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CHARACTERIZATION OF O-GLYCAN BINDING LECTIN FROM THE RED ALGA *HYDROPUNTIA EUCHEUMATOIDES*

Le Dinh Hung[1,✉], Tran Thi Hai Yen[2], Dinh Thanh Trung[1]

[1]*Nha Trang Institute of Technology Research and Application, Vietnam Academy of Science and Technology*
[2]*Le Quy Don High School for the Gifted, Khanhhoa*

✉ To whom correspondence should be addressed. E-mail: ledinhhungims@yahoo.co.uk

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SUMMARY

The red alga, *Hydropuntia eucheumatoides* is one of the algal genera from which agar is commercially extracted, and is the main source of agar in the world. The lectin HEL from the red alga *H. eucheumatoides* was isolated by a combination of aqueous ethanol extraction, ethanol precipitation, ion exchange and filtration chromatography. Lectin gave a single band with molecular mass of 17,000 Da in both non-reducing and reducing SDS-PAGE conditions, therefore lectin exists in monomeric form. The hemagglutination activities of HEL were stable over a wide range of pH from 3 to 10, temperature up 60 ^{o}C and not affected by either the presence of EDTA or addition of divalent cations, indicating that lectin requires no metal for biological activity. The hemagglutination activities of HEL were not inhibited by monosaccharides and glycoproteins, D-glucose, D-mannose, D-galactose, D-xylose, N-acety-D-mannosamine, transferin, fetuin and yeast mannan, but strongly inhibited by monosaccharides containing acetamido groups at equatorial C2 position, such as N-acetyl-galactosamine, N-acetyl-glucosamine, N-acetyl-neuraminic acid and glycoprotein porcine stomach mucin bearing O-glycans. Thus, lectin is specific for O-glycans and may recognize the sequences GalNAcαSer/Thr, GalNAc(α1-3)[Fuc(α1-2)]Gal(β1-4)GlcNAc(β1-3)GalNAc- and GluNAc(α1-4)Gal- under interacting with the acetamido groups at equatorial C2 position of the terminal sugar residues in oligosaccharide structures of O-glycans. The red alga *H. eucheumatoides* could promise to be a source of valuable lectins for application in biochemistry and biomedicine.

Keywords: *Carbohydrate binding specificity; Hydropuntia eucheumatoides; lectin; O-glycans; red alga*

INTRODUCTION

Lectins are proteins (or glycoproteins), other than antibodies and enzymes, that bind specifically and reversibly to carbohydrates, resulting in cell agglutination or precipitation of polysaccharides and glycoconjugates. Lectins are ubiquitous in the biosphere, show diversities in structures, and biological functions, mainly dependent on the originated organisms. The carbohydrate-binding properties of lectins are critically important not only to clarify their biological roles but also to develop them as carbohydrate probes or medicines (Sharon, Lis, 2003).

Characterization studies reveal that algal lectins may be a new fascinating group of lectins, because many of them reveal some common features; low-molecular weight, monomeric form, thermostable and metal-independent hemagglutination, and no affinity for monosaccharide but for glycoproteins (Hori *et al*., 1990; Rogers, Hori, 1993). Although physiological function(s) of lectins in algae is presently unknown, recent studies show that marine algal lectins are promising compounds as potential drugs for the prevention of transmission of various enveloped viruses. For example, lectins from the red algae *Eucheuma serra* (ESA-2) (Hori *et al*., 2007; Sato *et al*., 2015), *Kappaphycus alvarezii* (KAA-2) (Sato *et al*., 2011a; Hirayama *et al*., 2016), *Griffithsia* sp. (GRFT) (Mori *et al*., 2005) and from the green algae *Boodlea coacta* (BCA) (Sato *et al*., 2011b) and *Halimeda renschii* (HRL) (Mu *et al*., 2017), all of them showed strong anti-HIV and anti-influenza activities. Thus, marine algae may be dominant sources of useful lectins for basic research and applications.

Gracilaria eucheumatoides Harvey, 1860 was

changed name to be *Hydropuntia eucheumatoides* (Harvey) Gurgel & Fredericq, 2004 and is one of the algal species from which agar is commercially extracted and the main source of agar in the world (Oliveira *et al*., 2000). Lectins from several species of the genus *Gracilaria* including *G. bursa-pastoris*, *G. tikvahiae*, *G. verrucosa*, *G. cornea, G. ornata* and *G. salicornia* (Okamono *et al*., 1990; Chiles, Bird, 1990; Kakita *et al*., 1999; Lima *et al*., 2005; Leite *et al*., 2005; Le Dinh Hung *et al.,* 2013) have been isolated and characterized. The studies on biochemical properties of these lectins indicated that their hemagglutination activity was strongly inhibited by glycoproteins bearing O-glycans, and do not require divalent cations for their biological activity and among them showed biological effects on the Cattle tick *Boophilus microplus* and the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae) (Lima *et al*., 2005; Leite *et al*., 2005).

