Carbohydrate Polymers 110 (2014) 231–237

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation, characterization, and anti-*Helicobacter pylori* activity of Bi^{3+}-*Hericium erinaceus* polysaccharide complex

Yang Zhu [a], Yao Chen [b], Qian Li [c], Ting Zhao [a], Ming Zhang [a], Weiwei Feng [c], Mohammed Takase [a], Xueshan Wu [a], Zhaoxiang Zhou [a], Liuqing Yang [a,*], Xiangyang Wu [b,**]

[a] School of Chemistry and Chemical Engineering, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, China
[b] School of the Environment, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, Jiangsu, China
[c] School of Food and Biological Engineering, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, China

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form 8 March 2014
Accepted 20 March 2014
Available online 2 April 2014

Keywords:
Hericium erinaceus
Bismuth
Bi^{3+}-polysaccharide complex
Characterization
Anti-*Helicobacter pylori* activity

ABSTRACT

Two new Bi^{3+}-*Hericium erinaceus* polysaccharide (BiHEP) complexes were prepared using Bi^{3+} and two purified polysaccharides from *H. erinaceus* (HEPs), respectively. The complexes were characterized by elemental analysis, FT-IR, CD, SEM, AFM, XRD, and TG. The anti-*Helicobacter pylori* (*Hp*) activities *in vitro* by agar dilution assay of the complexes were evaluated. The molecular weights of HEPs were 197 and 20 kDa, respectively. All the analyses confirmed the formation of new BiHEP complexes with lower content of Bi^{3+} compared with colloidal bismuth subcitrate (CBS), the most utilized bismuth preparation clinically. Furthermore, HEPs themselves have definite inhibition effects on *Hp*, and BiHEP complexes have lower content of Bi exhibited strong inhibition effects on *Hp* (MIC = 20 μg/mL), similar to that of CBS with higher content of Bi. The study provides a basis for further development of multiple treatments of *Hp* infection or new medicines.

1. Introduction

Helicobacter pylori (*Hp*) is a gram-negative, flagellated, spiral-shaped, urease producing bacterium that lives in the microaerophilic environment of the stomach and duodenum. *Hp* is one of the most common bacterial pathogens in humans, colonizing the stomachs of more than half of the world population. It is the causative agent of acute and chronic gastritis, peptic ulcer, gastric carcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Kusters, van Vliet & Kuipers, 2006; Stamatis, Kyriazopoulos, Golegou, Basayiannis, Skaltsas, & Skaltsa, 2003). Several pathogenic determinants of *Hp* including adhesions, cytotoxins, and different enzymes: urease, catalase, lipase, protease, etc. have been proposed (Paulo, Oleastro, Gallardo, Queiroz, & Domingues, 2011).

Successful treatment of chronic *Hp* infections leads to the resolution of gastritis and a decrease of ulcer recurrence. Thus it is important to investigate novel therapeutic approaches that lead to control or treatment of *Hp* infection without causing drug-resistance problems (Gisbert, Gisbert, Marcos, Jimenez Alonso, Moreno Otero, & Pajares, 2008; Graham & Shiotani, 2008). Bismuth compounds such as colloidal bismuth subcitrate (CBS) and ranitidine bismuth citrate (RBC) have widely been used in clinics for the treatment of gastrointestinal disorders and *Hp* infection together with antibiotics (Briand & Burford, 1999). Sequential therapy, concomitant therapy, or bismuth-containing quadruple therapy has been tried in an effort to resolve clarithromycin resistance with metronidazole. Therapies in which a bismuth compound, usually the subsalicylate or subcitrate, is included as part of the treatment regimen have greater success rates since *Hp* strains with resistant to bismuth compounds have not yet been reported. On the other hand, an important argument is that the treatment with bismuth compounds does not require maintenance of a practically neutral stomach pH (Bland, Ismail, Heinemann & Keenan, 2004; Engstrand & Lindberg, 2013). A significant drawback of bismuth-based therapy is the amount of bismuth which is ingested. Bismuth toxicity causes delirium, psychosis, ataxia, myoclonus, and seizures and is reversible over several weeks or months, when bismuth intake is stopped (Gordon, Abrams, Rubin, Barr, & Correa, 1995; Recklinghausen et al., 2008). Greater activities at lower concentrations, combined with benign ligands such as polysaccharides and other natural sources, are principal targets in the development of new bismuth-based drugs.

* Corresponding author. Tel.: +86 511 88791800; fax: +86 511 88791800.
** Corresponding author. Tel.: +86 511 88791200; fax: +86 511 88791200.
E-mail addresses: yangliuqing@ujs.edu.cn (L. Yang), wuxy@ujs.edu.cn (X. Wu).

http://dx.doi.org/10.1016/j.carbpol.2014.03.081
0144-8617/

Hericium erinaceus (*H. erinaceus*) belongs to the Aphyllophorales, Hydnaecae, and Hericium families and is a well-known edible and medicinal mushroom in East Asia. The bodies of *H. erinaceus* have attracted much attention due to their health effects when used as a home remedy for gastric and duodenal ulcers as well as some other diseases (Jia, Liu, Dong, & Fang, 2004). In recent years, a growing interest in biologically active compounds, including flavonoids from plants and other natural sources, has been observed (Manyi Loh, Clarke, Munzhelele, Green, Mkwetshana, & Ndip, 2010; Paulo, Oleastro, Gallardo, Queiroz, & Domingues, 2011; Zaidi, Yamada, Kadowaki, Usmanghani, & Sugiyama, 2009). To the best of our knowledge, there is little or no report on mushroom polysaccharides used for gastrointestinal disorders, against *Hp*.

In this paper, two *H. erinaceus* polysaccharides (HEPs) were isolated and purified by enzyme-assisted extraction, deproteinisation, and ethanol precipitation. Two BiHEP complexes were prepared and analyzed through several methods including elemental analysis, FT-IR, CD, SEM, AFM, XRD, and TG. The HEPs and complexes' antimicrobial activities against *Hp* were also studied. To date, little research has been reported on this subject.

