Food Chemistry 201 (2016) 7–13

Contents lists available at ScienceDirect

Food Chemistry

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

A novel thyroglobulin-binding lectin from the brown alga *Hizikia fusiformis* and its antioxidant activities

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ARTICLE INFO

Article history:
Received 7 October 2015
Received in revised form 29 November 2015
Accepted 14 January 2016
Available online 15 January 2016

Keywords:
Brown alga
Hizikia fusiformis
Lectin
Thyroglobulin from bovine thyroid
Free radicals

ABSTRACT

A lectin (HFL) was isolated from the brown alga, *Hizikia fusiformis*, through ion exchange on cellulose DE52 and HPLC with a TSK-gel G4000PW$_{XL}$ column. SDS–PAGE showed that HFL had a molecular mass of 16.1 kDa. The HPLC (with a TSK-gel G4000PW$_{XL}$ column) indicated that HFL is a tetramer in its native state. The total carbohydrate content was 41%. Glucose, galactose and fucose were the monosaccharide units of HFL, and the normalized mol% values were 6, 14 and 80, respectively. HFL contains a large amount of the acidic amino acid, Asx. The β-elimination reaction suggested that the oligosaccharide and peptide moieties of HFL may belong to the *N*-glucosidic linkage. The amino acid sequences, of about five segments of HFL, were acquired by MALDI-TOF/TOF, and the sequences have no homology with other lectins. HFL was found to agglutinate sheep erythrocytes. The hemagglutination activity was inhibited by thyroglobulin, from bovine thyroid, but not by any of the monosaccharides tested. The lectin reaction was independent of the presence of the divalent cation Ca^{2+}. HFL showed free radical scavenging activity against hydroxyl, DPPH and $ABTS^{+}$ radicals.

1. Introduction

Lectins are multivalent, carbohydrate-binding, proteins. The study of the properties and functions of lectins may lead to the discovery of new medical and biological applications, as well as enhancing the understanding of carbohydrate-protein interactions. In recent years, lectinologists have focused much attention on marine algae as new sources of lectins. A large amount of lectins have been isolated and characterized from marine algae. For example, a mucin-binding lectin (HML) and a GalNAc-specific lectin (HCA) were isolated from the red marine algae, *Hypnea musciformis* and *Hypnea cervicornis*, with molecular masses of 9357 ± 1 Da and 9193 ± 2 Da, respectively (Nagano et al., 2007). A divalent cation-dependent (Ca^{2+} or Mn^{2+}) *N*-acetyl-D-galactosamine/D-galactose-specific lectin (78.9 kDa) was purified from the red marine alga, *Vidalia obtusiloba* C. Agardh (Melo et al., 2004). A lectin (17 kDa) was isolated from the red alga, *Gracilaria ornate*, using ammonium sulfate precipitation (70% saturation), DEAE-cellulose chromatography and affinity chromatography on a mucin-Sepharose 4B column (Leite et al., 2005). A galactose-specific lectin was isolated from the red marine alga, *Ptilota plumose*, through affinity chromatography on Sephadex G-200 (Sampaio et al., 2002). An asialofetuin/fetuin-binding hemagglutinin (71 kDa) was isolated from the red alga, *Gracilaria verrucosa*, through ammonium sulfate fractionation, ion exchange and gel filtration chromatography (Kakita, Fukuoka, Obika, & Kamishima, 1999). A galactose-specific lectin (PFL) was isolated through affinity chromatography on cross-linked guar gum from the red marine alga, *Ptilota filicina*. Gel filtration and SDS–PAGE revealed that PFL is a 56.9 kDa native protein and has one band representing a 19.3 kDa subunit (Sampaio, Rogers, & Barwell, 1998a). A galactose-specific lectin was isolated from the marine red alga, *Enantiocladia duperreyi* (C. Agardh), through ammonium sulfate precipitation, ion exchange and affinity chromatography on alpha-lactose-agarose. The molecular mass of the *E. duperreyi* lectin, determined by gel filtration, is 24.7 kDa, and SDS–PAGE exhibited one protein band with a molecular mass of 16 kDa (Benevides, Holanda, Melo, Freitas, & Sampaio, 1998). Two novel mannan- and L-fucose-binding lectins (EPL-1 and EPL-2) were purified from the green alga, *Enteromorpha prolifera*. SDS–PAGE showed that both lectins were 20–22 KDa single-chain, nonglycosylated proteins. Sedimentation-diffusion equilibrium showed that EPL-1 and EPL-2 had molecular masses of

