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CRYSTAL STRUCTURE OF METHYLTETRAHYDROFOLATE: CORRINOID/IRON-SULFUR PROTEIN METHYLTRANSFERASE FROM *CLOSTRIDIUM THERMOACETICUM* AT 2.2 Å RESOLUTION

by

Tzanko I. Doukov

A DISSERTATION

Presented to the Faculty of

The Graduate College at University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Chemistry

Under the Supervision of Professors: John J. Stezowski and Stephen W. Ragsdale

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DISSERTATION TITLE

Crystal Structure of Methyltetrahydrofolate:Corrinoid/Iron-Sulfur Protein Methyltransferase from Clostridium thermoaceticum at 2.2 A Resolution

BY

Tzanko Iordanov Doukov

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GRADUATE COLLEGE
UNIVERSITY OF NEBRASKA

CRYSTAL STRUCTURE OF METHYLTETRAHYDROFOLATE: CORRINOID/IRON-SULFUR PROTEIN METHYLTRANSFERASE FROM *CLOSTRIDIUM THERMOACETICUM* AT 2.2 Å RESOLUTION

Tzanko I. Doukov, PhD.

University of Nebraska, 1999

Advisors: John J. Stezowski and Stephen W. Ragsdale

The cytoplasmic methyltetrahydrofolate: corrinoid/iron-sulfur protein methyltransferase (MeTr) is a key protein in the Wood-Ljungdahl pathway of CO_2 fixation. It reversibly transfers the N^5– methyl group from methyltetrahydrofolate (CH_3-H4folate) to the Co(I) nucleophilic center of either free cob(I)alamin or its natural acceptor, the corrinoid/iron-sulfur protein in the reductive acetyl-CoA pathway for CO_2 fixation. No crystal structure of a methyltetrahydrofolate methyltransferase has been determined to date. The MeTr structure was determined at 2.2 Å resolution by multiwavelength anomalous diffraction methods. The overall architecture of MeTr is a TIM barrel. This represents a new functional class (number 20) of the versatile TIM barrel fold. The MeTr structure is surprisingly similar to the crystal structures of dihydropteroate synthetases despite sharing less than 20 % sequence identity. This includes extensive conservation of the pterin ring binding residues (D43, D75, N96, D160) in the bottom of the polar active sites of the methyltransferases and dihydropteroate synthetases. The biggest structural difference between these enzymes is in a loop structure above the active site. It is quite open for MeTr, suggesting a probable

cobalamin (or corrinoid) binding site. Such structural solution fits a general trend for cobaimide enzymes. A TIM barrel embeds the relatively unreactive substrate and the cobamide, bound to other protein moiety (subunit), closes the C-terminus top of the barrel forming an isolated reaction cavity. Our results are consistent with either a "front" or "back" side protonation of CH_3-H_4folate, a key step in the mechanism of MeTr.

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LIST OF ABBREVIATIONS

<I>	average structure factor intensity
Å	Ångstrom
AdoMet	S-adenosyl-L-methionine (see SAM)
Ala, A	alanine
Arg, R	arginine
Asn, N	asparagine
Asp, D	aspartic acid
avg.	average
bp	base pairs
BDE	bond dissociation enthalpy
C_α	alpha carbon
CC	correlation coefficient
CCP4	Collaborative Computational Project, Number 4
C/Fe-SP	corrinoid/iron-sulfur protein
CFe-SP	corrinoid iron-sulfur protein
CoFe-SP	corrinoid iron-sulfur protein
CH_3-B_{12}	methylcobalamin
CH_3-H_4F	N^5-methyltetrahydrofolate
CH_3-H_4MPT	N^5-methyltetrahydromethanopterin
CH_3-H_4SPT	N^5-methyltetrahydrosarcinopterin
CoA	coenzyme A
CoB	coenzyme B
CODH	carbon monoxide dehydrogenase
CoM	coenzyme M
Cys, C	cysteine
DOE	Department of Energy
DTT	dithiothreitol
E	normalized structure factor amplitude
eV	electron Volt
f'	dispersive component of the atomic scattering factor (real)
f''	anomalous component of the atomic scattering factor (imaginary)
F^+, F^-	structure factor amplitudes of Bijvoet related reflections
FAD	flavin adenine dinucleotide
F_c	calculated structure factor amplitude
F_H	calculated heavy atom structure factor amplitude
F_o	observed structure factor amplitude
f^0	normal atomic scattering factor
FOM	figure of merit
F_P	native structure factor amplitude
F_{PH}	heavy atom derivative structure factor amplitude
g	gram

Gln, Q	glutamine
Glu, E	glutamic acid
Gly, G	glycine
H_4F	tetrahydrofolate (see also THF)
HEPES	N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonate]
His, H	histidine
hr	hour
I_{calc}	calculated structure factor intensity
Ile, I	isoleucine
I_{obs}	observed structure factor intensity
I_{obs-d}	observed detwinned structure factor intensity
K	temperature Kelvin
k	kilo
kb	kilo base pairs
K_{cat}	rate of catalysis
kDa	kilo-dalton
K_d	equilibrium dissociation constant
K_m	Michaelis-Menton constant
KPi	potassium phosphate
L	liter
Leu, L	leucine
Lys, K	lysine
M	molar (concentration)
μ	micro
m	milli; meter
MAD	multiwavelength anomalous diffraction
max.	maximum
MES	2-[N-morpholino]ethanesulfonate
Met, M	methionine
MeTr	MTHF: corrinoid iron-sulfur protein methyltarsferase
MIR	multiple isomorphous replacement
MMA	methyl mercury acetate
MPD	2-methyl-2,4-pentanediol
MR	molecular replacement
MTHF	methyltetrahydrofolate
MTHMP	methyltetrahydromethanopterin
MTHSP	methyltetrahydrosarcinopterin
MW	molecular weight
NAD	nicotinamide dinucleotide
NADP	nicotinamide dinucleotide phosphate
NCS	noncrystallographic symmetry
NTP	nucleoside triphosphate
O.D.	optical density
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction

