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**The Refolding of
Recombinant Human Liver Methylmalonyl-CoA Mutase
from Inclusion Bodies Produced in *Escherichia coli***

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ABSTRACT

Human methylmalonyl-CoA mutase (hMCM) is an adenosylcobalamin-dependent enzyme that catalyses the structural rearrangement of (R)-methylmalonyl-CoA to succinyl-CoA as part of the catabolism of the branched chain amino acids valine, leucine and isoleucine, odd chain fatty acids and intermediates of cholesterol metabolism. Reactions that require adenosylcobalamin (AdoCbl) have been intensively studied, and the first step in the catalysis is widely agreed to involve homolytic cleavage of the unusual carbon-cobalt bond in the cofactor. A reliable source of recombinant hMCM would be useful in defining more fully the mechanistic pathway of AdoCbl-dependent enzymes.

Recombinant hMCM overexpressed in *E. coli* forms insoluble aggregates of inactive protein known as inclusion bodies. hMCM inclusion bodies were purified, solubilised and then several different *in vitro* refolding techniques were tested in attempts to produce active recombinant hMCM from purified solubilised inclusion body material. These methods included refolding by rapid dilution, refolding by dialysis, detergent-assisted refolding, refolding by gel filtration chromatography and chaperonin-assisted refolding. Chaperonin-assisted refolding necessitated the purification of recombinant *E. coli* chaperonins GroES and GroEL from the *E. coli* strain DH1/pGroESL.

Refolding by rapid dilution of the GdmHCl-solubilised inclusion bodies into a refolding buffer was judged to be the simplest and most effective method, however the refolding process was extremely inefficient. Refolding by rapid dilution was scaled up to 2 litres to produce as much active hMCM as possible. The refolded protein was concentrated by batch adsorption to and stepwise elution from hydroxyapatite, and further purified using a synthesised 5'adenosylcobalamin-agarose 'affinity' chromatography column. The final refolded hMCM preparation contained a single ~29 kDa contaminant protein, tentatively identified as *E. coli* branched-chain amino acid aminotransferase (FC 2.6.1.42), present in approximately equal amounts to the hMCM, and had a specific activity of ~3.11 units/mg.

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Thank God for methylmalonyl-CoA mutase; it is a stubborn but special little protein.

And finally thanks to anyone who reads this line and has picked up this thesis hoping that they will find the information they need. Good luck!

LIST OF ABBREVIATIONS

5'AdoCbl	5'deoxyadenosylcobalamin
Amp	Ampicillin
Avg	Average
bMCM	Bacterial methylmalonyl-CoA mutase
BSA	Bovine serum albumin
C-terminal	Carboxyl terminal
C₈-β-D Gluc	C ₈ -β-D Glucopyranoside
CAM	Chloramphenicol
CAPS	3-[Cyclohexylaminol]-1-propanesulfonic acid
cDNA	Complementary DNA
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CHAPSO	3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxypropane-1-sulfonate
CMC	Critical micelle concentration
CNCbl	Cyanocobalamin
CTAB	Cetyltrimethylammonium bromide
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
g	Gravitational field, unit of
GdmHCl	Guanidinium hydrochloride
hMCM	Human methylmalonyl-CoA mutase
HTP	Hydroxyapatite
kan	Kanamycin
KP	Potassium phosphate
LDAO	Lauryldimethylamine oxide
LM	Lauryl Maltoside
Lubrol PX	Polyoxyethylene (9) lauryl ether
MAP	Methionine aminopeptidase

MEGA-9	Nonanoyl-N-methylglucamide
MCM	Methylmalonyl-CoA mutase
Mr	Relative molecular mass
mt	Mitochondrial
NADH	Nicotinamide-adenine dinucleotide, reduced
Nonidet P40	Nonaethylene glycol octylphenyl ether
N-terminal	Amino terminal
PAGE	Polyacrylamide gel electrophoresis
PDI	Protein disulphide isomerase
PK	Protein Kinase
PMSF	Phenylmethylsulphonyl fluoride
PPI	Peptidyl-prolyl <i>cis-trans</i> isomerase
RNase	Ribonuclease
SDS	Sodium dodecyl sulphate
TCA cycle	Tricarboxylic acid cycle
TEMED	N, N, N', N'-tetramethylethylenediamine
Tris	Tris-(hydroxymethyl)-aminomethane
Triton X-100	Nonaethylene glycol octylphenyl ether
Tween 20	Polyoxyethylene sorbitan monolaurate
Z-3-XX	Zwittergent 3-XX series

THREE AND ONE LETTER AMINO ACID CODE

Ala	A	Alanine
Arg	R	Arginine
Asn	N	Asparagine
Asp	D	Aspartic acid
Cys	C	Cysteine
Gln	Q	Glutamine
Glu	E	Glutamic acid
Gly	G	Glycine
His	H	Histidine
Ile	I	Isoleucine
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Tyr	Y	Tyrosine
Val	V	Valine
Trp	W	Tryptophan

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