N10-formyltetrahydrofolate synthetase

R. Radfar[1], W. Minor[2], and L. Lebioda[1,*]
[1]University of South Carolina, Columbia, SC 29208 USA
[2]University of Virginia, Charlottesville, VA 22906 USA

Introduction

Tetrahydrofolate (THF) and its derivatives are the biologically active forms of folic acid, a four-electron oxidized form of THF. They are specialized cosubstrates for a variety of enzymes involved in one-carbon metabolism. THF reacts in an ATP-dependent manner with formate in the following reaction catalyzed by formyltetrahydrofolate synthetase (FTHFS) (EC 6.3.4.3).

HCOOH + MgATP + THF ->
10-formyl-THF + MgADP + Pi

The catalyzed reaction proceeds via the formation of a tightly bound formylphosphate intermediate produced by nucleophilic attack of formate on the γ-phosphate of MgATP. Subsequently, the N10 of THF affords nucleophilic attack on the formyl group of formyl phosphate resulting in the formation of the final product (10-formyl-THF) and the release of products. The enzyme, present at high levels in the acetogenic and purinolytic bacteria, is involved in a C1 carbon fixation process for cellular biosynthesis.

Methods and Materials

Data from multiwavelength anomalous diffraction (MAD) phasing of the Se-Met FTHFS were collected at the Structural Biology Center (SBC-CAT) beamline at the Advanced Photon Source (APS), Argonne National Laboratory (ANL). Data sets where collected at four different wavelengths, the low- and high-energy remote, the inflection point, and the peak of f". The data were collected as 1º oscillation frames from a single crystal frozen at 100 K. Cryogenic conditioning of the Se-Met FTHFS crystals was achieved by soaking the crystals in 22% glycerol-enriched mother liquor. All data were processed with the HKL 2000 system. There are 14 (seleno)methionines containing anomalous scatterers per subunit of *M. thermoacetica* FTHFS; two subunits per asymmetric part of the unit cell make it a 28-atom problem. To obtain the positions of Se atoms the four-wavelength data were combined together using the local scaling option of the SOLVE package. The estimates of structure factors of the anomalous scatterers (Fa's) were used as the input to the SHELXD program and yielded the positions of all 28 Se atoms. The average deviations of the original positions of the Se atoms from those from the final model were 0.25 Å for subunit A and 0.46 Å for subunit B. The preliminary MAD phases were calculated by the maximum-likelihood algorithm implemented in MLPHARE using the Se sites. Solvent flattening and histogram matching were applied to improve the initial phases using the DM program from the CCP4 package. The structure refinement was carried against the data set collected at 1.04189 Å, which had the best statistics.

Results

The reported FTHFS model for two subunits contains 8264 atoms, 11 sulfates, 2 cis-prolines (residue 121), and 269 water molecules.

The subunit of N10-formyltetrahydrofolate synthetase is composed of three domains organized around three mixed β-sheets. There are two cavities between adjacent domains. One of them was identified as the nucleotide binding site by homology modeling. The large domain contains a seven-stranded β-sheet surrounded by helices on both sides. Second domain contains five-stranded β-sheet with two α-helices packed on one side while the other two are a wall of the active site cavity. The third domain contains a four-stranded β-sheet forming a half barrel. The concave side is covered by two helices while the convex side is another wall of the large cavity. Arg 97 is likely involved in formylphosphate binding. The tetrameric molecule is relatively flat with the shape of the letter X, and the active sites are located at the end of the subunits far from the subunits interface.

Discussion

In order to determine the nucleotide binding site in the structure, the ATP binding domain of *Escherichia coli* ATPase (28) (residues 163–185) and *Azotobacter vinelandii* nitrogenase Fe protein (25) (residues 3–26) were superimposed onto FTHFS (residues 58–85). The superposition allowed us to establish the location of the active site and identification of the sulfate binding site that corresponds to the formylphosphate binding position. We were also able to propose the site for the monovalent cation binding and propose its role in the enzyme thermostability [1].

Acknowledgments

This work was supported by NSF grant MCB-9873606. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

*(crystallography)

References

[1] R. Radfar *et al*., *Biochemistry*, (in press).