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Preparation and evaluation of polysaccharide sulfates for inhibiting *Helicobacter pylori* adhesion

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ABSTRACT

In treatments of *Helicobacter pylori* infections, recrudescences were common because of an unfavorable bacterial eradication rate due to the ever increasing resistance to antibiotics. In this study, we chose pectin, guar gum and chitosan to synthesize their sulfates to inhibit adhesions of *H. pylori* and thus enhance the eradication rate. The introduction of sulfates was characterized using FT-IR and elemental analysis. Data from zeta-potential, hydrodynamic diameter, hydrolysis and rheological property demonstrated the sulfates were physicochemically stable. Inhibition assay of hemagglutination and adhesion indicated sulfates prevented *H. pylori* from adhering to erythrocytes and AGS cells. In binding assay, affinities of sulfates to *H. pylori* suggested sulfates could compete with target cells for bacteria and moderated the bacterial adhesion to hosts. A higher content of galactoses and 2,3-O-linked sulfates benefited this action. Thus polysaccharide sulfates can serve as potential adjuvants to raise the bacterial eradication rate by inhibiting adhesions of *H. pylori*.

1. Introduction

Helicobacter pylori (*H. pylori*), a spiral-shaped and gram-negative bacterium, was first isolated in 1982 (Marshall & Warren, 1984), and is a causative agent of chronic active gastritis, peptic ulcer disease and gastric cancer (Blaser, 1990; Marshall & Warren, 1984; Parsonnet et al., 1994). It is estimated to infect 50% of the world populations (Kobayashi, Lee, Nakayama, & Fukuda, 2009). A standard triple therapy combining a proton pump inhibitor (PPI) and two kinds of antibiotics, especially clarithromycin and amoxicillin has been widely used for the treatment of *H. pylori* infections (Malfertheiner et al., 2012). However, making use of a large dose of antibiotics has caused the ever increasing resistance to clarithromycin and the reduction in eradication rates (74–78%). Recently quinolones and rifabutin are used instead of clarithromycin, but the results have been mixed (Qian et al., 2012; Zullo et al., 2012). Hence new drugs are in demand.

Recent studies have indicated some native acidic polysaccharides can inhibit the adhesion of *H. pylori* on cells such as gastric cells or macrophages (Lutay, Nilsson, Wadström, & Ljungh, 2011; Sim et al., 2011; Wittschier, Faller, & Hensel, 2009; Xu et al., 2010). Other researches in adhesion mechanisms have demonstrated the adhesion of *H. pylori* to hosts is mediated by bacterial outer membrane proteins (OMPs) and particular oligosaccharide epitopes on gastric mucins (Kenny et al., 2012). The oligosaccharide epitopes always contain acidic groups (sialylated or sulfated), which are usually attached to lacNAc (Galβ1-4GlcNAcβ1-) or lacdiNAc (GalNAcβ1-4GlcNAcβ1-). Thus we deduce acidic polysaccharides rich in sialylated or sulfated galactoses may display a strong affinity to *H. pylori*, which compete with host cells or mucins for *H. pylori* and thus the adhesion of *H. pylori* to hosts is inhibited. The aim of this study was to synthesize polysaccharide sulfates with characteristics of binding affinity to *H. pylori* and inhibiting bacterial adhesion to hosts, which could raise the eradication rate of *H. pylori* and ultimately reduce the dose of antibiotics.

Polysaccharides are perfect adjuvants for *H. pylori* related diseases. Firstly, polysaccharides are difficult to be absorbed in the alimentary tract and thus avoid possible side effects caused by absorption. Secondly, polysaccharides can increase the viscosity of stomach contents, prolong the time of gastric emptying and reinforce the effect of time dependent antibiotics. In this paper, we choose three types of polysaccharides, pectin, guar gum and chitosan for sulfating. Pectin (PEC) is a linear chain of α-(1-4)-D-galacturonic acids. As for guar gum (GUA), the backbone is a

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linear chain of β-(1-4)-mannoses to which galactose residues are 1-6-linked at every two mannoses. Chitosan (CHI) is composed of β-(1-4)-D-glucosamines and N-acetyl-D-glucosamines. The sulfation occurs on hydroxides or aminos on the polysaccharides. Sulfates only can be grafted on 2,3-OH on galacturonic acids of PEC, while GUA, besides 2,3-O-linked sulfates, 4,6-O-linked sulfates can also be obtained. As for CHI, 2-N-linked and 3,6-O-linked sulfates can be gained by sulfation. The structural differences were employed to identify the distinctive effects of linkage modes of sulfates and galactose amounts on the adhesion inhibition.

Over the years, although anti-adhesion effects of polysaccharide on *H. pylori* have been reported, there are little reports on the binding affinity of polysaccharides to the bacteria, limited by the existing detecting techniques. The most widely employed method is thin layer radiochromatography (Takamura et al., 2012; Teneberg, 2009), but mechanical strength of silica gel thin layer plates are too poor to be appropriate for immobilization and isolation of polysaccharides. Isotope labeling also requires demanding operations and lab conditions. We modified the classic method using nylon membranes to immobilize polysaccharides and fluorescein isothiocyanate (FITC) to label bacteria instead of isotopes. The novel method met the qualification of immobilization and quantitative detection.

In this work, we obtained pectin sulfate (PECS), guar gum sulfate (GUAS) and chitosan sulfate (CHIS), and evaluated their physicochemical properties such as zeta potential, hydrodynamic diameter, rheological property and stability. Erythrocytes and AGS cells were exploited for *H. pylori* adhesion inhibition assay. To certify whether the adhesion inhibition is caused by competitive bindings of polysaccharide sulfates to *H. pylori*, a binding assay between polysaccharide sulfates and bacteria was conducted on nylon membranes. From the results above, we found out favorable polysaccharide structures for adhesion inhibition.