Vietnam is located in the tropical and subtropical zone with a long coast line of about 3,260 km, where there is a diversity of marine organisms (Huynh Quang Nang, Nguyen Huu Dinh, 1998). These species may be potential sources of biologically active compounds including lectins. However, very little information is known concerning lectins from Vietnamese marine organisms, except several reports on the screening results of hemagglutinins from Vietnamese marine algae and invertebrates (Le Dinh Hung *et al*., 2009a, 2012; Dinh Thanh Trung *et al*., 2017), purification and characterization of lectins from the red algae *Kappaphycus alvarezii*, *Kappaphycus striatum*, *Eucheuma denticulatum*, *Gracilaria salicornia* and from sponge *Stylissa flexibilis* (Le Dinh Hung *et al*., 2009b, 2011, 2013, 2015a, 2018a), the cDNA clones encoding lectins from *K. striatum* and *E. denticulatum* (Le Dinh Hung *et al*., 2015a, 2015b, 2016) and seasonal variations in lectin contents from the cultivated red algae *K. alvarezii* and *K. striatus* (Le Dinh Hung *et al*., 2009c, 2018b). Thus, the objective of present research was to report on the isolation, biochemical properties and carbohydrate-binding specificity of a new lectin from the red alga *Hydropuntia eucheumatoides*.

MATERIALS AND METHODS

Materials

The red alga, *Hydropuntia eucheumatoides* was collected at the coast of Ninh Hai district (109° 02′ 01″ E, 11° 35′ 23″ N), Ninh thuan province, Vietnam in April, 2018, and kept at - 20 °C until used. The species was identified by Dr. Le Nhu Hau (Nhatrang Institute of Technology Research and Application). Prepacked columns used were Sephacryl S-200 (1.6× 60 cm) and DEAE Sepharose fast flow ion exchange chromatographic column (1.6×20 cm) from GE Healthcare (Sweden). Animal blood was obtained from the Institute of Vaccine - Nhatrang, Vietnam and human A, B, and O bloods from Khanhhoa General Hospital, VietNam. The monosaccharides, D-glucose, D-mannose, D-galactose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, N-acetyl-D-galactosamine and glycoproteins, transferrin, fetuin, porcine thyroglobulin, and porcine stomach mucin (type III) were purchased from Sigma Chemical Co. Yeast mannan and N-acetyl-neuraminic acid was from Nacalai Tesque Chemical Co. Asialo-derivatives of transferrin, fetuin and porcine stomach mucin were prepared by hydrolysis of the parent sialoglycoprotein with 0.05 M HCl for 1 h at 80 °C, followed by dialysis against saline overnight.

Extract and purification of lectin

Algal material was ground to powder and extracted with 4 volume of 20 % cold ethanol and kept at 4 °C for 18 h with occasionally stirring. After filtration through a cheese cloth, the filtrate was centrifuged at 6,000 rpm for 20 min at 4 °C. The supernatant was collected and examined for hemagglutination activity. To supernatant, cold absolute ethanol (- 20 °C) was added to a final concentration of 83 % and the mixture was kept at 4 °C overnight. The precipitate was collected by centrifugation at 6,000 rpm for 20 min at 4 °C. The precipitate was washed three times by cold absolute ethanol (- 20 °C) and centrifuged at 6,000 rpm for 20 min at 4 °C and thoroughly dialyzed against 20 mM carbonate buffer (pH 9.0). The fraction in the dialyzed bag was applied to a DEAE Sepharose fast flow ion exchange chromatographic column (1.6×20 cm), equilibrated with the above buffer. Unbound proteins and pigments were eluted with above buffer at a flow rate of 10.0 mL min^{-1} until the column effluents showed absorbance of less than 0.002 at 280 nm, lectin was eluted with 0.5 M NaCl in 20mM carbonate buffer, pH 9.0; the active fractions were pooled, concentrated by ultrafiltration and dialyzed against 50 mM phosphate buffer containing 150 mM NaCl (pH 7.0). The concentrate was subjected to gel

filtration on a Sephacryl S-200 column (1.6×60 cm) equilibrated with 50 mM phosphate buffer containing 150 mM NaCl (pH 7.0). The column was eluted with the same buffer at a flow rate of 0.8 mL min^{-1} and the active fractions were collected. The eluate was monitored for absorbance at 280 nm for protein and for hemagglutination activity with trypsin-treated rabbit erythrocytes. Active fractions were pooled and subjected to further analysis.

