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BIOCHEMICAL ASPECTS OF *H. PYLORI* ADHESION

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In analogy with *Vibrio cholerae* and other toxigenic enteropathogens such as *Yersinia enterocolitica* it seems most likely today that *H. pylori* by specific surface proteins binds to epithelial cell receptors (*Table 1*) allowing the pathogen to deliver urease, vacuolating toxin and other toxic metabolites to cause tissue damage and inflammation (1—3). *H. pylori* as well as animal gastric pathogens as *H. mustelae* and *H. felis* have developed an efficient flagellar apparatus allowing rapid and efficient penetration of the gastric mucus layer (3). Most interestingly, flagellae seems to be synthesized in the late stage of growth before the switch of spiral-shaped organisms to coccoidal forms in a liquid medium.

Table 1. Receptor binding sites for *H. pylori*

1. Sialylated glycoproteins and glycolipids	
2. Sulphated glycoconjugates:	— glycosaminoglycans
	— sulphatides
3. Phospholipids:	— phosphatidylethanolamine
2. Blood group antigens:	— Lewis b
5. Extracellular matrix proteins:	— collagen type IV
	— laminin
	— vitronectin
	— plasminogen

Key words: *receptors, glycoproteins, H. pylori, flagellar apparatus*

INTRODUCTION

1. H. pylori interactions with the gastric mucus layer

It seems likely that the pathogen penetrates rapidly through the upper phospholipid-rich mucin layer. It has been reported that a haemagglutinin (Hap) identical to rich *V. cholerae* mucinase, may be a broad spectrum protease

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degrading fibronectin and structural proteins of the mucin layer in the small bowel (4). Our studies have not confirmed these findings, despite the attempts to activate *H. pylori* haemagglutinins by mild protease treatments. Furthermore, our recent findings show that *H. pylori* does not produce sialidase as it was reported earlier (5). It seems likely that *H. pylori* as well as *H. felis* and other *Helicobacters* will adapt to a life in the lower mucin layer of the stomach and has developed specific enzymes to allow the bacteria to digest nutrients besides efficient urea degradation. Sialic acid specific lectins (SALs) of *H. pylori* interact with sialylglycoconjugates rich in alpha-2,3-sialic acid linkages (6). Immunomagnetic bead-captured *H. pylori* cells from dental plaque scrapings and saliva samples interact with specific saliva glycoconjugates (7). These findings and detection of *H. pylori* in dental plaque indicate that SALs as well as other haemagglutinins may clump organisms in the oral cavity colonization process and allow such clumps to be better adapted to penetrate the gastric mucin layer upon swallowing saliva, giving *H. pylori* a better chance to colonize and infect the gastric mucosa. The discovery that *H. pylori* interact with Lewis b blood group substance found in saliva and other body secretions (8) will stimulate further studies on the oral cavity as a micro-environment for *H. pylori* to colonize and allow oral-oral transmissions. A better understanding of such a process in secretors and non-secretors of Lewis and other blood group substances seems important to define the complex process of how *H. pylori* cell surface adhesins interact with the gastric mucosa.

2. H. pylori cell surface lectins and their role in a gastric colonization process

Cell surface lectins play key roles in an adhesion and colonization process of bacterial pathogens. They function *in vivo* as adhesins interacting specifically with certain glycoconjugate structures. These interactions can be studied *in vitro* systems using intact bacteria. Haemagglutination and haemagglutination inhibition assays have been the classical methods to define surface proteins of bacteria (9). Recently, a novel optical biosensor technique, the IAsys (IAsys Applied Sensors, U.K.), based on detection of biomolecular interactions in real time and requiring no sample labeling, was used to characterize *H. pylori* strains on the expression of certain cell surface lectins (Hirmo *et al.*, in preparation). Our results indicate the potential of this technique to study specific cell interactions with appropriate glycoconjugates as a receptor analogues which were immobilized to the sensor surface (*Fig. 1*).

