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Agglutination of *Helicobacter pylori* coccoids by lectins

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Abstract

AIM To study the agglutination pattern of *Helicobacter pylori* coccoid and spiral forms.

METHODS Assays of agglutination and agglutination inhibition were applied using fifteen commercial lectins.

RESULTS Strong agglutination was observed with mannose-specific *Concanavalin A* (Con A), fucose-specific *Tetragonolobus purpureas* (Lotus A) and N-acetyl glucosamine-specific *Triticum vulgaris* (WGA) lectins. Mannose and fucose specific lectins were reactive with all strains of *H. pylori* coccoids as compared to the spirals. Specific carbohydrates, glycoproteins and mucin were shown to inhibit *H. pylori* lectin-agglutination reactions. Pr e-treatment of the bacterial cells with formalin and sulphuric acid did not alter the agglutination patterns with lectins. However, sodium periodate treatment of bacterial cells were shown to inhibit agglutination reaction with Con A, Lotus A and WGA lectins. On the contrary, enzymatic treatment of coccoids and spiral s did not show marked inhibition of *H. pylori* lectin agglutination. Interes tingly, heating of *H. pylori* cells at 60iæ for 1 hour was shown to augmen t the agglutination with all of the lectins tested.

CONCLUSION The considerable differences in lectin agglutination patterns seen among the two differentiated forms of *H. pylori* might be attributable to the structural changes during the events of morphological transformation, resulting in exposing or masking some of the sugar residues on the cell surface. Possibility of various sugar residues on the cell wall of the coccoids may allow them to bind to different carbohydrate receptors on gastric mucus and epithelial cells. The coccoids with adherence characteristics like the spirals could aid in the pathogenic process of *Helicobacter* infection. This may probably lead to different clinical outcome of *H. pylori* associated gastroduodenal disease.

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INTRODUCTION

Helicobacter pylori has established firmly as a human pathogen causing chronic active gastritis and peptic ulcer[1-3]. In addition, there are epidemiological data that support association between. *H. pylori* infection and the development of varieties of gastric cancers[4-6]. However, the pathogenetic mechanisms of *H. pylori* induced gastroduodenal disease are not well established[7-9].

The widespread prevalence of *H. pylori* infection indicates its infectivity. In general, the success of a pathogen depends on both its virulence and pathogenicity[8-9]. Of these, adherence to surface receptors is an essential step in the pathogenesis for many bacteria[10]. The attachment is mediated by adhesins or ligands which may be soluble or cell-associated and can be demonstrated by haemagglutination of various species of erythrocytes[10-11]. Previously, it was reported that the surface of *H. pylori* contains lectins or adhesins which may influence its adherence to the membrane of surface mucous cells[12]. Since lectins have the ability to bind to a wide variety of microbial substances containing simple or complex carbohydrates, they have been used to detect cell wall modifications, elucidate complex cell wall carbohydrates and detect intra-strain variations in cell wall carbohydrates or carbohydrate linkages[13-14].

The specificity of lectin binding to bacterial surface carbohydrates has been reported[15] and exploited as a tool for typing microorganisms, such as Neisseria[16,17], *Staphylococcus*[18], *Legionella*[19], *Bacillus*[20], *Campylobacter*[21-22], *Helicobacter*[23] and *Streptococcus species*[24-25]. Many

bacteria, including *H. pylori*, were found to have cell wall associated lectins which allow them to bind selectively to mucus and epithelial cells[8,12]. Emody *et al* (1988)[26] also showed that *H. pylori* lectins attach to red cells from various animal species. Moreover, it was suggested that bacterial surface haemagglutinins and lectins play a significant role in pathogenesis of many mucus-associated infections[11]. However, lectins were studied only on spiral forms of *H. pylori*. In this study, we applied lectin agglutination to characterize the carbohydrate residues on the cell wall of *H. pylori* coccoids as compared to spiral and to examine the agglutination profiles of *H. pylori* coccoids and spirals.

