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Crystallization and preliminary X-ray diffraction studies of 5-chlorolevulinate-modified bovine porphobilinogen synthase and the Pb^{II}-complexed enzyme

H. L. CARRELL, J. P. GLUSKER, L. SHIMONI, L. J. KEEFE,† C. AFSHAR, M. VOLIN AND E. K. JAFFE* *at Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA. E-mail: ek_jaffe@fccc.edu*

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Abstract

Bovine porphobilinogen synthase (PBGS) is an homo-octameric enzyme with four active sites. Each active site binds two Zn^{II} atoms whose ligands differ and two molecules of 5-aminolevulinate whose chemical fates differ. The asymmetric binding of two Zn^{II} atoms and two identical substrate molecules by a homodimeric active site is apparently unique. Modification by 5-chlorolevulinate can be used to differentiate the two substrate-binding sites; diffraction-quality crystals of 5-chlorolevulinate-modified PBGS have been obtained. Pb^{II} can be used to differentiate the two different Zn^{II}-binding sites; diffraction-quality crystals of the Pb^{II} complex of PBGS have been obtained. Preliminary diffraction data reveal an *I*422 space group, in agreement with a general model for the quaternary structure of PBGS.

1. Introduction

The enzyme porphobilinogen synthase (PBGS) catalyzes the asymmetric condensation of two molecules of 5-aminolevulinic acid (ALA) to give porphobilinogen (Shemin & Russell, 1953) (Fig. 1).

Porphobilinogen is the monopyrrole precursor of all biological tetrapyrroles, the porphyrins, corrins, chlorins and other cofactors. As such, this enzyme is essential to all cellular life. The primary structure of PBGS is highly conserved suggesting that the chemical mechanism is also conserved through evolution. PBGS is, however, a metalloenzyme and there is a phylogenetic variation in the use of metal ions for catalytic and other functions (Jaffe, 1995).

Bovine PBGS is a 280 kDa protein, each molecule consisting of eight identical subunits with four active sites. Each active site is, therefore, formed from two identical subunits so that it is homodimeric. Two functionally distinct types of Zn^{II} bind to each active site (Jaffe, Volin, Myers & Abrams, 1994). In addition, two ALA substrate molecules (whose chemical fates are different, as shown in Fig. 1) bind. The asymmetric binding of two Zn^{II} and two ALA by a homodimeric active site is apparently unique. A full understanding of the nature of the asymmetry of the binding in the active site must come from a determination of the X-ray crystal structure of the protein.

Differentiation of the two substrate-binding subsites in any one active site can be accomplished through the use of a substrate analog which is bound at only one of the two subsites. An analogue that is found experimentally to fit this requirement is the active-site-directed chemical modification reagent 5-chlorolevulinic acid (5-CLA) which modifies the protein preferentially at only one of the two subsites (Jaffe, Abrams, Kaempfen & Harris, 1992). Each active site is covalently modified at Cys223 with a levulinic acid moiety which occupies the binding site for the ALA molecule which becomes the acetyl half of porphobilinogen. The environmental toxin lead (Pb), known to bind tightly to PBGS (Gibbs & Jordan, 1981), is expected to help differentiate between the two Zn^{II}-binding sites if they have different properties. Only four lead ions bind to the octameric enzyme (Beyersmann, 1986).

† Present address: Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439, USA.

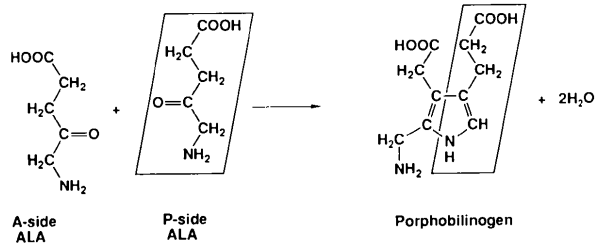

Fig. 1. The porphobilinogen synthase-catalyzed asymmetric condensation of two molecules of 5-aminolevulinate (ALA) to form porphobilinogen.