Preparation of a 2 % suspension of native or enzyme-treated erythrocytes

Each blood sample was washed three to five times with 50 volumes of 150 mM NaCl. After washing, a 2 % erythrocyte suspension (v/v) was prepared in 20 mM phosphate buffer containing 150 mM NaCl (pH 7.2) and used as native erythrocytes. Trypsin- or papain-treated erythrocytes were prepared as follows: One-tenth volume of 0.5 % (w/v) trypsin or papain solution was added to a 2 % native erythrocyte suspension, and the mixture was incubated at 37 °C for 60 min. After incubation, the erythrocytes were washed three to five times with saline and a 2 % suspension (v/v) of trypsin- or papain-treated erythrocytes was prepared as above (Le Dinh Hung *et al*., 2009a).

Hemagglutination assay

Hemagglutination assays were carried out using a microtiter method in a 96-well microtiter V-plate (Le Dinh Hung *et al*., 2009a). First, 25 µL amounts of serially two-fold dilutions of a test solution were prepared in 20 mM phosphate buffer containing 150 mM NaCl (pH 7.2) on a microtiter V-plate and incubated at room temperature for 1 h. To each well, 25 µL of a 2 % erythrocyte suspension was added and the mixtures gently shaken and incubated at room temperature for 2 h. A positive result was indicated by formation of a uniform layer of coagulant over the surface of the well. On the other hand, a negative test result was indicated by the formation of a discrete "button" at the bottom of the well. Hemagglutination activity was expressed as a titer, the reciprocal of the highest two-fold dilution exhibiting positive hemagglutination. The assay was carried out in triplicate for each test solution.

Hemagglutination-inhibition test

Hemagglutination-inhibition tests were carried according to the method previously described (Le Dinh Hung *et al*., 2009a). All tested inhibitors were dissolved in 150 mM NaCl at an initial concentration of 100 mM for monosaccharides and 2000 µg/mL for glycoproteins. First, 25 µL of serial two-fold dilutions of sugar or glycoprotein were prepared in 20 mM phosphate buffer containing 150 mM NaCl (pH 7.2). To each well, an equal volume of a lectin solution (4 doses of agglutination) prepared in 20 mM phosphate buffer containing 150 mM NaCl (pH 7.2) was added, and the plate was mixed gently and allowed to stand at room temperature for 1 h. Finally, 25 µL of a 2 % suspension of trypsin-treated rabbit erythrocytes was added to each well, and the plate was gently shaken and incubated for a further 1 h. Inhibition was observed macroscopically and inhibition activity was expressed as the lowest concentration of sugar or glycoprotein at which complete inhibition of hemagglutination was achieved. The assay was performed in triplicate per sugar and glycoprotein.

Preparation of trypsin-treated porcine stomach mucin

Porcine stomach mucin (10 mg) was dissolved in 5 mL of 20 mM phosphate buffer containing 150 mM NaCl (pH 7.2). Trypsin (5 mg) was added to the sample and the solution obtained was incubated at 37 °C for 24 h. Treated porcine stomach mucin was heated to 100 °C for 30 min then cooled (final reaction volumes were 10 mL) and further used as inhibitor (Xiong *et al*., 2006).

Effects on hemagglutination activity of divalent cations, pH, and temperature

To examine the effects of divalent cations on hemagglutination activity, the lectin solution was dialyzed at 4 °C overnight against 100 mL of 50 mM EDTA followed by dialysis against 20 mM phosphate buffer containing 150 mM NaCl (pH 7.5). The hemagglutination activity was determined in the absence or presence of $CaCl_2$. To examine the effect of temperature, each the lectin solution was treated at various temperatures (30 – 100 °C) for 30 min, then immediately cooled on ice, and hemagglutination activity was determined as above. To examine the effect of pH, each the lectin solution was dialyzed at 4 °C overnight against 100 mL of 50 mM buffers of various pH from 3 to 10 and then dialyzed against 150 mM NaCl solution. The following buffers were used; acetate buffer for pH from 3 to 5, phosphate buffer for pH from 6 to 7, and Tris-HCl buffer for pH from 8 to 10. Hemagglutination activity was determined with trypsin-treated rabbit erythrocytes. The assay was

carried out in triplicate for each test solution (Le Dinh Hung *et al*., 2009a).