MATERIALS AND METHODS

Bacterial strains and spiral forms of H. pylori

Two local strains, RH 54, V_2 and a standard strain, NCTC 11637 were grown on moist chocolate blood agar at 37℃ for 3 days in a humidified, 5% CO_2 incubator (Forma Scientific) to provide homogeneous spiral forms as described in Khin and Ho (1994)[27]. The spiral nature was observed under phase contrast microscope (Nikon Microphot-FXA, Japan).

Preparation of coccoid forms

Ageing coccoids were obtained as described in Khin *et al* (1996)[28]. Additionally, induced coccoids were also prepared by culturing the 3-day-old spirals in brain heart infusion medium supplemented with 10% horse blood and 0.4% yeast extract and exposed to amoxicillin (Sigma) in a final concentration of 5mg/L. The broth containing coccoid forms when inoculated onto moist chocolate blood agar and incubated in a humidified incubator (Forma Scientific, USA) at 37℃ with 5% carbon dioxide did not show any growth after 12 days of incubation.

Both spiral and coccoid forms were washed in PBS (pH 7.4) for 3 times. The bacterial cells were resuspended to a final concentration of 1×10^{10} cells/mL in PBS. Spirals and coccoids were enumerated in triplicates using a bacte ria counting chamber under phase contrast microscope.

Lectins

The lectins used (Table 1) were selected on the basis of their reported specificities, to cover the widest possible range of sugars. All are commercially availa ble from Sigma (St. Louis, USA). The lectins were resuspended at 1μg/L in PBS (pH 7.4) and stored at -20℃ until use.

Agglutination assay

Agglutination assay was carried out according to the method described by Ascencio *et al* (1990)[23]. The assay was performed in triplicates. Binding of lectins to coccoids and spirals were assayed using glass slides by mixing 20μL of bacterial suspension with an equal volume of lectin solution. A negative control was included for each strain by adding 20μL of PBS instead of lectin solution. The agglutination reaction was scored as follows:

3=strong positive reaction with large clumps; 2=strong- positive reaction with moderate size clumps; 1=positive agglutination with fine clumps; w=weak agglutination; -=no agglutination.

Table 1 Major sugar specificities of lectins used in this study

Lectin	Abbreviation	Carbohydrate specificity
Arachis hypogaea	PNA	β-D-Gal(1-3)GalNAc
Bandeirae simplicifolia	BS-I	α-Gal, α-GalNAc
Concanavalin A	Con A	α-D-Man, α-D-Glc
Datura stramonium	DSA	$(D\text{-}GlcNAc)_2$
Glycine max	SBA	D-GalNAc
Helix promatia	HPA	D-GalNAc
Lens culinaris	LcH	α-D-Man
Narcissus pseudonarcissus	NPA	α-D-Man
Pisum sativum	PEA	α-D-Man
Tetragonolobus purpureas	Lotus A	α-L-Fuc
Triticum vulgaris	WGA	$(D\text{-}GlcNAc)_2$,NeuNAc
Vicia faba	VFA	D-Man, D-Glc
Vicia sativa	VSA	D-Glc, D-Man
Vicia villosa	VVA	D-GalNAc
Vigna radiata	MBA	α-Gal

Gal=D-Galactose; GalNAc=N-acetyl galactosamine; GlcNAc= N- acetyl glucosamine; NANA=N-acetyl neuraminic acid; Man= Mannose; Glc=Glucose;Malt=Maltose; Suc=Sucrose; Fuc=L-Fucose.

Agglutination Inhibition Assay

To test whether the agglutination was inhibited by specific glycoproteins or carbohydrates, 20μL of each lectin solution was incubated with an equal volume of the test substance at 20℃ for 1 hour before adding 20μL of bacterial suspension. The agglutination reaction was scored accordingly.

Carbohydrates (Gal, GalNAc, GlcNAc, NANA, Man, Glc, Malt, Suc and Fuc) were dissolved at a final concentration of 0.1M in PBS (pH 7.4). Glycoproteins (bovine submaxillary mucin, porcine stomach mucin, fetuin, asialofetuin, bovine orosomucoid and gangliosides II) were dissolved at a final concentration of 0.1% in PBS. All these chemicals were purchased from Sigma.

