**Crystallization and Subunit Structure of Histidine Decarboxylase from Lactobacillus 30a**

(Received for publication, August 21, 1980)

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Histidine decarboxylase from *Lactobacillus 30a* has been crystallized in a variety of forms which together indicate a revised subunit structure for the native particle. Octahedral crystals of the wild type enzyme obtained at room temperature from ammonium sulfate solutions in microdiffusion cells belong to tetragonal space group I4,22 with a = b = 222 Å and c = 107.5 Å. Trigonal and hexagonal plates of prohistidine decarboxylase and activated proenzyme obtained at 4°C from polyethylene glycol solutions by vapor equilibration using the hanging drop technique belong to the trigonal space group P321 with a = b = 100 Å and c = 164 Å. The space group symmetries and unit cell contents of these crystals indicate 32 point group symmetry for the subunit structure of these enzymes. Sedimentation coefficients of wild type enzyme measured as a function of ionic strength at pH 7.0 indicate a rapid equilibrium between species varying from 6.9 S to 9.4 S. Sedimentation equilibrium analysis demonstrated the existence of a nearly homogeneous particle with $M_s \approx 208,000$ at ionic strengths above $I = 0.20$, while an additional species of approximately one-half that molecular weight is observed at very low ionic strengths ($I = 0.02$). At the pH optimum of the enzyme (pH 4.8), the larger species is dominant at all ionic strengths tested. Electron micrographs of native wild type enzyme show a dominant tetrahedral particle approximately 60 Å on an edge while similar micrographs of enzyme cross-linked with glutaraldehyde show a dumbbell-shaped particle approximately 60 Å in width and 120 Å in length. These results establish that: (a) the native enzyme has a $M_s \approx 208,000$ and a subunit composition (αβ)$_4$; (b) the proenzyme has a subunit composition (αβ)$_2$ and (c) stable (αβ)$_2$ and (αβ)$_4$ particles exist under certain conditions.

Histidine decarboxylase from *Lactobacillus 30a* differs from most other amino acid decarboxylases in that a covalently bound pyruvyl residue is present at the active center instead of pyridoxal 5'-phosphate (1–3). The pyruvyl moiety arises from an internal serine residue (Fig. 1) of a proenzyme subunit ($M_s \approx 37,000$) which splits during activation by an apparently intramolecular process into a small β subunit ($M_s \approx 9000$) and a larger α subunit ($M_s \approx 28,000$) that contains the newly formed pyruvyl residue in amide linkage to a phenylalanine residue at its NH$_2$-terminal end (3–7). Recsei and Snell (4) showed that the pyruvyl moiety participates in catalysis by forming a Schiff base intermediate with the substrate histidine and the product histamine in a manner analogous to that observed for interaction of pyridoxal phosphate-dependent enzymes with their substrates.

The subunit composition and physical characteristics of this enzyme have been studied extensively (5). Early sedimentation equilibrium measurements (8) indicated that the native particle has a $M_s \approx 190,000$. From this value and $M_s$ values for the α and β subunits, Riley and Snell (3) concluded that the native enzyme had the subunit composition (αβ)$_4$.

We have crystallized histidine decarboxylase from *Lactobacillus 30a* as a step toward determining its three-dimensional structure and comparing its mechanism of action with that of pyridoxal phosphate-dependent enzymes. The crystallographic results presented here demonstrate that proenzyme and wild type enzymes have 32 point group symmetry and the subunit stoichiometries of (αβ)$_4$ and (αβ)$_2$, respectively. Ultracentrifugation and electron microscopic studies support this conclusion and also indicate the existence of stable (αβ)$_4$, and (αβ)$_2$, particles under certain conditions. The latter findings relate this enzyme more closely to a pyruvate-dependent histidine decarboxylase from a *Micrococcus* which is reported to have an (αβ)$_3$ subunit structure (9).

**EXPERIMENTAL PROCEDURES**

**Materials**—Crystalline wild type histidine decarboxylase was obtained from *Lactobacillus 30a* by a slight modification of the method of Chang and Snell (10). Prohistidine decarboxylase from mutant 3 was purified as described by Recsei and Snell (7). All reagents and other materials were of commercial grade.

**Crystallization and Space Group Determination**—The octahedral crystals of histidine decarboxylase were grown at room temperature in microdiffusion cells (11) at a protein concentration of 5 to 15 mg/ml, pH 4.8, 0.20 M ammonium acetate buffer equilibrated against a 45% saturated ammonium sulfate solution in the same buffer. The trigonal crystals of the proenzyme and activated proenzyme were obtained by vapor equilibration via hanging drops (12) against 10% polyethylene glycol solutions in the cold at 4°C, pH 4.8, with 0.2 M ammonium acetate buffer. Space groups were determined from $M = 10^3$ precision photographs of all major diffraction zones.

**Crystal Density Measurements**—Histidine decarboxylase crystals were fixed in 0.1% glutaraldehyde for 6 h and subsequently rinsed in water. Crystal densities were determined in a xylene/bromobenzene-modified gradient by the method of Low and Richards (13) using amino acids as markers. Density measurements reported are averages of several measurements.