2. Experimental

2.1. Chemicals

PEC, GUA and CHI were obtained from Sigma–Aldrich (USA). All other chemicals unless otherwise stated, were of analytical grade.

2.2. Sulfation of polysaccharides

The sulfation of PEC, GUA and CHI was carried out according to the previous method (Vogl, Paper, & Franz, 2000). In brief, polysaccharide was soaked in dry DMF overnight and then mixed with SO_3-pyridine in DMF under agitation. For every mole of SO_3-pyridine, 1 mol pyridine was added to the mixture. After stirring under nitrogen atmosphere (120 °C, 4 h), the reaction was terminated in an ice bath and adding distilled water. To obtain soluble polysaccharides, the pH of solutions was adjusted to 6.0–7.0 using 1 mol/L NaOH. The sulfated polysaccharides were dialyzed against distilled water for 4 days with dialysis bags (MWCO: 3500 Da) and freeze-dried.

2.3. Structural characterization

The structure of polysaccharide sulfates was characterized using a FT-IR spectrometer (TENSOR 37, Bruker, Germany). An elemental analyzer (Vario EL cube, Elementar, Germany) was applied to determine the elemental composition of PECS, GUAS and CHIS.

2.4. Zeta potential and hydrodynamic diameter

The polysaccharide solutions (0.1 mg/mL) were used for zeta potential (Z_p) and hydrodynamic diameter (*Z*-average) determination. The experiment was conducted at 25 °C using a Malvern Zetasize (Zetasizer Nano ZS90, Malvern Instruments, UK).

2.5. Hydrolysis stability

The sulfated polysaccharides were dissolved in 0.1% HCl solution (pH 1.2) or acetate buffer (pH 4.0), shaken in water bath (37 °C, 100 rpm) and sampled at 1 h, 2 h, 4 h and 6 h. Content of sulfate was measured using an elemental analyzer (Vario EL cube, Elementar, Germany).

2.6. Rheological property

The polysaccharide solutions were prepared in distilled water at a concentration of 1% (w/v). All rheological measurements were conducted with a rotational rheometer (DV-III ULTRA, Brookfield, US) at 25 °C.

2.7. Bacterial and cell culture

H. pylori SS1 and *Lactobacillus bulgaricus* ATCC 10638 were used in the study. *H. pylori* was grown on fetal bovine serum (Gibco, USA) coated Columbia agar with 5% sheep blood (BD, USA) for 48–72 h under microaerophilic condition at 37 °C. *L. bulgaricus* was grown in MRS broth for 12–24 h under anaerobic condition at 37 °C. AGS cells were cultivated in culture dish using RPMI 1640 medium (Corning, USA) with 10% fetal bovine serum at 37 °C and subcultivated every 2 days at a ratio of 1:3.

2.8. FITC-labeling to bacteria

Bacteria were suspended in 10 mL PBS (6×10^8 cfu/mL) and incubated with 6 μL FITC (10 mg/mL in DMSO) at 37 °C in the dark for 12 h. The FITC-labeled bacteria were harvested by centrifugation (3500 rpm, 10 min) and washed three times in PBS containing 0.1% Tween-20 (Xu et al., 2010). The labeling efficiency of the bacteria was detected by a flow cytometer (EPICS XL, Beckman Coulter, USA) equipped with a 488 nm argon laser for excitation. The events collected were ten thousands and the data were analyzed through EXP032 software.

2.9. Binding assay

Nylon membranes (pore size 0.22 μm) were soaked in polysaccharide solutions (1 mg/mL) and incubated at 50 °C for 24 h. Then polysaccharides were coated on nylon membranes (pore size 0.22 μm) by pressure filtration to obtain PECS chips, GUAS chips and CHIS chips respectively, which were placed into a 96-well opaque plate. In direct binding assay, 100 μL FITC-labeled *H. pylori* or *L. bulgaricus* solution (6×10^8 cfu/mL) was added into each well, incubated for 1 h at 37 °C, and then washed three times in PBS containing 0.1% Tween-20. In presaturated binding assay, 100 μL unlabeled *H. pylori* solution (6×10^8 cfu/mL) was first added into each well and incubated to saturate the polysaccharides on the chips and then washed to remove the excess bacteria. After that, 100 μL FITC-labeled *H. pylori* solution (6×10^8 cfu/mL) was added. The following steps were the same as in the direct binding assay. Fluorescence intensity was determined by a microplate reader (Flex Station 3, Molecular Devices, US) using an excitation wavelength at 485 nm and a detection wavelength at 528 nm.

2.10. Hemagglutination and hemagglutination inhibition assay

Hemagglutination (HA) assay was improved based on the previous study (Belogortseva, Yoon, & Kim, 2000). Twenty-five microliter