With the respect to the haemagglutination activity and specificity, *H. pylori* strains are heterogeneous and can be grouped into sialic acid-dependent and sialic acid-independent classes (6). A fimbrial sialic acid specific haemagglutinin has been purified and cloned as a 20 kDa cell adhesin protein (10). Wadström *et al.* (11) using a mild acid glycin treatment (pH 2) were able to remove the cell

surface proteins from the *H. pylori* cells without cell-lysis and contamination of intracellular proteins. A sialic acid specific lectin as protein complex was purified and polypeptide identified by SDS-PAGE and crossed immunoelectrophoresis (mol. weights. 64, 56 and 20 kDa)(12). Huang *et al.* (13) purified a 59 kDa haemagglutinin.

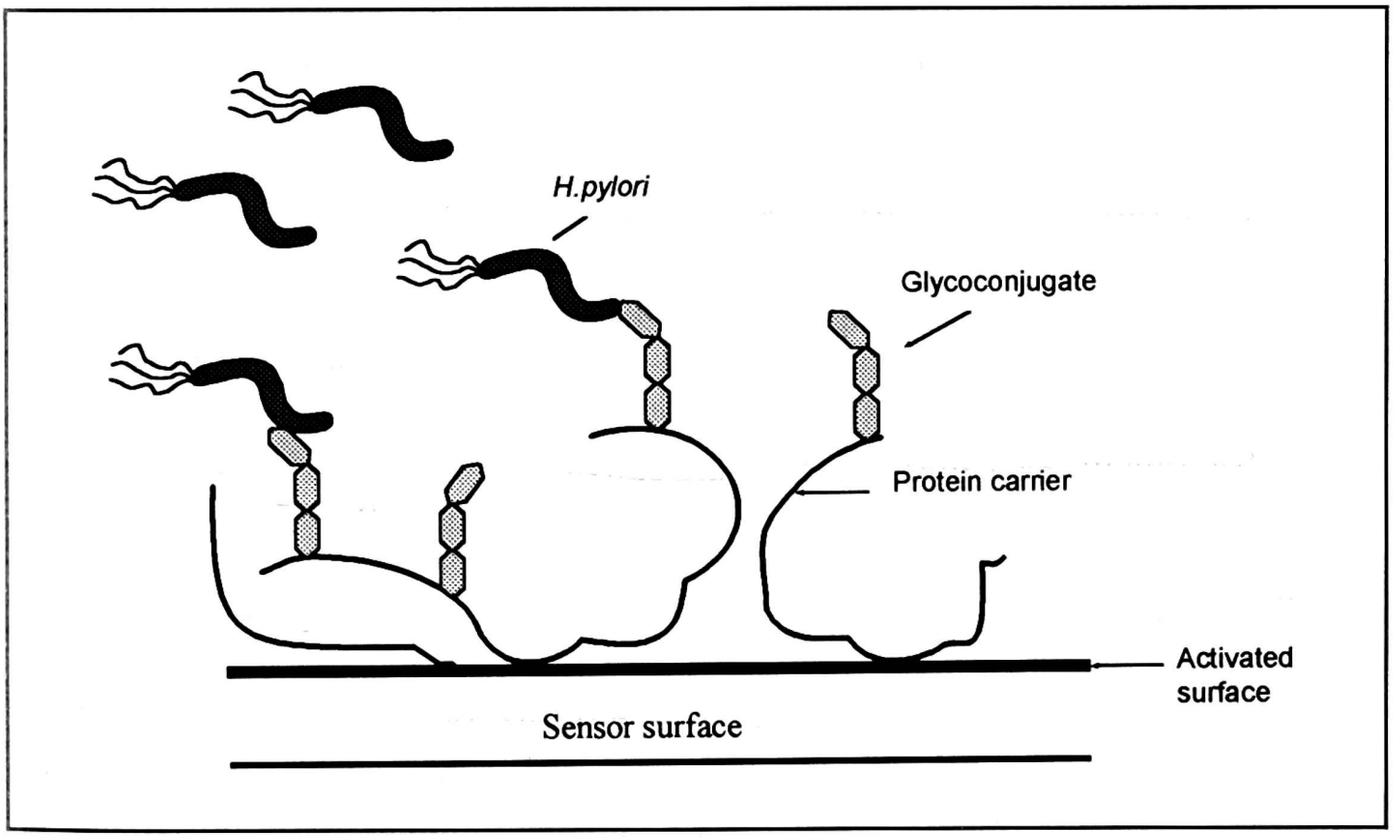

Fig. 1. The model system for the biosensor assay to study *H. pylori* cell interactions with receptor glycoconjugate structures. The appropriate oligosaccharide anchored to the protein carrier, so-called multivalent neoglycoproteins were covalently immobilized to the sensor surface. The increase in an signal is observed in the case of recognizing and binding of the bacteria to the glycoconjugate structure.

3. *H. pylori glycolipid and phospholipid binding surface proteins*

Saitoh *et al.* (14) identified glycolipid receptors of *H. pylori* by thin layer chromatography and immunostaining and defined the ganglioside GM3 and sulphatide(sulfogalactosylceramide) as putative gastric cell receptors for *H. pylori.* Gold *et al.* (15) demonstrated by TLC overlayer binding assays that *H. pylori* also bind to specific phospholipids such as phosphatidylethanolamine and gangliotetraosylceramide. Other investigators identified diglycosylceramides with phytosphingosine and 2D hydroxyfatty acids of 16 to 24 carbon atoms to bind to haemagglutinating and non-haemagglutinating *H. pylori* strains and acid glycine extracted surface proteins. Sialylated gangliosides such as GM1 and GM3 have been identified as specific host cell receptors for a number of mucosal bacterial pathogens including *H. pylori* (14, 16). It has been also reported that *H. pylori* bind to specific glycolipids of the ganglio-series but not of the lacto-series. More studies on cell surface

