Preliminary X-ray Data on Crystals of Mandelate Racemase*

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The recent development of a high-yield expression system and purification scheme for mandelate racemase has enabled us to produce sufficiently large quantities of pure enzyme to pursue x-ray crystallographic study. Large, single crystals of mandelate racemase have been grown from buffered polyethylene glycol (pH 8.0) in the presence of 10 mM magnesium chloride. The crystals grow in several habits, and we have identified two distinct tetragonal space groups in preliminary x-ray diffraction analysis. Crystals shaped as rectangular plates demonstrate 4/mmm Laue symmetry and systematic absences consistent with the space group 1422. They have cell dimensions of a = b = 153 Å and c = 181 Å. Octahedrally shaped crystals of mandelate racemase display 4/m Laue symmetry and systematic absences consistent with the space group 14. Cell dimensions for these crystals are a = b = 113 Å and c = 124 Å. Based on estimates of Vm and on the measured density of the 1422 form, we suggest that two subunits of mandelate racemase (38,570 daltons/subunit) occupy the asymmetric unit in both crystal forms. Crystals of both forms diffract to beyond 3.0-Å resolution. We are currently screening for isomorphous heavy-atom derivatives.

Mandelate racemase (EC 5.1.2.2) from Pseudomonas putida (ATCC 12633) belongs to a family of inducible enzymes that permits certain strains of bacteria to utilize either D- or L-mandelic acid (Scheme I) as sole carbon and energy source. Although its biological role is uncertain, this pathway has served as a prototype for the study of inducible enzymatic systems. Studies on the mandelate pathway helped demonstrate that multiple enzymes may be encoded within a single operon (1-4), and further studies have suggested that enzymes in this pathway may assemble to form an efficient catalytic machine in vivo (5).

In addition, studies on the mechanism of racemases are crucial to our understanding of how enzymes accommodate changes in stereochemical configuration along the reaction coordinates of the reactions they catalyze. Both mandelate racemase and bacterial alanine racemases are believed to utilize a single-base, stepwise mechanism (6, 7). In this mechanism, an active-site base abstracts the enantiomeric proton of the substrate to yield a planar, resonance-stabilized carbanionic intermediate (8-10). The same active-site base then delivers a proton randomly to either the original or opposite prochiral face of the intermediate. It seems apparent that in order for a single active-site base to interact with both faces of the planar intermediate, some alteration of active-site geometry must take place. Proposed schemes for this structural rearrangement include the "swinging door" mechanism (11) for enzymes utilizing a pyridoxal 5'-phosphate cofactor; however, no structural evidence exists to support this scheme.

We have undertaken structural characterization of mandelate racemase in the hope of establishing what types of structural rearrangements of the enzyme accompany racemization of substrate.

Crystallographic study of mandelate racemase augments ongoing structural studies of alanine racemase (12) since, while alanine racemase requires pyridoxal 5'-phosphate for activity, mandelate racemase has no organic coenzyme requirement. Rather, mandelate racemase requires a divalent metal ion for catalytic activity. Thus, it should be very enlightening to observe in detail the structural origins of catalytic power for these two enzymes that catalyze analogous reactions, yet must use markedly different means of orienting and stabilizing the carbanionic intermediate.

MATERIALS AND METHODS

Mandelate racemase was isolated from Pseudomonas aeruginosa transformed with an overproducing mutant of pSCR1, a plasmid containing the cloned gene of the enzyme from P. putida (13), and was purified to homogeneity (as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis) as previously described (13). The enzyme (2-20 mg/ml) was crystallized by hanging drop vapor diffusion (14) against 10-20% polyethylene glycol 8000 (Sigma) in a buffer consisting of 50 mM Tris (pH 8.0), 10 mM MgCl2, and 1 mg/ml NaN3. Crystals have also been obtained by batch precipitation both against polyethylene glycol and ammonium sulfate in the above buffer; however, these crystals were of low quality. For diffraction analysis, crystals were mounted in quartz capillaries. Zero-level precession photographs were recorded on Kodak DEF film and analyzed for reciprocal lattice spacings and symmetry (14).

Crystal density was measured on a water-saturated bromobenzene/xylene gradient calibrated with drops of aqueous cesium chloride. To avoid dehydration,
Crystals of Mandelate Racemase

FIG. 1. Photomicrograph of crystals of mandelate racemase, Form I. The largest crystal shown measures 0.5 × 0.5 × 0.2 mm.

FIG. 2. Photomicrograph of mandelate racemase crystals, Form II. The crystals shaped as regular octahedra, or truncated bipyramids, are representative of Form II. The largest crystal shown of this form measures about 0.1 mm on a side.

FIG. 3. hko precession photograph of I422 form of mandelate racemase crystals. The precession angle is 10°. Note the 4/mmm symmetry apparent in this zone. The h0l zone displays mm2 symmetry.

RESULTS AND DISCUSSION

We have characterized two distinct crystal forms of mandelate racemase. As demonstrated by both 15° precession photos and still photos, crystals of both forms diffract to at least 3.0-Å resolution. Form I crystals have the shape of rectangular plates (Fig. 1), whereas Form II crystals are octahedra (Fig. 2). The diffraction pattern of Form I crystals displays 4/mmm Laue symmetry (Fig. 3) and general extinctions of reflections with h + k + l ≠ 2n, consistent with a body-centered lattice; assignment of axial directions consistent with a primitive lattice does not yield a proper reduced cell. No additional extinctions are apparent. This uniquely identifies the space group as I422 (16). Cell dimensions are a = b = 153 Å and c = 181 Å. The diffraction pattern of Form II crystals displays 4/m Laue symmetry (Fig. 4) and general extinctions h + k + l ≠ 2n; no other extinctions are apparent. Thus, the space group of this form is I4 (16). Cell dimensions for this form are a = b = 113 Å and c = 124 Å. The two crystal forms coexist in crystallizations; however, Form I nucleates and grows preferentially at lower protein and/or precipitant concentrations than Form II. Conversely, at higher concentrations of either protein or precipitant, the octahedral habit (Form II) nucleates slowly but grows at the expense of the plate habit (Form I). Form II crystals typically attain a maximum length of 0.2 mm, whereas Form I crystals readily attain dimensions of 0.5 × 0.5 × 0.2 mm and occasionally grow as large as 1 mm in their longest dimension.

Assumption of two subunits as the contents of the asymmetric unit for each crystal form leads to calculated Vm values (unit cell volume in Å³/dalton of protein) of 2.6 and 3.4 for the I4 and I422 forms, respectively. Although both of these values are within the observed range for protein crystals of 1.8–3.5 as compiled by Matthews (17), a Vm of 3.4 suggests a relatively high solvent content (64%) for the I422 form. The measured density of Form I (I422) crystals is 1.11 g/ml. This is consistent with a high solvent content for these crystals (14) and confirms our proposal that two subunits of mandelate racemase occupy the asymmetric unit.

The immediate objective of these crystallographic studies is to determine the three-dimensional structure of mandelate racemase by the method of isomorphous replacement. If successful, this will pave the way to crystallographic studies of the mechanism of the enzyme; since mandelate racemase is a single-substrate, single-product enzyme, we hope to observe
crystallographically a productive substrate-enzyme complex (18). The structure of such a complex should be of great value in assessing the nature of structural rearrangements associated with catalysis.

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