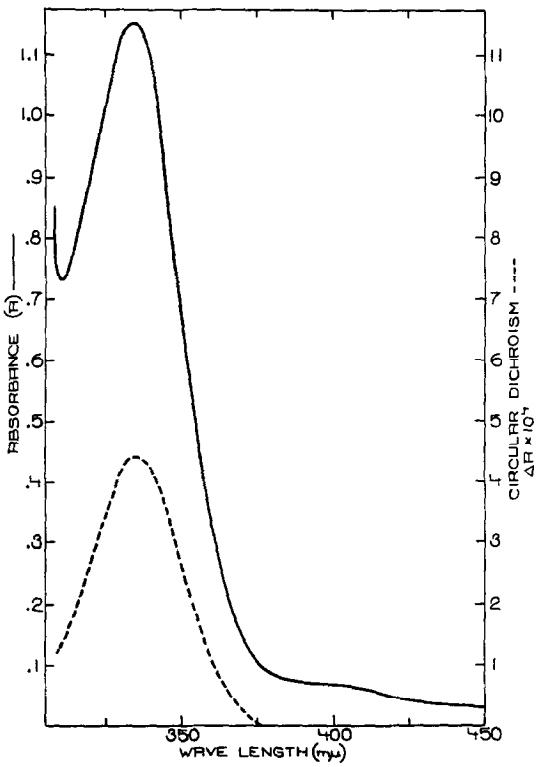

Figure 2 Absorbance and CD of reduced GAD at pH 6.3. The solid line and the dashed line represent absorbance and CD respectively. The pH 6.5 solution was obtained by dialysis against a 0.1M potassium phosphate buffer at that pH.

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to an equal volume of 3.3×10^{-3}M NH_2OH in 0.1M sodium acetate (pH 4.5) or to an equal volume of 3.3×10^{-3}M NH_2OH in 0.1M potassium phosphate (pH 6.5). The absorbance spectrum of the enzyme-NH_2OH complex was independent of pH, whereas the CD spectrum shows large changes with pH (Fig. 3 and Table 1). To determine if the pH dependent change in the sign of the 380 mμ CD peak is readily reversible or dependent solely upon the pH at which the complex was originally formed, the enzyme-NH_2OH complex at pH 6.3 was treated with sufficient 0.1N HCl to reduce

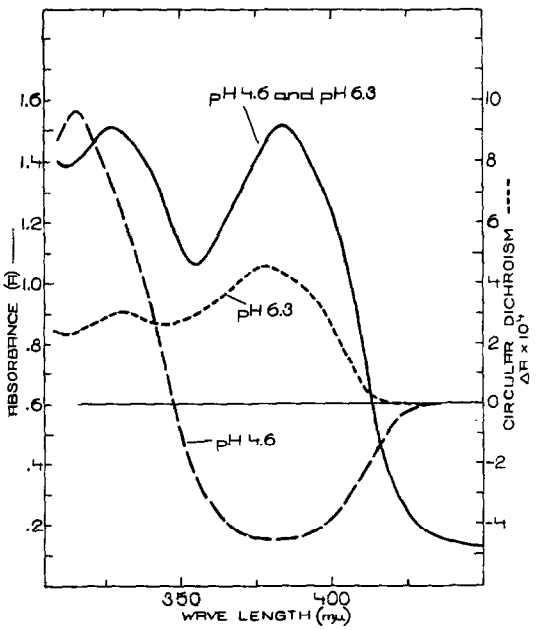

Figure 3 Absorbance and CD of the GAD-NH_2OH complex. The solid lines and the dashed lines represent absorbance and CD respectively. The pH 4.6 and pH 6.3 solutions were obtained as described in the text.

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the pH to 4.5. This pH change was accompanied by a reversal of the sign of the CD peak at 380 mμ, indicating that this pH-dependent change is, indeed, reversible. At pH 4.6, the CD of the NH_2OH derivative of GAD showed an apparent maximum at 316 mμ, while the absorbance maximum in this region was 327 mμ. The cause of this shift of the CD maximum to a shorter wavelength is not known. Addition of $NaBH_4$ to the enzyme-NH_2OH complex at pH 4.6 or pH 6.3 caused no change in the absorbance or CD spectra.

Johnson and Graves (1966) have shown that the active form of rabbit muscle phosphorylase has a Kuhn dissymmetry factor $\left(\frac{\Delta\epsilon}{\epsilon} = \frac{\Delta A}{A}\right)$ of 1.1×10^{-3} for the absorbance maximum at 333 mμ. Under various denaturing conditions, this maximum

disappears and is replaced by an absorbance peak at 415 mμ which they found to have no optical activity. This result is the opposite of that observed with GAD, which has a large CD peak for the 415 mμ absorbing form and very little CD for the species absorbing at 340 mμ. Both enzymes, however, have large Kuhn dissymmetry factors of comparable magnitude in their enzymatically active, native conformations.

Since free PLP in solution is not optically active, the CD of the PLP absorption bands of GAD must be due to the manner in which the coenzyme is bound to the protein. That this induced optical activity, at least in the case of reduced GAD, is due solely to non-covalent interactions, has been demonstrated by the addition of urea which destroys the conformational integrity of the protein and also causes loss of the CD of the covalently bound PLP moiety.

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