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http://dx.doi.org/10.1016/j.foodchem.2016.01.061
0308-8146/

60 kDa (EPL-1) and 59.5 kDa (EPL-2), indicating that EPL-1 and EPL-2 have a tendency to self-associate into higher order aggregates (Ambrosio, Sanz, Sanchez, Wolfenstein-Todel, & Calvete, 2003). The lectin from the subspecies of the green marine alga, *Codium fragile* (CFT), has a molecular mass of 60 kDa and is composed of four 15 kDa subunits joined by disulfide bonds, moreover, the lectin recognized the GalNAcα1 → Ser/Thr as carbohydrate side chains (Wu et al., 1997). The lectin that recognizes L-fucose, fucoidan, porcine stomach mucin and bovine submaxillary gland mucin was purified from the green marine alga, *Ulva lactuca*. The molecular weight determined by gel filtration was 8370 Da, and SDS–PAGE gave a single protein band with a molecular mass of 17120 Da (Sampaio, Rogers, & Barwell, 1998b). Unfortunately, there is limited data about lectins from brown algae in comparison with those from red and green algae, and only a few publications are available. Isolating protein from brown algae is difficult because of the viscous polysaccharides (Kim, Kong, & Kim, 2010).

Several new compounds from the brown alga, *H. fusiformis*, have been isolated and shown to have various bioactivities. Water-soluble polysaccharides possessing antioxidant activities were isolated from *H. fusiformis* (Wu, Wu, Qu, Li, & Yan, 2013), the protective effect of a glycoprotein from *H. fusiformis* on acetaminophen-induced liver injury has been investigated (Hwang, Kim, & Nam, 2008), and immune-modulating activities of polysaccharides from *H. fusiformis* were studied (Jeong, Jeong, Lee, & Kim, 2015). Although new compounds have been found in brown algae, *H. fusiformis*, the search for novel lectin remains a challenge.

In this report, we describe the purification, characterization, carbohydrate specificity and antioxidant activity of a new Ca^{2+}-independent thyroglobulin-binding lectin from the brown alga, *H. fusiformis*.

2. Materials and methods

2.1. Materials

Monosaccharides were obtained from Merck (Darmstadt, Germany). Porcine stomach mucin (PSM, type III), thyroglobulin from bovine thyroid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Chemical (USA). Sheep erythrocytes were obtained from the Houxin Biotechnology Company (Nanjing, China). Cellulose DE52 was obtained from the Solarbio Science and Technology Company (Beijing, China). TSK gel G4000PW_{XL} columns were purchased from TOSOH (Japan). The standard proteins used for apparent molecular mass estimation by SDS–PAGE were purchased from the Beijing Solarbio Science and Technology Company (Beijing, China). 3-Methyl-1-phenyl-2-pyrazolin-5-one (PMP) was obtained from J and K Science Ltd. (Beijing, China).

The brown alga, *H. fusiformis*, was collected manually along the coast of Zhoushan, East China Sea in May 2012. The sample was cleaned and air-dried prior to extraction.

2.2. Isolation and purification of HFL

A 50 g portion of the algal powder was homogenized in the same volume (w/v) of 0.9% NaCl. The homogenate was stirred for 16 h at 4 °C and centrifuged at 4000 rpm for 20 min. Crude saline extract (50 ml) was dialyzed for 10 h against water at 4 °C and lyophilized to yield powder (870 mg). The powdered sample (100 mg) was suspended in 2 ml of 0.01 M Tris–HCl buffer (TB), pH 7.4, and centrifuged at 4000 rpm for 20 min. The clear supernatant was applied to a cellulose DE52 column (3 × 11 cm) that had been previously equilibrated and eluted with TB. After unbound proteins were eluted in TB, adsorbed proteins were eluted with a NaCl gradient (0–1 M) in TB. The protein concentration and hemagglutination titer of each fraction were measured. Fractions containing hemagglutinating activity were pooled and lyophilized to yield powder (45 mg). The purified sample (50 μg) was suspended in 20 μL of 0.01 M TB, pH 7.4, and centrifuged at 8000 rpm for 20 min. The clear supernatant was also purified by high-performance liquid chromatography (HPLC) on a G4000PW_{XL} column (7.8 mm × 30 cm) and eluted with TB.

2.3. Measurement of the molecular mass of the purified native lectin and the subunit

The molecular mass of the purified native lectin was measured by passing it through a G4000PW_{XL} column (7.8 mm × 30 cm) in 0.01 M TBS, pH 7.4 (Tong et al., 2012). The standard proteins used were rabbit muscle phosphorylase B (97,000), bovine albumin V (68,000), egg albumin (45,000), and trypsin (23,300).

