

## **INFORMATION TO USERS**

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning  
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA

**UMI**<sup>®</sup>  
800-521-0600

PREVIEW

**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

Lincoln, Nebraska

December 1999

UMI Number: 9958391

**UMI**<sup>®</sup>

---

UMI Microform 9958391

Copyright 2000 by Bell & Howell Information and Learning Company.

All rights reserved. This microform edition is protected against  
unauthorized copying under Title 17, United States Code.

---

Bell & Howell Information and Learning Company  
300 North Zeeb Road  
P.O. Box 1346  
Ann Arbor, MI 48106-1346

DISSERTATION TITLE

Crystal Structure of Methyltetrahydrofolate:Corrinoid/Iron-Sulfur Protein

Methyltransferase from Clostridium thermoaceticum at 2.2 Å Resolution

BY

Tzanko Jordanov Doukov

SUPERVISORY COMMITTEE:

APPROVED

DATE

Signature

John J. Stezowski

Typed Name

12/10/99

Signature

Stephen W. Ragsdale

Typed Name

12/10/99

Signature

Lawrence J. Parkhurst

Typed Name

12/13/99

Signature

Mark A. Griep

Typed Name

12/10/99

Signature

Paul H. Blum

Typed Name

12/13/99

Signature

David S. Hage

Typed Name

12/13/99



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<sub>2</sub> fixation. It reversibly transfers the N<sup>5</sup>-methyl group from methyltetrahydrofolate (CH<sub>3</sub>-H<sub>4</sub>folate) 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<sub>2</sub> 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 cobamide 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  $\text{CH}_3\text{-H}_4\text{folate}$ , a key step in the mechanism of MeTr.

PREVIEW

## TABLE OF CONTENTS

<b>TABLE OF CONTENTS.....</b>	<b>i</b>
<b>ACKNOWLEDGMENTS.....</b>	<b>iv</b>
<b>LIST OF ABBREVIATIONS.....</b>	<b>ix</b>
<b>LIST OF APPENDICES.....</b>	<b>xii</b>
<b>LIST OF FIGURES.....</b>	<b>xiii</b>
<b>LIST OF TABLES.....</b>	<b>xiv</b>
<b>MATERIALS .....</b>	<b>xv</b>
<b><u>I. INTRODUCTION.....</u></b>	<b>1</b>
<b>A. Overview of the thesis.....</b>	<b>1</b>
<b>B. MeTr and Wood-Ljungdahl Acetyl-CoA pathway.....</b>	<b>2</b>
<b>C. Cobamide dependent methyltransferases.....</b>	<b>3</b>
<b>1. MT1 and MT2 reactions.....</b>	<b>3</b>
<b>2. Common features.....</b>	<b>4</b>
<b>3. Methyl group sources: CH<sub>3</sub>-X and pterin ring based carriers.....</b>	<b>4</b>
<b>a. CH<sub>3</sub>-X.....</b>	<b>4</b>
<b>b. Pterin ring based CH<sub>3</sub> carriers.....</b>	<b>5</b>
<b>4. Cob(I)amide properties.....</b>	<b>5</b>
<b>a. Intrinsic properties of cobalt.....</b>	<b>6</b>
<b>b. Cobamide forms.....</b>	<b>6</b>
<b>c. Free cobamides reactivity.....</b>	<b>7</b>
<b>5. MeTr.....</b>	<b>8</b>
<b>a. Properties.....</b>	<b>8</b>
<b>b. MeTr Substrates.....</b>	<b>9</b>
<b>c. Kinetics: rapid equilibrium random Bi-Bi mechanis.....</b>	<b>10</b>



