Structure and Mechanistic Implications of a Uroporphyrinogen III Synthase−Product Complex[†,‡]

Heidi L. Schubert,*[,§] John D. Phillips,[||] Annie Heroux,[⊥] and Christopher P. Hill[§]

Departments of Biochemistry and Internal Medicine, School of Medicine, University of Utah, Salt Lake City, Utah 84112, and Department of Biology, Brookhaven National Laboratory, Upton, New York 11973-5000

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ABSTRACT: Uroporphyrinogen III synthase (U3S) catalyzes the asymmetrical cyclization of a linear tetrapyrrole to form the physiologically relevant uroporphyrinogen III (uro'gen III) isomer during heme biosynthesis. Here, we report four apoenzyme and one product complex crystal structures of the *Thermus thermophilus* (HB27) U3S protein. The overlay of eight crystallographically unique U3S molecules reveals a huge range of conformational flexibility, including a "closed" product complex. The product, uro'gen III, binds between the two domains and is held in place by a network of hydrogen bonds between the product's side chain carboxylates and the protein's main chain amides. Interactions of the product A and B ring carboxylate side chains with both structural domains of U3S appear to dictate the relative orientation of the domains in the closed enzyme conformation and likely remain intact during catalysis. The product C and D rings are less constrained in the structure, consistent with the conformational changes required for the catalytic cyclization with inversion of D ring orientation. A conserved tyrosine residue is potentially positioned to facilitate loss of a hydroxyl from the substrate to initiate the catalytic reaction.

Tetrapyrrole cofactors such as heme, chlorophyll, cobalamin (vitamin B12), siroheme and coenzyme F430 are essential for oxygen transport, electron transport, photosynthesis, methionine synthesis in mammals, nitrite and sulfite assimilation, and methane production in methanogens. Tetrapyrrole cofactors share a multistep, branched biosynthetic pathway (*1, 2*). In mammals, the initial steps include the reaction of glycine and succinyl-CoA to produce 5-aminolevulinic acid (ALA[1]), condensation of two ALA molecules to form the basic pyrrole structure (porphobilinogen, PBG), and assembly of four PBG pyrroles into a linear tetraypyrrole (hydroxymethylbilane, HMB). Subsequently, uroporphyrinogen III synthase (U3S; E.C. 4.2.1.75) cyclizes HMB to produce uroporphyinogen III (uro'gen III), the last common precursor of all tetrapyrrole cofactors. Remarkably, U3S catalyzed cyclization occurs with inversion of the fourth pyrrole ring (D ring) to form the III isomer of uroporphyrinogen. As illustrated in Figure 1, the U3S catalyzed reaction is believed to proceed via loss of substrate hydroxyl to form an initial azafulvene intermediate, which proceeds to a spiropyrrolenine transition state in which carbons 16 and 20 form a covalent bond. A second azafulvene intermediate is resolved by bond formation between substrate carbons 19 and 15 to produce the uro'gen III product (*3–5*). In the scheme, the D ring of the proposed spiro-pyrolenine transition state is excluded from the primary ring by connection of the A−D and C−D bridge carbon atoms through a single carbon atom of the D ring, C16. In support of this mechanism, a spirolactam derivative of the proposed transition state (Figure 1) has been shown to competitively inhibit the enzyme (*6*).

Defects in tetrapyrrole biosynthetic enzymes are associated with human disease, and mutations in U3S cause congenital erythropoietic porphyria (CEP), which is transmitted as an autosomal recessive trait (*7, 8*). In the absence of U3S activity, HMB autocyclizes to uroporphyrinogen I, which is released from cells and is fully oxidized to uroporphyrin I, where it reaches elevated levels in the urine and defines the biochemical manifestation of the disease. The severe phenotype associated with CEP was one of the first disorders of porphyrin biosynthesis described by Günther in 1911, and is often referred to as Günthers disease (*9*). The disorder has been described in multiple ethnic groups with approximately 200 cases reported worldwide. The developing erythroblast is the primary site for the overproduction of porphyrins and fluorescence of the marrow is a uniform finding. The defective erythroblasts are fragile and prone to peripheral hemolysis leading to varying degrees of anemia and splenomegaly. Homozygotes or compound heterozygotes with a phenotype of CEP generally have less than 10% of normal U3S activity; however, patients with mild signs of

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[‡] Data deposition: Model coordinates and structure factors were deposited in the Protein Data Bank, www.rcsb.org (PDB ID codes 3D8N, 3D8R, 3D8S, 3D8T).

* To whom correspondence should be addressed. Tel: 801-585-9776. Fax: 801-581-7959. E-mail: heidi@biochem.utah.edu.

[§] Department of Biochemistry, University of Utah.

[||] Department of Internal Medicine, University of Utah.

[⊥] Brookhaven National Laboratory.

[1] Abbreviations: U3S, uroporphyrinogen III synthase; uro'gen III, uroporphyrinogen III synthase; ALA, 5-aminolevulinic acid; PBG, porphobilinogen; HMB, hydroxymethylbilane, RMSD, root-mean-square deviation; PDB, Protein Data Bank.

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