Determination of protein content

Protein contents were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Absorbance at 280 nm was also used to estimate protein contents in chromatography.

Determination of molecular mass

The molecular mass of purified lectin was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Briefly, the samples for SDS-PAGE were denatured at 100 °C for 5 min with or without 2 % 2-mercaptoethanol and then electrophored using a 10 % gel (Laemmli, 1970). After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 to detect proteins.

RESULTS AND DISCUSSION

Extract and purification of lectin

The ethanol extract of *H. eucheumatoides* strongly agglutinated trypsin- and papain-treated erythrocytes of rabbit, sheep and chicken, but showed no agglutination with erythrocytes of human A, B and O blood groups, even when erythrocytes were treated by enzymes (Table 1). This study is consistent with other reports on agglutinations preferentially towards the animal erythrocytes more than human ones for marine algal lectin extracts (Chiles, Bird, 1989; Hori *et al*., 1988, 1990; Ainouz *et al*., 1992; Freitas *et al*., 1997; Le Dinh Hung *et al*., 2009a, 2012). From the ethanol extract, after 83 % cold ethanol precipitation and dialysis, the precipitate gave a single active peak on DEAE Sepharose fast flow ion exchange chromatographic column when eluted with 0.5 M NaCl in 20mM carbonate buffer, pH 9.0 (Figure 1a). The active peak was further separated into two peaks in gel filtration chromatography on a Sephacryl S-200 column. The first peak (I) showed no hemagglutination activity, whereas the second peak (II) exhibited strong hemagglutination activity (Figure 1b). Thus, the purified lectin was designated as HEL. The results of purification are summarized in table 2

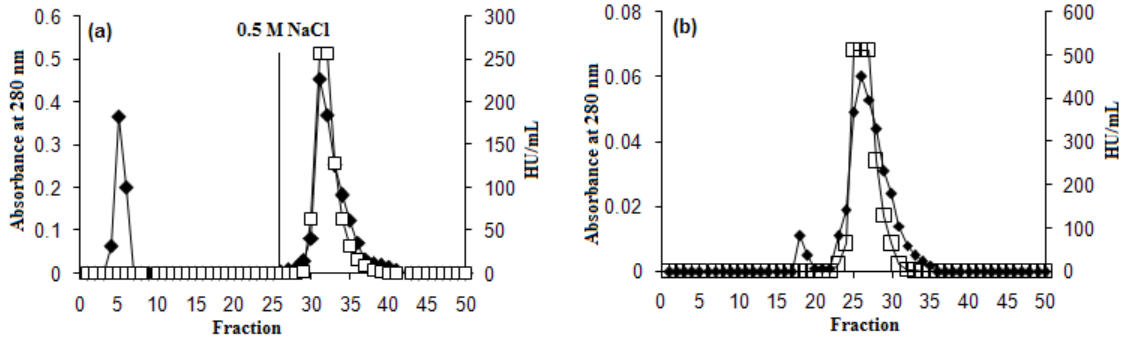

Figure 1. (a) Ion-exchange chromatography of the precipitated fraction obtained from crude extract of red alga, *H. eucheumatoides* on a DEAE Sepharose fast flow column. Lectin was eluted with 20 mM phosphate buffer containing 0.5 M NaCl, pH 9.0. **(b)** Gel chromatography on a Sephacryl S-200 column of the active peak obtained by ion-exchange chromatography. The column was eluted with 50 mM phosphate buffer containing 150 mM NaCl, pH 7.0. Fractions were collected and measured at absorbance of 280 nm (—◆—) for protein and for hemagglutination activity (—□—) with trypsin-treated rabbit erythrocytes. HU hemagglutination unit.

Table 1. Hemagglutination activity of the crude extract from red alga *H. eucheumatoides*. The hemagglutination activity is expressed as a titer that is the reciprocal of the highest two-fold dilution exhibiting positive agglutination.

Hemagglutination titer with erythrocytes																	
Rabbit			Sheep			Chicken			Human A			Human B			Human O		
N[a]	T[b]	P[c]	N	T	P	N	T	P	N	T	P	N	T	P	N	T	P
-	128	1024	-	512	512	-	128	128	-[d]	-	-	-	-	-	-	-	-

[a] Native erythrocytes; [b] Trypsin-treated erythrocytes; [c] Papain-treated erythrocytes; [d] No hemagglutination.

