GÖTEBORGS UNIVERSITET

INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Structural Insight Into the Bacterial Sialic Acid Catabolic Pathway

Rhawnie Caing-Carlsson
Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i Kemi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 23:e, November, 2018 kl. 13:15 i Ivan Ivarsson, institutionen för kemi och molekylärbiologi i, Medicinaregatan 3, Göteborg.

ISBN: 978-91-7833-225-0, tryckt version
ISBN: 978-91-7833-226-7, pdf nätet
Tillgänglig via http://handle.net/ 2077/57823

Abstract

A genetically diverse community of commensal and pathogenic bacteria thrive in the digestive system and urogenital tracts of animals. Many of these bacteria forage sialic acid from mucosal cell surfaces. Bacteria have evolved a system that utilizes host-derived sialic acid either as an alternative food source (catabolic pathway) or for molecular mimicry to evade the host's immune system (sialylation pathway). Their ability to utilize sialic acid confers a selective advantage by securing an ecological niche for colonization and persistence. Sialic acid catabolic and sialylation pathways are therefore potential targets for the development of novel antimicrobial therapies.

This thesis presents work aimed at determining X-ray structures of sialic acid catabolic enzymes and sialic acid transporters. An automated pipeline was developed to optimize the cloning, expression and purification of enzymes involved in the sialic acid catabolic and sialylation pathways. This led to the large-scale production and purification of Nan kinase from *Fusobacterium nucleatum* (Fn)NanK, an enzyme that phosphorylates ManNAc to ManNAC-6-P in the catabolic pathway. The apo structure of FnNanK was determined at 2.2 Å resolution and displays motifs characteristic of the repressor open reading frame kinase (ROK) superfamily. Despite lacking a zinc-binding region previously implicated in stabilizing the enzyme's active site, FnNanK conserved all structural features required for enzymatic activity. A broad-base strategy for the expression, solubilization and purification of a sialic acid TRAP transporter orthologues was pursued with the overriding goal of determining the crystal structure of a sialic acid TRAP transporter. Different constructs from four orthologues funneled down to the *Pasteurella multocida* TRAP transporter yielding crystals which diffracted to 11 Å resolution. New crystallization strategies or other structural approaches may be necessary to propel this project to structure determination. Finally, the crystal structure of the sialic acid transporter SiaT from *Proteus mirabilis* was determined at 1.9 Å with bound substrate. SiaT adopts an outward-facing conformation that provides novel insight into the alternate access mechanism employed by transporters with inverted topology. The crystal structure also reveals a second sodium-binding site that aids substrate binding and stabilizes the outward-facing conformation.