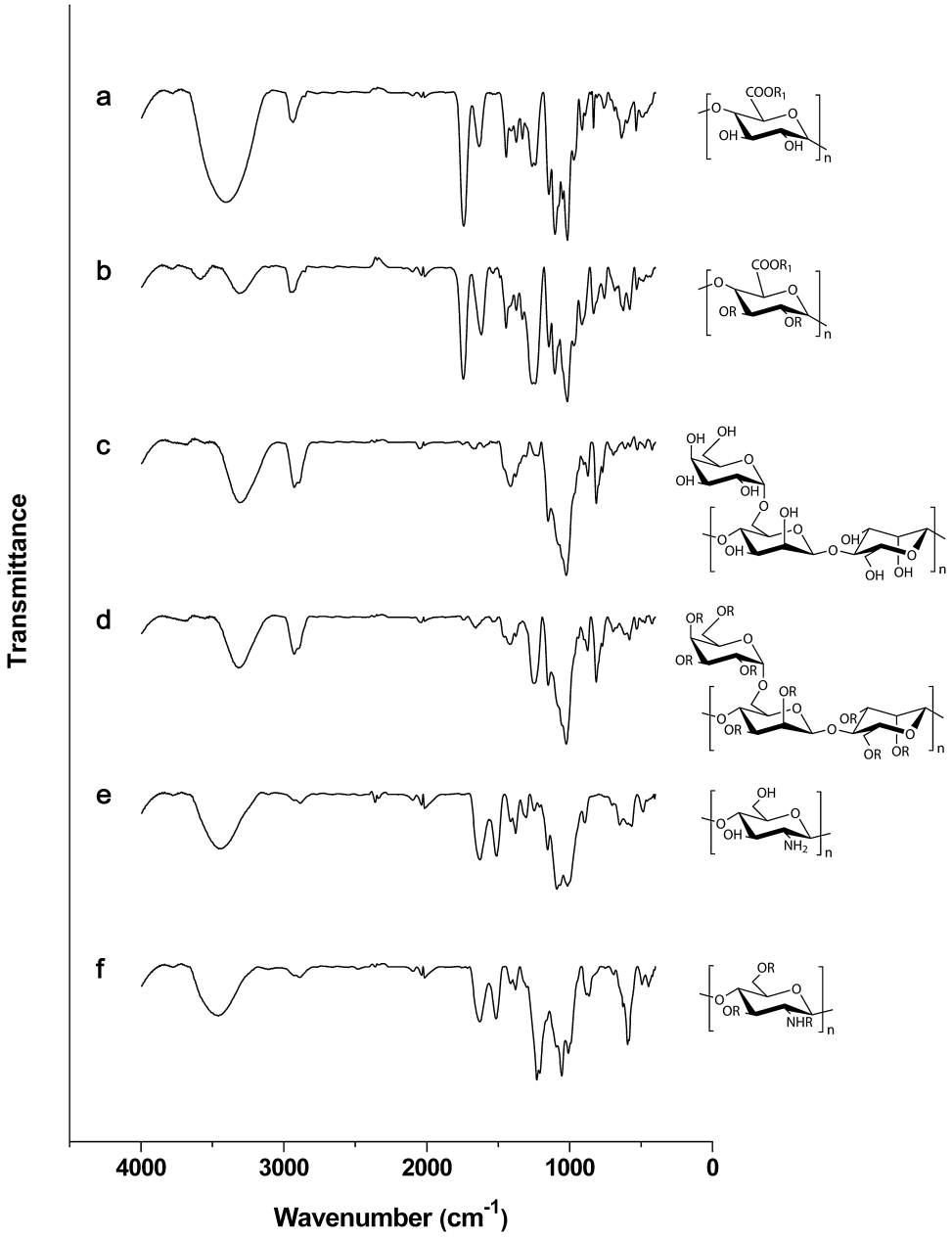

Fig. 1. FT-IR spectra of native and sulfated polysaccharides. (a) PEC, (b) PECS, (c) GUA, (d) GUAS, (e) CHI and (f) CHIS. $R_1 = CH_3$ or H, R = SO_3H, SO_3Na or H.

bacterial suspension (1.8×10^9 cfu/mL) was diluted in 2-fold dilution series against PBS in the V-shaped 96 microtiter plate, and 25 μL 1% rabbit erythrocytes (RBCs) in PBS were added in each well, followed by 25 μL PBS. The plate was covered with parafilm and incubated at 37 °C for 1 h, the agglutination of RBCs was visually evaluated and 50% HA concentration of *H. pylor*i was recorded as one HA unit (1 U). Heparin was used as a control.

For hemagglutination inhibition (HI) assays, 25 μL polysaccharide sulfates solutions were 2-fold diluted in PBS in microtiter plates and 25 μL standard 4 U bacterial suspension was added. After being incubated for 1 h, 25 μL of 1% RBCs were added and incubated for another hour. The inhibition was visually and microscopic inspected.

2.11. Adhesion inhibition assay

Adhesion inhibition assay was based on Beil's method (Beil & Kilian, 2007). AGS cells were seeded into 96-well plates at a density of 1×10^4 cells/well. AGS cell monolayer was infected with FITC-labeled *H. pylori* (5×10^6 cfu) in the presence of polysaccharide sulfates at 37 °C for 3 h. The concentrations of each polysaccharide sulfate were 200 μg/mL, 400 μg/mL and 800 μg/mL. The cell monolayer was washed with PBS for 1 time and then inspected via a fluorescence microscope (IX71, Olympus, US). Bacteria adhesion was quantified by fluorescence intensity after lysed in trypsin-EDTA for 2 h.

Table 1
Elemental analysis and DS of polysaccharide sulfates.

Sample	C (%)	H (%)	N (%)	S (%)	DS
PECS	31.70	4.892	1.69	7.23	0.49
GUAS	33.03	5.164	2.71	9.26	0.61
CHIS	21.28	4.428	3.98	14.13	1.10

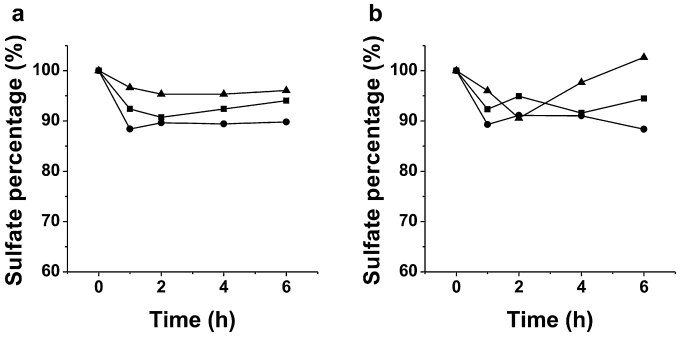

Fig. 2. Stability of polysaccharide sulfates in 0.1% HCl (a) and acetate buffer (b) at 37 °C. ■, ▲ and ● represent PECS, GUAS and CHIS respectively.