Pre-treatment of bacterial cells

H. pylori RH 54 spirals and coccoids were treated with sodium periodate (0.075 M), sulfuric acid (0.025N), formalin (1% w/v) and glycine hydrochlorid e buffer (pH 2.2). Bacterial cells were also heated at 60℃ for 1 hour. Alternatively, these spirals and coccoids were treated with neuraminidase, trypsin, pepsin, proteinase K and chymotrypsin each at 0.1μg/L for 1 hour at 37℃.

After treatment,bacterial cells were washed three times with PBS and resuspended to 10^{10} cells/mL in PBS and used for the aggl utination assay.

Reproducibility

Reproducibility of lectin agglutination was confirmed by repeating the test three times.

RESULTS

The coccoids and spirals of all three strains of *H. pylori* tested showed different agglutination reactions to the 15 lectins. The sugars recognized by *H. pylori* coccoids and spirals were Man, Fuc, Glc, Gal, GalNAc and $(GlcNAc)_2$ (Table 2). Among the 15 lectins tested, strong agglutination patterns were observed with Man-specific Con A, Fuc-specific Lotus A and $(GlcNAc)_2$specific WGA lectins (Table 2). All of the coccoids and spirals were agglutinated by the Man-specific Con A, PEA and VFA lectins,GalNAc-specific HPA and Glc-specific VSA lectins (Tables 2 and 3). RH 54 coccoids reacted with all the 15 lectins tested (Table 2) whereas NCTC 11637 coccoids were found to be non-reactive with WGA and VVA lectins. Similarly, SBA, VVA and MBA lectins were not reactive to V_2 coccoids (Table 2). The spirals were refractory to agglutination with Man-specific L cH and NPA lectins (Table 2). Interestingly, NCTC 11637 spirals were non-re active with Fuc-specific Lotus A, Gal-specific PNA and BS- I and $(GlcNAc)_2$ specific DSA and WGA lectins.

α-D-Man/Con A, LcH, NPA, PEA and VFA binding

It is interesting to note that Man was recognized by all the coccoids as compared to the spirals, since all the coccoids were agglutinated by the Man-specific Con A, LcH, NPA, PEA and VFA lectins (Tables 2 and 3). In contrast, three strains of spirals were agglutinated by Con A, PEA and VFA, but none of the spirals was reactive to LcH or NPA lectins (Tables 2 and 3).

α-L-Fuc/lotus A affinity

Three strains of *H. pylori* coccoids were agglutinated by Fuc-specific Lotus A lectin (Tables 2 and 3). All the spirals except NCTC11637 spirals reacted with Lotus A (Table 2). Strong agglutination pattern was shown by RH 54 and HpV_2 coccoids as well as spirals (Table 2).

$(D\text{-}GlcNAc)_2$/DSA and WGA binding

Both RH 54 and V_2 coccoids as well as spirals had an affinity for GlcNAc residue as shown by agglutination with DSA and WGA lectins. NCTC 11637 spirals were non-reactive to both DSA and WGA but their corresponding coccoids showed agglutination with DSA (Table 2).

D-GalNAc/HPA, SBA and VVA binding

Among the three strains of coccoids, only RH 54 coccoids reacted with GalNAc-specific HPA, SBA and VVA lectins. Among these three lectins, HPA was the only lectin which agglutinated all the coccoids tested. Interestingly, V_2 coccoids lacked the affinity to both SBA and VVA lectins (Table 2). On the other hand, N CTC 11637 coccoids were agglutinated by SBA but not by VVA lectins (Table 2). All the spirals tested were found to be agglutinated by HPA, SBA and VVA lecti ns (Table 3).

D-Glc, D-Man/VSA binding

Three strains of *H. pylori* coccoids and their corresponding spirals were observed to be agglutinated by VSA lectin (Tables 2 and 3). Strong agglutination was given by NCTC 11637 and V_2 coccoids (Table 2).

β-D-Gal or α-Gal/PNA, BS-I and MBA binding

The coccoids of RH 54 and NCTC 11637 showed affinity to Gal-specific PNA BS-I and MBA lectins but V_2 coccoids were non-reactive to MBA lectin (Table 2). On the other hand, MBA lectin agglutinated with all the spirals testes (Tables 2 and 3). Among the spirals, only NCTC 11637 spirals were non-reactive with PNA and BS-I lectins (Table 2).

