

Functional characterisation of the N-terminal region of Holocarboxylase synthetase

by

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Abstract

Biotin (vitamin H or B7) is an important micronutrient that is covalently attached to biotin-dependent enzymes by human biotin protein ligase (hBPL) or holocarboxylase synthetase (HCS). Patients with HCS deficiency are treated with oral biotin supplementation, which in most cases is able to reverse the clinical symptoms. However, some patients respond poorly to biotin therapy and have an extremely poor long-term prognosis. The molecular explanation for this is not understood. In this study HCS was investigated to improve our understanding of this key enzyme.

The catalytic region of all BPLs is contained in the conserved C-terminal region. HCS contains a long N-terminal extension that is not present in bacterial BPLs. The structure and function of the N-terminal region is yet to be determined. In order to delineate the domain structure of HCS limited proteolysis was performed previously in our laboratory. Two protease-sensitive linker regions were identified, one between residues 151-153, the other at amino acid 314. Of particular importance is the proposed structured domain containing residues 159-314, as amino acid substitutions in this region have been shown to compromise enzyme activity despite being distal to the C-terminally located active site. This thesis provides genetic evidence for a direct interaction between the N-terminal and C-terminal halves of HCS using a yeast two-hybrid assay. This interaction was mapped using a truncation study to the proposed structured domain of the N-terminal region of HCS (159-314 N-HCS).

HCS deficiency gives rise to the metabolic disorder multiple carboxylase deficiency (MCD). Mutations within the proposed structured domain 159-314 N-HCS give rise to MCD patients that are poorly responsive to the current therapy (biotin supplementation) and have an extremely poor long-term prognosis. In this thesis, a series of novel mutations in the

proposed structured domain 159-314 N-HCS were generated using “error prone” PCR. The catalytically inactive mutants were isolated from the library using an *in vivo* complementation assay. The mutants that were isolated were identified by DNA sequencing as L166R, L206P, W210R, L246M, L270S, H306R, F321S and the double mutant E181G, E327G. These residues are highly conserved within vertebrate species. These novel HCS mutants, together with the MCD mutants L216R HCS and L237P HCS, were employed to further characterise the function of the proposed structured N-terminal domain. Using the Yeast Two-hybrid assay, it was shown that the interaction between the two halves of HCS was not disrupted by the MCD mutants nor the novel mutants. Conversely, it was shown that the MCD mutants, and the majority of the novel HCS mutants, disrupted the interaction between HCS and its protein substrate the Pyruvate Carboxylase biotin domain hPC107.

Surface Plasmon Resonance was then employed to further characterise this observation. This study has demonstrated for the first time that although the association between HCS and its substrate was not compromised by mutation, the MCD mutants had a >15-fold increase in dissociation rate from the substrate compared to wild type HCS. This work provided a novel function for the proposed structural N-terminal domain. Furthermore, these data provide a molecular explanation for the HCS deficient patients that do not respond to biotin therapy.

Statement of Originality

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed

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Lungisa Mayende

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List of Publications

Published Manuscript

Lungisa Mayende, Rachel D. Swift, Lisa M. Bailey, Tatiana P. Soares da Costa, John C. Wallace, Grant W. Booker and Steven W. Polyak. (2012) A novel molecular mechanism to explain biotin-unresponsive holocarboxylase synthetase deficiency, *J. Mol Medicine*, 90(1): 81-88.

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Mayende, L., Swift, R.D., Bailey, L.M., Wallace, J.C., Booker, G.W. & Polyak, S.W. (2010) Mechanism to explain the juvenile metabolic syndrome Multiple Carboxylase Deficiency. Australian Society for Medical Research SA division Scientific Meeting, Poster 58.

Mayende, L., Swift, R.D., Bailey, L.M. Wallace, J.C., Booker, G.W. & Polyak, S.W. (2010) Domain mapping and functional analysis of the N-terminal region of Holocarboxylase synthetase. Adelaide Protein Group (APG) Student Award **Finalist**, Oral Presentation.

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Mayende, L., Swift, R. D., Bailey, L. M., Pardini, N. R., Wallace, J. C., Polyak, S. W. & Booker, G.W. (2008) Domain structure of human Holocarboxylase synthetase: evidence of an interaction between the N-terminal and C-terminal halves. 33rd Lorne Conference on Protein Structure and Function, Poster 255. **Awarded** student travel award.

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List of Abbreviations

1D	One-dimensional
2D	Two-dimensional
3D	Three-dimensional
Ab	antibody
ACC	acetyl CoA carboxylase
AD	activation domain
Amp	ampicillin
AMP	adenosine monophosphate
AP	alkaline phosphatase
Apo	unliganded enzyme
ATP	adenosine triphosphate
BirA	biotin inducible repressor A
BCCP	biotin carboxyl carrier protein
BLAST	basic local alignment search tool
BME	beta-mercaptoethanol
bp	base pair
BPL	biotin protein ligase
BSA	bovine serum albumin
C-	carboxyl-
cDNA	complementary deoxyribonucleic acid
C-HCS	C-terminal amino acids 315-726 of Holocarboxylase synthetase
D ₂ O	deuterium oxide
DMSO	dimethyl sulfoxide
DNA	deoxynucleotide triphosphate
DNA BD	DNA binding domain
dNTPs	deoxynucleotide triphosphates
DTT	dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylene diamine tetra-acetic acid
GST	glutathione-S-transferase
HCS	Holocarboxylase synthetase
Holo	ligand bound enzyme
hPC107	107 amino acids encoding the biotin domain of human pyruvate carboxylase

hr	hour
HRP	horseradish peroxidase
IP	immunoprecipitation
k_a	association rate constant
k_d	dissociation rate constant
K_D	equilibrium dissociation constant
K_M	Michaelis constant
kb	kilobase pair
kDa	kilodalton
LB	Luria broth
LiAc	Lithium Acetate
m	metre
M	molar
μ	micron
mA	milliampere
min	minute, minutes
MCC	methylcrotonyl-CoA carboxylase
MCD	multiple carboxylase deficiency
MOPS	3-morpholinopropanesulfonic acid
MtBPL	<i>Mycobacterium tuberculosis</i> BPL
MW	molecular weight
MWCO	molecular weight cut-off
n	nano
N-	amino-
N-HCS	N-terminal amino acids 1-314 of Holocarboxylase synthetase
NMR	nuclear magnetic resonance
OD _x	nm optical density at x nm wavelength
PCC	propionyl-CoA carboxylase
PBS	phosphate buffered saline
PBS-T	phosphate buffered saline and 0.05% (v/v) Tween-20
PC	pyruvate carboxylase
PCR	polymerase chain reaction
PDB	protein data bank
PhBPL	<i>P.horikoshii</i> BPL
PMSF	phenylmethylsulfonylfluoride

PVDF	polyvinyl difluoride
RMSD	root mean square deviation
RNA	ribonucleic acid
RT	room temperature
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
s	second
TBS	tris buffered saline
TBS-T	tris buffered saline and 0.1% (v/v) Triton-X
Tris	2-amino-2-hydroxymethylpropane-1,3-diol
Tween-20	polyoxyethylene-sorbitan monolaurate
U	units
V_{MAX}	maximum velocity
UV	ultra violet
WT	wild type
yBPL	Yeast (<i>S.cerevisiae</i>) biotin protein ligase