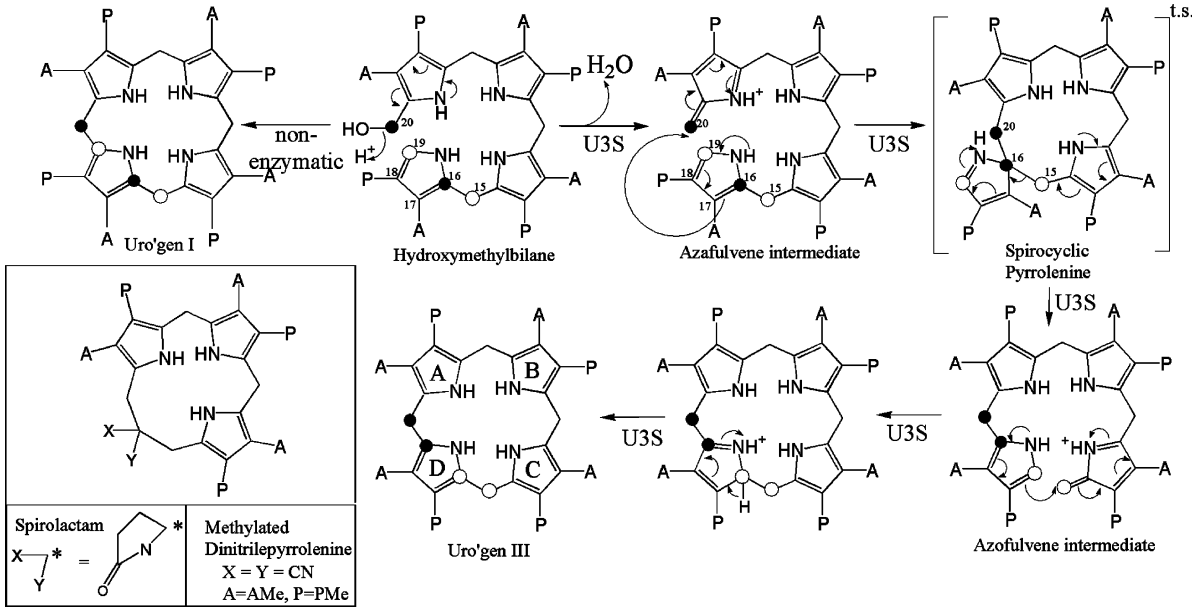

FIGURE 1: The proposed mechanism of U3S catalyzed formation of uro'gen III (*3*). This figure resembles that of Roessner et al. (*33*). Spirolactam, a transition state mimic, has been shown to be a competitive inhibitor of U3S (*36*), and the structure of a synthetic precursor, methylated dinitrilepyrrolenine, has been determined (*6*). A = CH_2CO_2, P = $CH_2CH_2CO_2$.

CEP may have up to 20% of normal activity (*10, 11*). Severe photosensitivity is common in patients, with skin fragility and cutaneous infections leading to photomutilation (*12*). The majority of known point mutations are expected to reduce enzyme activity by interfering with protein stability, such as by disrupting the packing of the hydrophobic core or by exposing hydrophobic residues to the solvent (*13*).

The current level of structural understanding of the U3S mechanism is provided by crystal structures and mutagenic/biochemical analysis of the human enzyme (*13*), and by two *Thermus thermophilus* (strain HB8) structures that were recently deposited in the Protein Data Base (*14*) (PDB codes 1WCW and 1WD7) by the RIKEN Structural Genomics/Proteomics Initiative (*15*). The U3S structure comprises two α/β domains that each contain a central 5-stranded β-sheet flanked by α-helices; although the two domains possess similar folds, they differ in the position and angles of the helices. The two strands connecting the domains, which are presumably flexible in solution, adopt an extended β-sheet conformation in the human U3S crystal structure and display a range of less regular conformations in the *T. thermophilus* structures. Consistent with these structures, it has been proposed that the active site lies between the domains and that a significant conformational change might occur upon substrate binding (*13*).

In an effort to better understand the mechanism of U3S we set out to obtain a ligand protein complex. This effort failed using the human enzyme, but we did succeed in crystallizing and determining the structure of *T. thermophilus* (strain HB27) U3S (TtU3S) in complex with the reaction product, uro'gen III. In addition, we determined additional structures of apo *T. thermophilus* U3S, which collectively display an impressive (90°) range of interdomain angles. The uro'gen III product is seen to bind between the two domains, and specific interaction of the ligand carboxylate groups suggests that the defined closed conformation is formed in both substrate and product complexes. There is a notable lack of interactions between the enzyme and the product's pyrrole rings, and the dominant use of enzyme main chain groups for hydrogen bonding interactions is consistent with the apparent lack of conserved active site residues (*13*). The precise coordination of A and B pyrrole carboxylate groups and solvent exposure of the D ring carboxylates is consistent with the model that the enzyme functions by restricting substrate conformations to productive orientations while allowing the D ring to rotate.

MATERIAL AND METHODS

The gene encoding the *T. thermophilus* (strain HB27) U3S (TtU3S) was obtained from a genomic DNA library (ATCC) and cloned into pET151/D-TOPO for expression with an N-terminal His-tag followed by a TEV protease cleavage site immediately preceding the full open reading frame. Recombinant protein expression in BL21(DE3)pLysS codon plus cells occurred by autoinduction (*16*). Protein was extracted from clarified whole cell lysate and purified over a Ni^{2+} affinity column (Qiagen). TEV cleavage was performed at room temperature overnight during dialysis into 20 mM Tris-HCl, 100 mM NaCl, pH 7.0 (all pH values were determined at room temperature, and all chromatography columns were run at 4 °C). Following size exclusion chromatography on a S200 column (Pharmacia) equilibrated in the same buffer supplemented with 1 mM DTT, the purified protein was concentrated to 8−12 mg·mL^{-1} for crystallization trials.

Four crystal structures were obtained: three apo (TtU3S1, TtU3S2, TtU3S3) and one product complex. The crystals typically grew in clusters that required manipulation to extract single crystals for data collection. All crystals were grown by vapor diffusion in sitting drop trays by mixing 2 μL of protein with 2 μL of precipitant solution and equilibrating the drop over 1 mL of precipitant solution. Small apo crystal forms grew at 4 °C within 1−7 days over the following reservoir solutions: TtU3S1, 1.2 M $NaPO_4$, 0.8 M K_2PO_4 (~20 μm × 50 μm × 100 μm; deltoid shape);