The molecular mass of the subunit of the purified lectin was measured by SDS–PAGE according to the Laemmli procedure using a 15% polyacrylamide separation gel and a 4% polyacrylamide stacking gel (Laemmli, 1970). The molecular mass of HFL was determined by SDS–PAGE in the presence and absence of dithiothreitol. Reduction of HFL was performed by heating at 100 °C for 5 min in sample buffer containing 2% SDS and 2.5% dithiothreitol (Tong et al., 2012). Gels were calibrated using the following standard proteins: phosphorylase B (94,000), BSA (67,000), ovalbumin (45,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and alpha-lactalbumin (14,400). Proteins and glycoproteins were stained with Coomassie brilliant blue and glycoprotein stain (Thermo® Pierce Glycoprotein Staining Kit), respectively.

Molecular masses were further investigated by MALDI-TOF mass spectrometry. The measurements were performed on a Bruker Autoflex time-of-flight mass spectrometer (Bruker, Bremen, Germany), equipped with a delayed ion-extraction device and a pulsed nitrogen laser (337 nm; 3 ns). An aqueous solution of lectin (16 nmol/ml) was diluted 1:1 with aqueous 50% acetonitrile containing sinapinic acid (11 mg/ml). One microliter of the mixture was deposited on the target and left to air dry. The MALDI was run in linear mode with an acceleration voltage of 25 kV and the lag pulse set to 3,200 V. Cytochrome C and BSA were used for external calibration. Typically, 50 laser shots were averaged per spectrum.

2.4. Hemagglutination assay

To assay hemagglutinating activity, HFL was 2-fold serially diluted with 0.01 M TBS (25 μl) in microtiter U-plates. An equal volume of a 2% suspension of sheep erythrocytes was added to each well, and the mixture was agitated. The hemagglutination was visually evaluated after 30 min (Belogortseva, Molchanova, Kurika, Skobun, & Glazkova, 1998; Qu et al., 2015).

For the hemagglutination inhibition assay, the aqueous solutions of various substances were 2-fold serially diluted with TBS. HFL (25 μl, 4 doses of agglutination) and a 2% erythrocyte suspension (25 μl) were successively added to each sample (25 μl). The mixture obtained was gently stirred by pipette and kept for 1 h. The minimal concentration of each substance required for complete inhibition was determined (Qu et al., 2015).

2.5. Effect of divalent cations

HFL was dialyzed for 24 h against 0.1 M TBS (pH 7.4), containing 50 mM $EDTANa_2$ or 50 mM $CaCl_2$. Sheep erythrocytes were used as indicator cells.

2.6. Amino acid analysis

The purified HFL (1.0 mg/ml) was hydrolyzed under argon in a sealed tube with 6 M HCl, at 100 °C for 24 h. The amino acid composition of HFL was determined with a Hitachi 835 amino acid analysis system.

2.7. Protein and carbohydrate content and N-terminal amino acid sequence analysis

The protein content of the samples was determined, according to the method of Lowry (Lowry, Rosenbrough, Farr, & Randae, 1951), using crystalline bovine serum albumin as the standard protein. The sugar content was estimated by the phenol–sulfuric acid method using D-glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Xiong et al., 2006).

The N-terminal amino acid sequence was determined using an automated 492 model sequencer (Applied Biosystems, Shanghai, China) at Shanghai Applied Protein Technology Co., Ltd.

2.8. HFL identification by mass spectrometry

HFL sequencing was performed by trypsin-digesting the lectin overnight at 37 °C and sequencing the different peptides using MALDI-TOF/TOF (5800 Applied Biosystems) at Shanghai Applied Protein Technology Co., Ltd. The obtained peptide sequences were analyzed by BLAST2 (http://www.ncbi.nlm.nih.gov/BLAST/).

2.9. β-Elimination reaction

Approximately 1.2 mg of HFL was dissolved in 0.3 ml of a 0.2 M NaOH solution. Then, the solution was incubated at 60 °C for 30 min. The solution was scanned from 200 to 400 nm using an ultraviolet (UV) spectrophotometer before and after treatment with NaOH (Hirohashi & Vacquier, 2002; Tong et al., 2012; Ye et al., 2009).