i. Precatalytic activation of MeTr.....	10
ii. Step 1 Substrate protonation.....	10
iii. Step 2 Rate determining step – methyltransfer.....	11
iv. Step 3 Rapid dissociation of the products.....	11
v. Last destination of the methyl group.....	12
6. MetH - Cobalamin dependent methionine synthase.....	12
7. MtrH - MTHMTP:CoM methyltransferase.....	13
8. O-Demethylase methyltransferase.....	14
9. Unifying approach in cobamide dependent methyltransfer.....	15
D. Cobamide independent methyltransfer reactions in nature.....	15
1. S-Adenosylmethionine (SAM).....	16
2. MetE: Cob(I)amide independent mechanism for activation.....	17
a. Homocysteine activation is conserved, involves Zn.....	17
b. MTHF/MTHMP activation.....	18
3. Betaine: homocysteine methyltransferase (BHMT).....	19
E. Importance of cobamide dependent methyl transfers.....	20
1. Basic biochemistry - C1 carriers.....	20
2. Environmental importance: the Wood-Ljungdahl pathway.....	20
3. Medical importance, methionine/SAM/folate metabolisms.....	21
Figure I-1 Wood-Ljungdahl pathway.....	22
Figure I-2 Methanogenic adaptation of the pathway.....	23
Figure I-3 Anaerobic methyl group transfer.....	24
<b>II. OBTAINING MeTr.....</b>	<b>25</b>
A. Experimental Methods.....	25
1. Molecular biology – subcloning.....	25
a. MeTr gene subcloning.....	25

b. Transformation into met- <i>E. coli</i> strain B834.....	26
2. Biochemistry - protein purification and characterization.....	26
a. MeTr purified from <i>C. thermoaceticum</i> .....	26
b. Heterologous expression of MeTr in <i>E. coli</i> .....	27
i. Cells growth, induction, and harvesting.....	27
ii. MeTr purification steps.....	27
iii. Sonication and ultracentrifugation.....	28
c. Expression of the Se-Met substituted MeTr.....	30
d. Unsuccessful Te-Met substitution.....	30
B. Results and conclusions.....	31
1. Molecular biology results summary.....	31
2. Biochemistry - protein purification summary.....	31
Figure II-1 The purity of the MeTr by Coomassie Blue stained gel.....	33
Figure II-2 Mass spectrometry on Se-substituted MeTr in <i>E. coli</i> .....	35
<b>III. CRYSTALLIZATION OF MeTr.....</b>	<b>36</b>
A. Protein crystallization.....	36
1. Overview of protein crystallization.....	36
a. Crystals.....	36
b. Biological crystals.....	37
2. Parameters affecting crystallization.....	37
a. Protein purity.....	37
b. Storage conditions.....	38
3. Crystallization methods.....	38
a. Vapor diffusion and hanging drop.....	39
b. Seeding.....	40
c. Screening.....	40

<b>B. General conditions for obtaining MeTr crystals.....</b>	<b>42</b>
<b>C. <i>C. thermoaceticum</i> MeTr crystals.....</b>	<b>43</b>
<b>D. Crystallizing MeTr overexpressed in <i>E. coli</i>.....</b>	<b>45</b>
<b>E. Crystallizing Se-Met substituted MeTr.....</b>	<b>46</b>
<b>Figure III-1 Crystals from <i>C. thermoaceticum</i> purified MeTr.....</b>	<b>48</b>
<b>Figure III-2 Yellow protein contaminant in <i>C. thermoaceticum</i>.....</b>	<b>49</b>
<b>Figure III-3 Crystals from <i>E. coli</i> purified MeTr.....</b>	<b>50</b>
<b>Figure III-4 CODH/ACS crystals.....</b>	<b>51</b>
<b>Appendix III-1 Fast screen.....</b>	<b>52</b>
<b>Appendix III-2 Ion screen.....</b>	<b>54</b>
<b>Appendix III-3 Crystallizing Other Proteins.....</b>	<b>58</b>
<b>Corrinoid/Iron-Sulfur Protein (CFeSP).....</b>	<b>58</b>
<b>Carbon Oxide Dehydrogenase/Acetyl-CoA Synthase.....</b>	<b>59</b>
<b>Condition 1.....</b>	<b>60</b>
<b>Condition 2.....</b>	<b>60</b>
<b><u>IV. MeTr STRUCTURE DETERMINATION AND MODELING.....</u></b>	<b>62</b>
<b>A. Methods and experiments.....</b>	<b>62</b>
<b>1. Overview.....</b>	<b>62</b>
<b>2. Cryocrystallography.....</b>	<b>63</b>
<b>a. Selecting conditions for MeTr cryoprotection.....</b>	<b>63</b>
<b>b. Growing MeTr in the presence of glycerol.....</b>	<b>64</b>
<b>c. Crystal annealing.....</b>	<b>64</b>
<b>3. Data collection and processing.....</b>	<b>65</b>
<b>a. Overview.....</b>	<b>65</b>
<b>b. Specific data collection procedures.....</b>	<b>67</b>
<b>i. At The University of Nebraska –Lincoln.....</b>	<b>67</b>