2. Materials and methods

Bovine PBGS was purified from freshly slaughtered beef liver (Jordan, 1986). Zn^{II} (10 μM) and 2-mercaptoethanol (10 m*M*) were included in all purification buffers. 5-CLA-modified PBGS was prepared as previously described using a 1 h modification protocol (Jaffe, Abrams, Kaempfen & Harris, 1992). The lead (Pb) complex of PBGS was prepared by ultrafiltration exchange of purified bovine PBGS into 0.1 *M* KP_i (pH 7.0), 10 m*M* 2-mercaptoethanol, 20 μM Pb^{II}.

Crystals of 5-CLA-modified PBGS were obtained by the vapor-diffusion method using hanging drops (McPherson, 1982). Crystals were grown by equilibrating drops containing ~8.3 mg ml^{-1} of protein solution, plus 0.67 *M* ammonium sulfate solution in 50 m*M* 2(*N*-morpholino)ethanesulfonic acid (MES, pH 6.2), against 1.36 *M* ammonium sulfate in 50 m*M* MES (pH 6.2). Crystals appeared approximately one year later at room temperature. The parallel attempts at 277 K gave no crystals. By altering the conditions slightly, crystals can now be grown in 2–4 months time, but their size is unpredictable.

Crystals of the lead-modified enzyme have recently been obtained under conditions similar to those described above. These crystals grow in much the same manner and appear after about three months. In addition, parallel crystallization experiments were carried out in a darkened box and a few crystals with slightly better diffraction qualities appeared.

3. Results

3.1. *Crystal and diffraction data*

Colorless crystals generally grow as square plates or truncated square plates. In general, the crystals are less than 0.15 mm in the maximum dimension, but some have grown as large as 0.4 × 0.4 × 0.15 mm. Precession photographs indicate that the crystals are tetragonal with a space group of *I*422. The unit-cell parameters obtained for the 5-CLA-modified enzyme are $a = 125.4\ (3)$ and $c = 200.6\ (4)$ Å. The diffraction data showed a cutoff, on film, of about 3.9 Å and the data measured with a Siemens multiwire area detector showed a similar cutoff. The area-detector data were processed with the *XENGEN* software package (Howard, Gilliland, Finzel, Poulos, Ohlendorf & Salemme, 1987). The average $I/\sigma(I)$ fell to 2.0 and R_{sym} for the data set was 0.098 for 29 122 reflections which gave 7603 unique data to 3.9 Å resolution. The value of V_m, the volume per mass unit (Matthews, 1968), is 2.8 Å^3 Da^{-1} for 32 monomers (four octamers) in the unit cell. Attempts to obtain isomorphous heavy-atom derivatives have been hampered by our inability to reproducibly grow diffraction-quality crystals.

The crystals of the lead-complexed enzyme grown in the light and in darkness exhibit diffraction and appear to have virtually identical unit-cell dimensions and the same space group, namely *I*422 with $a = 124.8\ (2)$, $c = 200.2\ (3)$ Å. The principal difference is that while the crystals grown under full light exposure diffract to about 3.6 Å resolution, the crystals grown in complete darkness diffract to 3.2 Å resolution. For the latter crystals, the data were measured as described previously. These area-detector data were processed with the *XDS* package (Kabsch, 1988*a*,*b*). In all 38 580 reflections were measured for the Pb^{II}–PBGS crystal to give 10 466 unique data with $R_{sym} = 0.096$. The data are 84% complete to 3.2 Å resolution.

4. Discussion

The first crystals of bovine PBGS were reported by the late David Shemin in a paper which demonstrated that bovine PBGS is an octamer of 35 kDa subunits (Shemin, 1976). Electron microscopy showed the subunits to be arranged at the corners of a cube (Wu, Shemin, Richards & Williams, 1974). Each subunit appeared spherical with a diameter of 44 Å (Shemin, 1976). It was also established that each octamer contains only four functional active sites (Bevan, Bodlaender & Shemin, 1980), a fact which we and others have confirmed (Jaffe & Hanes, 1986; Beyersmann, 1986).

