PRELIMINARY NOTES

BBA 61173

Electron-microscopic investigation of *Escherichia coli* glutamate decarboxylase

The activity of glutamate decarboxylase from *Escherichia coli* was shown by SHUKUYA AND SCHWERT[1] to undergo marked reduction on cooling of the purified enzyme in diluted solution, and to be restored on addition of pyridoxal phosphate. The conjecture seemed plausible that the macromolecular structure of the enzyme, at low concentration and in the cold, is subject to alterations similar to those observed in certain other enzymes under comparable conditions[2,3].

In the present study we have attempted to obtain information on the structural organization of glutamate decarboxylase, and on its relation to enzymatic activity, by means of electron microscopy.

Glutamate decarboxylase was prepared from cells of *E. coli*, strain 600, essentially by the procedure of SHUKUYA AND SCHWERT[4], and was crystallized once according to STRAUSBAUCH *et al.*[5]. The enzyme crystals were dissolved in 0.05 M phosphate buffer (pH 6.0). Such preparations had a specific activity of 33 000–37 000 μl CO_2 per 10 min per mg protein and displayed one sedimentation peak in the analytical Spinco E ultracentrifuge. According to STRAUSBAUCH *et al.*[5] the glutamate decarboxylase has a molecular weight of 360 000 and contains 5 moles of pyridoxal phosphate per 1 mole of the protein. A pyridoxal phosphate content of 6 moles was determined in our preparations.

The apoenzyme was prepared as follows: to a solution of glutamate decarboxylase containing 1 mg protein per ml, satd. $(NH_4)_2SO_4$ solution (adjusted to pH 3.3) was added to achieve 60% satn., and the mixture was kept 1 h in the cold; the precipitated protein was dissolved in 0.05 M phosphate buffer (pH 6.0).

Decarboxylase activity was measured according to ref. 4, and protein determinations were performed by the method of Lowry *et al.* For the experiments we used solutions of glutamate decarboxylase and of its apoenzyme diluted at room temperature to a protein content of 20–30 μg/ml. Activity measurements were performed (a) on these diluted enzyme solutions, (b) on the same solutions kept for 1 h at 1° and (c) on the latter samples following addition of pyridoxal phosphate. The activity was also measured of the apoenzyme (d) and the holoenzyme reconstituted with pyridoxal phosphate (e).

Specimens for electron microscopy from all the above solutions were prepared by using the negative staining method. A small drop of the enzyme solution under study was spread on a grid with carbon–collodion film; then a drop of 1% uranyl acetate solution was added, and the excess liquid was removed with filter paper. The specimens were examined in the JEM-7 electron microscope at a screen magnification of $\times$ 50 000 at 80 kV.

The specific enzymatic activities of the preparations studied are listed in Table I.

As seen from these data, low temperature treatment results in marked inactivation of the enzyme; addition of pyridoxal phosphate largely restores the activity.

TABLE I

SPECIFIC ACTIVITIES OF GLUTAMATE DECARBOXYLASE PREPARATIONS SUBJECTED TO ELECTRON MICROSCOPY

Solution assayed	*Specific activity (μl CO_2 per 10 min per mg)*
(a) Initial enzyme	37 000
(b) Enzyme kept 1 h at 1°	10 300
(c) Solution b + pyridoxal phosphate ($4 \cdot 10^{-4}$ M)	28 800
(d) Apoenzyme	8 850
(e) Apoenzyme + pyridoxal phosphate ($2 \cdot 10^{-4}$ M)	39 500

The apoenzyme had some residual activity and was reconstituted by adding pyridoxal phosphate to give the original level of activity.