2. Materials and methods

2.1. Materials

Fruits of *H. erinaceus* identified by Dr. Guanghua Mao were provided by FangGe Pharmaceutical Co., Ltd. in Zhejiang Province. Columbia Agar Base and calf serum were obtained from Hefei Bomei Chemical Agent Co., Ltd (Hefei, China). Colloidal bismuth subcitrate (CBS) was purchased from Hubei Prosperity Galaxy Chemical Co. Ltd (Hubei, China). *H. pylori* strain NTCC11637 was obtained from School of Medical Science and Laboratory Medicine of Jiangsu University. All other chemicals used were of analytical grade.

2.2. Methods

2.2.1. Extraction of H. erinaceus polysaccharide (HEP)

Crude HEP was extracted according to the procedures reported by our previous work (Zhu et al., 2014). Briefly, dried fruits of *H. erinaceus* samples were ground and extracted with distilled water (solid:liquid ratio of 1:30, w/v) in a round bottom flask at a pH of 5.7 (pH values of the mixtures were adjusted with 0.1 M HCl), a temperature of 52 °C, a time of 34 min, and enzyme concentration of 3.0% (cellulose:pectinase:trypsin the ratio of 2:2:1). After the enzymolysis process, samples were rapidly heated at 100 °C in boiling water with stirring for 3 h. The mixture was centrifuged (4000 r/min for 10 min), filtered, and the insoluble residue then treated again as mentioned above. The supernatants were incorporated and concentrated using a rotary evaporator under vacuum. The extracts were precipitated by addition of 95% (v/v) ethanol to a final concentration of 80% (v/v) and incubated for 12 h at 4 °C. The polysaccharide precipitates were collected by centrifugation (4000 r/min for 10 min) and lyophilized to obtain crude polysaccharides. The HEPs were re-dissolved and subjected to the trichloroacetic acid method for the removal of proteins.

2.2.2. Isolation of HEPs fractions by gradient ethanol precipitation

After extraction of crude polysaccharides from the raw materials of *H. erinaceus*, the purification technology was further performed using the ethanol precipitation method at a different final concentration. For preparation of HEP25, ethanol was added to HEP at a final concentration of 25% (v/v), centrifuged, and the precipitates dissolved in appropriate distilled water. The obtained supernatants of 25% ethanol solution (E25) were used to prepare HEP50. Ethanol was added to E25 at a final concentration of 50% (v/v) and the precipitates were dissolved in distilled water (HEP50) after centrifugation. Similarly, to obtain HEP75, ethanol was further added to the ethanol solution obtained in the process of preparing HEP50 at a final concentration of 75% (v/v). Polysaccharides were treated once more.

2.2.3. Determination of the molecular weight

The molecular weight distribution of HEPs were determined with a high performance gel permeation chromatography (HPGPC) on an LC-10ATvp instrument (Shimadzu, Tokyo, Japan), equipped with a precolumn of TSK-GUARD COLUMN PWH (7.5 × 75 mm, Tosoh corporation, Tokyo, Japan) and a column of TSK-GEL G4000PW (7.5 × 300 mm, Tosoh corporation, Tokyo, Japan). It was then eluted with a 0.003 mol/L CH_3COONa solution at a flow rate of 1.0 mL/min. The peaks were detected using a refractive index detector (Shimadzu RID-10A, Shimadzu). Standard dextran (Pharmacia) including T-10 (molecular mass, 1×10^4 Da), T-40 (4×10^4 Da), T-70 (7×10^4 Da), T-500 (5×10^5 Da), and T-2000 (2×10^6 Da) were used as molecular mass markers (Zhao et al., 2013).

2.3. Preparation of the BiHEP complexes

A mixture of 1.22 g of $Bi(NO_3)_3 \cdot 5H_2O$ and equivalence of mannitol in a beaker was magnetically stirred at 25 °C for 1 h. The pH of $Bi(NO_3)_3$ solution was adjusted to 11.0–12.0 with 10% KOH solution. A quantity of 0.5 g purified of HEP was added to 50 mL of distilled water and left to swell for several hours. The pH of HEP solution was then adjusted to 9.0–10.0. The $Bi(NO_3)_3$ solution was slowly added to the HEP solution and the resulting mixture stirred for about 30 min. The BiHEP complexes were then precipitated, filtered, washed several times with water and ethanol, and then lyophilized to obtain BiHEP complexes (Jin, Ling, He, Chen, Chen, & Zhang, 2008).

2.4. Characterizations

2.4.1. Elemental analysis

The content of Bi^{3+} was determined by complexometry (Pharmacopoeia of PRC, 2010). Elemental analysis of carbon, hydrogen, and nitrogen was carried using an EA-1112 CHN analyser (CE, Italy). 2.3 mg of sample was accurately weighed, heated to 1150 °C and the corresponding element determined using a thermal conductivity 10 detector.

2.4.2. Infrared spectra

The IR spectra of the polysaccharides were determined using a Fourier transform IR spectrophotometer (FT-IR) (Nicolet Avatar-370, USA). The BiHEP complexes and HEPs were ground with KBr powder and then pressed into pellets for FT-IR measurement in the frequency range of 500–4000 cm^{-1}.

2.4.3. Circular dichroism (CD) spectroscopy

The BiHEP complexes and HEPs were dissolved in deionized water at 100 μg/mL and the solution kept at 45 °C for 2 h. Circular dichroism was performed in a Jasco spectrophotometer using a 3 mL rectangular quartz cuvette (Jasco) with a 1 cm path length. The spectra were recorded at 25 °C with a JASCO J-815 spectropolarimeter in the wavelength (λ) range of 190–400 nm with the following setup: bandwidth of 2.5 nm; time constant of 2 s; scan rate of 50 nm/min.