Table 2 Lectin agglutination patterns of *H. pylori* coccoids and spirals

Lectin	RH 54		NCTC 11637		V_2	
	Coccoids	Spirals	Coccoids	Spirals	Coccoids	Spirals
Con A	2	1	3	2	2	1
LcH	2	0	2	0	1	0
NPA	1	0	1	0	1	0
PEA	2	1	2	W	1	1
VFA	1	1	2	1	1	1
Lotus A	3	3	1	0	3	3
DSA	1	2	1	0	1	2
WGA	2	3	0	0	2	3
HPA	1	2	1	1	1	1
SBA	1	1	1	1	0	3
VVA	1	1	0	1	0	1
VSA	1	1	2	1	2	1
PNA	1	1	1	0	1	2
BS-I	2	2	1	0	1	2
MBA	1	3	1	1	0	1

Sugar specificity / Lectins

Man- ConA=*Concanavalin* A; LcH=*Lens culinaris;* NPA=*Narcissus pseudonarcissus;* PEA=Pisum sativum; VFA=*Vicia faba*

Fuc- Lotus A=*Tetragonolobus purpureas;*

$(GlcNAc)_2$- DSA=*Datura stramonium*; WGA=*Triticum vulgaris*

GalNAc- HPA=*Helix promatia*; SBA=*Glycine max*; VVA=*Vicia villosa.*

Glc- VSA=*Vicia sativa*

Gal- PNA=*Arachis hypogaea*; BS-I=*Bandeirae simplicifolia* MBA=*Vigna radiata*

3=strong positive reaction with large clumps; 2=strong positive reaction with moderate size clumps; 1=positive agglutination with fine clumps; w=weak agglutination; -=no agglutination.

Table 3 Strains of *H. pylori* agglutinated by lectins of various specificities

Sugar-specificity/Lectin	No.of *H. pylori* strains agglutinated	
	Coccoids	Spirals
α-D-Man, α-D-Glc/Con A	3	3
α-D-Man/LcH	3	0
α-D-Man/NPA	3	0
α-D-Man/PEA	3	3
D-Man, D-Glc/VFA	3	3
α-L-Fuc/Lotus A	3	2
$(D\text{-}GlcNAc)_2$/DSA	3	2
$(D\text{-}GlcNAc)_2$/WGA	2	2
D-GalNAc/HPA	3	3
D-GalNAc/SBA	2	3
D-GalNAc/VVA	1	3
D-Glc, D-Man/VSA	3	3
β-D-Gal(1-3)GalNAc/PNA	3	2
α-Gal, α-GalNAc/BS-I	3	2
α-Gal/MBA	2	3

Reproducibility testing revealed that lectin patterns were not altered after subculture. Among the three *H. pylori* strains, RH 54 was chosen since it showed agglutination with at least 13 out of 15 lectins tested. Of the 15 lectins, Con A, Lotus A and WGA which gave strong agglutination were chosen for further studies on the specificity of these lectin agglutination (Tables 4 and 5). Specific sugars, glycoproteins and mucin altered the lectin-bacterial agglutination reactions. As shown in Table 4, bovine submaxillary mucin reduced RH 54-Con A agglutination but did not alter the Lotus A and WGA binding patterns. Of great interest was the porcine stomach mucin, which not only reduced the binding ability to Con A and Lotus A but also completely inhibited RH 54-WGA binding. Fetuin slightly reduced RH 54 coccoids-WGA binding and the spirals-Con A agglutination patterns. Other glycoproteins like asialofetuin, bovine orosomucoid and gangliosi des II display a considerable inhibitory ability on binding of RH 54 by Con A and WGA. More importantly, *H. pylori* Lotus A agglutination patterns were strong and were not inhibited by various glycoproteins with the exception of porcine stomach mucin that slightly reduced the coccoids-Lotus A binding capacity.

