Electron Microscopy of Aspartate Transcarbamylase and Its Catalytic Subunit†

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ABSTRACT: Aspartate transcarbamylase and its catalytic subunit have been examined by electron microscopy as negatively stained preparations. When the intact enzyme is dried in very thin stain, which was predeposited upon a hydrophilic carbon surface, it appears with its threefold axis normal to the plane of the specimen film. In this orientation it exhibits a structure composed of an inner, solid equilateral triangle and a circumscribing triangle rotated by 60°. The inner triangle appears identical in size and form with the particles appearing in electron micrographs of the catalytic subunit: edge length of 90–95 Å and a closed trimeric substructure. The arms of the circumscribing triangle are believed to represent the three regulatory dimers. When the enzyme is dried in thick stain it exhibits two prominent halves, suggesting that it dries with its threefold axis parallel to the specimen film. The electron microscopic appearance of the enzyme, coupled with physical–chemical data, leads to the structural model described in the following paper.

In the following paper the structure of aspartate transcarbamylase and its catalytic and regulatory subunits are discussed on the basis of results obtained from physical–chemical experiments and from electron microscopy. In this paper we describe the conditions under which the electron micrographs of the enzyme and its catalytic subunit were obtained and present the pictorial evidence leading to the structural model shown as Figure 8 of the following paper.

Experimental Section

Materials and Methods

Enzyme Preparations. Preparations of aspartate transcarbamylase and its purified catalytic subunit were kindly provided by Dr. H. K. Schachman. The material was stored at high concentration as a precipitate in NH_4SO_4. The proteins were solubilized for electron microscopic examination by dialysis against 0.001 M imidazole acetate–0.001 M EDTA (pH 7.0) and diluted to a concentration of 0.1–0.5 mg/ml with the same buffer.

Electron Microscopy. STAINS. Potassium phosphotungstate, uranyl acetate, ammonium molybdate, and sodium silicotungstate were used as negative stains. The last three did not produce results superior to the first; hence, only results obtained with potassium phosphotungstate are presented. It was neutralized to pH 7.0 with KOH prior to application to the specimen films by spraying from a nebulizer.

SPECIMEN FILMS. The specimen films were made of a thin (~200 Å) collodion film, coated with a carbon film about 50-Å thick, and supported on Siemens perforated platinum disks. In some cases (mentioned below) the films were subjected to a 2000-V, vacuum glow discharge immediately prior to use.

SPECIMEN DEPOSITION. Two methods of deposition were used. Method I. Glow-discharged (hydrophilic) films were sprayed with 2% potassium phosphotungstate, and the droplets were allowed to dry. They were then super-sprayed with the enzyme preparation at a concentration of 0.5 mg/ml. Method II. The enzyme, diluted to 0.5 mg/ml, was mixed 1:1 with 2% potassium phosphotungstate and immediately sprayed upon non-glow-discharged films.

ELECTRON MICROSCOPY. A Siemens 1-A electron microscope, utilizing an anticontamination device, was used under these conditions: 60-kV accelerating voltage, 50-μ objective aperture, X40,000–50,000 magnification. All micrographs were obtained under conditions of minimal beam exposure (Williams and Fisher, 1970).

Results

The enzyme molecule presented two distinctive appearances depending upon whether method I or II was used for specimen deposition. Electron micrographs of the enzyme deposited by method I showed it to have the form of an outer and an inner equilateral triangle (Figure 1). The outer triangle is delineated by thin lines 145 Å long, with the vertices relatively prominent. Inscribed within this otherwise hollow triangle, but rotated 60°, is a solid equilateral triangle with a 95-Å edge length. Close inspection of the micrographs shows that the inner triangle is somewhat too large to be completely inscribed within the larger one, and that, in some particles, it shows a closed trimeric substructure.

The appearance of enzyme when it was deposited by method II is shown in Figure 2. Many of the particles appeared to have two separated portions identical in size and shape. Each portion has a shape somewhat like a segment of a circle; *i.e.*, a convex outer edge and a straight, or slightly concave, inner edge. The length of the segment is 90 Å, the maximum width 40 Å, and the open space between segments, quite variable in width, is estimated to be in the range of 20–40 Å. Some of the particles seen in the micrographs show faint extensions of material beyond the ends of the segments, or connecting them.

The catalytic subunit of enzyme, deposited by method II, appeared as shown in Figure 3. Its form is approximately that of an equilateral triangle with a 90-Å edge length. On some of the particles there are indications of a closed trimeric substructure.