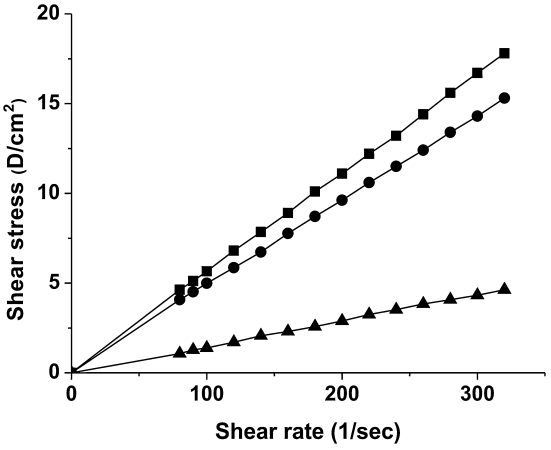

Fig. 3. Flow behavior of 1% polysaccharide sulfates solutions at 25 °C. ■, ● and ▲ represent PECS, GUAS and CHIS respectively.

2.12. Statistical analysis

The data obtained were means ± S.D. of at least three determinations, and *t*-test was used to determine any significance of differences ($^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$) between means in SPSS 17.0.

3. Results and discussion

3.1. Preparation and characterization of polysaccharide sulfates

The presence of sulfates in polysaccharides was confirmed by FT-IR and elemental analysis. In Fig. 1, peaks at 1261 cm^{-1} (S=O) and 832 cm^{-1} (C—O—S) were observed in PECS. Peaks at 1247 cm^{-1} (S=O) and 811 cm^{-1} (C—O—S) were observed in GUAS. Peaks at 1229 cm^{-1} (S=O) and 866 cm^{-1} (C—O—S) were observed in CHIS. The results indicated the presence of —O—SO_3— (Bae et al., 2009; Mestechkina, Egorov, & Shcherbukhin, 2006). The peak at 586 cm^{-1} was observed only in CHIS, which demonstrated the presence of C—N—S (Pires et al., 2013). The elemental composition and the degree of substitution (DS, the average number of sulfate groups attached to a monose unit) of each derivative were calculated (Table 1), which further confirmed polysaccharides were successfully sulfated.

During the polysaccharide sulfation, the step of alcohol precipitation was removed from the traditional method, because when alcohol was added, polysaccharide solutions formed into gel and was insoluble. When pH was adjusted to alkalescence, polysaccharide solution also formed into gel. In FT-IR spectra, peaks of C—O—S were not obvious, because of the overlap of peaks from the α-anomer form of pyranoid ring at about 834 cm^{-1} (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000).

3.2. Zeta potential and hydrodynamic diameter

Sulfated polysaccharides solutions at a concentration of 0.1% (w/v) acquired negative charges about −45.40 mV (PECS), −30.73 mV (GUAS) and −29.53 mV (CHIS) and possessed smaller sizes of hydrodynamic diameter about 518 nm (PECS), 409 nm (GUAS) and 344 nm (CHIS) compared to native ones (Table 2).

Table 2
Zeta potential (Z_p) and hydrodynamic diameter (*Z*-average) of native and sulfated polysaccharides.

	PEC		GUA		CHI	
	Native	Sulfated	Native	Sulfate	Native	Sulfate
Zeta-potential (mV)	−28.37 ± 0.23	−45.40 ± 2.18	−15.19 ± 3.34	−30.73 ± 3.59	12.65 ± 7.37	−29.53 ± 0.06
Size (nm)	619 ± 18	518 ± 11	1034 ± 23	409 ± 36	1123 ± 37	344 ± 15

$n = 3$.

