# 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 trucation 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

*Pubished 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.

*Communications and Conference Proceedings*

Mayende, L., Swift, R.D., Bailey, L.M. Wallace, J.C., Booker, G.W. & Polyak, S.W. (2010) Characterisation of the N-terminal region of Holocarboxylase synthetase. Ozbio Conference, Poster 255.

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.

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. 35$^{th}$ Lorne Conference on Protein Structure and Function, Poster 285. **Awarded** student travel award.

Mayende, L., Swift, R. D., Bailey, L. M., Pendini, N. R., Wallace, J. C., Booker, G.W. & Polyak, S. W. (2010) Protein biotinylation: An example of a post-translational modification of exceptional specificity. 3rd Annual Protein and Peptide Conference (Pep Con). Beijing, China.

Mayende, L., Swift, R.D., Bailey, L.M., Pendini, N.R., Wallace, J.C., Booker, G.W. & Polyak, S.W. (2008) Interaction and Mutagenesis studies on Holocarboxylase synthetase (HCS). Australian Society for Medical Research SA division Scientific Meeting, Oral Presentation.

Mayende, L., Swift, R. D., Bailey, L. M., Pendini, 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.

Mayende, L., Swift, R. D., Bailey, L. M., Pendini, N. R., Wallace, J. C., Polyak, S. W. & Booker, G.W. (2007) Evidence of an interaction between the N-terminal and C-terminal halves of Holocarboxylase synthetase. University of Adelaide, School of Molecular and Biomedical Sciences Research Symposium.

## 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 |
| $D_2O$ | deuterium oxide |
| DMSO | dimethyl sulfoxide |
| DNA | deoxynucleotide triphosphate |
| DNA BD | DNA binding domain |
| dNTPs | deoxynucleotide triphosphates |
| DTT | dithiothreitol |
| *E. coli* | *Escherichia coli* |
| 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 |
| *Mt*BPL | *Mycobacterium tuberculosis* 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 |
| ODx | 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 |
| *Ph*BPL | *P.horikoshii* 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 (*S.cerevisiae*) biotin protein ligase |