

## HIGH-MANNOSE TYPE N-GLYCAN BINDING SPECIFICITY OF A NOVEL LECTIN FROM THE RED ALGA (*BETAPHYCUS GELATINUS*)

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### SUMMARY

The red alga, *Betaphycus gelatinus* is one of carrageenan sources in the world. The lectin from the red alga *B. gelatinus* was isolated by a combination of aqueous ethanol extraction, ethanol precipitation, ion-exchange chromatography and gel filtration chromatography and was designated as BGL after the specific name of alga. Lectin gave a single band with molecular mass of about 19,000 Da in both non-reducing and reducing SDS-PAGE conditions, therefore lectin exists in monomer form. The hemagglutination activities of BGL were stable over a wide range of pH from 3 to 10, temperature up 60 °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 BGL were not inhibited by monosaccharides and glycoproteins, D-glucose, D-mannose, D-galactose, D-xylose, N-acetyl-galactosamine, N-acetyl-glucosamine, N-acetyl-neuraminic acid, N-acetyl-D-mannosamine, transferrin and fetuin, but strongly inhibited by glycoproteins bearing high-mannose type N-glycan, such as yeast mannan and porcine thyroglobulin. Lectin BGL is specific for N-glycans and may recognize terminal ( $\alpha$ 1-3) or ( $\alpha$ 1-6)-linked mannose residues in structure Man( $\alpha$ 1-6)[Man( $\alpha$ 1-3)]Man( $\alpha$ 1-6)[Man( $\alpha$ 1-3)]Man( $\beta$ 1-4)GlcNAc( $\beta$ 1-4)GlcNAc of N-glycans. High-mannose type N-glycan binding specificities of this lectin highly resemble with those of the anti-cancer, anti-virus and anti-bacteria lectins from the red algae, carrageenophytes, including *Euचेuma serra* (ESA-2), *Euचेuma denticulatum* (EDA-2), *Kappaphycus striatum* (KSA-2), *Kappaphycus alvarezii* (KAA-1 and KAA-2) and *Solieria filiformis* (SfL1 and SfL2). The red alga *B. gelatinus* could promise to be a good source of valuable lectins for application in biochemistry and biomedicine.

**Keywords:** *Betaphycus gelatinus*; carbohydrate-binding specificity; high-mannose type N-glycan; lectin; red alga; stable

### INTRODUCTION

Lectins, or carbohydrate-binding proteins, are present in various organisms from virus to mammal, and serve as recognition molecules between cells, cell and matrix, and organisms. Owing to the capability of discriminating carbohydrate structures, not only are lectins used as valuable biochemical reagents in many research fields, including glycomics, but they are promising candidates for medicinal and clinical application (Sharon, Lis, 2003).

Indeed, anti-human immunodeficiency virus (HIV) and/or anti-influenza virus lectins from bacteria, algae, fungi, and land plant have the common property of binding to high-mannose N-

glycans, thereby blocking the entry of viruses into host cells through binding to the mannose structures in the viral envelope, which are critical for the primary infection of viruses (Ziolkowska, Wlodawer, 2006; Balzarini, 2007). Recently, the high-mannose N-glycan-specific lectins from the marine algae, such as *Euचेuma serra* (ESA-2) (Sugahara *et al.*, 2001; Fukuda *et al.*, 2006; Hori *et al.*, 2007; Omokawa *et al.*, 2010; Hayashi *et al.*, 2012; Sato *et al.*, 2015), *Kappaphycus alvarezii* (KAA-2) (Sato *et al.*, 2011a; Hirayama *et al.*, 2016), *Boodlea coacta* (BCA) (Sato *et al.*, 2011b), *Kappaphycus striatum* and *Euचेuma denticulatum* (Le Dinh Hung *et al.*, 2011, 2015a, 2015b), *Halimeda renschii* (HRL) (Mu *et al.*, 2017) and *Solieria filiformis* (SFL) (Chaves *et al.*, 2018) showed strong anti-HIV, anti-influenza virus, anti-

cancer and anti-bacteria activities through binding to high-mannose chains on viral envelopes or on the surface of the carcinoma cells. Thus, high-mannose binding lectins from marine algae may become a useful interesting target for application in medicine.

