CRYSTALLIZATION AND PROPERTIES OF GLUTAMATE DECARBOXYLASE FROM ESCHERICHIA COLI STRAIN W+

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The present note describes the purification and crystallization of glutamate decarboxylase (L-glutamate 1-carboxy-lyase, E.C. 4.1.1.15) from E. coli strain w, and some of the properties of the purified enzyme. Purified glutamate decarboxylase was obtained as a by-product of the purification of pyruvate oxidase carried out according to the procedure of Williams and Hager (1966). Previous reports on the purification of this enzyme from various strains of E. coli include those of Umbreit and Gunsalus (1945), Ayenger, Roberts, and Ramasarma (1951), Najjar and Fisher (1954), Shukuya and Schwert (1960), Sukhareva and Torchinsky (1966), and Huntley and Metzler (1967).

METHODS

Enzymatic activity was measured by determining the rate of release of $[^{14}C]$-CO_2 from 1-$[^{14}C]$-glutamate obtained from New England Nuclear, Boston

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Mass. The assays were run in metabolic reaction flasks, (K-88230), manufactured by the Kontes Glass Co., Vineland, New Jersey; the [^{14}C]-CO_2 released was trapped in ethanolamine:monomethylethylene glycol (1:2) solution and counted in a Packard Tricarb scintillation counter according to Jeffay and Alvarex (1961). One unit of activity was defined as the amount of enzyme catalyzing the release of one μmole of CO_2 per minute in a 0.2 M pyridine HCl buffer, pH 4.5 at 38°; the specific activity was defined as one unit of enzyme activity per mg protein, determined by the biuret method of Weichselbaum (1946) and using crystalline bovine serum albumin as standard. Pyridoxal 5'-phosphate was determined by the phenylhydrazone method of Wada and Snell (1961). Disc gel electrophoresis was run in 6% small pore gels as described by Davis (1965). Sedimentation velocity analyses were performed in a Spinco Model E ultracentrifuge at 52,640 rpm.

RESULTS

Purification of Glutamate Decarboxylase. Purified glutamate decarboxylase was obtained as a by-product in the purification of pyruvate oxidase as described by Williams and Hager (1966), carried out up to but not including the final protamine sulfate fractionation. The last step consisted of a DEAE-Sephadex column chromatography in which two closely associated colored peaks were eluted (Figure 1). The first peak contained pyruvate oxidase while the second was found to contain glutamate decarboxylase at approximately 60% purity. The active fractions were collected, concentrated by precipitation in ammonium sulfate and frozen until further use. For crystalization, the thawed DEAE-Sephadex fraction (40 mg. protein/ml) was adjusted to pH 6.5 with sodium phosphate buffer, 0.05 M final concentration, and solid ammonium sulfate was added in small portions to the ice-cold solution over a period of five days. Crystals in the form of thin needles or flat plates appeared when the concentration of ammonium sulfate reached 15% w/v. Ammonium sulfate was slowly added over a period of two additional days to a final concentration of 20% w/v in order to complete the crystallization. The crystals were

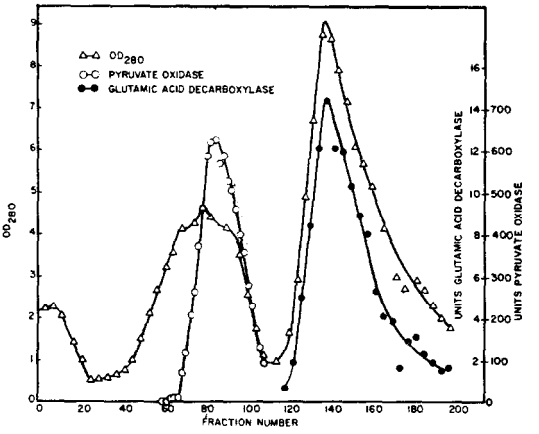

Figure 1. Elution diagram of a typical DEAE-Sephadex A-50 column employing a linear gradient between 0.02 M and 0.3 M potassium phosphate buffer, pH 5.7 and pH 5.3, respectively.

collected and dissolved in a minimum amount of 0.05 M phosphate buffer, pH 6.5, after which the overall crystallization process was repeated twice more (Fig. 2). Table I briefly summarizes the purification procedure for a typical preparation of the enzyme.

Figure 2. Crystals of glutamate decarboxylase, X 528.

