Table 1: Data Collection Statistics

	TtU3S1	TtU3S2	TtU3S3	product complex
space group	$P3_1$	$P2_12_12_1$	$P2_1$	$P2_1$
cell dimens	$a = b = 63.83$	$a = 40.86$	$a = 40.75$	$a = 35.74$
		$b = 63.24$	$b = 89.96$	$b = 66.48$
	$c = 58.58$	$c = 90.68$	$c = 74.98$	$c = 55.46$
			$\beta = 101.32$	$\beta = 91.76$
no. of obs refs	147186	75146	244799	73535
no. of unique refs	38923	14308	65660	19239
high res (Å)	2.07−2.0	2.12−2.0	1.66−1.60	1.97−1.90
completeness (%)	99.9 (100)	99.(100)	94.2(59.8)	94.4(66.7)
R_{sym}[a] (%)	8.6 (42.5)	9.1(49.5)	4.1 (35.1)	7.5(54.8)
av $I/\sigma(I)$	39.5 (7)	18.4(3.3)	30.3(2.2)	31.7(2.3)
mosaicity (deg)	0.2	0.47	0.35	0.73

[a] $R_{sym} = \sum|I - \langle I\rangle|/\sum I$, where $\langle I\rangle$ is the average intensity from multiple observations of symmetry related reflections.

Table 2: Refinement Statistics

	TtU3S1	TtU3S2	TtU3S3	uro'gen III complex
resolution range (Å)	30.0−2.0	30−2.0	30−1.6	30.0−1.9
no. of protein atoms	2005	1944	4055	1950
no. of solvent molecules	225	115	466	124
Nn. of ligand molecules	4 SO4	1 GOL	0	1 UR3
R_{factor} (%)[a]	18.0	20.4	18.9	19.3
R_{free} (%)	23.2	25.9	23.6	24.0
rmsd (bond lengths) (Å)	0.011	0.01	0.014	0.011
rmsd (bond angles) (deg)	1.24	1.22	1.41	1.39
$\langle B\rangle$ (Å^2)				
main chain	18.7	27.3	21.6	31.3
side chain	21.9	29.8	24.9	34.2
water molecules	35.7	34.3	31.4	41.7
ligands	50.2	44.9	-	48.3
no. of Φ/Ψ angles (%)				
most favored	94.8	93.8	94.2	92.8
allowed	5.2	5.8	5.7	7.2

[a] $R_{factor} = \sum||F_o| - |F_c||/\sum|F_o| \times 100$ over 95% of the data. R_{free} = same calculation on 5% data not used in refinement.

TtU3S2, 21−24% PEG MW 8000, 0.2 M Mg acetate, 0.1 M MES-HCl, pH 6.5, 0.2 M NaCl (100 μm × 200 μm; rectangular plate clusters, very thin in the third dimension); TtU3S3, 18−24% PEG MW 8000, 0.1 M Tris-HCl, pH 8.5, 0.2 mM $MgCl_2$ (30 μm × 30 μm × 100 μm; rod clusters).

Uro'gen III was produced by chemical reduction of uroporphyrin III (Frontier Scientific, Logan, UT) using a method developed by H. Bergoniam and J.D.P. (in preparation). Briefly, 2 mg of uroporphyrin III was resuspended in 200 μL of H_2O and 1,800 μL of methanol. The mixture was added to 5 mg of 10% palladium on activated carbon (PdC, Aldrich, St. Louis, MO) in a hydrogen atmosphere and stirred for 40−60 min. The PdC was removed using a glass fiber filter. The filtrate was dried under argon gas at 60 °C. The reduced uro'gen III was resuspended in 100 μL of 10 mg·mL^{-1} TtU3S in 20 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, pH 7.0 prior to setting down crystal trays. Despite multiple attempts, only one drop containing the product complex crystals was obtained. The crystals (~30 μm × 30 μm × 30 μm; cube) grew anaerobically (using degassed solutions and incubated in an anaerobic chamber) over a reservoir of 28% PEG MW8000, 0.1 M MES-HCl, pH 6.5, 0.2 M Mg acetate at 16 °C in 4 days.

All crystals were cryoprotected in a solution of the precipitant made up with 20% glycerol and cryocooled by plunging into liquid nitrogen prior to data collection on beamlines X25 and X29 at the National Synchrotron Light Source, Brookhaven National Laboratory. Data were processed using the HKL suite of programs (*17*). Molecular replacement was performed with PHASER (*18*) using the *T. thermophilus* (strain HB8) U3S structure deposited to the PDB by the RIKEN Structural Genomics/Proteomics Initiative (pdb code 1WD7) (*15*). Solutions for all structures were only found when the two α/β domains of U3S were separated into independent search models. The β-strand linker was rebuilt in all structures using O (*19*), and final modeling and structure validation were performed using the program COOT (*20*). The uro'gen III ligand and corresponding refinement constraints were built using the SKETCHER module of CCP4 (*21*). All refinements, including 10 segment TLS (*22*) refinements, were performed using REFMAC (*23, 24*). Crystallographic statistics are given in Tables 1 and 2.

RESULTS AND DISCUSSION

Structures of T. thermophilus U3S. The structure of apo U3S from *T. thermophilus* (HB27) (TtU3S) was determined in three different crystal forms, two of which contained one molecule in the asymmetric unit and one of which contained two molecules in the asymmetric unit, to give a total four crystallographically independent views of the unliganded enzyme. A crystal structure of TtU3S in complex with uro'gen III (product) was also determined. Attempts to obtain a complex between spirolactam (a kind gift of Alan Spivey) and the human U3S failed as did attempts to form a complex between *T. thermophilus* U3S and PBG. Curiously, all of the *T. thermophilus* crystal forms grew from very similar conditions, and the drops of apo enzyme often contained more than one form. The different apo and product complex structures were refined against data collected to 1.6−2.0 Å with R_{free} values of 18.0−20.4% and good geometry (Tables 1, 2).

As expected, TtU3S adopts the same overall fold as previously determined U3S structures (PDB codes 1JR2, 1WD7, and 1WCW) (*13, 15*). The first 8 and last 4 residues are generally disordered. Domain 1 comprises residues 8−39 and 168−260, domain 2 comprises residues 47−161, and the two domains are connected by two linker sequences comprising residues 40−46 and 162−167. The structures of domains 1 and 2 are each highly conserved between the different U3S structures. The overlap of multiple individual domains for the five unique TtU3S (HB27) molecules using the maximum likelihood approach and the program THESEUS (*25*) yields root-mean-square deviations (RMSDs) of 0.46 Å (domain 1; 123 Cα) and 0.77 Å (domain 2; 114 Cα), respectively. TtU3S (HB27) is 98% identical to TtU3S (HB8) with only 4 amino acid substitutions, all of which are within the first 10 residues. Pairwise comparisons typically display RMSDs of 0.4 Å and 0.6 Å for overlaps on domain 1 and domain 2, respectively. Despite a sequence identity between the *T. thermophilus* and human enzymes of only 14%, the structural alignment has a *Z*-score of 14.8 using the program DALI (*26*), and RMSDs on structurally equivalent residues of domain 1 (115/121 Cα) and domain 2 (113/127 Cα) are 2.7 Å and 2.5 Å, respectively.

The short linker segments between the two domains form an antiparallel β-ladder in the maximally extended human enzyme, but adopt less regular conformations in the TtU3S structures and are often poorly ordered in the apo structures. Linker flexibility facilitates an impressive array of relative