2.10. Monosaccharide composition analysis

The uronic acid content was determined by the carbazole-sulfuric acid method, using glucuronic acid as the standard (Bitter & Muir, 1962). The neutral monosaccharide composition of water-soluble polysaccharides was determined using the method of Fu and O'Neill with trichloromethane replacing butyl ether (Fu & O'Neill, 1995). Twenty microliters of the resulting aqueous phase of the PMP-labeled monosaccharides was analyzed on a C18 column (4.6 × 250 mm) connected to a Waters high-performance liquid chromatography (HPLC) system (1525 binary HPLC pump and 2487 dual λ absorbance detector). To separate the PMP-labeled monosaccharides, the buffer was 0.1 M PB (pH 6.7) with 17% acetonitrile, and the flow rate was set to 1 ml/min. The wavelength for UV detection was 245 nm (Fu & O'Neill, 1995).

2.11. Sulfate content

Sulfate content was assayed by turbidity measurement after hydrolysis of the corresponding samples in 4 N HCl and the addition of gelatin-$BaCl_2$ (Dodgson, 1961).

2.12. Fourier-transform infrared (FT-IR) spectroscopy

HFL was compressed into a KBR pellet, and the FT-IR spectra were recorded on a Frontier FT-IR spectrometer (PerkinElmer).

2.13. Scavenging of the hydroxyl radical

The hydroxyl radical scavenging activities of the lectin were measured according to the method of Chung et al. (Chung, Osawa, & Kawakishi, 1997). The hydroxyl radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity } (\%) = (A_{\text{blank}} - A_{\text{sample}}) \times 100/A_{\text{blank}} \quad (1)$$

2.14. Scavenging of the DPPH radical

HFL DPPH radical scavenging activities were measured according to the method of Singh with several modifications (Singh & Rajini, 2004). Briefly, 2 ml of DPPH solution (0.2 mM in 95% ethanol) was incubated with varying concentrations of HFL. The reaction mixture was shaken well and incubated for 30 min at room temperature, and the absorbance of the resulting solution was read at 517 nm against a blank. The DPPH radical scavenging activity was calculated using Eq. (1).

2.15. Scavenging of the $ABTS^+$ radical

The ABTS assay was based on the method of Re et al. (Re et al., 1999) with modifications. ABTS was dissolved in water to a 7 mM concentration. The $ABTS^+$ radical was produced by reacting ABTS solution with 2.45 mM potassium persulfate in the dark at room temperature for 12 h before use. The $ABTS^+$ radical solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. After the addition of 1.0 ml of diluted $ABTS^+$ radical solution to 50 μl of the HFL solution, the absorbance reading was taken at 30 °C, exactly 1 min after the initial mixing and was continued for up to 6 min. Appropriate solvent blanks were run in each assay. The $ABTS^+$ radical scavenging activity was calculated using Eq. (1).

2.16. Statistical analysis

Each experiment was carried out in triplicate. Values are given as the means ± SD.

3. Results and discussion

3.1. HFL purification

HFL was purified from *H. fusiformis* by ion exchange on cellulose DE52 (Fig. 1A) and HPLC on $G4000PW_{XL}$ (Fig. 1B). HFL migrated as a symmetric HPLC peak, and the hemagglutination activity corresponded to the protein content (data not shown). Only one hemagglutination activity fraction was separated in two purification steps, indicating there is only one kind of lectin in *H. fusiformis*, by this purification procedure. In order to simplify the purification procedure, fractions with the highest hemagglutination activity in every step were collected.

After two purification steps, HFL was purified to homogeneity. HFL was analyzed by SDS–PAGE and migrated as a major intense band with a relative molecular mass of approximately 16.1 kDa (Fig. 2, line 1). Reduction with dithiothreitol led to the appearance of a 15.2 kDa band (Fig. 2, line 3). This finding indicates that HFL has intramolecular disulfide bond(s). Several other proteins have been shown to change their electrophoretic mobility on SDS–PAGE, most likely because these proteins also had intramolecular disulfide bonds (Findik, Reuter, & Presek, 1990). Bovine serum albumin and HFL were stained with glycoprotein stain kit (Fig. 2, line 4 and 5). The result shows that HFL belongs to glycoprotein. The purified HFL was eluted from the TSK gel G4000PWXL column at (Ve − Vo)/