2.4.4. Scanning electron microscopy (SEM)

The shape and surface characteristics of the gold coated samples were observed and recorded using a scanning electron microscope (SEM; JSM-7001F, JEOL, Ltd. Japan). The dried and powdered BiHEP complexes and HEPs were placed on a specimen holder with

the help of double-sided adhesive tapes and sputtered with gold powder using a sputter coater. Each sample was observed at an accelerating potential of 20 kV under a high vacuum condition.

2.4.5. Atomic force microscopy (AFM)

The BiHEP complexes and HEPs were dissolved in deionized water at 100 μg/mL, and the solution kept at 45 °C for 2 h. Five μL of the solution was then dropped on to a freshly cleaved mica surface and allowed to dry at room temperature. Atomic force microscopy was performed in the tapping-mode using a MFP-3D instrument (Asylum Research, USA).

2.4.6. X-ray diffractometry (XRD)

The $Bi(NO_3)_3{\cdot}5H_2O$, HEPs, and BiHEP complexes were tightly packed into the sample holder and X-ray diffraction patterns recorded in the reflection mode at ambient temperature by a BDX3300 diffractometer, operated at a Cu Kα. Radiation from the anode was operated at 40 kV and 30 mA. The diffractometer was equipped with a 1° divergence slit, 0.3 mm receiving slit, a 1° scatter slit, scan speed 7° min^{-1}, and angular range of 5–90°.

2.4.7. Thermogravimetric (TG)

Thermal behaviour of the BiHEP complexes and HEPs were tested using STA449C thermal gravimetric analysis (TGA) (Netzsch, Germany). Approximately, 2.5 mg of sample was introduced into the sample pan and the TGA trace was obtained in the range 25–800 °C under nitrogen atmosphere with a flow rate of 25 mL/min at rise of 20 °C/min.

2.5. Determination of MICs of the HEPs and BiHEP complexes against Hp

The minimum inhibitory concentrations (MICs) of HEPs, BiHEP complexes, and CBS were determined by the agar dilution method (Asha et al., 2013). Bacteria were grown on Columbia Agar Base supplemented with 10% lysed calf serum and *Hp* antibiotic selective supplement. The plates were incubated for 72 h at 37 °C under microaerophilic conditions in anaerobic jars with CampyPak (BBL Beckton-Dickinson, Sparks, USA). The test compounds were dissolved in sterile distilled water and then further diluted in a twofold series. Final concentration of the bacteria used was 10^7 colony forming units (cfu)/mL by adjusting the suspension to match the McFarland No. 0.5 turbidity standard. MICs were then determined after 3 days of incubation at 37 °C under microaerophilic conditions. Colloidal bismuth subcitrate (CBS) was used as positive control in both methods. All experiments were performed in triplicate and at least three independent experiments were recorded. MIC was defined as the lowest tested treatment concentration that inhibits visible bacterial growth.

3. Results and discussion

3.1. Molecular weight determination of HEP

Crude polysaccharide was isolated from the fruits of *H. erinaceus* through enzyme-assisted extraction, deproteinized by the trichloroacetic acid method, ethanol precipitation, dialysed against water, and then dried. From HPGPC analysis, a calibration curve was obtained by using various Dextran T-series standards of known molecular weights. According to the calibration curve, average molecular weights of HEP25 and HEP75 were calculated to be 197 and 20 kDa, respectively.

Table 1
Elemental composition of BiHEP complexes.

	C (% w/w)	H (% w/w)	N (% w/w)	Bi (% w/w)
BiHEP25	25.44	3.94	–	25.65
BiHEP75	25.80	4.17	–	25.71

"–": Not detectable.

3.2. Characterization of BiHEP complexes

3.2.1. Elemental analysis

The results of the composition of elemental analysis are shown in Table 1. Analysis of elemental (C, H, N) composition of the BiHEP complexes indicated the content of C and H to be 25.44 and 3.94 (w/w %), respectively. Complexometry analysis showed content of Bi^{3+} to be 25.65% and 25.71%, respectively, and this value was lower than that of 37.5% of CBS. From the elemental analysis, there was no marked difference on the content of Bi^{3+} between the high molecular weight and low molecular weight complexes.

3.2.2. Infrared spectra analysis

FT-IR spectra of the complexes and corresponding starting materials of HEPs were measured (see Fig. 1). For HEP75, a strong and broad absorbent band centered at 3406 cm^{-1} is attributed to –OH asymmetrical stretching vibration. The signals at 1652 cm^{-1} was attributed to asymmetric and symmetric stretching of the carboxylate anion group (C=O). FT-IR spectrum of BiHEP75 showed