Table 2. The summary of purification of the lectin from *H. eucheumatoides*. The algal powder was used as a starting material.

Extraction and purification step	Total Protein (mg)	Total HA (HU[a])	Specific activity (HU mg^{-1})	Yield (%)
Extraction	156.9	58880	375.3	100
Ethanol precipitation	23.0	46080	2003.4	78.3
Ion exchange	8.6	17536	2039.0	29.8
Gel filtration	7.0	14336	2048.0	24.3

Note: [a] Total activity is shown by titer x volume.

Determination of molecular mass

The native molecular masses of HEL were estimated to be 17,000 Da in both reducing and non-reducing SDS-PAGE conditions (Figure 2).

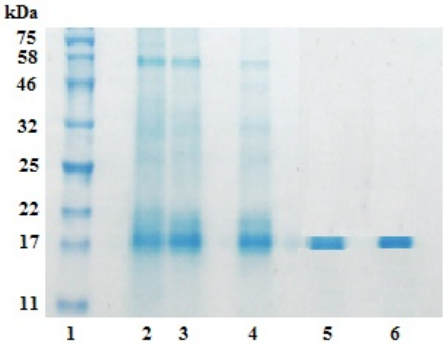

Figure 2. SDS-PAGE of the lectin isolated from red alga *H. eucheumatoides*. SDS –PAGE was carried using a 10% polyacrylamide gel. Protein bands were stained with Coomassie Brilliant blue R-250 reagent. Lane 1, mixture of reference proteins (New England BioLabs Inc); lane 2, crude extract; lane 3, 83 % ethanol precipitate; lane 4, active fractions obtained from ion-exchange chromatography; lane 5, active fractions obtained from gel filtration in non-reducing condition; lane 6, active fractions obtained from gel filtration in reducing condition by β-mercaptoethanol.

The similar results have been reported for lectins belonging to genus *Gracilaria*, such as Granin-BP from *G. bursa pastoris* (Okamono *et al*., 1990), GCL from *G. cornea* (Lima *et al*., 2005) and GOL from *G. ornata* (Leite *et al*., 2005), all of them were monomeric proteins and showed the resemblance highly among the lectins of the genus *Gracilaria*. However, the isolated lectin from *G. salicornia* exhibited difference in its molecular structure compared to those of lectins belonging to genus *Gracilaria*, it gave a single band with molecular mass of 45,000 Da in non-reducing SDS-PAGE condition, whereas it made a band of 22,500 Da in reducing one, indicating that lectin was a dimeric protein composed of two identical subunits of about 22,500 Da, which is linked by a disulfide bond (Le Dinh Hung *et al*., 2013).

Effects of temperature, pH, and metal ions on hemagglutination activity

The purified lectin is thermostable at 60 ^{o}C for 30 min, where they maintained 100% of its hemagglutination activity, whereas they gradually decreased as incubation temperature exceeded 60 ^{o}C (Figure 3a). Hemagglutination activity of HEL was stable in a wide range of pH from 3 to 10 (Figure 3b) and not affected by either the presence of EDTA or addition of divalent cations, indicating that lectin required no metal for biological activity. Thermostable and metal-independent hemagglutination activities have been reported for many lectins from marine algae (Hori *el al*., 1990; Kawakubo *et al*., 1997, 1999; Le Dinh Hung *et al*., 2009a, 2009b, 2011, 2012, 2015a).

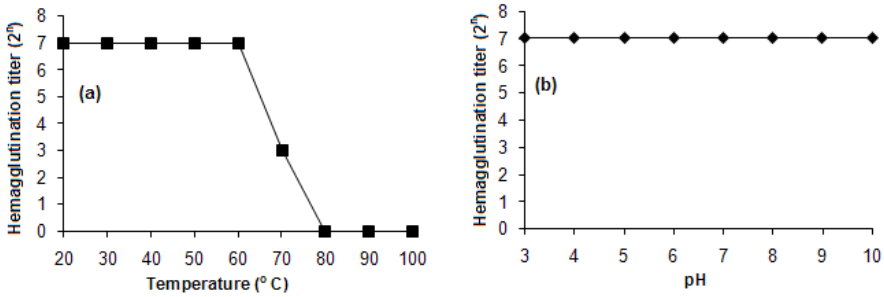

Figure 3. Effects of temperature **(a)** and pH **(b)** on haemagglutination activities of lectin HEL from red alga *H. eucheumatoides.*