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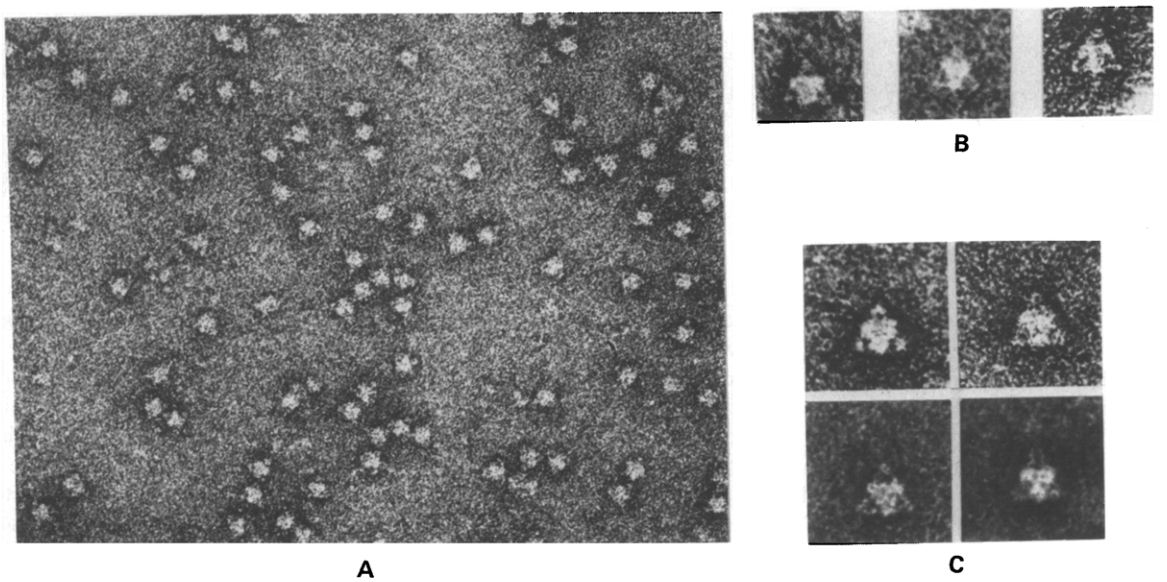

FIGURE 1: Aspartate transcarbamylase molecules, deposited by method I (see text). (a) Field at ×200,000. (b) Particles selected to show a solid triangular structure within a larger triangle mainly delineated by its edges. The former shows evidence of a closed trimeric substructure, ×400,000. (c) Composite views. Each view is a photographic montage of the images of five particles selected from the same electron micrograph, ×400,000.

Enzyme particles deposited by method I usually exhibited only the contours described above. Occasionally, however, electron micrographs contained particles showing, as well, contours like those obtained through use of method II.

Discussion

The micrographs of the intact enzyme preparation deposited by method I show particles which have two visually distinguishable portions: the material in the solidly filled, inscribed triangle, and the material in the arms of the circumscribing triangle (Figure 1). When the enzyme preparation is deposited by method II many of the particles appear to consist of two prominent and identical portions with indistinct extensions beyond their limits (Figure 2). The catalytic subunit alone exhibits a contour which is close to that of an equilateral triangle. The edge lengths of the inner triangles in Figure 1 and of the catalytic subunit are almost identical (90–95 Å); the length of the prominent segments seen in Figure 2 falls within this range of dimensions. From these data, and from the well-established evidence (Meighen *et al.*, 1970; Rosenbusch and Weber, 1971; Cohlberg *et al.*, 1972) that the

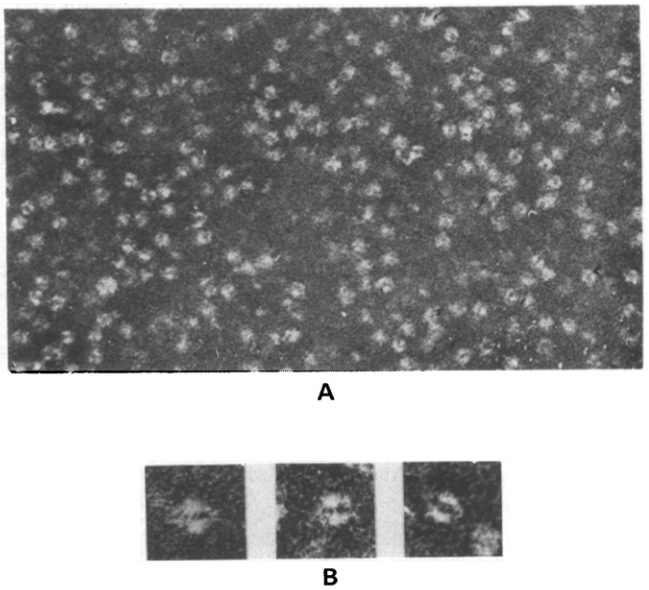

FIGURE 2: Aspartate transcarbamylase molecules, deposited by method II (see text). (a) Field at ×180,000. (b) Particles selected for their appearance of twofold symmetry. Faintly evident is material extending beyond the limits of the dense portions, ×360,000.