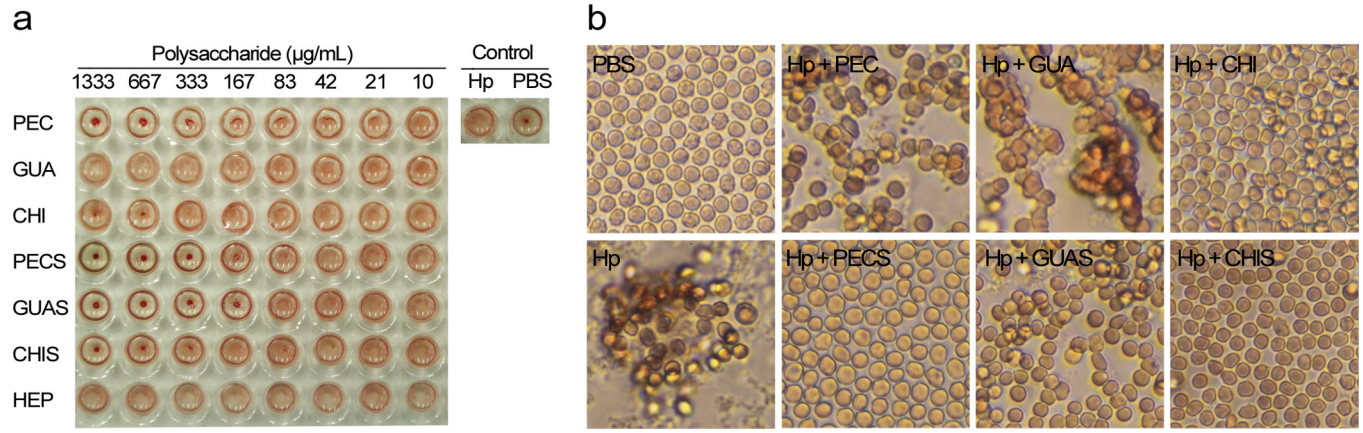

Fig. 4. Inhibitory effects of polysaccharide sulfates on hemagglutination induced by *H. pylori*. (a) HI assay conducted in a V-shaped 96 microtiter plate and inspected visually. (b) Micrographs of HI caused by polysaccharide sulfates at a concentration of 1333 μg/mL (the magnification is 400×).

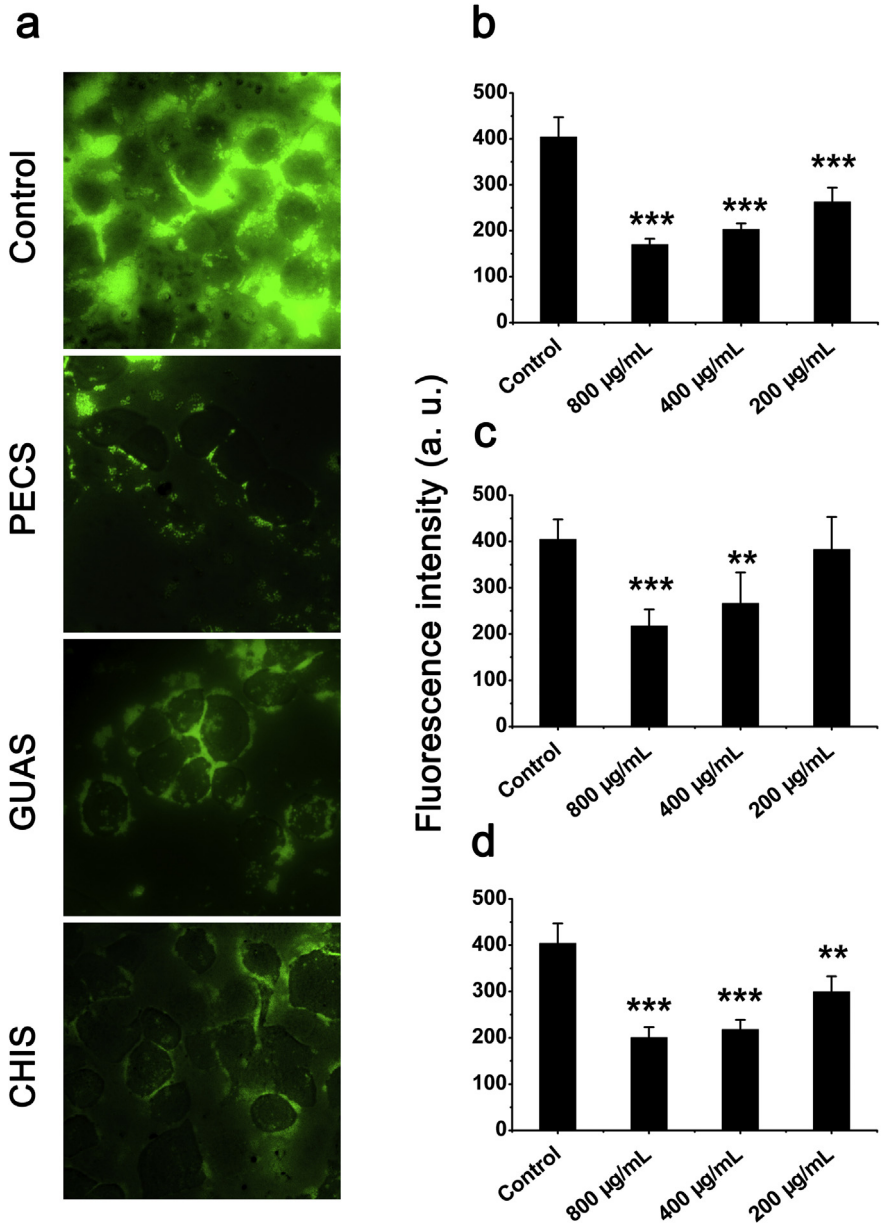

Fig. 5. Inhibition of *H. pylori* adhesion to AGS cells by polysaccharide sulfates. (a) Photomicrographs of bacterial adhesion to AGS cells inhibited by polysaccharide sulfates. The control group (control), bacteria interacted with AGS in RPMI1640 without sulfates. For sulfates group (PECS, GUAS and CHIS), bacteria interacted with AGS in RPMI1640 containing 400 μg/mL PECS, GUAS and CHIS, respectively (the magnification is 400×). Adhesion of *H. pylori* to AGS cells in different concentrations of PECS (b), GUAS (c) and CHIS (d). All values represent mean ± SD. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the control group. $n = 6$.

Negative charges reflected the introduction of sulfates. Increases in absolute value of charge and decreases in hydrodynamic diameter demonstrated the good stability of polysaccharide solutions.

3.3. Hydrolysis stability

The PECS and GUAS were hardly hydrolyzed in 0.1% HCl solution (pH 1.2) or acetate buffer (pH 4.0) at 37 °C within 6 h since the degradation of three polysaccharide sulfates were less than 20% (Fig. 2).

The stability is enough for polysaccharide sulfates to work efficiently either *in vitro* or *in vivo*. The CHIS appeared to be less stable since N—SO_3 can be hydrolyzed under acidic condition.