Electron-microscopic investigation of the initial glutamate decarboxylase solution revealed that the enzyme consists of macromolecular particles mostly having the aspect of a hexagonal disc with low mass density in the centre (Fig. 1a). On the basis of inspection of many electron micrographs and of biochemical data we consider a regular polyhedric structure less likely. The diameter of these particles is 130–140 Å. The central low-density area has a diameter of 18–20 Å. On careful investigation of the electron micrographs, each hexagonal disc appears to consist of a number of discrete morphological subunits. One group of subunits, each of 25–30 Å width, forms an inner ring around the central low-density area. The other, outer morphological subunits, 6 in number and with a diameter about 30 Å, are attached to the inner ring; it is possible that each of them is connected with one of the morphological subunits of this ring. The inner ring presumably consists of 6 subunits; however, more frequently only 3–5 are seen on the micrographs, possibly owing to very dense packing of these units.

The structure of the enzyme in solutions exposed to cold treatment is strikingly different from the original one (Fig. 1b). The large hexagon-shaped forms occur very rarely. The preparation mainly consists of smaller particles of variable shape. Some of them look like rings about 70 Å in diameter with low density in the centre; in some of the particles (indicated by arrows in Fig. 1b), the discrete subunits forming the ring are clearly visible. Other particles have the shape of open or incomplete rings. Still others are S-shaped, like twisted ring fragments. Moreover, sometimes 1–3 of the 6 outer subunits remain attached to such particles. It thus appears that inactivation of the enzyme by cold treatment is associated with a disorganization of its macromolecular structure, resulting in dissociation of the outer and, in part, of the inner subunits.

Following the addition of pyridoxal phosphate to the cold-inactivated solution, the initial form of the enzyme is completely reconstituted (Fig. 1c). The particles regain the regular shape of hexagons of 130–140 Å width with clearly visible, central, low-density zones.

Micrographs of the apodecarboxylase (Fig. 1d) show its structure to be closely similar to that of the cold-treated enzyme. In the presence of pyridoxal phosphate the large hexagonal particles of active holoenzyme are reconstituted (Fig. 1e).