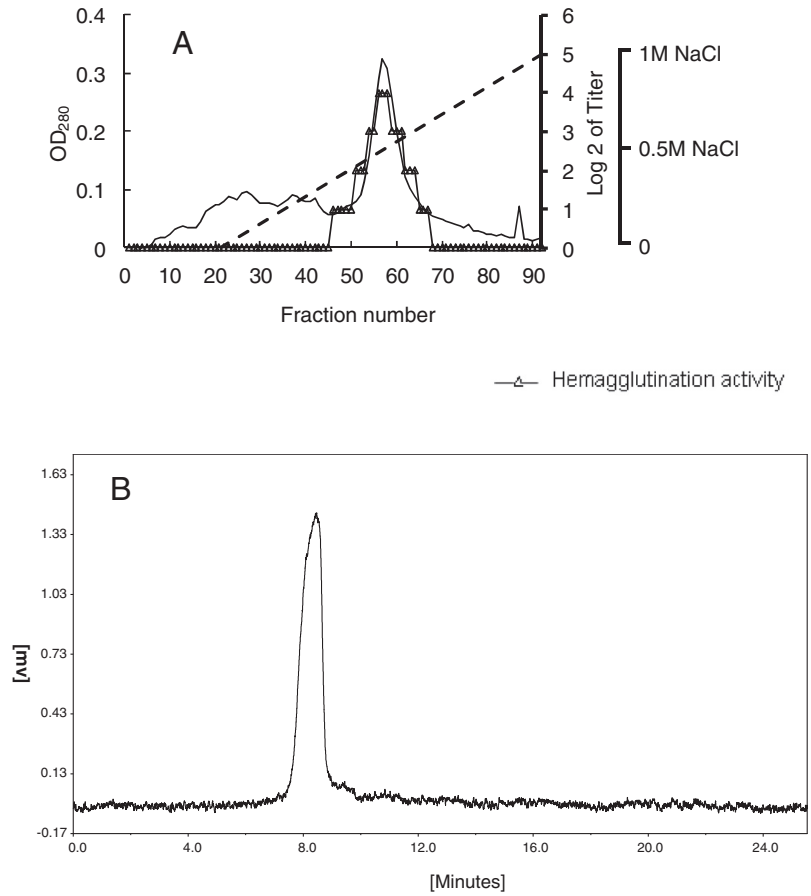

Fig. 1. (A) Ion-exchange chromatography of a crude extract of the brown alga *H. fusiformis* on cellulose DE52. (B) HPLC of purified HFL on a TSK gel G4000PW$_{XL}$ column.

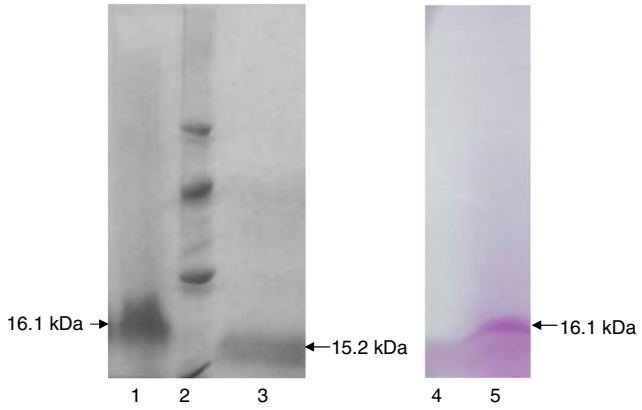

Fig. 2. SDS–PAGE. Lane 1 – HFL, $M_r \sim 16{,}100$. Lane 2 – marker proteins: rabbit actin (43,000), bovine carbonic anhydrase (31,000), trypsin inhibitor (20,100), and hen egg white lysozyme (14,400). Lane 3 – HFL treated with dithiothreitol. Lane 4 – Bovine serum albumin for glycoprotein staining (Thermo® Pierce Glycoprotein Staining Kit) as a negative control for glycoprotein. Lane 5 – HFL glycoprotein staining.

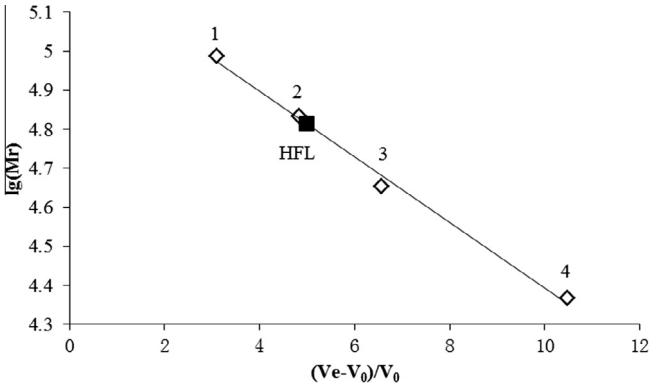

Fig. 3. Estimation of the molecular mass of HFL by HPLC on a G4000PW$_{XL}$ column (7.8 mm × 30 cm). 1. Trypsin (23,300); 2. Albumin egg (45,000); 3. Albumin bovine V (68,000); 4. Rabbit muscle phosphorylase B (97,000).

