Supporting Online Material

Crystal Structure of Biotin Synthase, an *S*-Adenosylmethionine-Dependent Radical Enzyme

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Materials and methods

ecBioB was purified and anaerobically reconstituted in the presence of AdoMet and DTB as previously described (*1*). Crystals were grown using hanging drop vapor diffusion techniques at room temperature in a Coy anaerobic chamber under a 95% Ar / 5% H_2 atmosphere. Protein solution (20 mg/mL ecBioB, 25 mM Tris hydrochloride pH 8.0) was mixed in a 2:1 ratio with precipitant solution (0.1 M Tris hydrochloride, 0.1 M glycine, 0.2 M $MgCl_2$, 20% w/v polyethylene glycol 1000; final pH ~6.5) and equilibrated over a well solution of 0.5 mL 2.5 M NaCl. Crystals were cryoprotected by soaking in precipitant solution with 20% glycerol added, and belong to spacegroup $P3_121$, with 2 molecules per asymmetric unit (ASU), and a = b = 155.69, c = 90.88 Å. Data were collected at the Advanced Light Source beamline 5.0.2 and the National Synchrotron Light Source beamline X25, both equipped with a charge-coupled device detector (Area Detector Systems Corp). Data were processed and scaled with DENZO, SCALEPACK, and XDS (*2, 3*). MAD data were collected using an inverse beam method, and the Fe sites were found with CNS (*4*) and refined with SOLVE (*5*). The experimental electron density map was subjected to solvent-flattening and two-fold non-crystallographic symmetry

averaging in DM (*6*), resulting in a 3.7 Å resolution map of high enough quality to begin model building. Iterative rounds of model building in O (*7*) and refinement in CNS resulted in the final model at 3.4 Å resolution. The refined structure of the dimeric enzyme contains residues 4-315 (molecule A) and 3-315 (molecule B), two DTB, two AdoMet, two Fe_4S_4 clusters, two Fe_2S_2 clusters, and one Tris molecule. There is no electron density for the N-terminal histidine tag, the first 2 or 3 residues at the N-terminus and the last 31 residues at the C-terminus. The final model has 99.3 % of all residues residing in the allowed region of the Ramachandran plot with 79.8% (molecule A) and 79.8% (molecule B) in the most favored region, 18.3% (molecule A) and 17.9% (molecule B) in the additional allowed regions, 1.5% (molecule A) and 1.5% (molecule B) in generously allowed regions, and 0.7% (molecule A) and 0.5% (molecule B) in disallowed regions, as calculated by PROCHECK (*8*). One of the residues in the disallowed regions, Asn311, is at the conformationally flexible C-terminus. The other, Asp155, is in position to interact with the ribose moiety of AdoMet, and therefore its unusual geometry is likely to be functionally significant.

Supplemental References

1. N.B. Ugulava, K.K. Frederick, J.T. Jarrett, *Biochemistry* **42**, 2708 (2003).
2. W. Kabsch, *J. Appl. Crystallogr.* **26**, 795 (1993).
3. Z. Otwinowski, W. Minor, *Meth. Enzymol.* **276**, 307 (1997).
4. A.T. Brunger *et al.*, *Acta Crystallogr.* **D55**, 905 (1998).
5. T.C. Terwilliger, J. Berendzen, *Acta Crystallogr.* **D55**, 849 (1999).
6. K.D. Cowtan, K.Y.J. Zhang, *Prog. Biophys. Mol. Biol.* **72**, 245 (1999).
7. T.A. Jones, J.-Y. Zou, S.W. Cowan, M. Kjeldgaard, *Acta Crystallogr.* **A47**, 110 (1991).
8. R.A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, *J. Appl. Crystallogr.* **26**, 283 (1993).

Table S1. Data collection and refinement statistics

Wavelength (Å)	Resolution range (Å)	Unique reflections	Redundancy	Completeness (%)	I/sigma(I)	R_{sym}
1.30000	100 - 3.7	26,018	3.4	99.5 (100.0)	11.8 (2.3)	6.7 (37.5)
1.73827	100 - 4.1	19,002	3.1	98.3 (99.7)	14.6 (1.9)	6.4 (29.7)
1.74150	100 - 4.5	14,030	2.8	97.1 (95.7)	14.0 (2.6)	8.2 (36.7)
1.10000*	100 - 3.4	17,465	5.4	98.1 (87.9)	15.79 (3.78)	6.6 (25.9)

Non-hydrogen atoms in ASU	4,984	
Resolution range (Å)	44.5 - 3.4	
Number of reflections (working set / test set)	16,222 / 1242	
R_{work} (%)	25.6	
R_{free} (%)	30.0	
RMS deviations of protein from ideal geometry		
Bonds (Å)	0.009	
Angles (°)	2.3	
	Molecule A	Molecule B
Overall *B* (Å^2) (all atoms)	87.8	93.3
Group *B* (Å^2)		
AdoMet		
Methionine	130.5	146.3
Ribose	84.6	84.6
Adenine	68.3	88.6
Dethiobiotine	47.4	61.8
Fe_4S_4	50.8	71
Fe_2S_2	35.7	43

$R_{sym} = [\sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|] / \sum_{hkl}\sum_i I_i(hkl)$ for *n* independent reflections and *i* observations of a given reflection, with no σ cutoff. $\langle I(hkl)\rangle$ is the average intensity of the *i*th observation. Values for data in the highest resolution bin are listed in parentheses. *For this wavelength, Friedel pairs were merged during data processing.