Expression, Purification and Characterization of the Biotin Transporter from *Staphylococcus aureus*

By

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July 2013

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STATEMENT OF ORIGINALITY

This thesis contains no material that has been accepted for the award of any degree or diploma by any University and, to the best of my knowledge, contains no material that has been previously published by any other person, except where due reference has been made in the text.

This thesis is presented for examination in the School of Molecular and Biomedical Science. Parts of this thesis contain material of a commercially sensitive nature and may not be disclosed without prior consent of the author.

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July 31, 2013

ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to my supervisors, Associate Prof. Grant Booker and Dr. Steven Polyak, for your patient supervision, guidance and constant encouragement throughout this year. Thank you Grant for providing me opportunity to study in your lab, I really appreciate this experience, not to mention all your inspiring advice on both the experiments and presentations. Steven, thank you so much for all your enlightening talks and support, without you, I could not finish my exchange master program and the thesis in the present.

I would like to thank you Al for all the help in framing my project and help in experiments. Wanisa, it is a great pleasure to be your friend and you help me a lot to get used to a new culture environment. And a big thankyou goes to Ashileigh, for your help to get familiar with the new lab and for taking your time to read through my thesis. Jiulia, it is really enjoyable to eat chocolate with you and you are such a great labmate. To the other Booker lab members: Kate, Jorinda thank you for the help you have given me this year. I'd also like to extend thanks to Christopher McDevitt for giving advice on membrane protein purification, Miranda Ween for training me on ultracentrifugation and those advice on handling tricky membrane proteins, and Victoria Lewis for your wonderful advice (I always feel welcomed for asking questions).

My eternal gratitude also goes to my friends far and near, my familiy members who have supported me throughout this year. I will always love you. To Pengcheng Li and Lu Zeng who also involved in this program, I wish you two all the best with your future. Thank you Mama, for your patient listening and tolerating my bad temper when I feel discouraged. Thank you Dad, for always making me feel free and no burden. Being away from you two has been hard but you always make me feel beloved and never give me up.

ABSTRACT

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ECF transporters are a group of newly defined ABC-like modular transporters and they are composed of three main elements: 1) a high-affinity membrane-embedded substrate binding protein (S component), 2) a membrane-spanning protein (T component), and 3) two identical or homologous ATPases (A, A' components) which resemble the nucleotide binding domains in ABC transporters. *Staphylococcus aureus* biotin transporter (*Sa*BioMNY) belongs to the subgroup II ECF transporters which are characterized by their shared use of energy coupling module (AT module) by several S components, with each having a different substrate preference. Therefore, characterizing the S families in ECF transporters are important for us to gain new knowledge about the mechanism of subgroup II ECF transporters. Besides, laboratory has developed a series of biotin analogues with antibacterial activity against *S. aureus*. Previous studies have demonstrated that these compounds were capable of binding to the S component of *S. aureus* (i.e. *Sa*BioY). It was reasonable to speculate that these biotin analogues were transported across the *S. aureus* cells by the biotin transporter BioY. To further improve the antibacterial potency and selectivity, the binding and translocation mode of these compounds across the bacterial membrane via *Sa*BioY needs to be defined.

By utilizing a filter disk diffusion assay, I determined that the susceptibility of *E. coli* BL21 to antibiotics (erythromycin, streptomycin and chloramphenicol) was significantly increased when wild type *Sa*BioY was heterologously overexpressed in the cells. A library of *Sa*BioY mutants was also screened in this assay and the overexpression of all the mutants surprisingly increased the sensitivity of *E. coli* cells to all three antibiotics compared to the un-induced one. One exceptional mutant was the D157K/K160E that was able to restore the tolerance of cells to the antimicrobial agents. I reasoned recombinant *Sa*BioY adopted a functional channel in the membrane of *E. coli* for low molecular weight antibiotics to diffuse through. In

addition, I also found that R75, D157 and K160 are essential to the surrogate transport pathway since a single amino acid change can dramatically alter the sensitivity of *E. coli* cells to antibiotics compared to the wild type one.

To further characterize the biotin core transporter *Sa*BioY, I attempted to purify recombinant *Sa*BioY from *E. coli* BL21 (DE3). The optimized conditions for expressing *Sa*BioY were determined to be 1) culturing cells at 25°C, 2) using the richer potassium buffered TB growth medium and 3) using a high concentration of IPTG (0.8 mM). I have also developed a system for the scalable purification of this integral membrane protein using SDS as a solubilizer. 9.7 mg of SDS-solubilized *Sa*BioY (with expected molecular weight of 19,492 Da) was obtained from 2 liters of culture after IMAC purification, with 90% purity determined by Commassie staining gel. A small panel of available mild detergents was subsequently tested for their efficiency of extracting membrane protein from natural lipids with TrionX-100 giving the best extraction efficiency. This present study paves the way for further detergent screening and purification of *Sa*BioY.

ABBREVIATIONS

ABC transporters	ATP-binding cassette containing transporters
ACC	Acyl-CoA carboxylase
Amp	Ampicillin
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BioY	Biotin substrate binding protein
BioMNY	Intact biotin transporter
BPL	Biotin protein ligase
BSA	Bovine serum albumin
°C	Degrees Celsius
C-	Carboxyl-
CA-MRSA	Community acquired methicillin resistant *Staphylococcus aureus*
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
ECF	Energy-coupling factor
EDTA	Ethylene diamine tetra-acetic acid
g	Gram
HA-MRSA	Hospital acquired methicillin resistant *Staphylococcus aureus*
hSMVT	Human sodium-dependent multivitamin transporter
kDa	Kilo Dalton
LB	Luria broth
m	Milli-
M	molar

MDR	Multidrug-resistant
MCC	3-methylcrotonyl-CoA carboxylase
MCT1	Monocarboxylate transporter 1
Min	Minute, minutes
MW	Molecular weight
n	Nano-
N-	Amino-
NBDs	Nucleotide binding domains
OD_{xnm}	Optical density at x nm wavelength
PC	Pyruvate carboxylase
PCC	Propionyl-CoA carboxylase
PCR	Polymerase carboxylase
PMSF	phenylmethylsulfonylfluoride
*Sa*BPL	*Staphylococcus aureus* biotin protein ligase
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Standard erroe of the mean
SMVT	Sodium-dependent multivitamin transporter
TB	Terrific broth
TBS	Tris buffered saline
TMDs	Transmembrane domains
Tris	2-amino-2-hydroxymethylpropane-1,3-diol
µ	Micro-
V	Voltage
VISA	Vancomycin intermediate resistant *Staphylococcus aureus*
VRSA	Vancomycin resistant *Staphylococcus aureus*
wt	Wild type