Vo = 5.00 (Fig. 3). The apparent molecular weight of HFL estimated from HPLC was approximately 65 kDa.

The HFL molecular mass was further determined by MALDI-TOF mass spectrometry. The HFL spectrum contained peaks corresponding to singly charged $(M+3H)^{3+}$ molecular ions from the subunit at m/z 6.6 kDa (Fig. 4A).

In summary, molecular mass determination by SDS–PAGE and MALDI-TOF mass spectrometry showed that HFL has a single polypeptide chain with a molecular mass of 16.1 kDa. Gel filtration of HFL on TSK-gel G4000PW$_{XL}$ indicates that it exists as a tetramer in its native state. According to the hemagglutination assay, HFL does not require the divalent cation Ca^{2+} for lectin activity (data not shown).

Edman degradation of HFL was unsuccessful, which suggested that its N-terminus is blocked. HFL was digested with trypsin and analyzed with MALDI-TOF/TOF. Five peptide sequences (ASESEAIK, SVEDLTNK, QEFESLSRK, LQAHSELERAK and DEAEAARAALAEAEQK) were obtained and subjected to a BlastX search (http://www.ncbi.nlm.nih.gov/BLAST/). BlastX revealed that the

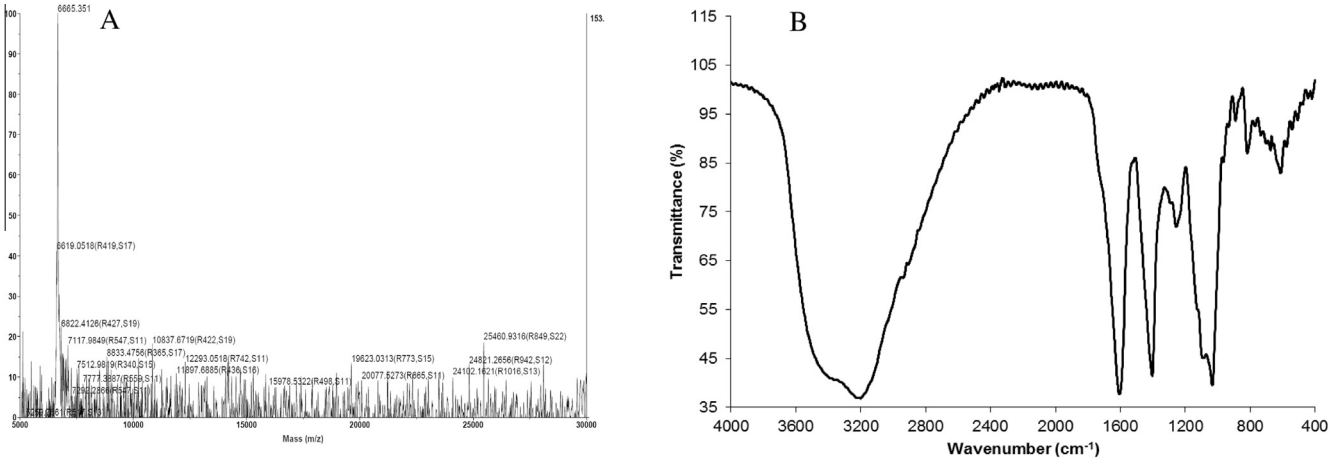

Fig. 4. (A) MALDI-TOF mass spectrum of HFL. The mass spectrum was obtained on a Bruker Autoflex time-of-flight mass spectrometer using a sinapinic acid matrix as detailed in Section 2; (B) FTIR spectrum of HFL.

five peptides did not show any significant homology with other lectins of the marine alga species listed in the BLAST output.

3.2. Carbohydrate composition and amino acid composition

As shown in Table 1, the total carbohydrate content of HFL is 41%. Accordingly, the HFL molecular mass of 16.1 kDa is due to the polypeptide chain and the carbohydrates. Glucose, galactose and fucose are the monosaccharide units of HFL, accounting for 6%, 14% and 80%, respectively. The uronic acid content of HFL is 5.91%, and no sulfate content was detected. Purified HFL contains relatively high amounts of the apolar amino acids Gly, Thr and Ala and the polar amino acids Glx and Asx.