**Sedimentation Velocity and Sedimentation Equilibrium Measurements**—Sedimentation velocity experiments were run in a titanium rotor at speeds between 40,000 and 60,000 rpm with Schlieren optical
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FIG. 1. Relationship of the precursor z subunit sequence of prohistidine decarboxylase to that of the a and b subunits of activated proenzyme. Activated proenzyme and wild type histidine decarboxylase are indistinguishable on the basis of a number of criteria and apparently differ in primary structure by only one or a few amino acids.

measurements made at 546 nm. The movement of the Schlieren peak was measured by hand on a Nikon comparator and the sedimentation coefficients calculated from a plot of ln r versus time. Sedimentation equilibrium molecular weights were determined by the meniscus depletion method of Yphantis (14). The interference photographs were measured with an automatic densitometer (15) and the data evaluated by a computer program (16). Molecular weights were calculated from the intercepts of 1/Ms vs. c plots using e = 0.73 ml/g calculated from amino acid analyses by the method of Cohn and Edsall (17).

Electron Microscopy—Negatively stained specimens of native enzyme in solution were prepared as described by Oliver (18) using sodium phosphotungstate or methylamine tungstate stains. Negatively stained specimens of protein crystals were prepared by grinding crystals with a glass rod at 4°C in 12% polyethyleneglycol mother liquor, fixing for 2 h in the cold with 3% glutaraldehyde, followed by repeated rinsings and resuspensions in 0.125% methylamine tungstate stain. Specimens were examined in Siemens Elmiskop model I electron microscope operated at 80 kV. All micrographs were taken at 10,000.

RESULTS

Crystallization and Implied Molecular Symmetry

Eight distinct crystal forms of histidine decarboxylase have been obtained under various conditions. A tetragonal form of the wild type enzyme and the isomorphous trigonal forms of proenzyme and activated proenzyme have been selected for further crystallographic analysis. These crystals typically grow to 0.3 to 0.5 mm in size and diffract to 2.8 Å resolution.

The major diffraction zones of the octahedral crystals obtained from ammonium sulfate solutions are shown in Fig. 2, A and B. These crystals are tetragonal, space group 1422 with a = b = 222 Å and c = 107.5 Å. Density measurements of cross-linked, water-soaked crystals averaged 1.15 g/cm³, indicating that 8 molecules (Mₛ = 200,000) occupy the 16 general positions of the unit cell, i.e., one-half molecule per asymmetric unit. This observation implies that the molecule has 2-fold molecular symmetry and contains an even number of subunits.

Diffraction patterns of the h0k0 and h0l zones of the trigonal plates of proenzyme obtained from polyethyleneglycol solutions are shown in Fig. 3, A and B. The trigonal form of the proenzyme is isomorphous with that of the activated proenzyme (not shown). The space group of these crystals is P321; a = b = 100 Å and c = 164.5 Å. The density of these crystals averaged 1.14 g/cm³, indicating that there are only 2 molecules in the unit cell or one-third molecule per asymmetric unit. The molecules are positioned on the two 3-fold axes of the unit cell and therefore must have 3-fold molecular symmetry.

Ultracentrifugation Behavior of Histidine Decarboxylase

Sedimentation Velocity—At pH 4.8 (the optimum pH for enzyme activity) wild type histidine decarboxylase sediments as a single peak at both high salt (I = 0.22) and low salt (I = 0.02) concentrations (Table I). At pH 7.0 the enzyme also sedimented as a single peak, but the sedimentation coefficient varied from 6.9 S in the low salt condition (I = 0.02) to 9.4 S at high salt (I = 0.25). These findings demonstrate the capacity of the enzyme to dissociate. The equilibrium composition of this apparently rapidly equilibrating heterogeneous system depends upon ionic strength and pH of the solvent.

Sedimentation Equilibrium—Sedimentation equilibrium experiments of wild type enzyme at pH 4.8 showed little evidence of heterogeneity and a species of Mₛ = 208,000 was observed under both high and low salt conditions. At pH 7.0, a salt effect was again apparent. Under high salt conditions (I = 0.22) a dominant particle with Mₛ = 208,000 was present toward the bottom of the cell where the protein concentration is highest. Under low salt conditions (I = 0.02) and low protein concentrations a dominant particle with Mₛ = 104,000 was observed. Heterogeneity was observed under both these conditions at the higher pH as illustrated in Fig. 4.

Electron Microscopy—A variety of image forms, many with distinctive subparticle detail, were observed on negative stain micrographs of wild type histidine decarboxylase (Fig. 5A). The projected dimensions of a major portion of the images are in the range of 55 to 65 Å. Volume and mass estimates
The conditions imposed by the negative stain procedure are low ionic strength, neutral pH, and low protein concentrations. The concentration was based on sedimentation equilibrium analysis. Initial protein concentration was 2 mg/ml in A, 0.01 M sodium phosphate, pH 7.0, or B, 0.02 M sodium acetate, pH 4.8. In each case, the solid circle represents no additional salt added, whereas the cross represents the results obtained in the presence of 0.20 M NaCl. Note the heterogeneity evident in experiments at pH 7.0 and that the ordinate is linear in 1/M, although it is labeled with M, values for convenience. All runs were made at 20°C.