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 algae (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), the purification and characterization of lectins from the red algae *K. alvarezii*, *K. striatum*, *E. denticulatum*, *Gracilaria salicornia*, *Hydropuntia eucheumatoides* and from marine sponge *Stylissa flexibilis* (Le Dinh Hung *et al.*, 2009b, 2011, 2013, 2015a, 2018a, 2018b), 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, 2019). Thus, the objective of present research was to report on the isolation, biochemical properties and high mannose N-glycan binding specificity of a new lectin from the red alga *Betaphycus gelatinus*, which can provide valuable information regarding lectins from the carrageenophyte algal family.

## MATERIALS AND METHODS

### Materials

The red alga, *Betaphycus gelatinus* (Esper) Doty ex P.C.Silva was collected at the coast of Ninh Hai district (109°02'01"E, 11°35'23"N), Ninh Thuan province, Viet Nam in April, 2018, and kept at - 20 °C until used. The species were identified by Le Nhu Hau (Nha Trang Institute of Technology Research and Application). Prepacked columns used were Sephacryl S-200 (1.6x60 cm) and DEAE Sepharose fast flow ion exchange chromatographic column (1.6x20 cm) from GE Healthcare (Sweden). Animal blood was obtained from the Institute of Vaccine-Nha Trang, Viet Nam and human A, B and O blood from Khanh Hoa General Hospital, Viet Nam. 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 were 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

Lectin was purified as method described previously (Le Dinh Hung *et al.*, 2018a). Algal material was extracted with 20% cold ethanol and kept at 4°C for 18 h with occasionally stirring. The filtrate was centrifuged at 6,000 rpm for 20 min at 4°C and was precipitated by cold absolute ethanol (-20°C). The precipitate was collected by centrifugation 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 (1.6x20 cm), equilibrated with the above buffer. Lectin was eluted with a linear gradient between 0 to 0.5 M NaCl in 20 mM carbonate buffer, pH 9.0 for 20 min; 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 applied to gel filtration chromatography on a Sephacryl S-200 column (1.6x60 cm). The column was eluted with 50 mM phosphate buffer containing 150 mM NaCl (pH 7.0) 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 solution 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). Briefly, First, 25  $\mu$ L 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. To each well, 25  $\mu$ L 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.

#### **Carbohydrate binding specificity**

Carbohydrate binding specificity were carried according to the method previously described (Le Dinh Hung *et al.*, 2009a). Briefly, first, 25  $\mu$ L of serial two-fold dilutions of sugar or glycoprotein were prepared in 20 mM phosphate buffer containing 150 mM NaCl (pH 7.2). An equal volume of a lectin solution (4 doses of agglutination) was added to each well of plate and plate was mixed gently and allowed to stand at room temperature for 1 h. Finally, 25  $\mu$ L 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. Carbohydrate binding specificity was expressed as the lowest concentration of sugar (mM) or glycoprotein ( $\mu$ g/mL) at which complete inhibition of hemagglutination (titer 4) was achieved. The test 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 an inhibitor (Xiong *et al.*, 2006).

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

Effects on hemagglutination activity of divalent cations, pH, and temperature were carried according to the method previously described (Le Dinh Hung *et al.*, 2009a). 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<sub>2</sub>. 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 including 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.

#### **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 *B. gelatinus* 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 cold ethanol precipitation and dialysis,

the precipitate gave a single active peak in ion-exchange chromatography on DEAE Sepharose fast flow column (Figure 1a). The active peak was further separated into two peaks in gel 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 BGL after the specific name of alga. The results of purification are summarized in Table 2.