3.3. Carbohydrate-binding specificity

The HFL carbohydrate-binding specificity was examined by a hemagglutination inhibition test. The HFL hemagglutination activity was not inhibited by any of 100 mM D-glucose, D-galactose, D-mannose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, D-fucose, fructose, ribose, erythrose, talose, or lactose, as well as 2 mg/ml PSM, whereas, it was inhibited by 0.125 mg/ml glycoprotein thyroglobulin from bovine thyroid. The results of the hemagglutination inhibition study suggest that HFL is a thyroglobulin-binding lectin. The carbohydrate side chains of thyroglobulin from bovine thyroid are *N*-glycosidically linked through Man/GlcN to the Asn of the protein core (Rawitch, Pollock, & Yang, 1993). The Man/GlcN-type units of the thyroglobulin are asymmetrically distributed toward the C-terminus of the protein. The HFL hemagglutination activity was strongly inhibited by thyroglobulin-bearing *N*-glycans, such as the lectin from the red alga *Kappaphycus striatum* (Carrageenophyte) (Hung, Sato, & Hori, 2011) and the lectin from *Phaseolus vulgaris* L. (Bonorden & Swanson, 1992).

Table 1
Carbohydrate and amino acid compositions of HFL.

Items	Data
Carbohydrate (w%)	41
Sulfated groups (w%)	0
Uronic acids (w%)	5.91
Glycosyl composition (*mol%*)	
Glc	6
Gal	14
Fuc	80
Amino acid composition (*mol%*)	
Asx	16.32
Thr	9.12
Glx	8.56
Pro	3.99
Gly	11.13
Ala	8.37
Ile	4.59
Leu	4.97
Tyr	2.36
Phe	4.40
His	3.77
Lys	1.46
Arg	1.90
Met	1.29
Cys	0.10

3.4. FT-IR spectroscopy and determination of HFL glycosylation

FT-IR spectroscopy analysis indicated that HFL showed peaks that were atypical for a glycoprotein and similar to those of acidic polysaccharides (SFPS-1 and SFPS-2) from *Sargassum fusiforme* (Fig. 4B) (Zhou, Hu, Wu, Pan, & Sun, 2008). The FT-IR spectrum of HFL has characteristic absorption bands at 3408, 2939, 1606, 1403, 1255, 1036, 890, 817 and 612 cm^{-1}. The strong peak at 3408 cm^{-1} represents the stretching and angular vibration of the O–H linkage. The peak at 2939 cm^{-1} is attributed to the stretching and angular vibration of the C–H linkage. The peaks at 1606 and 1403 cm^{-1} are due to the asymmetric and symmetric stretch vibrations of the $-COO^-$ of uronic acid (Singthong, Cui, Ningsanond, & Goff, 2004). The peak at 1255 cm^{-1} is assigned to the stretching vibration of C=O groups (Cui et al., 2014). The peak at 890 cm^{-1} is ascribed to the β-configuration of glycosidic linkages (Zhou et al., 2008). The peak at 817 cm^{-1} is due to the characteristic absorption of mannuronic acid (Tian & Zhang, 2005). The peak at 612 cm^{-1} is attributed to N–H bending vibrations of the amide groups (Cui et al., 2014).

According to the principle of the β-elimination reaction, the glycoprotein N-glucosidic linkage is always stable under low concentration alkaline conditions, and no change in absorption at 240 nm is observed (Tian & Zhang, 2005). In this research, the absorption curve at 240 nm was very similar to the curve before treatment with 0.2 M NaOH at 60 °C for 30 min. This result suggests that the linkage between the oligosaccharide moiety and the peptide moiety of HFL may be an *N*-glucosidic linkage.

3.5. Free radical scavenging activity of HFL

The DPPH radical scavenging activity of HFL is shown in Fig. 5A. The DPPH radical scavenging activity of HFL was concentration dependent. For an HFL concentration of 1.6 mg/ml, the DPPH radical scavenging activity was 77.23%. The hydroxyl radical scavenging activities of HFL were measured using the Fenton reaction. The hydroxyl radical scavenging activity of HFL is shown in Fig. 5B. For HFL, the hydroxyl radical scavenging activity was concentration dependent. For a HFL concentration of 1.6 mg/ml, the hydroxyl radical scavenging activity was 33.65%. Fig. 5C shows the ABTS radical scavenging activity of the extract. The ABTS radical scavenging ability of HFL was concentration dependent. The clearance rate reached 68.97% at an HFL concentration of 4 mg/ml.