Properties of Crystalline Glutamate Decarboxylase. The purified enzyme has a specific activity (extrapolated to infinite substrate concentration) of

TABLE I

PURIFICATION OF GLUTAMIC ACID DECARBOXYLASE

Fraction	Volume (ml)	Protein (mg)	Enzyme Activity		
			Total Units 1×10^{-4}	Specfic Activity	Recovery %
Ammonium Sulfate+ 0.25 - 0.75 Saturation	1730	45,500	48.5	10.6	100
Protamine Sulfate Supernatant	2520	39,000	42.8	11.0	88
Ammonium Sulfate Supernatant 0.36 Saturation	2750	28,900	38.4	12.9	79
Ammonium Sulfate Precipitate 0.36 - 0.55 Saturation	400	22,700	36.0	15.8	74
Supernatant Fraction After Heat Treatment	890	15,400	39.9	26.0	83
DEAE Sephadex Fractions	1200	6,340	32.9	52	68
Aliquot of Concentrated DEAE Sephadex Fraction Used for Crystallization	3.5	138	0.7	51.9	(68)*
1st Crystals	1.1	51	0.4	79	(38.8)*
2nd Crystals	1.4	23.2	0.23	100	(22.3)*
3rd Crystals	1.5	13.6	0.16	115	(15.6)*

+ Starting material was 1400 gm. of E. coli cell paste; see Williams and Hager (1966) for details.

* Recovery in crystallization steps is based on the overall purification procedure.

110 ± 5 μmoles CO_2 released per min/mg protein at pH 4.5, 38°, and a K_M for glutamate of 5.4 X 10^{-4} M. The optimum activity of the enzyme is at pH 3.8 when determined in 0.2 M pyridine·HCl (pH 3.5 to 6) and 0.2 M glycylglycine buffers (pH 2 to 4).

The three-times crystallized enzyme preparation gives a single band on disc gel electrophoresis carried out at pH 7.9; however, when large amounts of material are applied a second faint, fast-migrating band can be observed. Since this latter component increases upon treatment of the enzyme with hydroxylamine, it has been tentatively ascribed to the presence of traces of apoenzyme in the crystalline preparation; alternatively it could be due to dissociation of the holoenzyme to lower forms of aggregation.

The enzyme preparation gives a single symmetrical peak (as determined in a Dupont 310 curve resolver) in the ultracentrifuge in a 0.1 M pyridine·HCl buffer, pH 4.5 and at 20° (Fig. 3). Rectilinear extrapolation of the data by the method of least squares from six ultracentrifuge runs to zero protein concentration gave an $S^{\circ}_{20,w}$ of 12.72 S; the sedimentation constant decreased

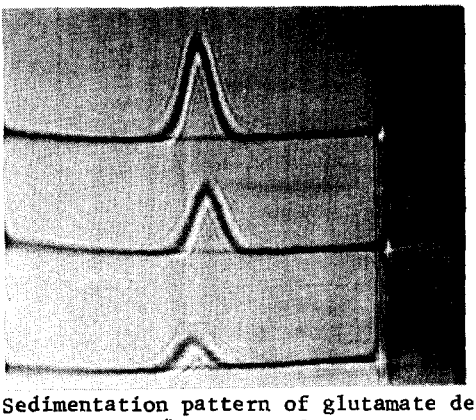

Figure 3. Sedimentation pattern of glutamate decarboxylase after 32 minutes at 52,640 rpm, 20° C. The meniscus is to the right of the photograph. The patterns are shown for 5.50 mg/ml, 3.66 mg/ml, and 1.79 mg/ml solutions of the enzyme in 0.1 M pyridine·HCl, pH 4.5.

by 0.07 S per mg with increasing protein concentration. A diffusion constant, $D_{20,w}$ of 3.06 X 10^{-7} cm^2 sec^{-1} was determined for a 5.44 mg/ml solution of protein. A value of 365,000 was calculated for the molecular weight of the enzyme, using a $\bar{v}$ value of 0.73 obtained from total amino acid composition. The latter was determined on duplicate runs carried out on four-times of hydrolysis.

The enzyme has been found to contain one mole of pyridoxal 5'-phosphate per 75,000 g of protein, based on the molar absorbancy index ϵ = 6700 l·$mole^{-1}$ cm^{-1} for pyridoxal 5'-phosphate in 0.1 N HCl (Peterson and Sober, 1954).

References

Davis, C.H., Ph.D. Thesis, University of Washington (1965).
Huntley, T.E., and Metzler, D.E., Biochem. Biophys. Res. Comm. 6, 109 (1967).
Jeffay H., and Alvarez, J., Anal. Chem. 33, 612 (1961).
Najjar, V.A., and Fisher, J., J. Biol. Chem. 206, 215 (1954).
Peterson, E.A., and Sober, H.A., J. Am. Chem. Soc. 76, 169 (1954).
Shukuya, R., and Schwert, G.W., J. Biol. Chem. 235, 1649 (1960).
Sukhareva, B.S., and Torchinsky, Y.M., Biochem. Biophys. Res. Comm. 25, 585 (1966)
Umbreit, W.W., and Gunsalus, I.C., J. Biol. Chem. 159, 333 (1945).
Wada, H., and Snell, E.E., J. Biol. Chem. 236, 2089 (1961).
Weichselbaum, T.E., Am. J. Clin. Path. Tech. Sect. 10, 40 (1946).
Williams, F.R., and Hager, L.P., Biochem. Biophys. Acta 116, 168 (1966).