ii. MAD data collection at SSRL.....	68
iii. At The University of Michigan and OCC.....	69
c. Data Processing.....	70
i. Overview.....	70
ii. Steps in data processing.....	70
4. Phasing from MAD data.....	71
5. Model building.....	71
6. Refinement.....	72
7. Modeling, sequence, structure analysis.....	74
<b>B. Results.....</b>	<b>75</b>
1. Overall structure.....	75
2. Structural Similarities.....	77
3. Active Site.....	78
a. Modeling MTHF/THF binding site.....	78
b. Conserved residues.....	79
4. Modeling cobamide binding.....	83
<b>Figure IV-1 Experimental electron density quality.....</b>	<b>85</b>
<b>Figure IV-2 Overall structure of MeTr.....</b>	<b>86</b>
<b>Figure IV-3 Representation of the dimer interface.....</b>	<b>87</b>
<b>Figure IV-4 Sequence and structure homologs.....</b>	<b>88</b>
<b>Figure IV-5 MeTr:MTHF binary complex model.....</b>	<b>94</b>
<b>Figure IV-6 MeTr active site.....</b>	<b>95</b>
<b>Figure IV-7 Electrostatic surface of the pterin ring binding site.....</b>	<b>96</b>
<b>Table IV-1 Native datasets statistics.....</b>	<b>97</b>
<b>Table IV-2 Heavy atom derivatives.....</b>	<b>98</b>
<b>Table IV-3 Room temperature datasets.....</b>	<b>101</b>

<b>Table IV-4</b>	<b>MAD diffraction data and phasing.....</b>	<b>102</b>
<b>Table IV-5</b>	<b>Data Collection and Refinement Statistics.....</b>	<b>103</b>
<b><u>V. DISCUSSION</u></b>	<b>.....</b>	<b>105</b>
<b>A. Cobamide binding site.....</b>		<b>105</b>
<b>B. MTHF activation.....</b>		<b>106</b>
<b>C. Kinetic mechanism.....</b>		<b>109</b>
<b>1. Random binding of the substrates.....</b>		<b>109</b>
<b>2. Ordered binding of the substrates.....</b>		<b>109</b>
<b>3. Summary on MeTr kinetic mechanism.....</b>		<b>109</b>
<b>D. Conclusions.....</b>		<b>110</b>
<b>Figure V-1A</b>	<b>MeTr + B12 + MTHF: a common structural motif.....</b>	<b>112</b>
<b>Figure V-1B</b>	<b>Methylmalonyl-CoA mutase.....</b>	<b>114</b>
<b>Figure V-1C</b>	<b>Diol dehydrase.....</b>	<b>115</b>
<b>Figure V-2</b>	<b>Two ways for MTHF activation.....</b>	<b>116</b>
<b>Figure V-3</b>	<b>Water mediated protonation at N<sup>5</sup>.....</b>	<b>117</b>
<b><u>LITERATURE CITED</u></b>	<b>.....</b>	<b>118</b>

## ACKNOWLEDGMENTS

First, I would like to thank my two advisors, Dr. John Stezowski and Dr. Steve Ragsdale for their support, encouragement, and guidance throughout the Ph.D. program. I would also like to thank my committee members, Dr. Stezowski, Dr. Ragsdale, Dr. Parkhurst, Dr. Griep, Dr. Blum, and Dr. Hage, for their help and guidance.

During the years I was very lucky to work with wonderful colleagues and friends from Dr. John Stezowski's group: Ali Rashid, Johanna Mazlo, Eric Haas, Joanna Clark, Dr. Peter Goebel, Ann Yen, Dr. Bill Parker, Joel Roberts, Dr. Ulrich Biederman, Fan Yang, Dave Nelis, Dr. Tom Brett, Jen Alexander, Dr. Charles R. Ross II, Scott Tremain, Randy Olsen, and Nathan Hood. I especially value the friendship with Ali and Johanna. You were so kind, giving, and supportive! It was a lot of fun to hang out in and out the lab with you. I will not forget the latin dances you taught me. You are wonderful friends! I am going to miss our everyday interactions.

