Göteborg, 2023

STRUCTURAL ANALYSES OF CARBOHYDRATE RECEPTORS FOR ENTEROTOXINS AND ADHESINS OF ENTEROTOXIGENIC ESCHERICHIA COLI

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Arvid Carlsson, Medicinaregatan 3, den 1 december, klockan 13.00.

av Dani Zalem

Fakultetsopponent:
Professor Peter Påhlsson
Linköpings Universitet, Sverige

Avhandlingen baseras på följande delarbeten

I. **Zalem D**, Ribeiro JP, Varrot A, Lebens M, Imberty A, Teneberg S. Biochemical and structural characterization of the novel sialic acid-binding site of Escherichia coli heat-labile enterotoxin LT-IIb. Biochemical Journal. 2016 Nov 1;473(21):3923-3936.

II. Von Mentzer A, **Zalem D**, Chrienova Z, Teneberg S. Colonization factor CS30 from enterotoxigenic Escherichia coli binds to sulfatide in human and porcine small intestine. Virulence. 2020 Dec;11(1):381-390.

III. **Zalem D**, Juhás M, Terrinoni M, King-Lyons N, Lebens M, Varrot A, Connell TD, Teneberg S. Characterization of the ganglioside recognition profile of Escherichia coli heat-labile enterotoxin LT-IIc. Glycobiology. 2022 Apr 21;32(5):391-403.

IV. **Zalem D**, Lebens M, Teneberg S, Karlsson G. *Structural and dynamic studies of Escherichia coli heat-labile enterotoxin LT-IIb B-subunits by NMR spectroscopy.* Manuscript.

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN

GÖTEBORGS UNIVERSITET

Göteborg, 2023

STRUCTURAL ANALYSES OF CARBOHYDRATE RECEPTORS FOR ENTEROTOXINS AND ADHESINS OF ENTEROTOXIGENIC ESCHERICHIA COLI

Dani Zalem

Avdelningen för medicinsk kemi och cellbiologi, institutionen för biomedicin, Sahlgrenska akademin, Göteborgs universitet, Sverige, 2023.

Abstract

Carbohydrate-binding proteins expressed by microbes are key determinants in initiating and sustaining infections that account for millions of deaths each year. This thesis focused on proteins integral to infections instigated by enterotoxigenic *Escherichia coli* (ETEC); estimated as the largest bacterial cause of diarrhea in the world with hundreds of millions of cases each year. ETEC infections are mediated by two primary carbohydrate-binding proteins; 1) Colonization factors (CF), which facilitate host cell attachment, and 2) Enterotoxins, which penetrate host cells to induce a potentially lethal diarrheal response. By employing biochemical techniques, such as chromatogram binding assays, mass spectrometry and NMR, we dissected the precise mechanisms fundamental for the interactions of ETEC carbohydrate-binding proteins.

In the presented papers, the novel colonization factor CS30, and the enterotoxins LT-IIb, and LT-IIc where investigated. Our findings identified the sulfatide glycosphingolipid as the principal receptor for CS30 and emphasized the significance of the carbohydrate-presenting lipid moiety in binding. The diarrhea-inducing toxins, LT-IIb and LT-IIc, demonstrated distinct binding specificity to sialic acid presenting glycosphingolipids, and the presence of such receptors were confirmed in the human intestine. Lastly, structural studies detailed the atomic framework of these binding interactions and quantified the binding affinities.

By revealing the specific carbohydrate interactions underpinning both adhesion and toxin action, our study uncovers the intricate processes governing pathogenic infection mechanisms, which may inform the design of next-generation anti-bacterial therapeutics, vaccines and diagnostical tools.

Keywords: Carbohydrate recognition, glycosphingolipid, diarrheagenic *E. coli*, ETEC, heat-labile enterotoxin, colonization factor

ISBN: 978-91-8069-533-6 (TRYCK)
ISBN: 978-91-8069-534-3 (PDF)