Bovine PBGS forms microcrystals rather readily, but these crystals are typically not suitable for diffraction studies. We find that freshly purified bovine PBGS contains product bound at about 30% of the active sites. This lack of uniformity in the composition of active sites may account for a general failure to obtain good quality crystals for the native holoenzyme. The 5-CLA-modified enzyme form which is reported here contains added substrate analog (5-CLA) in order to ensure that the active sites are uniform. This 5-CLA-modified protein contains no porphobilinogen (product), but the binding site for the ALA molecule which becomes the propionyl half of porphobilinogen is vacant (Jaffe, Abrams, Kaempfen & Harris, 1992). The active sites of the Pb^{II}–PBGS are vacant, and we have shown that product cannot bind to this modified enzyme (Jaffe, 1995).

Small-angle X-ray scattering data provided some more detail about the quaternary structure of bovine PBGS (Pilz, Schwarz, Vuga & Beyersmann, 1988). The resulting model is a square arrangement of four stacks, each stack composed of two monomeric subunits with an edge of 97 Å for the square; the height of the stacks is 70 Å. This corresponds to a V_m measurement of 2.5 Å^3 Da^{-1}. Each stack of two subunits was interpreted in this model to represent one catalytic unit (one active site).

The early electron micrographs, the small-angle X-ray scattering data, and much physical and chemical data on PBGS from a variety of species (Wu, Shemin, Richards & Williams, 1974; Pilz, Schwarz, Vuga & Beyersmann, 1988) led us to propose a general model for the quaternary structure of PBGS (Jaffe, Ali, Mitchell, Taylor, Volin & Markham, 1995). This model depicts four pairs of subunits arranged around a fourfold symmetry axis. The relationship between the two subunits in the dimer is now indicated by the self rotation function,

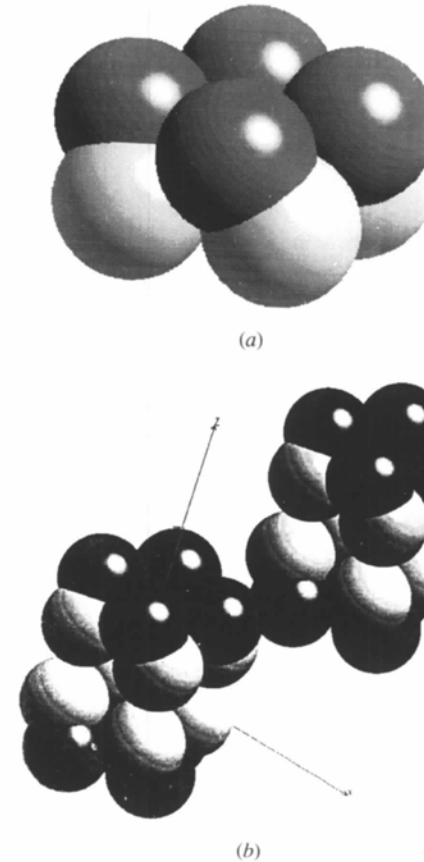

Fig. 2. A model of the quaternary structure of PBGS showing (*a*) one octamer where each monomer is represented by a sphere of diameter 44 Å (derived from the EM data); the two monomers of a functional dimer (depicted as one light and one dark sphere) overlap to give the octamer the dimensions ~90 × 90 × 70 Å consistent with the small-angle X-ray data. The placement of the non-crystallographic twofold axis and the angle of rotation about that axis is set arbitrarily to optimize the packing of the octamers in the cell as illustrated in (*b*). These drawings were prepared using the program *Cerius2 Visualizer* (Molecular Simulations, 1994).

using the program *ALMN* from the *CCP*4 program package (Collaborative Computational Package, Number 4, 1994), to be a non-crystallographic twofold rotation axis perpendicular to the *c* axis and 22.5° from the *a* or *b* axis. A model of the octamer can be constructed in such a way as to be consistent with the model proposed by Pilz, Schwarz, Vuga & Beyersmann (1988) and with the current single-crystal diffraction data. Fig. 2 illustrates such a model of the octameric molecule as well as the packing of the octamer.

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