I was taught biochemistry by Dr. Ragsdale and his group: Dr. Michaela Simianu, Dr. Manoj Kumar, Dr. Sander Arendsen, Dr. Don Becker, Dr. Asma El Kasmi, Dr. Saurabh Menon, Eisuke Murakami, Cristina Furdui, Dr. Devandra Naidu, Rama Dihanyamraju, Dr. Sudha Krishnan, and Yih-Chern Horng. My research on MeTr is a continuation of the MeTr project, which included efforts of the following people: Dr. Dave Roberts, Dr. Shaying Zhao, Dr. Javier Seravalli, and Onur Dunbar. I was fortunate to communicate with many experts at UN-L: Dr. Banerjee and her group, on B<sub>12</sub> chemistry, Dr. Veniamin Lapko and Dr. Gautam Saurath on proteins, and Dr. Ventzislav Vassilev on molecular biology.

The research described here would have not been completed if the following institutions and colleagues had not gotten involved. I am indebted to many: (1) I would like to thank Henry Bellamy from SSRL and Dr. Charles Ross II for their help during the data collection at beam line 1-5 at SSRL, which is supported by US DOE and by the NIH, Biotechnology Resource Program, Division of Research Resources; (2) Crystallographic data was also collected in Prof. Martha Ludwig's laboratory at The University of Michigan, Ann Arbor, MI, and in the Ohio Crystallographic Consortium, University of Toledo, Toledo, OH, under the supervision of Dr. Ewa Skrzypczak-Jankun; (3) I am also indebted to Prof. George Sheldrick using Se-Met MeTr data as a (successful) test case for his new program SHELXD, and Dr. Eric de la Fortelle for his guidance in using SHARP; (4) Preliminary sequence data was obtained from The Institute for Genomic Research website at <http://www.tigr.org>; (5) This work has been supported by the NIH GM39451 and the NSF-EPSCoR Metallobiochemistry grants; (6) I would like to acknowledge Warren F. and Edith R. Day for their student travel award. Being in graduate school is quite a stress, but I was lucky to meet Steve and Susana Sall, Rowena Sherman, Abdullah Can, Keiko and Ryoichi Teruyama, Ali Rashid, Chris Reade, Tadashi Mizusaki, Janice and Eraldo Zanella, and the rest of our soccer club "Porto Internationale", who brought a lot of joy, laughs, good times, and kept me sane. I would also like to extend these personal thanks to the friends from the small, but wonderful Bulgarian community in Lincoln.

Finally, I would like to thank my sisters Tzvetanka, Svetla and especially my parents Bojoura and Iordan Doukovi for their unconditional support and love. Thank you all!

## LIST OF ABBREVIATIONS

$\langle I \rangle$	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 <sub>α</sub>	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 <sub>3</sub> -B <sub>12</sub>	methylcobalamin
CH <sub>3</sub> -H <sub>4</sub> F	N <sup>5</sup> -methyltetrahydrofolate
CH <sub>3</sub> -H <sub>4</sub> MPT	N <sup>5</sup> -methyltetrahydromethanopterin
CH <sub>3</sub> -H <sub>4</sub> SPT	N <sup>5</sup> -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 <sup>+</sup> , F <sup>-</sup>	structure factor amplitudes of Bijvoet related reflections
FAD	flavin adenine dinucleotide
F <sub>c</sub>	calculated structure factor amplitude
F <sub>H</sub>	calculated heavy atom structure factor amplitude
F <sub>o</sub>	observed structure factor amplitude
$f^0$	normal atomic scattering factor
FOM	figure of merit
F <sub>P</sub>	native structure factor amplitude
F <sub>PH</sub>	heavy atom derivative structure factor amplitude
g	gram



Gln, Q	glutamine
Glu, E	glutamic acid
Gly, G	glycine
H <sub>4</sub> F	tetrahydrofolate (see also THF)
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonate]
His, H	histidine
hr	hour
I <sub>calc</sub>	calculated structure factor intensity
Ile, I	isoleucine
I <sub>obs</sub>	observed structure factor intensity
I <sub>obs-d</sub>	observed detwinned structure factor intensity
K	temperature Kelvin
k	kilo
kb	kilo base pairs
K <sub>cat</sub>	rate of catalysis
kDa	kilo-dalton
K <sub>d</sub>	equilibrium dissociation constant
K <sub>m</sub>	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 methyltransferase
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
$R_{\text{free}}$	free R-factor
rmsd	root-mean-square deviation
rmsF	root mean square structure factor amplitude
rpm	rounds per minute
$R_{\text{work}}$	working R-factor
s	second
$\sigma$	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-hydroxymethyl-1,3-propanediol; Tris(hydroxymethyl)aminomethane
Trp, W	tryptophan
TTBS	TBS + 0.05% Tween-20 detergent
Tyr, Y	tyrosine
U/mg	$\text{nmol mg}^{-1} \text{ min}^{-1}$
V	Volt
v/v	volume per volume