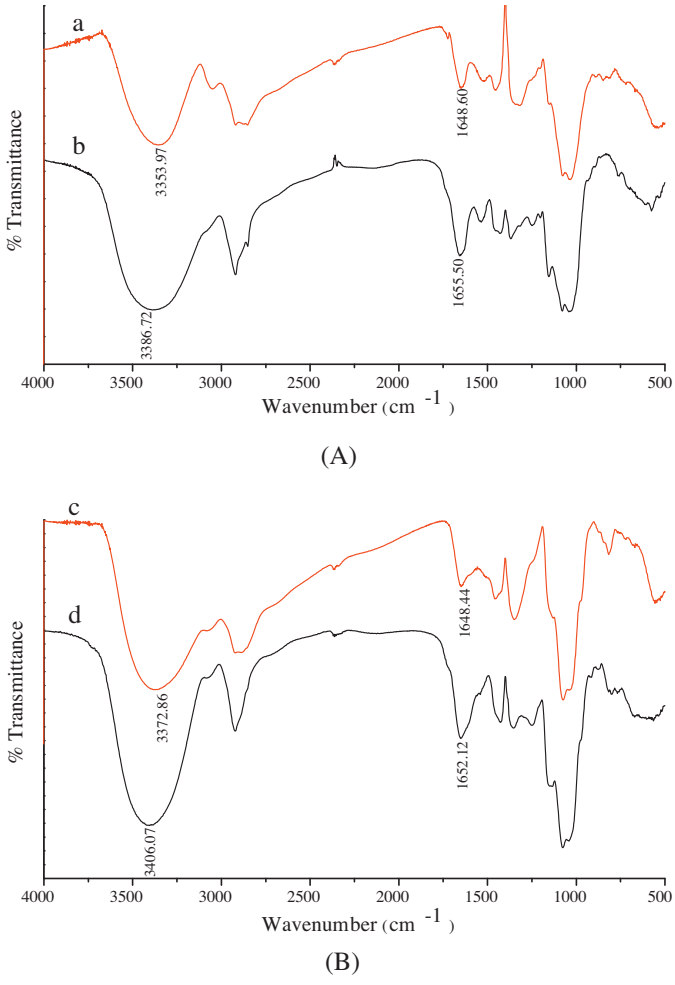

Fig. 1. Infrared spectra of BiHEP25 (a), HEP25 (b), BiHEP75 (c), and HEP75 (d) in the frequency range of 500-4000 cm^{-1}.

similar bands of HEP75, which exhibited an intense set of bands in the low-frequency region. These spectra show some spectral feature changes due to the interaction of the HEP75 samples with bismuth. Among these modifications, two deductions could be mentioned: (1) The wide peak at 3406 cm^{-1}, corresponding to the stretching vibration of –OH group, shifted to lower frequency (3372 cm^{-1}), indicating that the –OH group were involved in complexation. The band changes associated with –OH group, which suggested an increase in number of oscillation modes that could be attributed to the presence of new hydrogen bonding interactions. (2) The shift of the band at 1652–1648 cm^{-1} might be related to the loss of the amide (C=O) group and the formation of a new (C=O) group due to the polymer chain breakage, which indicated the bonding of Bi^{3+} to the carboxyl group (Ou et al., 2010). Furthermore, the end-carbonyl of sugar and Bi^{3+} were linked together through chemical covalent bond, and then caused deformation vibration of hydroxyl in end of sugar ring inside (Geng et al., 2014).

3.2.3. Circular dichroism (CD) spectroscopy analysis

Circular dichroism was an effective method to investigate the three-dimensional structure of compounds. The CD spectra of the polysaccharides and complexes in the range of 190–400 nm are shown in Fig. 2. It shows that the BiHEP complexes changed appreciably in the CD properties before the range of 250 nm when compared with HEPs. Particularly, strong negative Cotton effect appeared in 203 nm, which suggested that HEP25 had ordered helical structure in water solution (Fig. 2b). After complexing with Bi^{3+}, BiHEP25 showed positive Cotton effect at 222 nm, indicating that HEP25 was ordered and formed complexes with Bi^{3+} (Fig. 2a). The optically active absorption bands of the substituted chromophores arising from the n–π* transitions were located in the range of 200–250 nm (Kabat, Lloyd, & Beychok, 1969). The addition of HEP could lead to the formation of hydrogen bonds between Bi^{3+} and HEP, which possibly enhanced the intermolecular interaction.

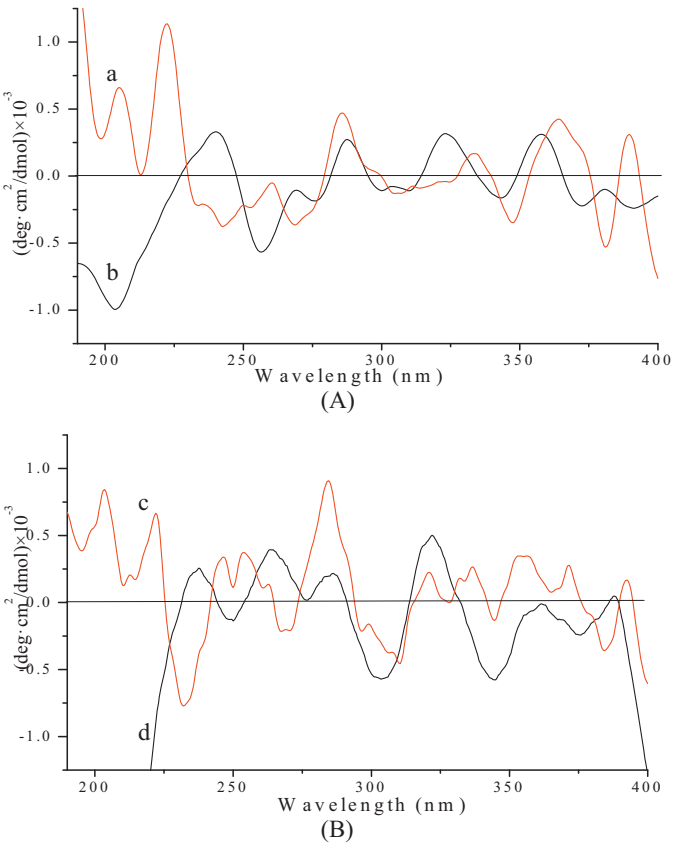

Fig. 2. CD spectra of BiHEP25 (a), HEP25 (b), BiHEP75 (c), and HEP75 (d) in the wavelength (λ) range of 190–400 nm.