In the same way, Gal, GalNAc, GlcNAc, NANA, Glc and Suc affected inhibition on *H. pylori* lectin agglutination reactions except Lotus A agglutination pattern (Table 4). It was interesting to note that Man and Malt completely inhibited coccoids and spirals-binding to Con A. On the other hand, the strong agglutination of Lotus A with coccoids and spirals was completely inhibited by fucose (Table 4). In general, agglutination to Con A was either weaken or completely inhibited by carbohydrates. In contrast, WGA agglutination remained unaffected with *H. pylori* spirals whereas the agglutination with coccoids reduced.

RH 54 spirals and coccoids exhibited different agglutination patterns according to their pre-treatment (Table 5). Pre-treatment of the bacterial cells with formalin (1.0%), glycine HCl buffer (pH 2.2) and sulphuric acid (0.025 N) did not alter the agglutination patterns with lectins. In contrast, sodium periodate treatment of bacterial cells was shown to entirely inhibit agglutination reaction with Lotus A and WGA lectins, but reduce the agglutination with Con A. Of inte rest was the heating of *H. pylori* cells at 60℃ for 1 hour which was shown to augment the agglutination reaction with all three lectins (Con A, Lotus A and WGA) tested (Table 5).

Enzymatic treatment of coccoids and spirals did not show marked inhibition of helicobacter-lectin binding patterns (Table 5). Neuraminidase, trypsin, pepsin, proteinase K and chymotrypsin did not change bacterial cells-Lotus A or bacterial cells-WGA agglutination. However, prior treatment of RH 54 coccoids with enzymes decreased agglutination with Con A.

Table 4 *H. pylori* lectin-binding inhibition

Inhibitor	RH 54 coccoids			RH 54 spirals		
	Con A	Lotus A	WGA	Con A	Lotus A	WGA
Glycoproteins						
Bovine submaxillary mucin	1	3	2	0	3	3
Porcine stomach mucin	1	2	0	1	3	0
Fetuin	2	3	W	W	3	3
Asialofetuin	1	3	1	W	3	3
Bovine orosomucoid	1	3	W	1	3	2
Gangliosides II	1	3	W	0	3	2
Carbohydrates						
Gal	1	3	1	W	3	3
GalNAc	1	3	1	W	3	3
GlcNAc	W	3	1	0	3	3
NANA	1	2	1	1	3	3
Man	0	3	1	0	3	3
Glc	W	3	1	0	3	3
Malt	0	3	1	0	3	3
Suc	W	3	1	0	3	3
Fuc	1	0	2	0	0	3
Control	2	3	2	1	3	3

The grade score to assess the reaction corresponds to Table 2.

Table 5 Effect of chemical and enzymatic treatments on *H. pylori* lectin binding

Inhibitor	RH 54 coccoids			RH 54 spirals		
	Con A	Lotus A	WGA	Con A	Lotus A	WGA
$NAIO_4$(0.075M)	W	0	0	W	0	0
H_2SO_4(0.025N)	1	3	2	1	3	3
Formalin(1.0%)	2	3	2	1	3	3
Glycine HCl buffer(pH 2.2)	1	3	2	1	3	3
60℃(1 hour)	3	3	3	3	3	3
Neuraminidase	1	3	2	1	3	3
Trypsin	1	3	2	1	3	3
Pepsin	1	3	2	1	3	3
Proteinase K	1	3	2	1	3	3
Chymotrypsin	1	3	2	1	3	3
Control	2	3	2	1	3	3

The grade score to assess the reaction corresponds to Table 2.

DISCUSSION

In this study the carbohydrate residues on the cell wall of *H. pylori* spirals and coccoids were characterized by lectin agglutination performed on the glass slides. The observations that the use of glass slides for helicobacter-lectin agglutination is simple, cost-effective and rapid, was supported by Davidson *et al* (1982)[18] and Wong *et al* (1986)[21] in their studies on *Staphylococcus* and *Campylobacter* species, respectively. The microtitre plates were also used to study the interaction between bacterial cells and lecti ns for testing large numbers of strains[20,29-30]. However, slide assays have the advantages of yielding results in less than wto minutes. This finding concurred with that of Wong *et al*. (1986)[21]who reported that slid e agglutination assay was the procedure of choice for determining lectin reactio n patterns among *Campylobacter jejuni* and Campylobacter coli. More impor tantly, interference from auto-agglutination is less frequent because bacterial cells-lectin agglutination occurs rapidly on glass slides before non-specific agglutination in the controls become evident.