proteins of haemagglutinating and non-haemagglutinating *H. pylori* strains are now needed to define the relation of surface lectins recognizing these structures and similar structures on glycoproteins and components in the gastric mucins, saliva and human milk. Research in this area will most likely define candidates for anti-adhesion prophylaxis.

It seems likely that sulphated cell surface structures are the natural targets for sulphate-recognizing surface proteins of *H. pylori.* Heparin and heparan sulphate binding surface proteins (HeBPs) show a high affinity for these molecules (17). However, we also showed that a mixture of sulphatides but not heparin, blocks binding to *H. pylori* to a monolayer of human gastric carcinoma cells (Kato III cells) (18). Further studies might show the identity of HeBPs with sulphatide-binding proteins and weather these are involved in close cell adhesion and cellular uptake of *H. pylori* into gastric epithelial cells.

Borén *et al.* (19) have developed elegant methods to demonstrate direct binding of *H. pylori* to the biopsies of the human gastric epithelium, including the natural surface mucin layer by using histochemical and immunohistochemical stainings and electron microscopy.

4. H. pylori interactions with extracellular matrix (ECM)

After initial colonization of the gastric mucin layer probably involving binding to Lewis blood group like glycoproteins and glycolipids, it seems likely that *H. pylori* interact with subcellular ECM components. *H. pylori* interact with type IV collagen, laminin, vitronectin and plasminogen has been described (20—23). It has been shown that growth conditions are extremely important for optimal binding to ECM proteins (20,24). Binding to the highly glycosylated laminin is sialidase sensitive and involves a specific *H. pylori* 25 kDa protein (25). Further studies are needed to identity the proteins involved and the possible role in tissue penetration since *H. pylori* penetrates intracellular junctions containing sialylglycoconjugate-rich cell adhesion molecules and ECM molecules. It seems likely that SAL and HeBP interact with sialylated and sulphated glycoconjugates in inflammed tissues and on cels of the human immune system.

The expression of binding ECM structures seems to be upregulated in stationary phase cells (24).