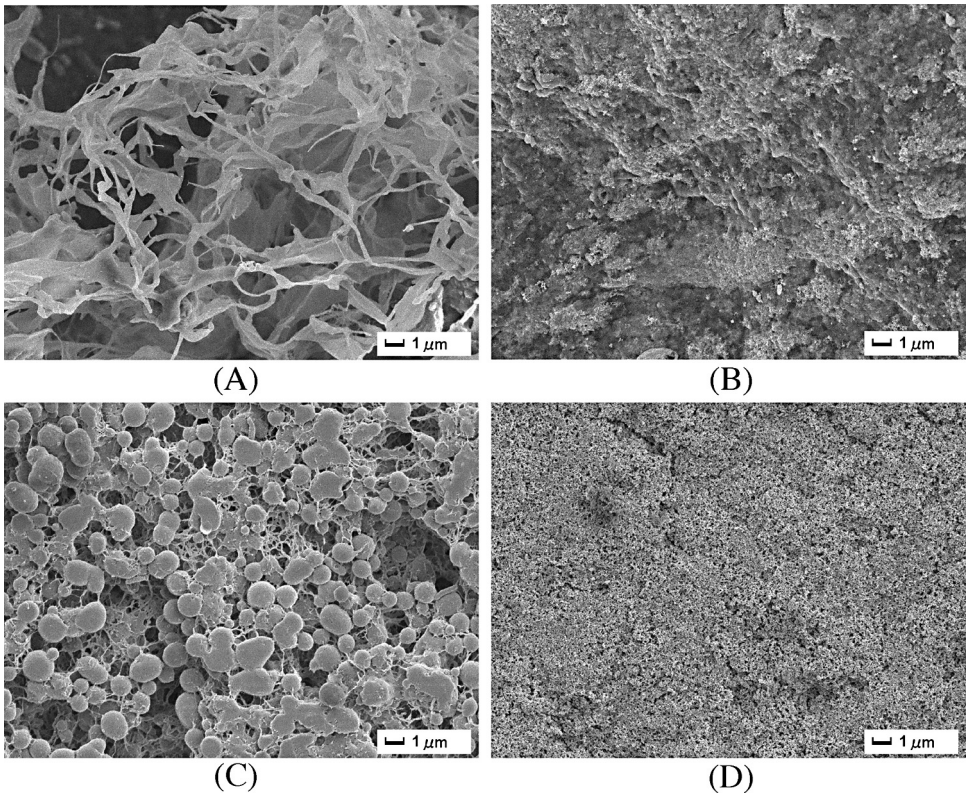

Fig. 3. Lyophilized samples were gold coated and analyzed by a JSM-7001F microscope. SEM micrographs of HEP25 (A), BiHEP25 (B), HEP75(C), and BiHEP75 (D).

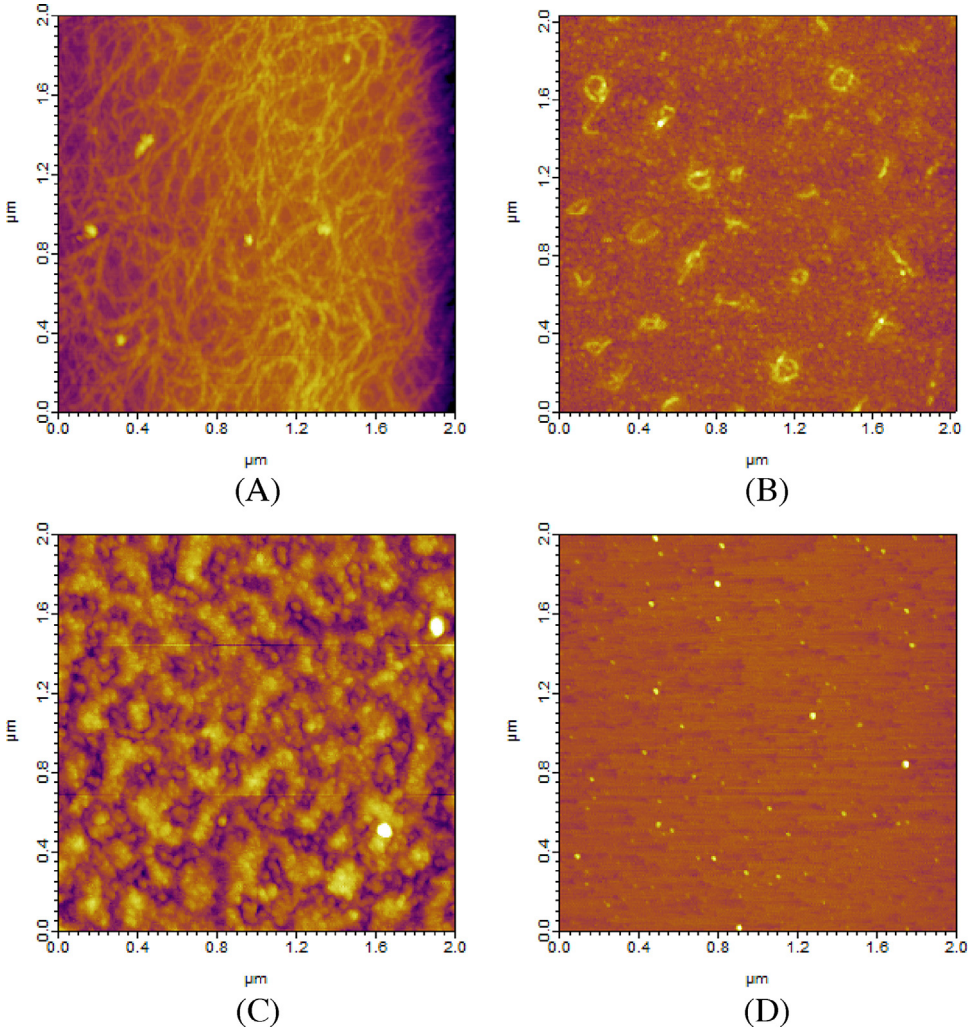

Fig. 4. The AFM images of the polysaccharides and complexes (HEP25 and BiHEP25 (A and B); HEP75and BiHEP75 (C and D)) were obtained with an MFP-3D atomic force microscope (Asylum Research, American) and collected by tapping mode.

The difference in CD properties also indicated the conformational change owing to the formation of the complexes.

3.2.4. Scanning electron microscopy (SEM) analysis

Analysis of surface morphology by scanning electron microscopy (SEM) is a qualitative tool to characterize fungal polysaccharides and assess morphological differences of complexes. The SEM images of the HEPs and BiHEP complexes are shown in Fig. 3. The results indicated that Bi^{3+} induced different physical changes. HEP25 is fiber netted structure (Fig. 3A) and HEP75 is spherical shape with netted structure (Fig. 3C), while BiHEP25 and BiHEP75 have close-up structure (Fig. 3B and D). The change of morphology and shape of BiHEP complexes suggests that inter- and intra-molecular hydrogen bonds of original HEP had been greatly increased. This variation on morphology may be related to the formation of the BiHEP complexes.