The fifteen lectins evaluated in this study were selected because their major specificities were for common saccharides present on the bacterial surface. Typing of bacterial strains in epidemiological studies using plant lectins was found to be a powerful method for differentiating strains of related species[18,20-21]. In this study, RH 54, NCTC 11637 and V_2 spirals were refractory to agglutination with LcH and NPA lectins, specific for mannose residues, implying that the spirals might have less affinity for mannose (Table 2). On the other hand, NCTC 11637 spirals did not react with Fuc-specific Lotus A, Gal-specific PNA and BS-I and $(GlcNAc)_2$-specific DSA and WGA lectins. It indicates that different lectin agglutination pattern shown by NCTC 11637, may be useful for discriminating it from RH 54, V_2 and other *H. pylori* strains.

Moreover, among *H. pylori* coccoids, RH 54 reacted with all lectins while NCTC 11637 was non-reactive with GalNAc-specific VVA and $(GlcNAc)_2$-specific WGA lectins (Table 2). In contrast, V_2 coccoids did not show reactivity to Gal-specific MBA and GalNAc-specific SBA or VVA lectins. The possible reason of different lectin agglutination patterns seen among the spiral and coccoid forms of three *H. pylori* strains studied, might be attributable to the considerable differences among different strains as well as the two morphological forms of the same strain in the affinity for the carbohydrate receptors of the host. This may eventually influence the adherence capatilities of *H. pylori* in gastric colonization resulting in different gastroduodenal pathology. It is suggested that lectins can also be used for characterization of *H. pylori* strains which will confer as a useful method for epidemiological studies to determine the natural history and mode of transmission of *H. pylori* as was also reported by Ascencio *et al* (1990)[23].

Lectins have been used to identify and distinguish microorganisms based on different sugar components on their cell surfaces[14]. All the coccoids tested were shown to recognize Man-specific lectins, in contrast to the spirals (Table 3), suggesting that the coccoid forms of *H. pylori* might have predilection for Man-receptors of the gastric tissues. This could possibly be due to acquisition of Man-residues which may become available for binding gastric tissues during transformation from spirals to coccoids. Baczako *et al*. (1995)[31] reported the differences in lectin binding properties of the antral and body surface mucosa of the human stomach and questioned whether it may be relevant for *H. pylori* affinity. The lectins from the jack bean, Con A as well as fava bean, VFA known to have an affinity for Man>Glc residues and another mannos e-specific lectin, PEA reacted with three atrains of *H. pylori* coccoids and spirals (Table 4). It indicates that Man/Glc residues present on the cell surface may contribute to their binding. Lectin histochemistry studies showed t hat the staining for mannose and glucose (bound by succinylated Con A) was negat ive in normal mucosa but was positive in *H. pylori* infected mucosa[31]. It is therefore possible that *H. pylori* coccoid form having stronger affinity for mannose residues (Tables 3 and 4) might mediate its adherence to the corresponding mannose receptors existing in the gastric mucous cells for its colonization. It is suggested that the two differentiated forms of *H. pylori* might have various forms of Man-associated lectins which allow them to bind selectively to the mucous and epithelial cells thereby facilitating gastric colonization.

The specificity of bacterial agglutination by lectins resides in the unique cell surface structures of the bacteria interacting with the carbohydrate-specific lectins[18,32-33]. The lectin from asparagus pea, Lotus A shown to an affinity for Fuc residues were recognized by three strains of *H. pylori* have coccoids and spirals with the exception of NCTC 11637 spirals (Table 2). It was reported that fucose was more likely to function as a receptor for *H. pylori* related adhesion[34]. It is possible that both the coccoid and spiral forms might have different Fuc specificities on the surface of the cells, contributing to the various degrees of *H. pylori* adhesion. Such different affinities might mediate their binding to gastric mucosa resulting in various forms of gastroduodenal pathology.