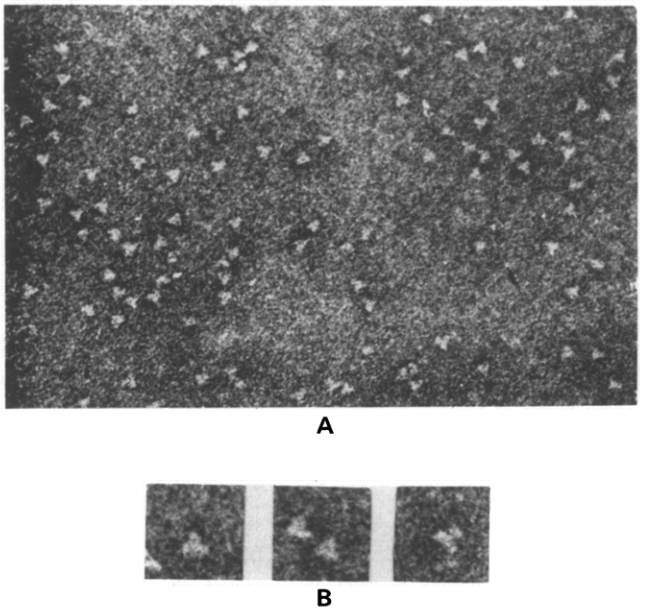

FIGURE 3: Catalytic subunit of aspartate transcarbamylase. (a) Field at ×180,000. (b) Particles selected to show triangular form and evidence of closed trimeric substructure, ×360,000.

ntact enzyme contains two catalytic subunits, we conclude hat the particles seen in Figures 1 and 2 are orthogonal views of the same structure, and that the dense portions represent he catalytic subunit. It seems reasonable to interpret the visual signs of a trimeric array within this subunit as repreenting the three known polypeptides within the catalytic ubunit (Meighen *et al.*, 1970; Rosenbusch and Weber, 1971; Cohlberg *et al.*, 1972). If these conclusions are correct, the orientation of the enzyme particles deposited by method I Figure 1) is such that one catalytic subunit lies directly above he other. Since a clearly demarcated triangle is seen, with fairly lear signs of trimeric subdivision, and no six-pointed or sided figures are seen, it would follow that the upper subunit eclipses the lower; *i.e.*, the two are in angular register, or nearly so.

The material in the arms of the larger triangles seen in Figure 1 is identified, by inference, with the three known egulatory dimers of the enzyme (Meighen *et al.*, 1970; Rosenbusch and Weber, 1971; Cohlberg *et al.*, 1972). Electron micrographs of preparations of purified regulatory subunits have been obtained, but these showed only small particles vith no typical shape; not surprisingly, since this subunit has a molecular weight of only 34,000. But since the known nass of the catalytic trimers is reasonably well accounted for Cohlberg *et al.*, 1972) by the dimensions of the structures dentified above as such trimers, and since three regulatory limers are known to exist, it seems likely that the "arms" ire these dimers.

An interesting feature of the observations reported here is he apparent orthogonality of particle orientation in the preparations deposited by method I and deposited by method II. Apparently, when the stain dries in a thick film on a relatively hydrophobic support surface (method II) the forces of surface tension and/or absorptive forces between enzyme and the surface are such as to cause the molecule to become immobilized with its threefold symmetry axis parallel to the substrate film, or nearly so. On the other hand, the dried stain resulting from method I is extremely thin, evidently causing the enzyme to assume a position in which its threefold axis is normal to the film.

A distinctive feature of the particles deposited by method II is their variability in appearance. This may be associated with variations in the orientation of the threefold axis or with distortions upon drying. The same phenomena may explain the variability in the width of the stain-filled region between the dense segments. The relative faintness of the extensions (believed to be the regulatory dimers) is puzzling, but possibly it is due to embedment in thick stain.

Despite the fact that regulatory dimers of the enzyme have been identified on the micrographs with a lesser degree of directness than have the catalytic trimers, we believe from our observations, coupled with physical–chemical data, that the model in Figure 8 of the following paper represents the most likely structure of aspartate transcarbamylase.

References

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