3.2.5. Atomic force microscopy (AFM) analysis

AFM images of HEPs and BiHEP complexes are shown in Fig. 4. In both cases, linear filamentous structures were present with a small proportion of them possessing branches. Furthermore, some aggregates, distinguishable by height measurements were also present even at low sample dilutions when very few individual polymers were present in the samples. This observation suggests that they are not simply superpositions or entanglements of polymers caused by the reduction of solvent volume during drying down on to the substrate, but are multi-polymer complexes held together by intermolecular interactions (Posé, Kirby, Mercado, Morris, & Quesada, 2012). As indicated by the inset height bars in Fig. 4, the molecules which rise above the surface are brighter in color. The molecules appear compact and somewhat asymmetric in shape. Fig. 4A shows that the polysaccharide chains were branched and entangled with each other. Remarkably, as shown in Fig. 4B, many of the smaller compact molecules have aggregated into linear, circular or combined linear-circular skeletal structures. Nevertheless, many of the smaller compact molecules remain visible.

3.2.6. X-ray diffractometry (XRD) analysis

The X-ray diffraction spectra of $Bi(NO_3)_3{\cdot}5H_2O$, HEPs, and BiHEP complexes are shown in Fig. 5. $Bi(NO_3)_3{\cdot}5H_2O$ have 11 typical crystal peaks at 2θ values of about 10.82°, 12.72°, 14.78°, 15.70°, 16.22°, 21.60°, 21.98°, 24.44°, 32.58°, 34.48°, and 43.88° as well as numerous small peaks between 5° and 90° (Fig. 5A). HEP25 consists of three peaks at 2θ of 19.82°, 46.53°, and 53.84°, among which the peak at 19.82° is relatively sharper. BiHEP complexes contain broad diffraction peaks at 2θ of 27.33° which are hardly appreciable (Fig. 5B). Thus, complexation of bismuth leads to very significant changes in the morphology of HEPs, indicating complete disruption of the inter-polymer bonds.

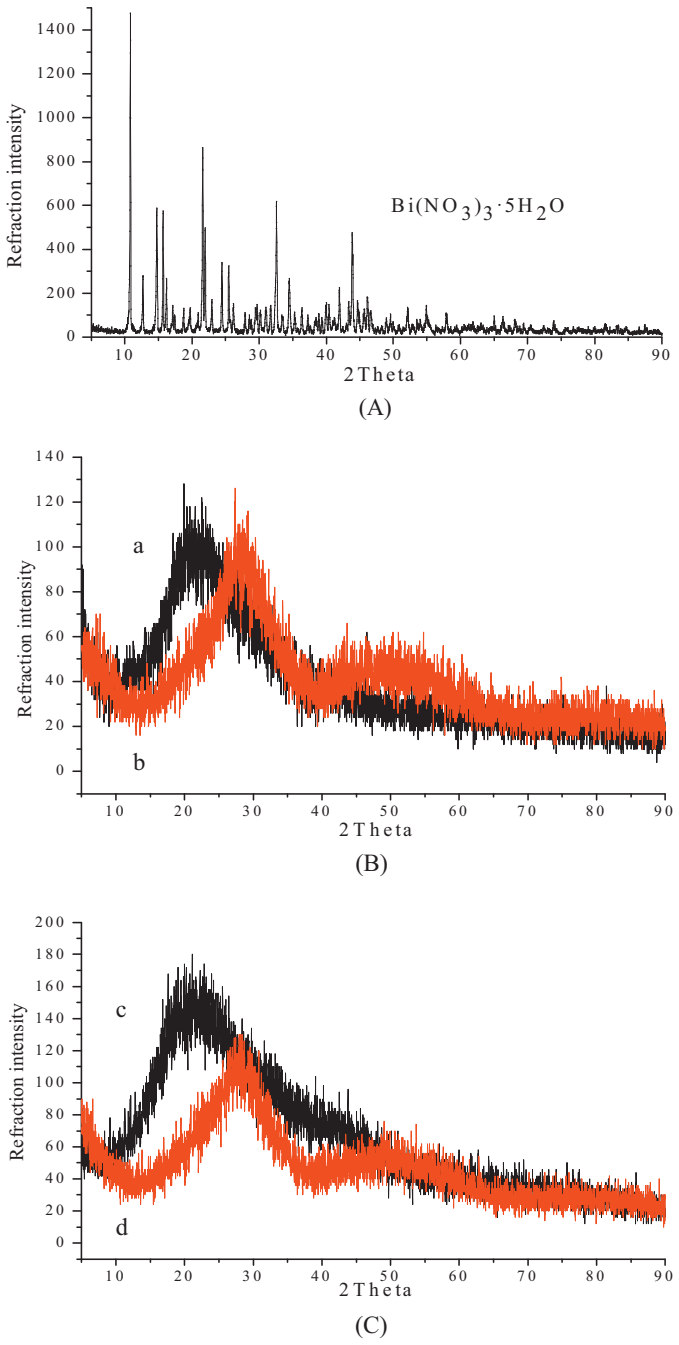

Fig. 5. X-ray diffraction patterns of $Bi(NO_3)_3{\cdot}5H_2O$, HEP25 (a), BiHEP25 (b), HEP75 (c), and BiHEP75 (d).