3.4. Rheological property

As shown in Fig. 3, the direct linear relationship between shear stress and shear rate exhibited Newtonian-like behavior for all polysaccharide sulfates. The values of viscosity were 5.64 cp (PECS), 4.83 cp (GUAS) and 1.43 cp (CHIS).

Heating and stirring during reaction broke down the polysaccharide sulfate chains so that the viscosities were lowered compared with native ones (data not shown). The rather low viscosity excluded the non-specific adhesion of bacteria to polysaccharide caused by viscous solution.

3.5. Hemagglutination inhibition assay

As shown in Fig. 4, polysaccharide sulfates inhibited the agglutination of RBCs caused by *H. pylori* (RBCs formed a small button),

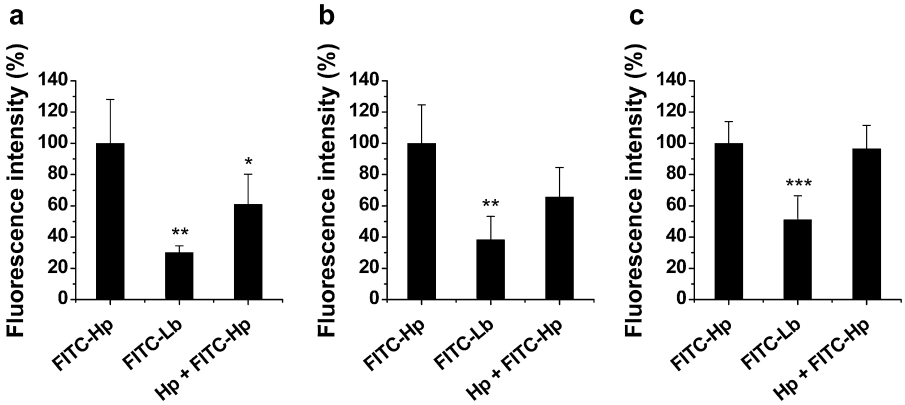

Fig. 6. Binding of *H. pylori* to PECS (a), GUAS (b) and CHIS (c). The intensity was calculated by comparison with the corresponding control group (FITC-Hp). Column 1 and 2 shows the direct binding affinity of FITC-labeled *H. pylori* and *L. bulgaricus* to polysaccharide chips respectively. As for column 3, polysaccharide chips were presaturated by unlabeled *H. pylori* and then incubated with FITC-labeled *H. pylori*. All values represent mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the control group. $n \geq 3$.

while native polysaccharides showed little effects (RBCs formed a large circle or button). PECS and GUAS exhibited a stronger hemagglutination inhibition (HI) action at a minimum inhibitory concentration of 167 μg/mL, while CHIS was 333 μg/mL (Fig. 4a). The inspection of *H. pylori* infected RBCs in polysaccharides solutions (1333 μg/mL) under a microscope was shown in Fig. 4b. RBCs in the PECS solution distributed uniformly, turning out the inhibition of bacterial adhesion to RBCs. Most of RBCs in the GUAS solutions were scattered but some masses also could be seen, displaying weaker HI action at the same concentration compared with PECS.

As we know, *H. pylori* exhibits a broad spectrum of hemagglutinins (Aspholm et al., 2006; O'Toole et al., 1995; Taylor, Hasubski, Fox, & Lee, 1992), which bind to erythrocytes (RBCs) and cause formation of a lattice. This phenomenon is called hemagglutination (HA). The mechanism of HA is similar to adhesion of *H. pylori* to host cell surfaces. Hence, RBCs can be used as a model to study adhesion (Koster, Putten, Rauws, & Tytgat, 1991). From our study, all the polysaccharide sulfates could inhibit adhesion of *H. pylori* to RBCs, but different glycosyl residues and location of sulfates showed different effects. PECS basically composed of galacturonic acids and 2,3-O-linked sulfates exhibited a better adhesion inhibition ability. CHIS without galactose only showed inhibition at a relative high concentration. GUAS, though one third of the composition of which were galactoses, sulfates located not only on 2,3-OH but also 4,6-OH, the inhibition of which was weaker than that of PECS at the same concentration. It is interesting that heparin, the most common disaccharide unit of which is composed of a 2-O-sulfo iduronic acid and 6-O-sulfo-N-sulfated glucosamine, could not inhibit the RBCs coagulation induced by *H. pylori*, which agreed with the results of early researches that binding of *H. pylori* was specific for 3-O-linked sulfates (Namavar, Sparrius, Veerman, Appelmelk, & Vandenbroucke-Grauls, 1998; Veerman et al., 1997). Therefore, we concluded the larger amount of galactoses and 2,3-O-linked sulfates, especially 3-O-linked sulfates played a more important role in HI.

3.6. FITC-labeling to bacteria

After incubated the bacteria with FITC in PBS at 37 °C for 12 h, the labeling efficiency of *H. pylori* was 99.85%, while the labeling efficiency of *L. bulgaricus* was 98.71%, manifesting the success in FITC-labeling. The ratio of mean fluorescence intensities (*H. pylori*/*L. bulgaricus*) was 1.62:1 (Table S1 and Fig. S1).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.12.063.

3.7. Adhesion inhibition assay

PECS, GUAS and CHIS showed no significant cytotoxicities to AGS cells at concentrations below 1 mg/mL (data not shown). The inhibition of *H. pylori* adhesion to AGS cells was inspected both by a fluorescence microscope and a microplate reader. Under the inspection of microscope, the fluorescence intensity was observed most on the surface of cell membranes (Fig. 5a), which certified bacteria adhered on cell surfaces. The fluorescence intensity reduction in the sulfate groups revealed the less adhesion of *H. pylori*. Quantitative results in Fig. 5b–d showed that the inhibition was in a concentration-dependent manner. The Amount of *H. pylori* attached to AGS cells was reduced by PECS notably even at a concentration of 200 μg/mL, while reduction of *H. pylori* in GUAS and CHIS were moderate.