Hemagglutination-inhibition test

The lectin, HEL, showed the hemagglutination-inhibition profiles with a series of sugars and glycoproteins (Table 3). Hemagglutination activities of HEL were not inhibited by monosaccharides, D-glucose, D-mannose, D-galactose, D-xylose, N-acety-D-mannosamine and glycoproteins, such as transferin, fetuin and yeast mannan, but strongly inhibited by monosaccharides containing acetamido groups at equatorial C2 position of sugars, such as N-acetyl-galactosamine, N-acetyl-glucosamine and N-acetyl-neraminic acid and glycoprotein porcine stomach mucin and its asialo derivative bearing O-glycans, indicating that the lectin is specific for O-glycans.

Transferrin bearing only complex type N-glycans and fetuin bearing both complex type N-glycans and O-glycans were not inhibitory. However, elimination of sialic acid residues of these glycoproteins increased inhibitory potential of parental glycoproteins.

The yeast mannan, which bearing high mannose N-glycans with the (α1-6) linkage in its backbone and (α1-3) linkage in the side chains did not show any inhibitory activity even at concentration of 2 mg/mL, indicating that HEL could not recognize the (α1-6) and (α1-3)-linked Man residues in structures of yeast mannan. Porcine thyroglobulin exhibited strongly inhibitory activity. This glycoprotein bears both high mannose type (unit A-type) and complex type (unit B-type) oligosaccharides. Among the unit A-type of porcine thyroglobulin, the common structure of high mannose type N-glycans is Man5GlcNAc2Asn with (α1-6)Man and (α1-3)Man residues branched from (α1-6)Man arm of the core pentasaccharide (Tsuji *et al*., 1981). Among the unit B-type, the major N-glycans contain at least 9 different structures consisting of mono- and disialylated (α1–6) fucosylated bi-, triantennary structures terminated either with (α2–3) or (α2–6)-linked sialic acid residues (Yamamoto *et al*., 1981), therefore HEL could recognize terminated either with (α2–3) or (α2–6)-linked sialic acid residues in structures of porcine thyroglobulin.

Table 3. Hemagglutination-inhibition test of *H. eucheumatoides* lectin (HEL). The value indicates the lowest concentration of sugar (mM) and glycoprotein (μg/mL) at which complete inhibition of hemagglutination (titer 4) was achieved.

Sugars	Minimum inhibitory concentration (mM or μg/mL)	Sugars and glycoproteins	Minimum inhibitory concentration (mM or μg/mL)
Sugar (mM)		D-xylose	-
D-galactose	-[a]	***Glycoproteins (μg/mL)***	
N-acetyl-D-galactosamine	6.25	Transferrin	-
P-Nitrophenyl-galactopyranoside	-	Asialo-transferrin	250.0
D-glucose	-	Fetuin	-
N-acetyl-D-glucosamine	12.5	Asialo-fetuin	1,000.0
P-Nitrophenyl-glucopyranoside	-	Yeast mannan	-
D-mannose	-	Porcine thyroglobulin	15.6
N-acetyl-D-mannosamine	-	Porcine stomach mucin	0.49
P-Nitrophenyl-mannopyranoside	-	Asialo-porcine stomach mucin	0.49
N-acetyl neuraminic acid	12.5	Trypsin treated porcine stomach mucin	7.8

Note: [a] Indicates no inhibition at 100 mM for monosaccharides and 2,000 μg/mL for glycoproteins.

The strongest inhibitor for hemaggulination activity of HEL was porcine stomach mucin and its asialo derivatives. The mucin type O-glycans has 8 core structures. The structural variability of O-linked glycans of the mucin already starts at the level of their core. All are based on the core residue GalNAcα1-, which can be further substituted at C3, C6, or at both positions with the monosaccharides β-Gal at C3, β-GlcNAc at C3 and/or C6, and α-GalNAc at C3 or C6 (Wopereis, 2006). The neutral