3.2.7. *Thermogravimetric analysis (TGA)*

The thermal stability is an important characteristic of materials that may have biological applications, considering the possible need of sterilisation by heating. Thermal stability of the polysaccharide sample was studied using thermal gravimetric analysis, as it can provide a quantitative measurement of mass change in materials associated with dehydration, decomposition and oxidation of a sample with time and temperature (Xie et al., 2013). The thermal stability and degradation behavior of HEP25 were evaluated by TGA under nitrogen atmosphere (Fig. 6A). The first mass loss, taking place between 25 and 140 °C, may be attributed to the loss of adsorbed and structural water of biopolymers or due to desorption of moisture as hydrogen bound water to the polysaccharide structure. The second weight loss event, with an onset of over 140 °C,

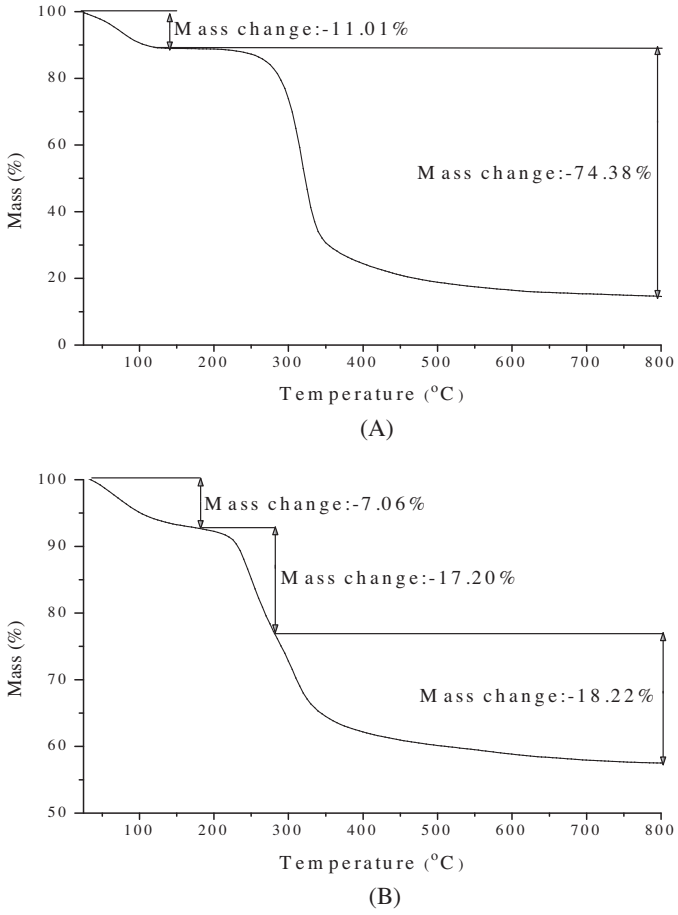

Fig. 6. Thermogravimetric analysis (TGA) of HEP25 (A) and BiHEP25 (B) from 25 to 800 °C, performed at a heating rate of 20 °C/min, in a nitrogen atmosphere.

resulted in a weight loss of about 74.38%, could be attributed to thermal decomposition of the polysaccharide and is described by a weight loss onset and oxidation temperature.

As for the complex, the thermal characteristic was different from that of HEP. BiHEP25 decomposed in three stages (Fig. 6B). Between 25 and 160 °C, BiHEP25 lost 7.06% of its total weight, including the loss of water. In the stage of 160–280 °C, BiHEP25 lost 17.20% of its total weight. When the test ended at 800 °C, the sample had lost approximately half of the starting mass. This high thermostability, with the consequent high solid residue content, might be due to the complex and heterogeneous molecular structure of the HEP (Mota et al., 2013). Moreover, TG curves showed that the temperatures for the rapid weight loss of HEP appeared at 140 and 800 °C. However, the corresponding temperatures for the rapid weight loss of BiHEP complexes were 280 and 800 °C, indicating that the complex is more stable as compared to HEP. The enhanced thermal stability of BiHEP was due to the grafted HEP chains.

3.3. *Testing for anti-Hp activity*

As shown in Table 2, the BiHEP complexes showed valid anti-*Hp* activity. The MICs of the complexes against *Hp* strains (NTCC11637)

Table 2
MICs of BiHEP complexes, HEPs, and CBS.

	BiHEP25	BiHEP75	HEP25	HEP75	CBS[a]
MIC (μg/mL)	20	20	320	160	20

[a] The Bi ion content of CBS is 37.5%.

are both identical with that of CBS, the most used bismuth preparation in eradication treatment of *Hp*, despite the high molecular weight and complicated structure of the complexes. For HEPs, because of their high molecular weights, high apparent viscosity, and poor water solubility, the polysaccharides are difficult to pass through organizational barriers and enter the interior of the cell, which limits their biological activity.

4. Conclusion

Two new Bi^{3+}-polysaccharide complexes were prepared with Bi^{3+} and two purified polysaccharides from *H. erinaceus*, respectively. The complexes were characterized by elemental analysis, FT-IR, CD, SEM, AFM, XRD, and TG. The anti-*H. pylori* activities *in vitro* by agar dilution assay were also evaluated. The molecular weights of HEPs were 197 and 20 kDa, respectively. All the analyses confirmed the formation of new BiHEP complexes with lower content of Bi^{3+} when compared with colloidal bismuth subcitrate (CBS), the most utilized bismuth preparation clinically. Furthermore, HEPs themselves have definite inhibition effects on *Hp* and BiHEP complexes with lower content of Bi^{3+} exhibited strong inhibition effects on *Hp* (MIC = 20 μg/mL), similar to that of CBS with higher content of Bi^{3+}. The study provides a basis for further development of multiple treatments of *Hp* infection or new medicines. Research on the possible anti-Hp mechanism of BiHEP complexes is worthy of future study.

Acknowledgments

The authors gratefully acknowledge the financial supports by Practice Innovative Projects in China (2012JSSPITP1240). We also appreciate Dr. Samuel Jerry Cobbina for language polishing.

References

Asha, M. K., Debraj, D., Prashanth, D., Edwin, J. R., Srikanth, H. S., Muruganantham, N., et al. (2013). In vitro anti-*Helicobacter pylori* activity of a flavonoid rich extract of *Glycyrrhiza glabra* and its probable mechanisms of action. *Journal of Ethnopharmacology*, *145*(2), 581–586.

