SOME OPTICAL PROPERTIES OF BACTERIAL GLUTAMATE DECARBOXYLASE

B.S.Sukhareva and Yu.M.Torchinsky

Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, U.S.S.R.

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The first high-purity preparations of glutamate decarboxylase were obtained from E.coli 26 by Shukuya and Schwert (1960). These authors showed that the catalytically active form of glutamate decarboxylase, existing in solution at pH $\leq$ 5.0, has a spectral absorption maximum at 415 mμ. Adjusting of the enzyme solution from pH 5.0 to pH 6.5 results in a diminution in absorbancy at 415 mμ and in appearance of a new peak at 340 mμ. Anderson and Chang (1965) concluded, on the basis of experiments with sodium borohydride, that the peak at 415 mμ is due to an intramolecular pyridoxal phosphate (PLP) aldimine which is converted to a substituted aldamine upon a pH change from 5.0 to 6.5.

The present paper deals with some optical features (absorption spectra and circular dichroism) of glutamate decarboxylase and its complexes with hydroxylamine and with α-methyl glutamate.

METHODS. Glutamate decarboxylase activity was assayed manometrically at 37° in the Warburg apparatus, as described by Shukuya and Schwert (1960). Specific activity of the preparations is defined as microliters of CO_2 released in 10 minutes per 1 mg of protein. Protein was determined by the method of Lowry et al.

Glutamate decarboxylase was isolated from acetone powder of E.coli 600 by a slight modification of the method of Shukuya and Schwert. Circular dichroism was measured with a Rousel-Jouan dichrograph in 2 cm cells with a sensitivity of 1.5×10^{-4} extinction units.

PROPERTIES OF THE ENZYME PREPARATIONS. The specific activity of our preparations of glutamate decarboxylase ranged from 10,000 to 16,000. Added PLP did not substantially increase the enzymatic activity. The K_M value for L-glutamate is 2×10^{-3}M. The purity of the enzyme preparations ranged from 50 to 90 per cent, as indicated by the results of sedimentation analysis and disc electrophoresis.

Fig. 1A shows the absorption spectra of glutamate decarboxylase; these spectra are similar to those described by Shukuya and Schwert (1960). In the absorption band around 415 mμ we observed positive circular dichroism (induced Cotton effect) (Fig.1B). The absorption band at 340 mμ was found to be optically inactive. These findings indicate that the change in type of linkage between PLP and the apoenzyme (i.e. conversion of the enzyme species with the absorption peak at 415 mμ into the species with the 340 mμ peak) is associated with conformational change in the active site.

INTERACTIONS OF GLUTAMATE DECARBOXYLASE WITH HYDROXYLAMINE AND α-METHYL GLUTAMATE. We studied the action of hydroxylamine, which is a potent inhibitor of the decarboxylase, upon the absorption spectrum and circular dichroism of the enzyme at pH 4.8. As shown in Fig. 2A, addition of an excess of hydroxylamine to the enzyme solution results in a diminution of absorbancy at 415 mμ and in appearance of a new peak at 380 mμ . This new