**Table 1.** Hemagglutination activity of the crude extract from red alga *Betaphycus gelatinus*.

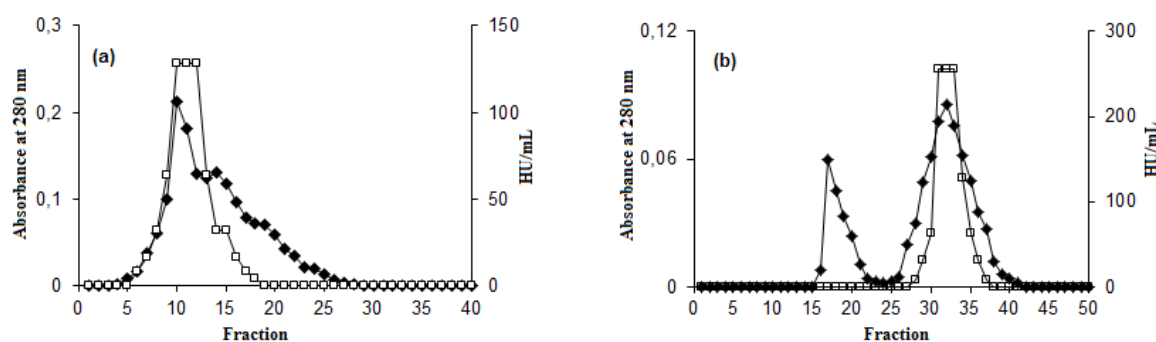
Hemagglutination titer with erythrocytes																	
Rabbit			Sheep			Chicken			Human A			Human B			Human O		
N <sup>a</sup>	T <sup>b</sup>	P <sup>c</sup>	N	T	P	N	T	P	N	T	P	N	T	P	N	T	P
-	128	128	-	128	128	-	32	32	- <sup>d</sup>	-	-	-	-	-	-	-	-

<sup>a</sup> Native erythrocytes; <sup>b</sup> Trypsin-treated erythrocytes; <sup>c</sup> Papain-treated erythrocytes; <sup>d</sup> No hemagglutination.

**Table 2.** The summary of purification of the lectin from *B. gelatinus*.

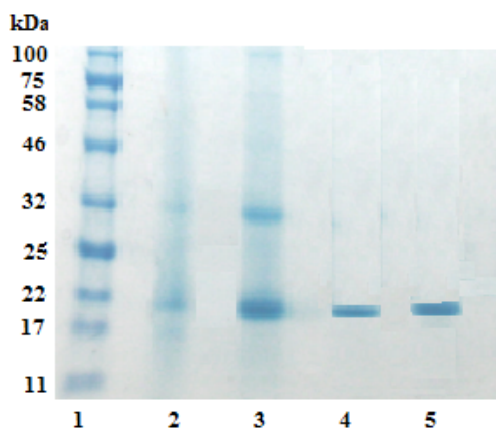
Extraction and purification step	Total Protein (mg)	Total (HU <sup>a</sup> )	activity	Specific activity (HU mg <sup>-1</sup> )	Yield (%)
Extraction	1141	89600		78.5	100
Ethanol precipitation	154.7	31488		203.6	35.1
Ion exchange	59.6	13920		233.6	15.5
Gel filtration	17.7	7040		396.8	7.8

<sup>a</sup>Total activity is shown by titer x volume.



**Figure 1.** (a) Ion-exchange chromatography of the precipitated fraction obtained from crude extract of red alga, *B. gelatinus* on a DEAE Sepharose fast flow column. Lectin was eluted with a linear gradient between 0 to 0.5 M NaCl in 20mM carbonate buffer, pH 9.0. (b) Gel filtration 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.