O-linked glycans of mucin include the human blood groups A and H, Tn antigen GalNAcα1-, Sialyl-Tn antigen Siaα2-6GalNAcα-1, the core1 Galβ1-3GalNACα1-, core2 GlcNAcβ1–6(Galβ1-3)GalNAcα1-, core3 GlcNAcβ1–3GalNAcα1-, core4 GlcNAcβ1-6(GlcNAcβ1-3)GalNAcα1-, core5 GalNAcα1-3GalNAcα-1, core6 GlcNAcβ1-6GalNAcα1-, core7 GalNAcα1-6GalNAcα1- and core8 Galα1-3GalNAcα1- (Van Halbeek *et al*., 1982; Karlsson *et al*., 1997). The results indicated that lectin HEL recognized preferentially the terminal GalNAcα1 and GlcNAcβ1 residues in porcine stomach mucin (Figure 4). Inhibition by the porcine stomach mucin that is related to GalNAc/GluNAc binding specificity reported for many lectins from red algae, such as GTA from *G. tikvahiae* (Chiles, Bird, 1990), GCL from *G. cornea* (Lima *et al*., 2005), GOL from *G. ornata* (Leite *et al*., 2005), HCA from *Hypnea cervicornis* and HML from *H. musciformis* (Nagano *et al*., 2005). HCA and HML binds GalNAc/Gal substituted through 1-3, 1-4, or 1-2 linkages in O-linked mucin-type glycans, but showed no fine discrimination for α and β linkage. Lectin HEL may preferably bind to nonsialylated terminal GalNAc/GluNAc residues in the structures of O-glycans and the acetamido group at equatorial C2 position of the terminal sugar residues in O-glycans was critical for lectin binding.