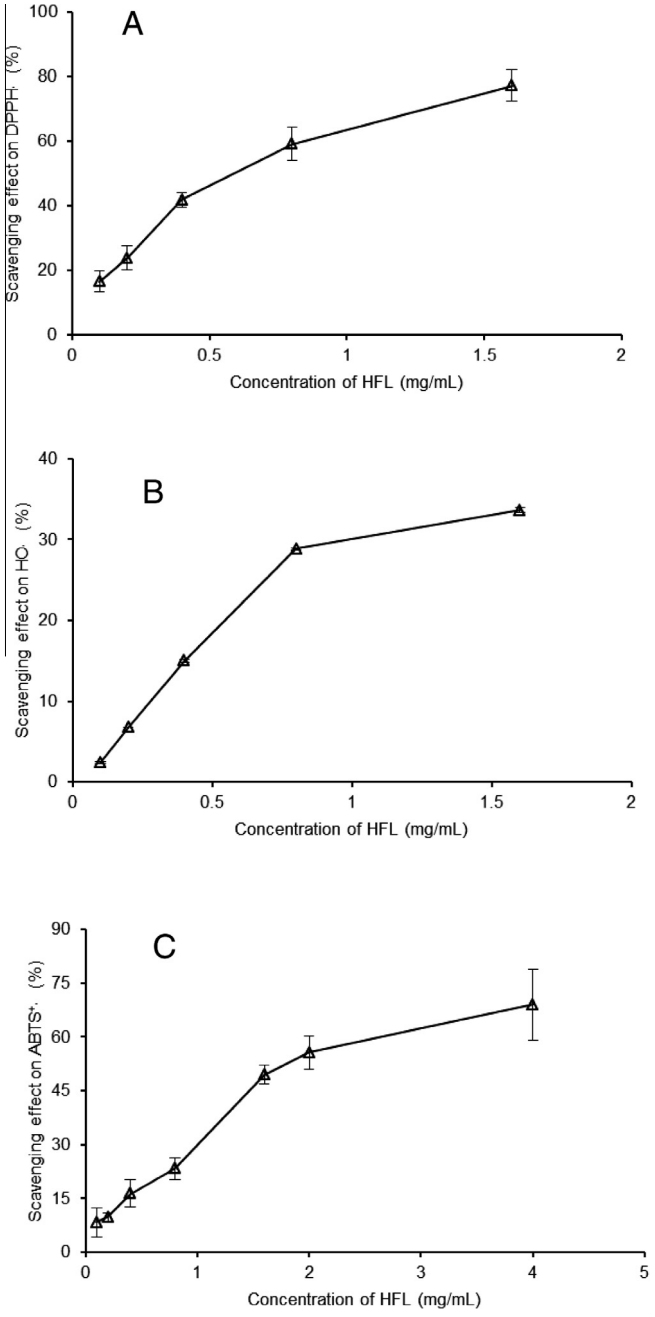

Fig. 5. Scavenging activity of HFL against the DPPH radical (A), hydroxyl radical (B) and ABTS radical (C). Values are the means ± SD ($n = 3$).

Antioxidative properties are not uncommon in marine algae. Several compounds with antioxidant activity have been isolated from brown algae, and most of them belonging to the phenolic fraction, polysaccharide and protein (Balboa, Conde, Moure, Falqué, & Domínguez, 2013). HFL contains polysaccharides and polypeptides. These findings suggest that HFL has potential antioxidant activity. The DPPH, hydroxyl and ABTS radical assays were used to evaluate the antioxidant activities of HFL. DPPH is a stable free radical used to evaluate algal compounds because of its stability, simplicity and reproducibility (Kang et al., 2003). The hydroxyl radical is an extremely reactive and harmful reactive oxygen species in living cells (Balboa et al., 2013). ABTS is used to measure the antioxidant capacity in water-soluble and lipid-soluble samples (Huang, Ou, & Prior, 2005). HFL exhibited better scavenging activities for DPPH and ABTS radicals than for hydroxyl radicals. Antioxidant activity is very rare for algal lectins. The observed properties and functions of HFL are promising for the discovery of new medical and biological applications.

4. Conclusion

A novel lectin (HFL) was isolated from the brown alga, *H. fusiformis*, and its preliminary structure was elucidated by HPLC, FT-IR, and the β-elimination reaction. The 65 kDa native HFL is a homotetrameric glycoprotein. The oligosaccharide and peptide moieties of HFL may be linked by *N*-glucosidic bonds. The carbohydrate specificity of HFL indicated that it is a thyroglobulin-binding lectin. HFL showed free radical scavenging activity against hydroxyl, DPPH and $ABTS^{+}$ radicals. Further studies should focus on the structure–activity relationships of HFL.

Acknowledgment

This work was supported by a grant from the National Natural Science Foundation of China (31470430).

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