View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Ghent University Academic Bibliography

Binding of the F18 fimbrial adhesin FedF to piglet intestinal epithelium involves specific receptor recognition and non-specific electrostatic attraction with the phospholipid membrane

Kristof Moonens[1,2], Julie Bouckaert[1,2], Maia De Kerpel[1,2], Annelies Coddens[3], Eric Cox[3], Thao Tran[1,2], Han Remaut[1,2], Henri De Greve[1,2]

[1] Department Molecular and Cellular Interactions, VIB, Brussels, Belgium
[2] Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[3] Department of Veterinary Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

Post-weaning diarrhoea and oedema disease are serious infectious diseases of piglets caused by pathogenic *E. coli* strains, including enterotoxigenic *E. coli* (ETEC) and Shiga toxin-producing *E. coli* (STEC). These strains account for substantial economical losses in the pig industry (Bertschinger *et al*., 1994; *E. coli in Domestic Animals and Humans* (Gyles, C. L. ed) pp. 193, CAB, Wallingford, Oxon, UK).
The first and crucial step during most of these pathogenic infections is the recognition and adhesion of these pathogens to a specific host tissue. Adhesins mediating such an interaction are often presented to the host cell receptors by pili or fimbriae. We examined FedF, the adhesive tipsubunit of F18 fimbriae expressed by STEC which infect recently weaned piglets. Recently, the carbohydrates interacting with the FedF adhesin were identified by Coddens *et al*. (2009, J. Biol. Chem. 284, 9713). The authors showed that FedF is interacting with ABH blood group type 1 determinants.

The structure of the lectin domain of FedF was determined for the apoprotein and in complex with blood group A type 1 antigen hexasaccharide. FedF adopts an immunoglobulin-like fold, common to most lectin domains. Ligand binding is located at one side of the FedF lectin domain and involves both β-strands as well as surface exposed loops. No large structural arrangements take place upon binding the ligand. Important residues interacting with the ligand are Tyr49, making hydrophobic stacking interactions with the α2-linked fucose; His88, which forms a hydrogen bond with the fucose and Arg117, making extensive hydrogen bonds with the galactose and glucose residues at the reducing end.

Mutating His88 and Tyr49 to alanine resulted in loss of adherence of mutant F18$^+$ *E. coli* to piglet villous enterocytes. In contrary the substitution of two positively charged lysine residues, located next to the ligand binding site, by both neutral and negatively charged amino acids of the same size (respectively two asparagines and two aspartic acids) did abolish binding. However, interchanging these lysines with positively charged arginine residues fully restored binding *in vitro* to piglet enterocytes. This result suggests the docking of the F18 receptor in the binding site is not sufficient but an additional electrostatic interaction is needed between positively charged residues on the adhesin surface and negatively charged phospholipids of the cellular membrane.