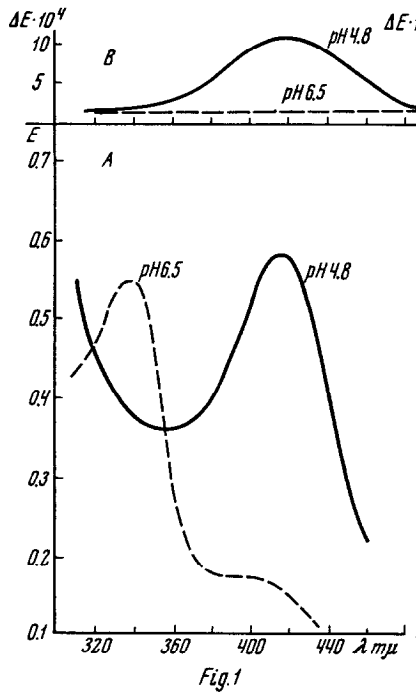

Fig. 1

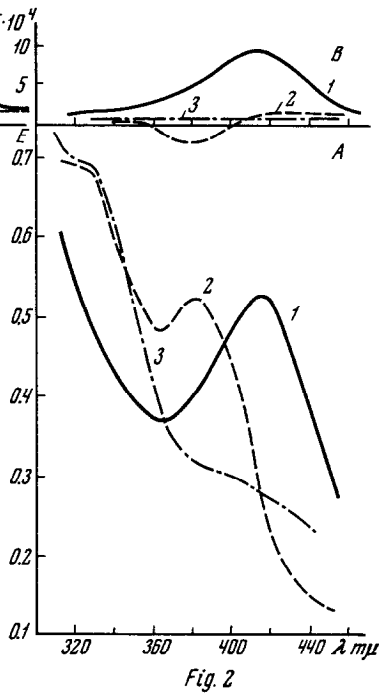

Fig. 2

Fig. 1. Absorption spectra (A) and circular dichroism (B) of glutamate decarboxylase at pH 4.8 and pH 6.5. ΔE - the observed values of circular dichroism. Protein concentration = 4.2 mg/ml.

Fig. 2. Effects of hydroxylamine and α-methyl glutamate on the absorption spectrum (A) and circular dichroism (B) of glutamate decarboxylase. Curve 1 - solution of enzyme at pH 4.8. Curve 2 - same as 1, upon addition of 1 x 10^{-2}M hydroxylamine, at pH 4.8. Curve 3 - same as 1, one hour after addition of 0,1 M α-methyl DL-glutamate at pH 4.8.

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peak evidently belongs to the protein-bound PLP aldoxime. A slight shoulder at 330 mμ simultaneously appears in the absorption curve. We may infer from the spectral data that hydroxylamine splits the aldimine bond between the aldehyde group of PLP and the NH_2 group of the enzyme protein. As seen from Fig.2B,

the rupture of this bond is associated with obliterating of the initial positive circular dichroism and appearance of a very small negative circular dichroism in the absorption band of the protein-bound PLP aldoxime.

Shukuya and Schwert (1960) have shown that addition of an excess of L-glutamate to the decarboxylase at pH 4.6 leads to instantaneous reduction of absorbancy at 420 mμ and to an increase at 330 mμ . These spectral changes are fully reversed within several seconds. We wanted to explore the nature of the intermediates responsible for the mentioned spectral shifts. To this end, we employed α-methyl glutamate, a substrate analogue that acts as an inhibitor of glutamate decarboxylase (Roberts, 1953). Addition of a large excess of α-methyl DL-glutamate to the enzyme solution at pH 4.8 leads to the disappearance of the absorption band and circular dichroism at 415 mμ . At the same time, there appears a shoulder at 330 mμ in the absorption spectrum of the enzyme; no circular dichroism is observed around 330 mμ (Fig.2). The alterations caused by α-methyl glutamate in the enzyme spectrum are similar to those observed by Shukuya and Schwert in the presence of L-glutamate. However, the spectral shift caused by α-methyl glutamate occurs at a considerably slower rate (the drop in absorbancy at 415 mμ usually requires at least 30 minutes for completion) and is not reversed on further incubation.

The enzyme-quasisubstrate complex obtained under the conditions described above is completely deprived of catalytic activity; activity is not restored even following pre-incubation of this complex during 1.5 hour at 37° with a 1×10^{-1}M L-glutamate. Reactivation was observed after incubation of the complex (without removal of the excess of α-methyl glutamate) at 37°

during 10 minutes with PLP at 4 x 10^{-3}M concentration, prior to the activity assay with glutamate. Under such conditions decar - boxylase activity was restored to the extent of 73 per cent of the initial value. When the enzyme-quasisubstrate complex was pre-treated with $NaBH_4$, its capacity to undergo reactivation in the presence of PLP was almost unaffected (in this case, 66 per cent of the original activity was restored). The nature of the enzyme-quasisubstrate complex is under further investigation.

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