Structure-function relationships of the biotin transporters from *Staphylococcus aureus*

By

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Abstract

The clinically relevant human pathogen *Staphylococcus aureus* employs an energy coupling factor (ECF) transporter to import the important micronutrient biotin. Like the well characterised ABC transporters, the ECF transporters utilise the hydrolysis of ATP to move substrates across biological membranes. However, the ECF transporters do not use a solute-binding protein to bind substrate instead employing a membrane embedded protein to fulfil this role. In certain bacteria, the substrate binding protein required for binding biotin is known as BioY. The aim of this thesis was to investigate protein structure and function relationships involving the BioY protein from *S. aureus* (*Sa*BioY).

S. aureus has functional import and export processes that result in a biphasic profile of biotin uptake. Active translocation of biotin was temperature-dependent, optimum at 30 minutes, and inhibited by biotin or structural analogues of the vitamin. The study demonstrated that recombinant *Sa*BioY could be expressed in *E. coli*, and was localised to the membrane fraction as observed by Western blot analysis on fractionated cell lysates and fluorescence microscopy. Importantly, a convenient ligand binding assay was developed that facilitated deeper analysis of the *Sa*BioY structure and function.

*Sa*BioY primarily recognises the ureido ring of biotin for substrate capture, but an intact thiophene ring also aids binding. Although a variety of functional groups can be appended onto the carboxyl group of the biotin moiety, the linker used to connect the molecules and the chemical property of functional group can impact binding to *Sa*BioY.

This knowledge can be exploited for developing biotin-based analogues with applications in antibiotic drug discovery.

Since an X-crystal structure of *Sa*BioY is not available, membrane topology predictions and computational modelling were used to generate a molecular module of *Sa*BioY. This yielded a model containing 5 transmembrane domains, 3 extracellular loops, and 1 intracellular loop with intracellular N- and C-termini. Whilst the model was in good agreement with known crystal structures of other known S components, it possessed an additional V-shaped membrane embedded helix. Conserved amino acid residues in BioY were identified using the web-based Clustal-W alignment program and then mapped onto the *Sa*BioY model. A series of 24 *Sa*BioY mutants were then generated using random and site-directed mutagenesis approaches. Fluorescence polarisation based competitive-binding assays using a fluorescent-biotin tracer revealed several conserved (R75, D157 and K160) and non-conserved (N38, T54, F81, F88 and D128) residues important for biotin binding. Interestingly, a double mutant D157K/K160E completely abolished biotin binding. A filter disk diffusion assay using a panel of antibiotics showed recombinant expression of *Sa*BioY increased *E. coli* antibiotic sensitivity to streptomycin, erythromycin and chloramphenicol, probably by forming a pore through channel by dimerisation requiring a dynamic cooperative interaction. Expression of all of the *Sa*BioY mutants increased sensitivity to the three antibiotics. The D157K/K160E double mutant was an exception, as it had no effect upon antibiotic sensitivity. We proposed that D157 and K160 together play an essential role in *Sa*BioY activity.

In conclusion, this study successfully characterised the *Sa*BioY transporter in both its native state and using a recombinant *E. coli* expression system. Substrate specificity of the transporter was determined, as was the channel gating potency of *Sa*BioY for certain antibiotics when expressed in *E. coli*. Computational modelling and a novel FP based competitive assay also provided useful tools for biochemical analysis of *Sa*BioY structure and function relationships. Further studies are now required to determine the *Sa*BioY X-ray crystal structure, transport mechanism and regulation as well as to explore possible application of the transporter as a novel drug target or an alternative gating system new antibiotic agents.

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 in my name 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 in my name, 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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Al Azhar

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List of Publications

Published Manuscripts

Wanisa Salaemae, **Al Azhar**, Grant W. Booker and Steven W. Polyak (2011) Biotin biosynthesis in *Mycobacterium tuberculosis*: Physiology, biochemistry, and molecular intervention. *Protein Cell* 2(9): 691-695.