### Determination of molecular mass



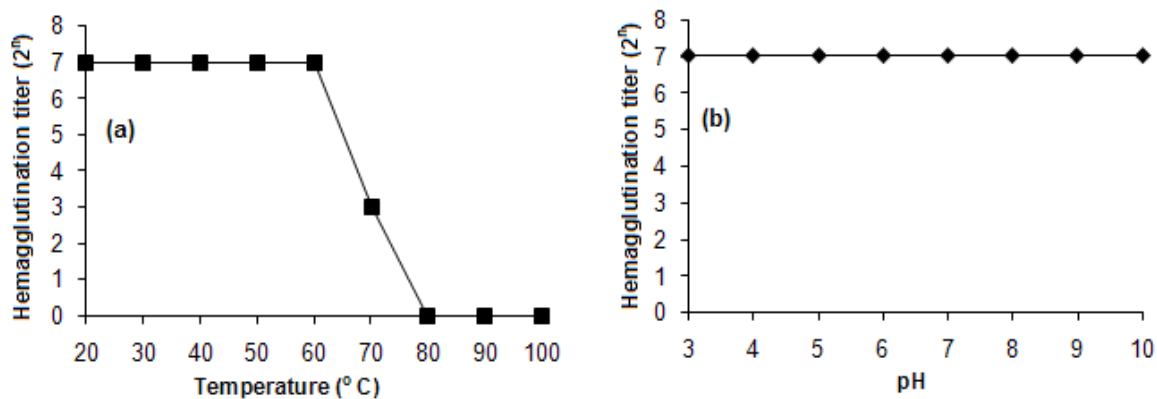
**Figure 2.** SDS-PAGE of the lectin isolated from red alga *B.gelatinus*. SDS –PAGE was carried using a 10% polyacrylamide gel. Protein bands were stained with Coomassie Brilliant blue R-250 reagent. Lane 1, mixture of reference proteins (New England BioLabs Inc); lane 2, 83 % ethanol precipitate; lane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

The molecular masses of lectin BGL were estimated to be 19,000 Da in both reducing and non-reducing SDS-PAGE conditions (Figure 2). The

similar results have been reported for lectins from red algae, carrageenophytes, such as *E. serra* (ESA-2) (Hori *et al.*, 2007; Sato *et al.*, 2015), *K. alvarezii* (KAA-2) (Le Dinh Hung *et al.*, 2009b; Sato *et al.* 2011a), *K. striatum* and *E. denticulatum* (Le Dinh Hung *et al.*, 2011, 2015a) and *S. filiformis* (SFL) (Chaves *et al.*, 2018), all of them were monomeric proteins in both reducing and non-reducing SDS-PAGE conditions, indicating that there was resemblance highly among the lectins of the genus *Eucheuma*.

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

The purified lectin is thermostable at 60 °C for 30 min, where they maintained 100% of its hemagglutination activity, whereas they gradually decreased as incubation temperature exceeded 60 °C (Figure 3a). Hemagglutination activity of BGL 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 *et al.*, 1990; Kawakubo *et al.*, 1997, 1999; Le Dinh Hung *et al.*, 2009a, 2011, 2012, 2015a).



**Figure 3.** Effects of temperature (a) and pH (b), respectively, on haemagglutination activities of lectin BGL from red alga *B. gelatinus*.

### Carbohydrate binding specificity

The lectin, BGL, showed the carbohydrate binding specificity profiles with a series of sugars and glycoproteins (Table 3). Hemagglutination activities of BGL were not inhibited by

monosaccharides, such as D-glucose, D-mannose, D-galactose, D-xylose, N-acetyl-glucosamine, N-acetyl-D-mannosamine, N-acetyl-galactosamine, N-acetyl-neraminic acid and glycoproteins, such as transferrin, asialo-transferrin and fetuin, but strongly inhibited by glycoproteins bearing high-mannose type N-

glycan, such as yeast mannan and porcine thyroglobulin, indicating that the lectin is specific for high-mannose type N-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 fetuin increased inhibitory potential compared with parental glycoprotein.