**LIST OF APPENDICES.....xii****Appendix III-1 Fast screen.....52****Appendix III-2 Ion screen.....54****Appendix III-3 Crystallizing Other Proteins.....58****Corrinoid/Iron-Sulfur Protein (CFeSP).....58****Carbon Oxide Dehydrogenase/Acetyl-CoA Synthase.....59****Condition 1.....60****Condition 2.....60**

PREVIEW

**LIST OF FIGURES.....xiii**

<b>Figure I-1</b>	<b>Wood-Ljungdahl pathway.....</b>	<b>22</b>
<b>Figure I-2</b>	<b>Methanogenic adaptation of the pathway.....</b>	<b>23</b>
<b>Figure I-3</b>	<b>Anaerobic methyl group transfer.....</b>	<b>24</b>
<b>Figure II-1</b>	<b>The purity of the MeTr by Coomassie Blue stained gel.....</b>	<b>33</b>
<b>Figure II-2</b>	<b>Mass spectrometry on Se-substituted MeTr in <i>E. coli</i>.....</b>	<b>35</b>
<b>Figure III-1</b>	<b>Crystals from <i>C. thermoaceticum</i> purified MeTr.....</b>	<b>48</b>
<b>Figure III-2</b>	<b>Yellow protein contaminant in <i>C. thermoaceticum</i>.....</b>	<b>49</b>
<b>Figure III-3</b>	<b>Crystals from <i>E. coli</i> purified MeTr.....</b>	<b>50</b>
<b>Figure III-4</b>	<b>CODH/ACS crystals.....</b>	<b>51</b>
<b>Figure IV-1</b>	<b>Experimental electron density quality.....</b>	<b>85</b>
<b>Figure IV-2</b>	<b>Overall structure of MeTr.....</b>	<b>86</b>
<b>Figure IV-3</b>	<b>Representation of the dimer interface.....</b>	<b>87</b>
<b>Figure IV-4</b>	<b>Sequence and structure homologs.....</b>	<b>88</b>
<b>Figure IV-5</b>	<b>MeTr:MTHF binary complex model.....</b>	<b>94</b>
<b>Figure IV-6</b>	<b>MeTr active site.....</b>	<b>95</b>
<b>Figure IV-7</b>	<b>Electrostatic surface of the pterin ring binding site.....</b>	<b>96</b>
<b>Figure V-1A</b>	<b>MeTr + B12 + MTHF: a common structural motif.....</b>	<b>112</b>
<b>Figure V-1B</b>	<b>Methylmalonyl-CoA mutase.....</b>	<b>114</b>
<b>Figure V-1C</b>	<b>Diol dehydrase.....</b>	<b>115</b>
<b>Figure V-2</b>	<b>Two ways for MTHF activation.....</b>	<b>116</b>
<b>Figure V-3</b>	<b>Water mediated protonation at N<sup>5</sup>.....</b>	<b>117</b>

**LIST OF TABLES.....xiv**

<b>Table IV-1</b>	<b>Native datasets statistics.....</b>	<b>97</b>
<b>Table IV-2</b>	<b>Heavy atom derivatives.....</b>	<b>98</b>
<b>Table IV-3</b>	<b>Room temperature datasets.....</b>	<b>101</b>
<b>Table IV-4</b>	<b>MAD diffraction data and phasing.....</b>	<b>102</b>
<b>Table IV-5</b>	<b>Data Collection and Refinement Statistics.....</b>	<b>103</b>

## 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. Isopropylthiogalactoside (IPTG) was bought from GIBCO. The met<sup>-</sup> 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<sub>12</sub> –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<sub>2</sub>, 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<sub>2</sub> fixation (**Figure I-1**)(1)(2)(3)(4)(5)(6)(7). This protein catalyzes methyl group transfer from (6*S*)-methyltetrahydrofolate (CH<sub>3</sub>-H<sub>4</sub>folate) to cob(I)amide. The resulting metal bound intermediate CH<sub>3</sub>-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