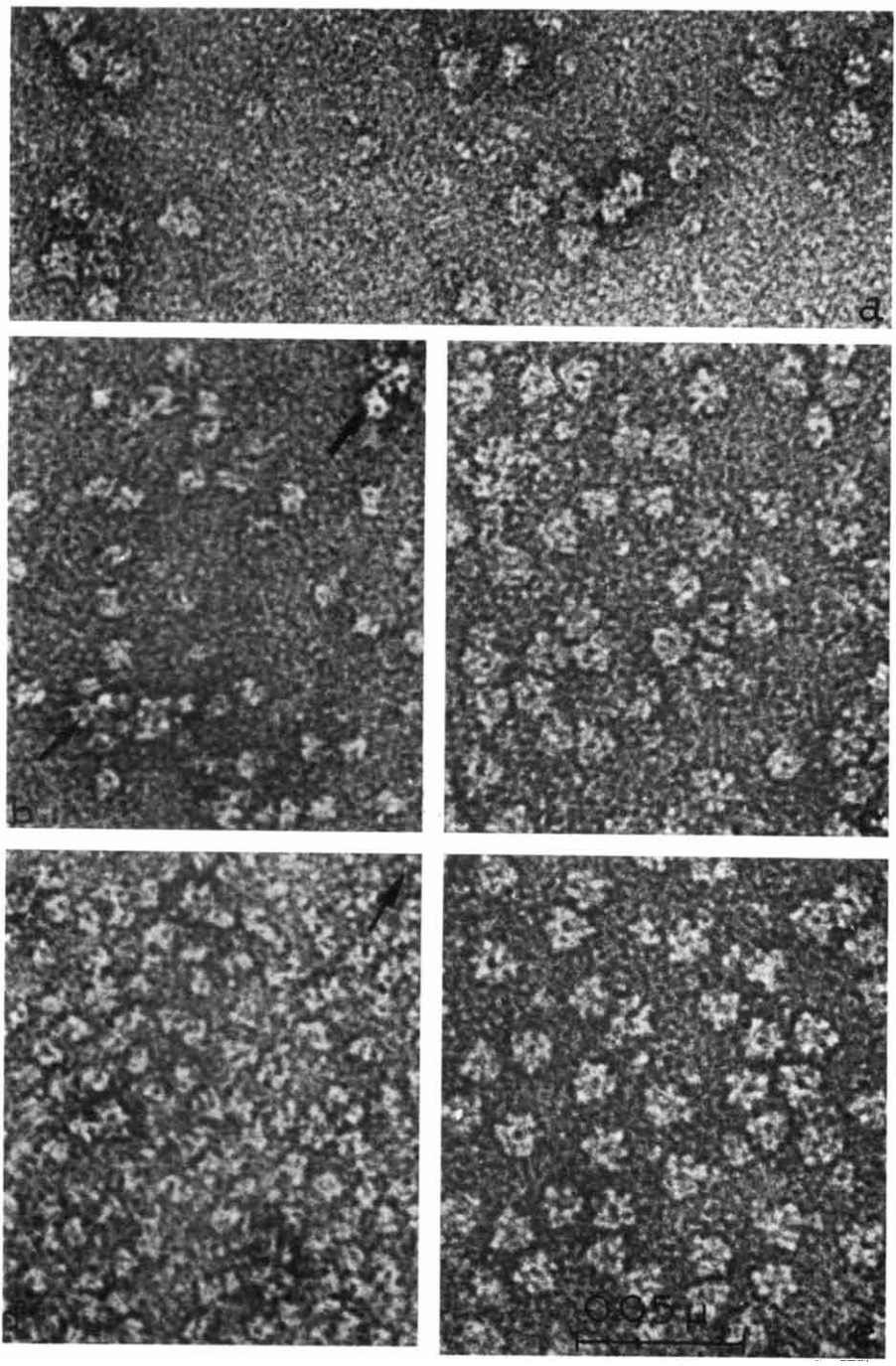

Fig. 1. Electron micrographs of: initial solution of intact glutamate decarboxylase (a); cold-treated solution of the enzyme (b); cold-treated enzyme with added pyridoxal phosphate (c); apodecarboxylase (d), and apodecarboxylase reactivated with pyridoxal phosphate (e). Magnification × 450 000. Experimental details and specific activities of the respective enzyme solutions are indicated in Table I and in the text.

The results reported above demonstrate a close correlation between the structural organization of glutamate decarboxylase and its specific catalytic activity. The active enzyme macromolecules have the aspect of hexagonal disc-shaped particles. Marked reduction in activity, both of the apoenzyme and of the enzyme exposed to cold in dilute solution, is associated with destruction of the initial shape of the active enzyme, *i.e.* with disorganization of its quaternary structure. Both the activity and structure of glutamate decarboxylase are restored on addition of pyridoxal phosphate. The experimental data thus indicate the role of bound coenzyme in stabilizing the structure of the holoenzyme.

Detailed investigation of the quaternary structure of glutamate decarboxylase by electron-micrographic and other methods is in progress.

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Received July 5th, 1968

Biochim. Biophys. Acta, 167 (1968) 476–479

BBA 61169

Properties of matrix supported acetylcholinesterase

The use of polymeric matrices to insolubilize proteins with catalytic activity has been described by several authors[1–6]. The interactions between the protein and the matrix is defined in terms of covalent[1–4] or ionic bonds[5] in certain instances while in others the protein is simply entrapped within the lattice of the polymer[6,7]. Those bonding situations involving known linkages hold the greater theoretical interest in the investigation of the relationship between enzyme-membrane binding and enzymatic activity; however, the matrix-entrapped systems may be of significant practical value because they are easily prepared with high activities and may demonstrate some unusual modification of enzyme behavior. In this study, Silastic-entrapped acetylcholinesterase has been prepared and certain of its properties relative to the free enzyme investigated. The Silastic resin employed has the general chemical structure where the