5. Possible regulation of H. pylori surface proteins

Lewis b blood group binding is expressed mainly in late stationary phase cells, unlike haemagglutination activities, ECM binding and adhesion to Kato III cells. Urease activity decline in stationary phase cells. Attempts to

develop *in vivo* like growth conditions to study expression of surface proteins is underway and is important for isolation in order to prepare specific antibodies to use as a tools to identify adhesin genes in gene-libraries. Immunization of rabbits with a specific cell surface protein complex such as cell surface haemagglutinins have clearly demonstrated several polypeptides in this protein complex. Colloidal gold staining of this complex has demonstrated that the sialic acid binding proteins are surface exposed as a non-fimbrial protein-complex (26).

6. H. pylori adhesion to host cells

The first steps of *H.pylori* host colonization and infection involves the penetration through the gastric mucin layer, adhesion to epithelial cells by surface lectins and direct damage cells by generation of ammonia including indirect immune mediated damage of these target cells (27). The Vac A toxin precursor, about 140 kDa protein, and the cytotoxin associated proteins (Cag A, Cag B and Cag C) seem also to be surface located and may act as putative cell adhesins (28, 29).

We have stained target Kato III gastric cell monolayers by neutral red and trypan blue after adhesion of HA and non HA *H. pylori* strains without detecting a cytotoxic effect. The staining of the cells with the Phalloidin method did not demonstrate dramatic changes in the cytosceleton.

It may be that surface expression of molecules need growth under *in vivo*-like conditions. Growth in an iron limited broth as well as an agar media with significant changes in surface protein profiles in SDS PAGE and Western blot indicate that iron may play a major role in the pathogenicity of *H. pylori* infection. (30).

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REFERENCES

1. Telford JL, Covacci A, Ghiara P, Montecucco C, Rappuoli R. Unravelling the pathogenic role of *Helicobacter pylori* in peptic ulcer: potential for new therapies and vaccines. *Trends Biotechnol* 1994; 12: 420—426.
2. Moran A, Wadström T. Bacterial pathogenic factors. The year of *Helicobacter pylori* 1994. In: Malfertheiner P, Mégraud F, Michetti P, Price A, eds. *Current Science* 1994: 17—21
3. Suerbaum S, Wadström T. Bacterial pathogenic factors. *Helicobacter pylori* year 1995. In: Malfertheiner P, Mégraud F, Michetti P, Price A, eds. *Current Science* 1995: 13—21.
4. Hurtado A, Chahal B, Owen RJ, Smith AW Genetic diversity of the *Helicobacter pylori* haemagglutinin/protease = (hap) gene. *FEMS Microbiol Lett* 1994; 123:173—178.