Steven W. Polyak, Lisa M. Bailey, **Al Azhar**, Grant W. Booker (2012) Biotin (Vitamin H or B7). *In* Micronutrients: Sources, Properties and Health Effects. AI Betancourt and HF Gaitan (Eds.). NOVA Science Publisher Inc., New York, pp: 65-95.

Communications and Conference Proceedings

Azhar, A., Polyak, S.W. and Booker, G.W. (2012) Structure and function studies on the biotin transport system from *Staphylococcus aureus*. *Proceedings of Annual Scientific Meeting of Australian Society for Medical Research South Australia Division Scientific Meeting.* Oral Presentation. O7

Azhar, A., Wiridja, A., Baker, P., Polyak, S.W. and Booker, G.W. (2012) Investigation into the biotin transport system in *Staphylococcus* aureus. *Proceedings of the 37th Lorne Conference on Protein Structure and Function, Poster 101*

Azhar, A., Polyak, S.W., Wiridja, A. and Booker, G.W. (2012) Substrate specificity of biotin transporter from *Staphylococcus aureus. Adelaide Protein Group. Poster 101*

Azhar, A., Wiridja, A., Baker, P., Polyak, S.W. and Booker, G.W. (2012) Investigation into the structure and function relationships for biotin transporter from *Staphylococcus aureus*. *Proceeding ComBio 2012. Poster 033*

A. Azhar, SW Polyak and GW Booker (2012) Structure and function studies on the biotin transport system from *Staphylococcus aureus*. *Proceedings of Annual Scientific Meeting of Australian Society for Medical Research South Australia 2012.* Oral Presentation.

A. Azhar, SW Polyak and GW Booker (2013) Investigating structure-function of the biotin transporter from *Staphylococcus aureus*. *Proceedings of Annual Scientific Meeting of Australian Society for Medical Research South Australia 2013.* Poster P4.

List of abbreviations

ABC	ATP binding cassette
Amp	ampicillin
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BC	biotin carboxylase
BCA	bicinchoninic acid
BCCP	biotin carboxyl carrier protein
BirA	biotin inducible repressor A
BLAST	Basic Local Alignment Search Tools
BME	beta-mercaptoethanol
bp	base pair
BPL	biotin protein ligase
BSA	bovine serum albumin
C-	carboxyl-
CT	carboxyltransferase
CO_2	carbon dioxide
Da	Dalton
DTT	dithiotriethol
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphates
E. coli	*Escherichia coli*
ECF	energy coupling factor

EDTA	ethylene diamine tetra-acetic acid
FB	fluorescein biotin
FP	fluorescence polarisation
hr(s)	hour(s)
IPTG	isopropyl β-D-1-thiogalactopyranoside
kb	kilobase pair
kDa	kilodalton
K_M	Michaelis constant
LB	Luria broth
m	metre
M	molar
µ	micron
mA	milliampere
MCT1	monocarboxylate transporter-1
min(s)	minute(s)
mL	milliliter
mM	millimolar
MW	molecular weight
MSM	mineral salt media
n	nano
NBD	nucleotide binding domain
nM	nanomolar
ODx	nm optical density at x nm wavelength
p	pico
PBS	phosphate buffered saline

PBS-T	phosphate buffered saline and 0.1% (v/v) Tween-20
PCR	polymerase chain reaction
PDB	protein data bank
PVDF	polyvinyl difluoride
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT	room temperature
s	second
S. aureus	*Staphylococcus aureus*
SBP	substrate binding protein
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEC-MALLS	size exclusion chromatography-multi angle light scaterring
SEM	standard error of the mean
SMVT1	sodium multivitamin transporter 1
TBS	Tris buffered saline
TBS-T	Tris buffered saline and 0.1% (v/v) Tween-20
Tris	2-amino-2-hydroxymethylpropane-1,3-diol
Tween-20	polyoxyethylene-sorbitan monolaurate
VHT1	vitamin H transporter 1
WT	wild type