H. pylori coccoid forms may have retained the

lectin binding sites for Fuc determinants, in an unmodified form or could have been the components of the surface of the coccoids. Support of this view comes from the observations that sever al *Bacillus cereus* expressed different lectin binding sites on spores and vegetative cells[20]. In addition, the lectin-staining pattern of surface mucous cells demonstrates, that there are mainly neutral carbohydrates (fucose and galactosamine) localised within the mucous granules[12]. Therefore, it is possible that cell wall of *H. pylori* coccoids and spirals bearing different affinity for Fuc residues might influence the binding to different gastric mucous cells receptors to obtain various pathogenic potentials in *Helicobacter* infection.

N-acetylglucosamine specific lectins from wheat germ, WGA and jimson weed, DSA showed agglutination with RH 54 and V_2 spirals and coccoids (Tables 2 and 3). On the other hand, DSA agglutinated only NCTC 11637 coccoids. Interestingly, the refractory of NCTC 11637 spirals and coccoids agglutination with WGA suggests either masking of GlcNAc-residues, absence of these units, or exposure of these units may differ between *H. pylori* strains. RH 54 and V_2 spirals showed st ronger agglutination reaction with WGA lectins than the coccoids (Table 2). This could be due to the acquisition of thick polysaccharide capsule on the coccoidal cells[35] resulting in weaker agglutination with WGA lectin. A similar mechanism was demonstrated on *Neisseria* species in which WGA was shown to specifically agglutinate *Neisseria gonorrhoeae* but not the encapsulated *N. meningitidis* suggesting that agglutination with WGA is due to the lack of a capsule on the gonococci[16].

All the spirals studied were shown to recognize GalNAc-specific lectins from HPA, SBA and VVA (Table 3) as was shown earlier by Ascencio *et al* (1990)[23]. *H. pylori* coccoids showed weaker affinity for GalNAc residues (Tables 2 and 3) suggesting that structural modifications might occur on the cell wall of the coccoids during morphological transformation from the spirals. It is proposed that *H. pylori* coccoids, a morphological variant of the spirals, might bind with different capacities to galactosamine receptors localised withi n gastric mucous granules, as was also observed for the spirals by Bode *et al*. (1988)[12]. After binding to the host cells, it may then take part in various mechanism of adhesion resulting in the gastroduodenal disease. Adhesion is considered as one of the virulence factors that allow *H. pylori* spirals to survive in the hostile gastric environment. Its ability to adhere to gastric mucosal cells and mucus[7,9,36] suggests that the coccoids may also use the same adherence mechanism for gastric colonization.

The ability of three strains of coccoids as compared to all spirals in agglutinating Glc-specific lectin (VSA) (Table 3) could be due to the retention of Glc residues on the cell surface of the coccoid forms during morphological conversion. Since the cell wall is an important virulence factor in many bacteria[25], the cell wall of *H. pylori* coccoids bearing similar Glc residues like the spirals, may be attributable to the virulence.

The absence of agglutination with PNA, which detects β-D-Gal (1-3) GalNAc and with BS-I, which detects α-Gal and α-GalNAc, suggests that these carbohydrates are not present on the reactive surface of NCTC 11637 spirals (Tables 2 and 3). This agglutination pattern might aid in differentiating *H. pylori* spirals. It was also shown by Ascencio *et al*. (1990)[23] who developed the lectin typing system of 50 *H. pylori* strains for epidemiological studies. On the other hand, Gal-specific MBA lectin did not agglutinate V_2 coccoids (Table 2) indicating the lack of this sugar on the cell wall of the coccoids.

Porcine stomach mucin completely inhibited RH 54-WGA binding (Table 4) verifying that the agglutination is specific and this glycoprotein has the highest affinity for that lectin. Inhibition studies with various carbohydrates suggest that Glc, Fuc, Man, Gal and GlcNAc are present on the surface of *H. pylori* coccoids and spirals indicating that this application may provide valuable information about the specific sugars present on the cell surface of the two differentiated forms of *H. pylori*. In an earlier study, Facinelli *et al*. (1994)[30] stated that *Listeria*-lectin binding inhibition studies were useful for characterization of specific sugars present at the cell surface of an organism.

