Structural Analysis of Prostaglandin H Synthase-1 Crystals

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Introduction

Prostaglandin H synthase (PGHS) catalyzes the committed step in the biosynthesis of prostaglandins, including prostacyclin and thromboxanes [1]. The PGHS isozymes 1 and 2 are integral membrane enzymes of the endoplasmic reticulum and nuclear envelope that convert arachidonic acid (AA; a 20:4 fatty acid) into prostaglandin H_2 (PGH_2) in two sequential reactions. The PGHS isozymes are also the targets of action for nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin and ibuprofen. The first step of the conversion of AA to PGH_2 takes place in the cyclooxygenase (COX) active site, where two molecules of oxygen insert into AA to form the bicyclic peroxide intermediate PGG_2. The monotopic membrane binding domain (MBD) of PGHS not only anchors the enzyme to the bilayer but also forms the mouth of the COX active site [1]. This structural feature allows AA to enter from the hydrophobic compartment of the bilayer directly into the COX active site — a long (25-Å), hydrophobic channel that extends from the MBD to Tyr 385 [1]. How AA binds within the active site of native PGHS-1 is now known [2], and the crystal structures of native PGHS-1 complexed with other fatty acids have also been determined [3, 4]. These structural results arose from the previous runs at the APS.

We have continued this work and are focusing on interpreting mutagenic studies [3-5] that could give us a much clearer view of the variation of substrate binding in PGHS-1 and its impact on the biosynthesis of series 1, 2, and 3 prostaglandins. However, all the crystal structures to date do not reveal the conformational transitions that must occur during substrate binding. We are now focusing on obtaining crystals of ovine PGHS-1 and selected mutants that would allow us to investigate, at higher resolution, the conformational aspects of catalysis in PGHS. We have recently developed crystallization protocols for native and recombinant ovine PGHS-1 that should allow us to reach that goal.

Methods and Materials

Conditions that produce two new crystal forms (Types III and IV) of ovine PGHS-1, reconstituted with either heme or cobalt-protoporphyrin IX, have been identified (Figs. 1 and 2). Type III crystals grow at 1.0 M potassium tartrate (pH 8.4), 100 mM imidazole, 200 mM NaCl, and 0.5% octyl glucoside; this form has not yet been characterized. The Type IV crystal form, which grows in 2.0-2.5 M sodium formate, is a very interesting variant of the Type II ($P6_522$): While the packing of the PGHS-1 dimers is the same in the unit cell, the hexagonal symmetry is broken, and the space group is now C2 (a = 182.2 Å, b = 315.7 Å, c = 104 Å, beta = 90.01). We can flash-freeze these crystals at 120K and have collected data to 2.5-Å resolution. The successful data sets show that Type IV PGHS-1 crystals have low mosaicity (0.6-0.8°).

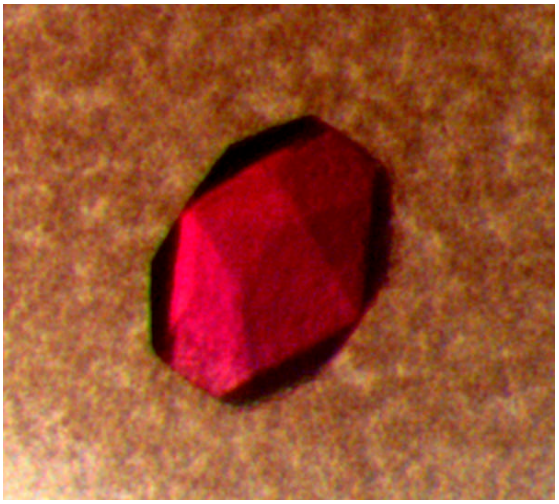

FIG. 1. A Type III crystal of ovine PGHS-1.

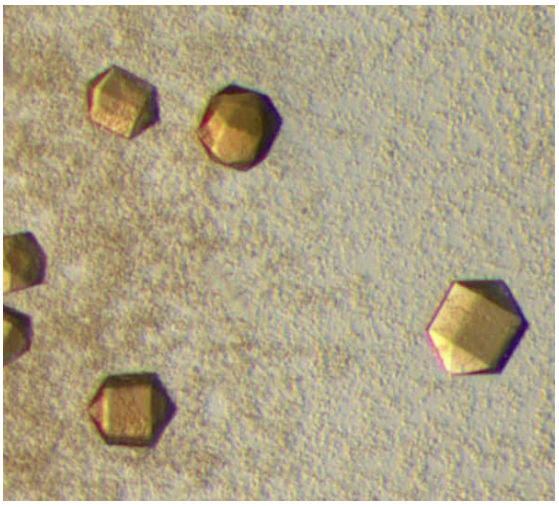

FIG. 2. Type IV crystals of ovine PGHS-1.

Results and Discussion

Although Type IV crystals have three dimers in the asymmetric unit (~29,000 nonhydrogen atoms), these crystals routinely diffract to at least 2.6-Å resolution. The best diffraction we observe has been out to 2.44-Å resolution. Precise and reproducible methods for freezing these crystals have not been worked out. Thus, higher-resolution diffraction may be obtainable. Moreover, Type IV crystals are more tolerant to buffer changes. We have been able to soak hydrogen peroxide into these crystals and retain diffraction to at least 2.9-Å resolution. We can now explore the initial stages of free radical damage to PGHS-1 during the peroxidase reaction.

We have collected data sets on several Type IV crystals liganded with the nonsteroidal anti-inflammatory drugs (NSAIDs) flurbiprofen, meloxicam, and SC-560. Refinement of these structures is underway, but early results suggest that the Type II $P6_522$ symmetry is subtly broken in the Type IV crystals due to drug-induced conformational changes *at the peroxidase active site*. This is the first time such conformational transitions have been observed. Preliminary results also suggest that mutant PGHS-1 will also crystallize in the Type IV crystal form. These new crystals may allow us to explore the structural changes, at high resolution, that occur throughout the enzyme as fatty acids or NSAIDs bind to the COX active site.

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