Based on these dimensions indicate the imaged particles contain but one-third to one-half the mass of the native enzyme. The conditions imposed by the negative stain procedure are low ionic strength, neutral pH, and low protein concentrations. During ultracentrifugation under similar conditions, the native aggregate incorporates a dyad axis. Studies of the trigonal crystals, P321, cited above indicate that 2 molecules of the enzyme (αβ)3 are positioned on the 3-fold axes of the unit cell. This interpretation implies that the molecules have at least 3-fold molecular symmetry. From these considerations, and with knowledge of the morphology of the molecules, the arrangement of mass in the crystal (to a low resolution) could be predicted. This model of the crystal is loosely packed (Vₚ = 3.4 Å³/dalton) and has large solvent channels at the lattice origin positions which could fill with negative stain. The model was confirmed by negative stain micrographs of fragments of the crystal, where lattice spacings were determined for projections of the crystal from measured optical diffractions of the micrographs. Fig. 6 is a micrograph of a crystal fragment for which the aspect is near [001]. The 3-fold packing of the molecules in the crystal is evident. The lattice spacing in the micrograph is ≈ 96 Å, a 4% shrinkage from the lattice spacing of the hydrated crystals, a = b = 100 Å.

Molecular weight determinations have indicated that the native histidine decarboxylase consists of five or six (αβ) subunits. The microscopic observations suggest its stoichiometry is (αβ)₅. Stable, finite aggregates comprised of an odd number of identical asymmetric units must be organized about a cyclic point group and this rotational symmetry axis may be evident in the distribution of mass in the aggregate revealed by some of its projections in negative stain. None of the observed histidine decarboxylase images indicate a 5-fold axis is present in the aggregate. Stable, finite aggregates comprised of an even number of identical asymmetric units may be organized about either a cyclic or a dihedral point group. None of the images suggest that a 6-fold axis is present in the aggregate. The apparent dyad axis which is observed indicates that the aggregate possesses dihedral symmetry.

Evidence for the presence of a 3-fold symmetry axis in the aggregate was provided by studies of the images of the particles which contain half the mass of the native enzyme and of fragments of the trigonal crystals of the enzyme. The population of images which derive from the postulated trimer (αβ)3 includes many images (Fig. 5B) which distinctly present triangular profiles. Some of these images are interpreted as projections along the 3-fold axis of a particle with cyclic 3-fold symmetry. The polyhedral morphology of the particle is revealed by other views (Fig. 5C and D) to be tetrahedral; that is, the particle is apparently comprised of four lobes of about equal mass (Fig. 5D). Since the combined mass of three β subunits approximates the mass of α subunit, a reasonable interpretive model of the particle places the three β subunits in contact to give the appearance of a single lobe of the four-lobed structure with an individual α subunit contained in each of the other three lobes. The dumbbell morphology of the native enzyme suggests that self-association of this trimer occurs by an interaction at the β subunits to form the native enzyme structure (αβ)₅ with 32 molecular symmetry.

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Subunit Structure of Histidine Decarboxylase

Electron micrographs of the wild type enzyme from Lactobacillus sp. 30a confirmed the existence of trimer and hexamer forms of this enzyme. Histidine decarboxylase previously cross-linked in solution with glutaraldehyde exhibits a "double-beaded," dumbbell appearance (Fig. 5E) corresponding to the hexamer, while the uncross-linked protein apparently dissociates to the stable trimer (Fig. 5A) under these staining conditions.

Crystal packing analysis further supports the above model for the hexamer of histidine decarboxylase. A dumbbell-shaped, dihedral particle of $\approx 120$ to 130 Å in length and $\approx 60$ to 65 Å in width can be conveniently packed in either unit cell, while unit cell parameters and placement of symmetry operators prohibit any packing arrangement involving the larger diameter particles necessitated by a cyclic arrangement of $\alpha\beta$ subunits. The molecular packing arguments were confirmed directly using electron microscopy for the trigonal crystal form (Fig. 5E) and by model studies for the tetragonal crystal form.

The preparation of heavy atom derivatives of the crystal forms reported here is in progress. Three heavy atom derivatives have been obtained for the octahedral crystal form. The trigonal crystal form, however, has obvious advantages since its asymmetric unit is smaller and isomorphic crystals of both proenzyme and activated proenzyme are obtained in this space group. These structural studies should help eventually to explain not only the mechanisms of catalytic action and proenzyme activation of this enzyme but also to illustrate the similarities and differences between the $\alpha$-keto acid-dependent and pyridoxal phosphate-dependent classes of enzymes.

Acknowledgments—We wish to thank William Lopatin, Lynda Lindsay, and Beth Hoffman for skilled technical assistance and Rita TheBerge for help in preparation of this manuscript.

REFERENCES