Bland, M. V., Ismail, S., Heinemann, J. A., & Keenan, J. I. (2004). The action of bismuth against *Helicobacter pylori* mimics but is not caused by intracellular iron deprivation. *Antimicrobial Agents and Chemotherapy*, *48*(6), 1983–1988.

Briand, G. G., & Burford, N. (1999). Bismuth compounds and preparations with biological or medicinal relevance. *Chemical Reviews*, *99*(9), 2601–2657.

Engstrand, L., & Lindberg, M. (2013). Helicobacter pylori and the gastric microbiota. *Best Practice & Research in Clinical Gastroenterology*, *27*(1), 39–45.

Geng, X., Cui, B., Li, Y., Jin, W., An, Y., Zhou, B., et al. (2014). Preparation and characterization of ovalbumin and carboxymethyl cellulose conjugates via glycosylation. *Food Hydrocolloids*, *37*, 86–92.

Gisbert, J. P., Gisbert, J. L., Marcos, S., Jimenez Alonso, I., Moreno Otero, R., & Pajares, J. M. (2008). Empirical rescue therapy after *Helicobacter pylori* treatment failure: A 10-year single-centre study of 500 patients. *Alimentary Pharmacology &Therapeutics*, *27*(4), 346–354.

Gordon, M. F., Abrams, R. I., Rubin, D. B., Barr, W. B., & Correa, D. D. (1995). Bismuth subsalicylate toxicity as a cause of prolonged encephalopathy with myoclonus. *Movement disorders*, *10*(2), 220–222.

Graham, D. Y., & Shiotani, A. (2008). New concepts of resistance in the treatment of *Helicobacter pylori* infections. *Nature Clinical Practice Gastroenterology & Hepatology*, *5*(6), 321–331.

Jia, L. M., Liu, L., Dong, Q., & Fang, J. N. (2004). Structural investigation of a novel rhamnoglucogalactan isolated from the fruiting bodies of the fungus *Hericium erinaceus*. *Carbohydrate Research*, *339*(16), 2667–2671.

Jin, Y., Ling, P., He, Y., Chen, L., Chen, J., & Zhang, T. (2008). Preparation, characterization and anti-*Helicobacter pylori* activity of Bi^{3+}-hyaluronate complex. *Carbohydrate Polymers*, *74*(1), 50–58.

Kabat, E. A., Lloyd, K., & Beychok, S. (1969). Optical activity and conformation of carbohydrates. 11. Optical rotatory dispersion and circular dichroism studies on immunochemically reactive oligo- and polysaccharides containing amino sugars and their derivatives. *Biochemistry*, *8*(3), 747–756.

Kusters, J. G., van Vliet, A. H., & Kuipers, E. J. (2006). Pathogenesis of *Helicobacter pylori* infection. *Clinical Microbiology Reviews*, *19*(3), 449–490.

Manyi Loh, C. E., Clarke, A. M., Munzhelele, T., Green, E., Mkwetshana, N. F., & Ndip, R. N. (2010). Selected South African honeys and their extracts possess in vitro anti-*Helicobacter pylori* activity. *Archives of Medical Research*, *41*(5), 324–331.

Mota, R., Guimaraes, R., Buttel, Z., Rossi, F., Colica, G., Silva, C. J., et al. (2013). Production and characterization of extracellular carbohydrate polymer from *Cyanothece* sp. CCY 0110. *Carbohydrate Polymers*, *92*(2), 1408–1415.

Ou, C., Zhang, C., Li, S., Yang, L., Dong, J., Mo, X., et al. (2010). Thermal degradation kinetics of chitosan–cobalt complex as studied by thermogravimetric analysis. *Carbohydrate Polymers*, *82*(4), 1284–1289.

Paulo, L., Oleastro, M., Gallardo, E., Queiroz, J. A., & Domingues, F. (2011). Anti-*Helicobacter pylori* and urease inhibitory activities of resveratrol and red wine. *Food Research International*, *44*(4), 964–969.

Posé, S., Kirby, A. R., Mercado, J. A., Morris, V. J., & Quesada, M. A. (2012). Structural characterization of cell wall pectin fractions in ripe strawberry fruits using AFM. *Carbohydrate Polymers*, *88*(3), 882–890.

Recklinghausen, U. V., Hartmann, L. M., Rabieh, S., Hippler, J., Hirner, A. V., Rettenmeier, A. W., et al. (2013). Methylated bismuth, but not bismuth citrate or bismuth glutathione, induces cyto- and genotoxic effects in human cells in vitro. *Chemical Research in Toxicology*, *21*(6), 1219–1228.

Stamatis, G., Kyriazopoulos, P., Golegou, S., Basayiannis, A., Skaltsas, S., & Skaltsa, H. (2003). In vitro anti-*Helicobacter pylori* activity of Greek herbal medicines. *Journal of Ethnopharmacology*, *88*(2–3), 175–179.

Xie, J. H., Liu, X., Shen, M. Y., Nie, S. P., Zhang, H., Li, C., et al. (2013). Purification, physicochemical characterisation and anticancer activity of a polysaccharide from *Cyclocarya paliurus* leaves. *Food Chemistry*, *136*(3–4), 1453–1460.

Zaidi, S. F. H., Yamada, K., Kadowaki, M., Usmanghani, K., & Sugiyama, T. (2009). Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against *Helicobacter pylori*. *Journal of Ethnopharmacology*, *121*(2), 286–291.

Zhao, T., Mao, G., Mao, R., Zou, Y., Zheng, D., Feng, W., et al. (2013). Antitumor and immunomodulatory activity of a water-soluble low molecular weight polysaccharide from *Schisandra chinensis* (Turcz.) Baill. *Food and Chemical Toxicology*, *55*(0), 609–616.

Zhu, Y., Li, Q., Mao, G., Zou, Y., Feng, W., Zheng, D., et al. (2014). Optimization of enzyme-assisted extraction and characterization of polysaccharides from *Hericium erinaceus*. *Carbohydrate Polymers*, *101*, 606–613.