5. Hirmo S, Kelm S, Wadström T, Schauer R. Lack of evidence for sialidase activity in *Helicobacter pylori*. *FEMS Immunol Med Microbiol* 1997; 17: 67—72.
6. Hirmo S, Kelm S, Schauer R, Nilsson B, Wadström T. Adhesion of *Helicobacter pylori* strains to -2,3-linked sialic acids. *Glycoconj J* 1996; 13: 1005—1011.
7. Ferguson A Jr, Li C, Patel R, Mayberry R, Chi D, Thomas E. Isolation of *Helicobacter pylori* from saliva. *J Clin Microbiol* 1993; 31: 2802—2804.
8. Borén T, Normark S, Falk P. *Helicobacter pylori*: molecular basis for host recognition and bacterial adherence. *Trends Microbiol* 1994; 2: 221—228.
9. Wadström T, Trust TJ. Bacterial surface lectins. *Med Microbiol* 1994; 4: 286—300.
10. Evans DG, Karjalainen TK, Evans DJ, Graham DY, Lee C-H. Cloning, nucleotide sequence, and expression of a gene encoding an adhesion subunit protein of *Helicobacter pylori*. *J Bacteriol*. 1993; 175: 674—683.
11. Wadström T, Guruge JL, Wei S, Aleljung P, Ljungh A. *Helicobacter pylori* haemagglutinins — Possible gut mucosa adhesions. In: Malfertheiner P, Ditschunheit H, eds. *Helicobacter pylori*, Gastritis and Peptic Ulcer. Berlin: Springer-Verlag 1990: 96—103.
12. Lelwala-Guruge J, Kreger AS, Ljungh A, Wadström T. Immunological properties of the cell surface haemagglutinins (sHAs) of *Helicobacter pylori* strain NCTC 11637. *FEMS Immunol Med Microbiol* 1995; 11: 73—78.
13. Huang J, Keeling PWN, Smyth CJ. Identification of erythrocyte-binding antigens in *Helicobacter pylori*. *J Gen Microbiol* 1992; 138: 1503—1513.
14. Saitoh T, Natomi H, Zhao W *et al.* Identification of glycolipid receptors for *Helicobacter pylori* by TLC-immunostaining. *FEBS Lett* 1991; 282: 385—387.
15. Gold BD, Huescesca M, Sherman PM, Inglewood C. *Helicobacter mustelae* and *Helicobacter pylori* binding to common lipid receptors in vitro. *Infect Immun* 1993; 61: 263—268.
16. Slomiany BL, Piotrowski J, Samanta A, VanHorn K, Murty VLN, Slomiany A. *Campylobacter pylori* colonization factor shows specificity for lactosylceramide sulfate and GM3 ganglioside. *Biochem Int* 1989; 19: 929—936.
17. Ascencio F, Fransson L, Wadström T. Affinity of the pathogen *Helicobacter pylori* for the N-sulphated glycosaminoglycan heparan sulphate. *J Med Microbiol* 1993; 38: 240—244.
18. Wadström T, Hirmo S, Novak H *et. al.* Sulfatides inhibit binding of *Helicobacter pylori* to the gastric cancer Kato III cell line. *Curr Microbiol* 1997; 34: 267—272.
19. Borén T, Falk P, Roth KA, Larson G, Normark S. Attachment of *Helicobacter pylori* to gastric epithelium mediated by blood group antigens. *Science* 1993; 262: 1892—1895.
20. Valkonen KH, Ringnér M, Ljungh A, Wadström T. High affinity binding of laminin by *Helicobacter pylori*: evidence for a lectin-like interaction. *FEMS Immunol Med Microbiol* 1993; 7: 29—38.
21. Valkonen K, Wadström T, Moran A. Interaction of lipopolysaccharides of *Helicobacter pylori* with basement membrane protein laminin. *Infect Immun* 1994; 62: 3640—3648.
22. Ringnér M, Valkonen KH, Wadström T. Binding of vitronectin and plasminogen to *Helicobacter pylori*. *FEMS Microbiol Immunol* 1994; 9: 29—34.
23. Ljungh A, Wadström T. Binding of extracellular matrix proteins by microbes. *Meth Enzymol* 1995; 263: 501—514.
24. Khin MM, Ringnér M, Aleljung P, Wadström T, Ho B. Binding of human plasminogen and lactoferrin by *Helicobacter pylori* coccoid forms. *J Med Microbiol* 1996; 45: 433—439.
25. Valkonen KH, Wadström T, Moran AP. Identification of the N-acetylneuraminyllactose-specific laminin-binding protein of *Helicobacter pylori*. *Infect Immun* 1997; 65: 916—923.

26. Lelwala-Guruge J, Ascencio F, Kreger AS, Ljungh A, Wadström T. Isolation of a sialic acid-specific surface haemagglutinin of *Helicobacter pylori* strain NCTC 11637. *Zbl Bact* 1992; 280: 93—106.
27. Mobley HLT. The role of *Helicobacter pylori* urease in the pathogenesis of gastritis and peptic ulceration. *Aliment Pharmacol Ther* 1996; 10 (Suppl 1): 57—64.
28. Figura N. *Helicobacter pylori* exotoxins and gastroduodenal disease associated with cytotoxic strain infection. *Aliment Pharmacol Ther* 1996; 10 (Suppl 1): 79—96.
29. Blaser MJ. Role of *vacA* and *cagA* locus of *Helicobacter pylori* in human disease. *Aliment Pharmacol Ther* 1996; 10 (Suppl 1): 73—78.
30. Dhaenens L, Szczebara F, Husson MO. Identification, characterization and immunogenicity of the lactoferrin-binding protein from *Helicobacter pylori. Infect Immun* 1997; 65: 514—518.

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