The porcine stomach mucin bearing O-glycans and its asialo derivatives showed inhibitory activity. However, elimination of sialic acid residues of porcine stomach mucin decreased inhibitory potential compared with parental glycoprotein. The O-glycan mucin type has 8 core structures. All are based on the core residue GalNAc $\alpha$ 1-, which can be further substituted at C3, C6, or at both positions with the monosaccharides  $\beta$ -Gal at C3,  $\beta$ -GlcNAc at C3 and/or C6, and  $\alpha$ -GalNAc at C3 or C6 (Wopereis, 2006).

**Table 3.** Carbohydrate binding specificity of *B. gelatinus* lectin (BGL).

Sugars and glycoproteins	Lectins					
	BGL	KAA-2 <sup>a</sup>	KSA-2 <sup>b</sup>	EDA-2 <sup>c</sup>	ESA-2 <sup>d</sup>	HRL <sup>e</sup>
<b>Sugar (mM)</b>						
D-galactose	- <sup>f</sup>	-	-	-	-	-
N-acetyl-D-galactosamine	-	-	-	-	-	-
D-glucose	-	-	-	-	-	-
N-acetyl-D-glucosamine	-	-	-	-	-	-
D-mannose	-	-	-	-	-	-
N-acetyl-D-mannosamine	-	-	-	-	-	-
N-acetyl neuraminic acid	-	-	-	-	-	-
D-xylose	-	-	-	-	-	-
<b>Glycoproteins (<math>\mu</math>g/mL)</b>						
Transferrin	-	-	-	-	-	-
Asialo-transferrin	-	-	-	-	-	>1000
Fetuin	-	31.2	31.2	62.5	62.5	>1000
Asialo-fetuin	125.0	31.2	31.2	125.0	31.2	>1000
Yeast mannan	7.8	3.9	1.9	3.9	3.9	15.6
Porcine thyroglobulin	7.8	1.9	0.9	1.9	3.9	62.5
Porcine stomach mucin	125.0	-	-	-	ND	ND
Asialo-porcine stomach mucin	250.0	-	-	-	ND	ND
Trypsin treated porcine stomach mucin	250.0	-	-	-	ND	ND

<sup>a</sup>Lectin KAA-2 from *Kappaphycus alvarezzi* alga (Le Dinh Hung *et al.*, 2009b); <sup>b</sup> Lectin KSA-2 from *Kappaphycus striatum* alga (Le Dinh Hung *et al.*, 2011); <sup>c</sup> Lectin EDA-2 from *Eucheuma denticulatum* alga (Le Dinh Hung *et al.*, 2015a); <sup>d</sup> Lectin ESA-2 from *Eucheuma serra* alga (Kawakubo *et al.*, 1997); <sup>e</sup> Lectin HRL from *Halimeda renschii* alga (Mu *et al.*, 2017). <sup>f</sup> Indicates no inhibition at 100 mM for monosaccharides and 2,000  $\mu$ g/mL for glycoproteins. ND not determined.