RH 54 coccoids and spirals were found to be non-reactive with Con A, Lotus A and WGA lectins after sodium periodate treatment (Table 5) suggesting that $NaIO_3$ probably had destroyed the surface carbohydrate structures of these cells. A similar observation was made by Ascencio *et al*. (1990)[23]. However, the reactions were shown to be resistant to sulphuric acid (0.025N),glycine hydrochloride (pH 2.2) and formalin (1.0%) indicating that these chemicals wer e not effective to alter the *H. pylori* lectin binding.

Treatment of *H. pylori* cells at 60℃ for 1 hour was shown to give stronger agglutination capacities with Con A, Lotus A and WGA lectins (Table 5). It could be possible that heating releases the concealed carbohydrate residues to be exposed onto the surface of *H. pylori* spirals and coccoids. Heating was found to eliminate interference of non-specific cell agglutination. A comparable finding was reported on Campylobacter jejuni and *C. coli* that bacterial interaction with lectins was greatly

enhanced by heating the cultures to 100℃ and holding for 30 to 60 minutes[21]. They found that heating had made it feasible to type most rough and auto-agglutinating strains as well as smooth cultures. Pre-treatment may therefore be a prerequisite for agglutination with lectins. Pre-treatment was shown to augment the agglutination reactions of *Streptoc occus* species[25] although it increases the time required to carry out the assay[24,37].

RH 54 coccoids-Con A agglutination patterns changed after treatment with enzymes such as, neuraminidase, trypsin, pepsin, proteinase K and chymotrypsin (Table 5). It could be due to the partial destruction of the bacterial surface structures by enzymes resulting in weaker agglutination reactions. However, Lotus A and WGA lectins did not alter the agglutination patterns, indicating that pretreatment with enzymes could probably be necessary for the lectin typing assays. It agrees with the studies on streptococci that when trypsin or other proteolytic enzymes partially hydrolyse proteoglycans, some carbohydrate residues may become available for binding[25,37]. At the same time, other carbohydrate residues may be destroyed or modified. This study shows that lectin-bacterial agglutination patterns may differ according to the pre-treatment of the bacteria. In addition, the bacterial surface structures might be partially destroyed by enzyme treatment as carbohydrate residues dissolve in the buffer while others, previously hidden, then become exposed on the surface[25]. Enzymatic treatment may therefore be useful for typing *H. pylori* as was also reported by Ascencio *et al*. (1990)[23].

Lectin reactions provide an attractive alternative method for typing microorganisms with minimum specialised facilities. In addition, lectin agglutination assay is rapid, inexpensive, reproducible, require no special equipment, simple to perform and a useful method for epidemiological studies. This study shows that lectins are stable and easy to store, commercially available and active at low concentration. However, lectin agglutination may be affected by culturing conditions, growth on solid or liquid media and is affected by specific treatment such as trypsin treatment or boiling before assay. More importantly, amoxicillin-induced coccoids tend to give auto-agglutination reaction. This could explain the divergence of structural changes on the cell surface of the coccoids due to the antibiotics or aging process.

It is apparent that the surface of *H. pylori* contains a variety of carbohydrates which may play a role in adherence phenomenon. Comparison of lectin agglutination reactions between the coccoids and spirals show that different kinds of sugar residues might be present on the two forms which might provide evidence for the complexity of the adherence process. It is possible that *H. pylori* coccoids and spirals, bearing different affinities for carbohydrate receptors on the cell surface may account for the variation in the adhesive properties of the organism for antral mucous cells in the stomach. This may probably reflect different strategies used by the two differentiated forms of *H. pylori* in colonizing gastroduodenal tissues, resulting in different disease profiles such as acute and chronic gastritis or peptic ulcer.

With regard to bacterial surface lectins, which often play a role in the initial step of immune defence against phagocytosis, it is assumed that both spiral and coccoid forms may involve in the interaction of *H. pylori* host immune mechanisms. As a rule, *H. pylori* infection is almost always associated with inflammation[9]. However, peptic ulcer disease and gastric carcinoma occur only in a subset of individuals infected chronically with *H. pylori*[9]. Therefore, it is proposed that, like spirals, coccoid forms also play an important pathogenic role in *H. pylori* infection.

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