Results of AGS cells were similar with those of RBCs, and further explained galactoses as well as 2,3-O-linked sulfates enhanced the adhesion inhibitory effect more efficiently. The degree of sulfates substitution (DS) was not the most important factor since a higher DS may lead to steric hindrances and excess of negative charged densities. As *H. pylori* is also negative charged *in vitro* (Smith, Drumm, Neumann, Policova, & Sherman, 1990), electrostatic repulsions may occur between sulfates and bacteria, which is unfavorable for the interaction of *H. pylori* and polysaccharides. The most optimal DS of sulfates should be studied in following researches.

3.8. Binding assay

The mechanism of the adhesion inhibition caused by sulfates was primarily investigated by a direct binding assay via polysaccharide chips and results were shown in Fig. 6. Three polysaccharide sulfates studied in this work showed affinity to FITC-*H. pylori* and the fluorescence intensity was recorded as 100%. When *L. bulgaricus*, a widely known probiotic with no reports about its binding affinity to sulfates, was used as a substitution test bacterium, the binding was reduced significantly ($P < 0.05$). When polysaccharide chips were pre-incubated with unlabeled *H. pylori*, the binding of FITC-*H. pylori* to polysaccharide chips was inhibited, but only the PECS group showed significant differences ($P < 0.05$). The

fluorescence intensities of different bacteria were adjusted by the ratio of mean fluorescence intensities described in section 3.6.

Results of the binding assay were in correspondence with those of the adhesion inhibition assay. We substantiated that polysaccharide sulfates bond to bacterial outer membrane proteins (OMPs) and occupied their adhesion site, thus OMPs cannot interacted with target cells (e.g. gastric cells and erythrocytes) or mucins and the adhesion to hosts was inhibited. Strong bindings to sulfates were only observed in *H. pylori* groups not *L. bulgaricus* groups, which can be explained by the specific binding affinity of sulfates to *H. pylori*. The binding of *H. pylori* to CHIS and GUAS were not strong enough and may be reversible since pretreated CHIS and GUAS chips with unlabeled *H. pylori* could not inhibit the binding of FITC-*H. pylori* at a notable degree.

4. Conclusions

In this study, PEC, GUA and CHI were subjected to sulfation via SO_3-pyridine method. PECS, GUAS and CHIS were Newton liquids and the solutions of which were stable and difficult to hydrolyze. The adhesion inhibition assay proved that polysaccharide sulfates inhibited the adhesion of *H. pylori* to RBCs and AGS cells. PECS rich in galactoses and 2,3-O-linked sulfates was more efficient than GUAS and CHIS with less galactoses and 4-O-linked, 6-O-linked or 2-N-linked sulfates apart from 2,3-O-linked sulfate. Results of the binding assay showed affinities of sulfates to *H. pylori*, and PECS exerted a stronger binding affinity compared with the other two. The results in keeping with those of the adhesion inhibition assay suggested that the adhesion inhibition of *H. pylori* to hosts was induced by the binding of sulfates to the bacteria, which counteracted the bacterial interaction to hosts. A higher content of galactoses and 2,3-O-linked sulfates promoted this action. We propose that polysaccharide sulfates, especial PECS will serve as potential drugs to keep the gastric wall off the bacterial load and thus enhance the bacterial eradication rate of *H. pylori*.

Acknowledgments

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References

Aspholm, M., Olfat, F. O., Norden, J., Sonden, B., Lundberg, C., Sjostrom, R., et al. (2006). SabA is the *H. pylori* hemagglutinin and is polymorphic in binding to sialylated glycans. *PLoS Pathogens*, *2*(10), 989–1001.

Bae, I. Y., Joe, Y. N., Rha, H. J., Lee, S., Yoo, S. H., & Lee, H. G. (2009). Effect of sulfation on the physicochemical and biological properties of citrus pectins. *Food Hydrocolloids*, *23*(7), 1980–1983.

Beil, W., & Kilian, P. (2007). EPs (R) 7630, an extract from *Pelargonium sidoides* roots inhibits adherence of *Helicobacter pylori* to gastric epithelial cells. *Phytomedicine*, *14*, 5–8.

Belogortseva, N. I., Yoon, J. Y., & Kim, K. H. (2000). Inhibition of *Helicobacter pylori* hemagglutination by polysaccharide fractions from roots of *Panax ginseng*. *Planta Medica*, *66*(3), 217–220.

Blaser, M. J. (1990). *Helicobacter pylori* and the pathogenesis of gastroduodenal inflammation. *Journal of Infectious Diseases*, *161*(4), 626–633.

de Koster, E., Putten, J. P. M., Rauws, E. A. J., & Tytgat, G. N. J. (1991). Adhesion properties of *Helicobacter pylori*. In H. Menge, M. Gregor, G. N. J. Tytgat, B. J. Marshall, & C. A. M. McNulty (Eds.), *Helicobacter pylori 1990* (pp. 15–18). Berlin: Springer Berlin Heidelberg.

Kacurakova, M., Capek, P., Sasinkova, V., Wellner, N., & Ebringerova, A. (2000). FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers*, *43*(2), 195–203.