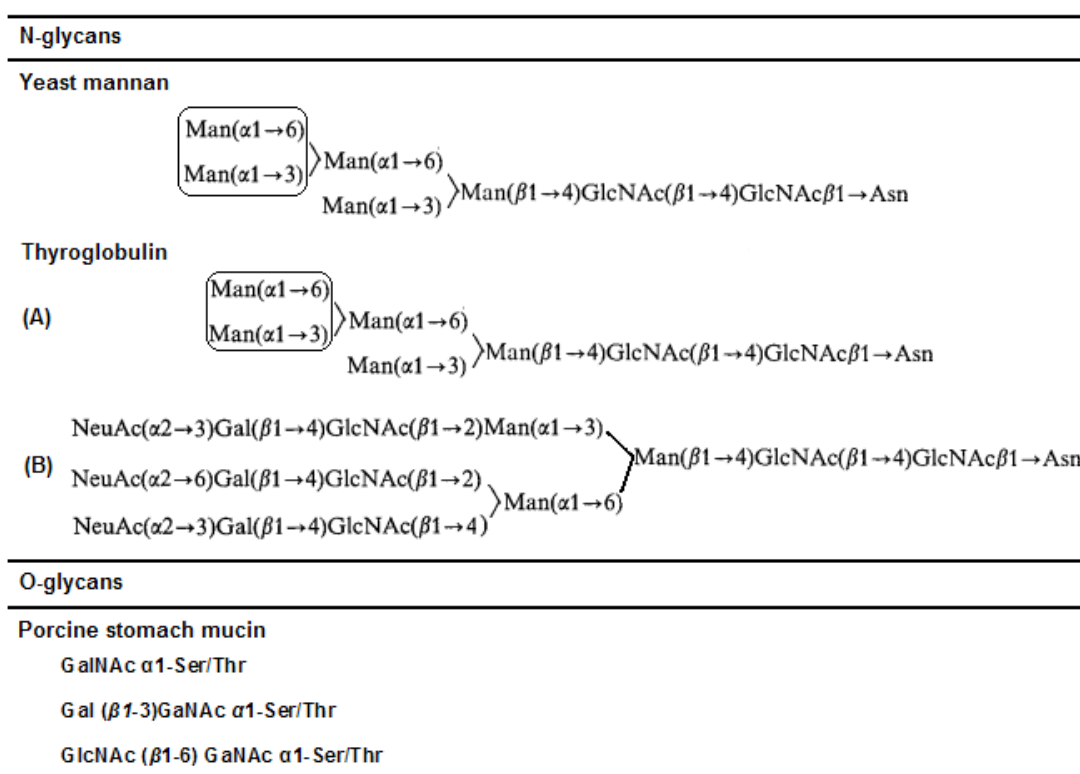
The yeast mannan, which bearing high mannose N-glycans with the ( $\alpha$ 1-6) linkage in its backbone and ( $\alpha$ 1-3) linkage in the side chains showed strongly inhibitory activity, indicating that lectin BGL could recognize the ( $\alpha$ 1-6) and ( $\alpha$ 1-3)-linked Man residues in structures of yeast mannan (Figure 4). 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 Man<sub>5</sub>GlcNAc<sub>2</sub>Asn with ( $\alpha$ 1-6)Man and ( $\alpha$ 1-3)Man residues branched from ( $\alpha$ 1-6)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 ( $\alpha$ 1-6) fucosylated bi-, triantennary structures terminated either with ( $\alpha$ 2-3) or ( $\alpha$ 2-6)-linked sialic acid residues (Yamamoto *et al.*, 1981). The results

indicated that BGL could recognize terminated either with (α1-3) or (α1-6)-linked mannose residues in structure  $\text{Man}(\alpha 1-6)[\text{Man}(\alpha 1-3)]\text{Man}(\alpha 1-6)[\text{Man}(\alpha 1-3)]$   $\text{Man}(\beta 1-4)\text{GlcNAc}(\beta 1-4)\text{GlcNAc}$  of yeast mannan and porcine thyroglobulin (unit A-type) (Figure 4).

Inhibition by yeast mannan and porcine thyroglobulin that is related to high-mannose type N-glycan binding specificity reported for many anti-HIV, anti-influenza virus, anti-cancer and anti-bacteria lectins from marine algae, such as *E. serra*

(ESA-2) (Fukuda *et al.*, 2006; Hori *et al.*, 2007; Omokawa *et al.*, 2010; Hayashi *et al.*, 2012; Sato *et al.*, 2015; ), *K. alvarezii* (KAA-2) (Sato *et al.*, 2011a; Hirayama *et al.*, 2016), *B. coacta* (BCA) (Sato *et al.*, 2011b), *K. striatum*, *E. denticulatum* (Le Dinh Hung *et al.*, 2015a, 2015b), *H. renschii* (HRL) (Mu *et al.*, 2017) and *S. filiformis* (SFL) (Chaves *et al.*, 2018), all of them were specific for high-mannose type N-glycans. Thus, high-mannose binding lectins from marine algae may become a useful interesting target for application in medicine.