PDB	Protein Data Bank
PEDD	platinum ethylene diamine dichloride
PEG	polyethylene glycol
PEGmme	polyethylene glycol monomethyl ether
Phe, F	phenylalanine
Pro, P	proline
R_{free}	free R-factor
rmsd	root-mean-square deviation
rmsF	root mean square structure factor amplitude
rpm	rounds per minute
R_{work}	working R-factor
s	second
σ	sigma level
SAM	S-adenosyl-L-methionine
SDS	sodium dodecyl sulfate
SDS PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SeMet	selenomethionine
Ser, S	serine
SIR	single isomorphous replacement
SIRAS	single isomorphous replacement with anomalous scattering
TBS	20 mM Tris-HCl, pH 7.5, 0.5 M NaCl
TEMED	N,N,N′,N′-Tetra-methylethylenediamine
THF	Tetrahydrofolate
THMP	Tetrahydromethanopterin
Thr, T	threonine
THSP	Tetrahydrosarcinopterin
TMLA	trimethyl lead acetate
TRIS	2-amino-2-hydroxylmethyl-1,3-propanediol; Tris(hydroxylmethyl)aminomethane
Trp, W	tryptophan
TTBS	TBS + 0.05% Tween-20 detergent
Tyr, Y	tyrosine
U/mg	nmol mg^{-1} min^{-1}
V	Volt
v/v	volume per volume

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MATERIALS

(D,L)-Selenomethionine was purchased from Sigma. (6S,6R)-Methyltetrahydrofolate was purchased from Schircks Laboratories, Switzerland. 6S-Methyltetrahydrofolate was a gift from SAPEC SA, Lugano, Switzerland. Isopropylthiogalactaside (IPTG) was bought from GIBCO. The met$^-$ B834 cells and pET3a expression vector were purchased from Novagen, restriction enzymes from New England Biolabs, Perkin-Elmer machine and PCR kit including the *taq* DNA polymerase from Perkin-Elmer, synthetic oligonucleotides from Ransom Hill Bioscience, Ramona, CA, Luria-Bertani (LB) medium ingredients from DIFCO. All other material were purchased either from Sigma or Fluka and used without further purification.

I. INTRODUCTION

A. Overview of the thesis

This thesis summarizes the path toward the successful structure determination of methyltetrahydrofolate: corrinoid iron-sulfur protein methyltransferase (MeTr) from *C. thermoaceticum*. That was made possible through successfully completing numerous tasks: (a) subcloning the MeTr gene into a pET3a expression vector; (b) improving the purification of MeTr by a heat treatment step; (c) fully substituting Se-methionine into the protein; (d) performing multiwavelength anomalous (MAD) experiments at the Stanford synchrotron (SSRL); and (e) determining the MeTr crystal structure by MAD phasing. In the course of this work, homologs of MeTr were identified. It was also possible to develop a model of how MeTr interacts with its substrate, the corrinoid iron-sulfur protein (CFeSP). These steps are important in understanding the mechanism of methyl transfer for this enzyme and in this class of B_{12} –dependent methyltransferases in general.

The thesis is therefore divided into the following chapters: (1) Introduction, (2) Obtaining MeTr, (3) Crystallization of MeTr, (4) MeTr Structure Determination and Modeling, and (5) Discussion. The Introduction will familiarize the reader with MeTr from *C. thermoaceticum*, its role in the acetyl-CoA pathway of anaerobic fixation of CO_2, its relation to other cob(I)amide dependent methyltransferases, and all methyl transfers, the importance of understanding the reaction chemistry, and its implications on basic biochemistry, environmental and medical applications. Chapter 2 will summarize the

protocol for obtaining highly pure and active MeTr. Chapter III will introduce the reader to the art of protein crystallization and the specific experiments for obtaining crystals from MeTr, CFeSP, and CODH/ACS. Chapter IV will describe all the steps in the experimental determination of the MeTr structure, starting from data collection, phasing, refinement of the model, description of MeTr structure, and an outline of the modeling studies. Chapter V will emphasize the on meaning of the results.

Some common experimental protocols are included in the different Appendices following the appropriate part. In addition, an electronic copy of the complete thesis, structure model coordinates, datasets, scripts, and additional material will be saved on a CD-ROM disk.

B. MeTr and Wood-Ljungdahl Acetyl-CoA pathway

Methyltetrahydrofolate: corrinoid/iron-sulfur protein methyltransferase (MeTr) catalyzes formation of the first organometallic intermediate in the Wood-Ljungdahl pathway of anaerobic CO_2 fixation (**Figure I-1**)(1)(2)(3)(4)(5)(6)(7). This protein catalyzes methyl group transfer from (*6S*)-methyltetrahydrofolate (CH_3-H4folate) to cob(I)amide. The resulting metal bound intermediate CH_3-Cob(III)amide is then transferred to another metal center in the bifunctional enzyme carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS). CODH/ACS combines the methyl group, CO, CoA to form acetyl-CoA. The overall stoichiometry is one acetyl-CoA molecule formed from two