Kenny, D. T., Skoog, E. C., Lindén, S. K., Struwe, W. B., Rudd, P. M., & Karlsson, N. G. (2012). Presence of terminal N-acetylgalactosamineβ1-4*N*-acetylglucosamine residues on *O*-linked oligosaccharides from gastric MUC5AC: Involvement in *Helicobacter pylori* colonization? *Glycobiology*, *22*(8), 1077–1085.

Kobayashi, M., Lee, H., Nakayama, J., & Fukuda, M. (2009). Roles of gastric mucin-type O-glycans in the pathogenesis of *Helicobacter pylori* infection. *Glycobiology*, *19*(5), 453–461.

Lutay, N., Nilsson, I., Wadström, T., & Ljungh, A. (2011). Effect of heparin, fucoidan and other polysaccharides on adhesion of enterohepatic helicobacter species to murine macrophages. *Applied Biochemistry and Biotechnology*, *164*(1), 1–9.

Malfertheiner, P., Megraud, F., O'Morain, C. A., Atherton, J., Axon, A. T., Bazzoli, F., et al. (2012). Management of *Helicobacter pylori* infection—The maastricht IV/florence consensus report. *Gut*, *61*(5), 646–664.

Marshall, B. J., & Warren, J. R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*, *1*(8390), 1311–1315.

Mestechkina, N. M., Egorov, A. V., & Shcherbukhin, V. D. (2006). Synthesis of galactomannan sulfates. *Applied Biochemistry and Microbiology*, *42*(3), 326–330.

Namavar, F., Sparrius, M., Veerman, E. C. I., Appelmelk, B. J., & Vandenbroucke-Grauls, C. M. J. E. (1998). Neutrophil-activating protein mediates adhesion of *Helicobacter pylori* to sulfated carbohydrates on high-molecular-weight salivary mucin. *Infection and Immunity*, *66*(2), 444–447.

O'Toole, P. W., Janzon, L., Doig, P., Huang, J., Kostrzynska, M., & Trust, T. J. (1995). The putative neuraminyllactose-binding hemagglutinin HpaA of *Helicobacter pylori* CCUG 17874 is a lipoprotein. *Journal of Bacteriology*, *177*(21), 6049–6057.

Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A. B., Warnke, R. A., Jellum, E., et al. (1994). *Helicobacter pylori* infection and gastric lymphoma. *New England Journal of Medicine*, *330*(18), 1267–1271.

Pires, N. R., Cunha, P. L. R., Maciel, J. S., Angelim, A. L., Melo, V. M. M., de Paula, R. C. M., et al. (2013). Sulfated chitosan as tear substitute with no antimicrobial activity. *Carbohydrate Polymers*, *91*(1), 92–99.

Qian, J., Ye, F., Zhang, J., Yang, Y. M., Tu, H. M., Jiang, Q., et al. (2012). Levofloxacin-containing triple and sequential therapy or standard sequential therapy as the first line treatment for *Helicobacter pylori* eradication in China. *Helicobacter*, *17*(6), 478–485.

Sim, J. S., Hahn, B. S., Im, A. R., Park, Y., Shin, J. E., Bae, E. A., et al. (2011). Inhibition of *Helicobacter pylori* adhesion to Kato III cells by intact and low molecular weight acharan sulfate. *Glycoconjugate Journal*, *28*(6), 411–418.

Smith, J. I., Drumm, B., Neumann, A. W., Policova, Z., & Sherman, P. M. (1990). In vitro surface properties of the newly recognized gastric pathogen *Helicobacter pylori*. *Infection and Immunity*, *58*(9), 3056–3060.

Takamura, A., Ito, M., Boda, T., Matsumoto, Y., Tanaka, S., Yoshihara, M., et al. (2012). High expression of gastrin receptor protein in injured mucosa of *Helicobacter pylori*-positive gastritis. *Digestive Diseases and Sciences*, *58*(3), 634–640.

Taylor, N. S., Hasubski, A. T., Fox, J. G., & Lee, A. (1992). Haemagglutination profiles of *Helicobacter* species that cause gastritis in man and animals. *Journal of Medical Microbiology*, *37*(5), 299–303.

Teneberg, S. (2009). The multiple carbohydrate binding specificities of *Helicobacter pylori*. *Topics in Current Chemistry*, *288*, 121–138.

Veerman, E. C. I., Bank, C. M. C., Namavar, F., Appelmelk, B. J., Bolscher, J. G. M., & Nieuw Amerongen, A. V. (1997). Sulfated glycans on oral mucin as receptors for *Helicobacter pylori*. *Glycobiology*, *7*, 737–743.

Vogl, H., Paper, D., & Franz, G. (2000). Preparation of a sulfated linear (1-4)-β-D-galactan with variable degrees of sulfation. *Carbohydrate Polymers*, *41*(2), 185–190.

Wittschier, N., Faller, G., & Hensel, A. (2009). Aqueous extracts and polysaccharides from liquorice roots (*Glycyrrhiza glabra* L.) inhibit adhesion of *Helicobacter pylori* to human gastric mucosa. *Journal of Ethnopharmacology*, *125*(2), 218–223.

Xu, C., Ruan, X. M., Li, H. S., Guo, B. X., Ren, X. D., Shuang, J. L., et al. (2010). Anti-adhesive effect of an acidic polysaccharide from *Aloe vera* L. var. *chinensis* (Haw.) Berger on the binding of *Helicobacter pylori* to the MKN-45 cell line. *Journal of Pharmacy and Pharmacology*, *62*(12), 1753–1759.

Zullo, A., de Francesco, V., Hassan, C., Ridola, L., Repici, A., Bruzzese, V., et al. (2012). Modified sequential therapy regimens for *Helicobacter pylori* eradication: A systematic review. *Digestive and Liver Disease*, *45*(1), 18–22.