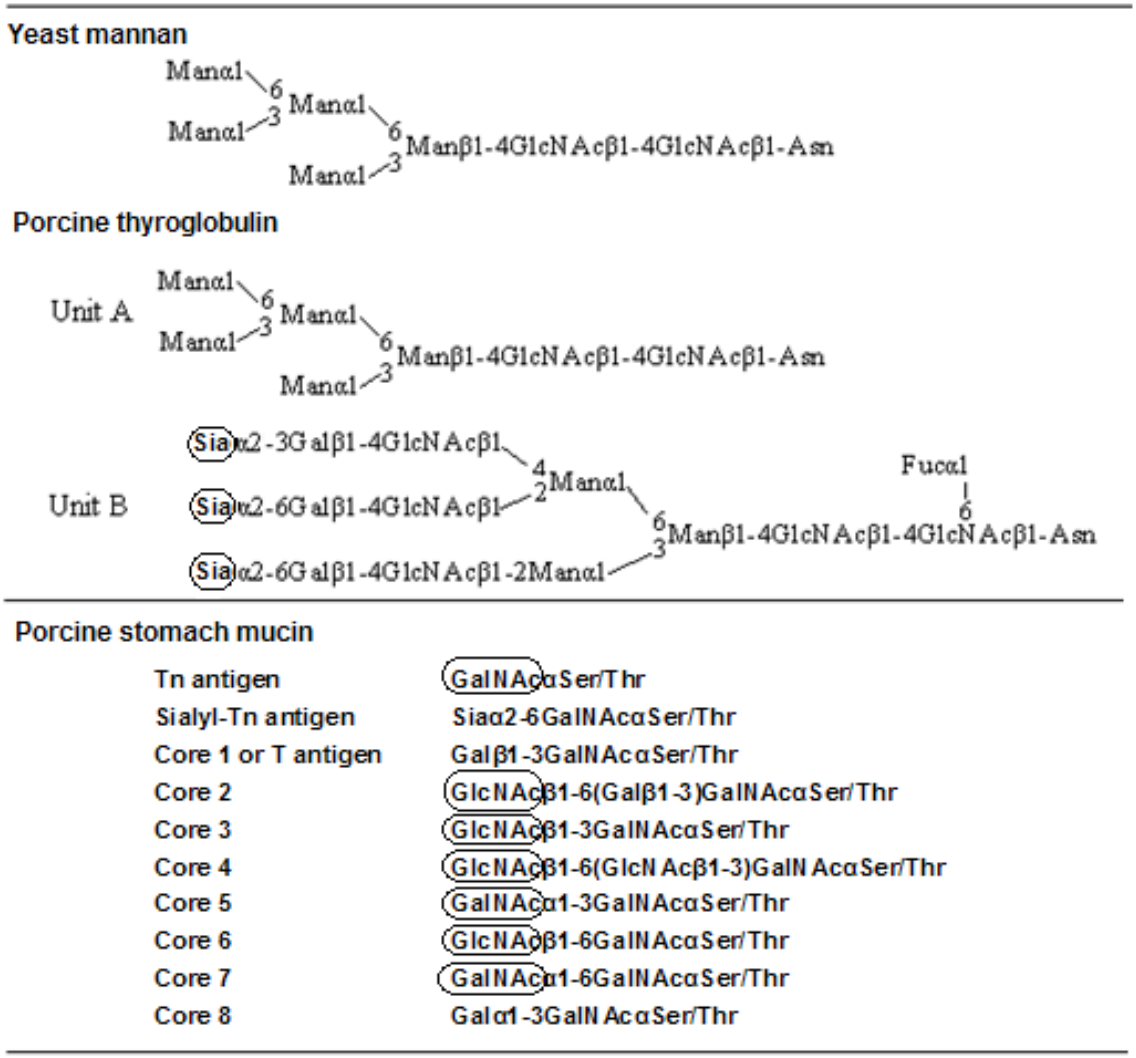

Figure 4. The putative epitopes recognized by lectin from red alga *H. eucheumatoides* are highlighted in oval. GlcNAc: N-acetyl-D-glucosamine; GalNAc: N-acetyl-D-galactosamine, Sia: N-acetylneuraminic acid; Ser/Thr: Serine/Threonie.

CONCLUSION

The lectin HEL from red alga *H. eucheumatoides* showed novel properties, including molecular mass, monomeric form, hemagglutination-inhibition profile, and stable over a wide range of pH and temperature. HEL had affinity for monosaccharides containing acetamido groups at equatorial C2 position of pyranose ring and preferably bond to terminal GalNAc/GluNAc/NeuNAc residues in the structures of O-glycans, indicating that the acetamido groups at equatorial C2 position of the terminal sugar residues in O-glycans were critical for lectin binding. The red alga *H. eucheumatoides* could promise to be a new natural lectin source for the elucidation of O-glycan structures.

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MÔ TẢ ĐẶC TÍNH CỦA LECTIN LIÊN KẾT O-GLYCAN TỪ RONG ĐỎ (*HYDROPUNTIA EUCHEUMATOIDES*)

Lê Đình Hùng[1], Trần Thị Hải Yến[2], Đinh Thành Trung[1]

[1]Viện Nghiên cứu và Ứng dụng công nghệ Nha Trang, Viện Hàn lâm Khoa học và Công nghệ Việt Nam
[2]Trường Trung học phổ thông chuyên Lê Quý Đôn, Khánh Hòa

TÓM TẮT

Rong đỏ, *Hydropuntia eucheumatoides* là một trong số chi rong được dùng để sản xuất agar trên thế giới. Lectin, HEL, từ mẫu rong này đã được tách chiết bằng sự kết hợp dịch chiết ethanol, kết tủa ethanol, sắc ký trao đổi ion và sắc ký lọc gel. Lectin có khối lượng phân tử khoảng 17.000 Da trong cả hai điều kiện điện di gel polyacrylamide không biến tính (SDS-PAGE) và biến tính, chỉ ra rằng lectin tồn tại ở dạng monome. Hoạt tính ngưng kết hồng cầu của HEL bền trong một phạm vi rộng của nhiệt độ, pH và không bị ảnh hưởng bởi sự có mặt của EDTA hoặc thêm cation hóa tri hai như Ca^{2+} và Mg^{2+}, vì vậy hoạt tính sinh học của lectin không phụ thuộc vào kim loại. Hoạt tính ngưng kết hồng cầu của lectin HEL không bị ức chế bởi các đường monosaccharide và glycoprotein, D-glucose, D-mannose, D-galactose, D-xylose, N-acety-D-mannosamine, transferin, fetuin and yeast mannan, nhưng bị ức chế mạnh bởi các đường đơn chứa nhóm acetamido ở vị trí C2 equatorial của vòng pyranose như N-acetyl-galactosamine, N-acetyl-glucosamine, N-acetyl-neraminic acid, và porcine stomach mucin và dẫn xuất asialo của nó mang dạng O-glycan. Kết quả cho thấy lectin HEL đặc hiệu cho O-glycan và có thể nhận biết các trình tự GalNAcαSer/Thr, GalNAc(α1-3)[Fuc(α1-2)]Gal(β1-4)GlcNAc(β1-3)GalNAc- và GluNAc(α1-4)Gal- qua tương tác với các nhóm acetamido ở vị trí C2 equatorial của các gốc đường cuối cùng trong cấu trúc oligosaccharide của O-glycan. Rong đỏ *H. eucheumatoides* hứa hẹn trở thành nguồn lectin gía trị cho sử dụng trong hóa sinh và y sinh.

Từ khóa: *Đặc tính liên kết carbohydrate; Hydropuntia eucheumatoides; O-Glycan; Lectin; Rong đỏ*