**Figure 4.** The putative epitopes recognized by lectin from red alga *B. gelatinus* are highlighted in rectangle. Man: Mannose; Gal: Galactose; Fuc: Fucose; GlcNAc: N-acetyl-D-glucosamine; GalNAc: N-acetyl-D-galactosamine, NeuAc: N-acetylneuraminic acid; Asn: Asparagine; Ser/Thr: Serine/Threonine.

## CONCLUSION

The lectin BGL from red alga *B. gelatinus* showed novel properties, including molecular mass, monomeric form, hemagglutination-inhibition profile, and stable over a wide range of pH and temperature. BGL preferably bond to terminal (α1-3) or (α1-6)-linked mannose residues in structure  $\text{Man}_5\text{GlcNAc}_2\text{Asn}$  of N-glycans, indicating that the terminal (α1-3) or (α1-6)-linked mannose residues

in N-glycans were critical for lectin binding. Thus, the red alga *B. gelatinus* promises to be a source of novel lectin (s) for application in biochemistry and biomedicine.

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## ĐẶC TÍNH LIÊN KẾT N-GLYCAN DẠNG HIGH-MANNOSE CỦA LECTIN MỚI TỪ RONG ĐỎ *BETAPHYCUS GELATINUS*

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### TÓM TẮT

Rong đỏ, *Betaphycus gelatinus* là một trong những nguồn carrageenan trên thế giới. Lectin BGL được đặt tên theo tên của mẫu rong 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 thể hiện một dải đơn với khối lượng phân tử khoảng 19.000 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 BGL 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 trị hai như  $\text{Ca}^{2+}$  và  $\text{Mg}^{2+}$ , vì vậy hoạt tính sinh học của lectin không phụ thuộc vào kim loại. Hoạt tính ngưng kết hồng cầu của lectin BGL không bị ức chế bởi các đường monosaccharide và glycoprotein, D-glucose, D-mannose, D-galactose, D-xylose, N-acetyl-galactosamine, N-acetyl-glucosamine, N-acetyl-neuraminic acid, N-acetyl-D-mannosamine, transferrin and fetuin, nhưng bị ức chế mạnh bởi các glycoproteins mang N-glycan dạng high-mannose như yeast mannan và porcine thyroglobulin. Lectin BGL đặc hiệu với N-glycan và có thể nhận biết các gốc mannose được liên kết ( $\alpha$ 1-3) hoặc ( $\alpha$ 1-6) ở vị trí cuối cùng trong cấu trúc  $\text{Man}(\alpha$ 1-6)[ $\text{Man}(\alpha$ 1-3)]  $\text{Man}(\alpha$ 1-6)[ $\text{Man}(\alpha$ 1-3)] $\text{Man}(\beta$ 1-4) $\text{GlcNAc}(\beta$ 1-4) $\text{GlcNAc}$  của N-glycan. Đặc tính liên kết N-glycan dạng high-mannose của lectin BGL tương tự với đặc tính liên kết N-glycan dạng high-mannose của các lectin có hoạt tính kháng virus, kháng ung thư và kháng vi khuẩn từ các loài rong đỏ, carrageenophyte, như *Eucheuma serra* (ESA-2), *Eucheuma denticulatum* (EDA-2), *Kappaphycus striatum* (KSA-2), *Kappaphycus alvarezii* (KAA-1 and KAA-2) và *Solieria filiformis* (SfL). Rong đỏ *B. gelatinus* hứa hẹn là một nguồn lectin giá trị để sử dụng trong hóa sinh và y sinh.

**Từ khóa:** *Betaphycus gelatinus*; Đặc tính liên kết carbohydrate; Lectin; N-glycan dạng high-mannose; Rong đỏ