HIGH-MANNOSE TYPE N-GLYCAN SPECIFIC LECTINS FROM RED MARINE ALGAE, CARRAGEENOPHYTES

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SUMMARY

The red algae *Kappaphycus striatum* and *Eucheuma denticulatum* are economically important species as a source of carrageenan. The isolated lectins from these algae shared the same properties in hemagglutination activity, hapten-inhibition profile of hemagglutination, and equivalent molecular mass of a monomeric protein (about 28,000 Da). The hemagglutination activities of isolated lectins were inhibited strongly by glycoproteins bearing high-mannose type N-glycan as porcine thyroglobulin and yeast mannan so far as tested. Their activities were not affected by either the presence of EDTA or addition of divalent cations, stable in a wide pH range from 3 to 10, and not changed by incubation at 60^{o}C for 30 min. Thus, these algal species can be a potential source of the useful lectins for development of strictly specific probes of high mannose N-glycans.

Keywords: *Carbohydrate-binding specificity, Eucheuma denticulatum, Hemagglutinins, Kappaphycus striatum, Lectins, Molecular weight*

INTRODUCTION

Glycoproteins are usually defined as proteins bearing one or more covalently linked carbohydrate moieties. These biomolecules are widely distributed in nature from virus to human. The occurrence of oligosaccharide chains covalently attached to the peptide backbone is the feature that distinguishes glycoproteins from other proteins and accounts for some of their characteristic chemical and biological properties (Kornfeld, Kornfeld, 1980). Cell surface glycoproteins have been shown to play important roles in pinocytosis,

differentiation, carcinogenesis, intercellular recognition, inflammation, and adhesion, as receptors for hormones and viruses, mediators of immunological specificity or as carriers of specific biologically signal transduction. Since the carbohydrate moieties of glycoproteins are responsible for many biological functions, a great deal of effort has been devoted to determine the glycostructures of the oligosaccharide chains. In order to understand the function of carbohydrates on glycoproteins, it is important to elucidate structures of oligosaccharide chains and their distributions. Lectins, agglutinins or hemagglutinins are carbohydrate-binding proteins or glycoproteins of non-immune origin, and are a versatile tool for categorizing carbohydrates because they can discriminate differences in carbohydrate structures and reveal various biological activities through binding to carbohydrates (Montreuil *et al*., 1995; 1997). Unlike antibodies, lectins show diversity in their molecular structures and carbohydrate-binding specificities, depending on their organisms of origin. In view of their special chemical and biological properties, lectins have applications in several research fields such as biochemistry, immunology, cell biology and cancer research (Sharon, Lis, 2003).

Algal lectins differ from higher plant lectins in a variety of properties. In general, marine algal lectins have common characteristics of low molecular weight, monomeric molecules, thermostability, having high affinity for glycoproteins, especially those found in animals, but not for monosaccharides, and no metal requirements for hemagglutination (Hori *et al*., 1990; Rogers, Hori, 1993). These features do not exclude algal lectins from various biological functions such as anti-tumor, mitogenic, and anti-virus activities.

The red algae *Kappaphycus alvarezii, K. striatum* and *Eucheuma denticulatum* are the economically important species of carrageenophytes. The colour strains (brown, red and green) of these algae were cultivated on a large scale in Vietnam. There will be considerable quantities of raw materials not only as a source of carrageenans but also as a source of valuable bioactive compounds for biochemical and medicinal uses. In previous results of the Vietnamese algal screening for hemagglutinins (lectins), the strong bioactivity in the extracts of the cultivated *K. alvarezii, K. striatum* and *E. denticulatum* (Hung *et al*., 2009a) were detected. In addition, the seasonal change in the lectin contents of *K. alvarezii*

was determined by enzyme-linked immunosorbant assay (ELISA) using an anti-serum raised against the lectin (KAA-2) isolated from *K. alvarezii* (Hung *et al*., 2009b), and lectins from this alga were also isolated and characterized (Hung *et al*., 2009c). However, at present, there is no general report on biochemical properties and biological activities of lectins from these algal species, which will be very important for application. Thus, the purpose of this work was to report on properties of the lectins from the red algae *K. striatum* and *E. denticulatum* as representatives the genera *Kappaphycus* and *Eucheuma* cultivated in Vietnam.

MATERIALS AND METHODS

The red marine algal strains, *Kappaphycus striatum* and *Eucheuma denticulatum*, were collected from Camranh Bay, Khanhhoa Province, Vietnam, in November, 2009. The Superdex R-75 HR 10/30 and TSK-GEL DEAE-5PW columns were obtained from Pharmacia Biotech (Uppsala, Sweden) and Tosoh Corporation (Nanyo, Japan), respectively. A G.P SENSOR kit for detection of carbohydrate was purchased from Seikagaku Corporation (Tokyo, Japan). Fucoidan, transferrin, fetuin, bovine thyroglobulin, porcine thyroglobulin, bovine submaxillary mucin and porcine stomach mucin were purchased from Sigma (St. Louis, MO). Bovine thyroglobulin was from WAKO (Osaka, Japan). Monosaccharides: D-glucose (Glc), D-mannose (Man), D-galactose (Gal), L-rhamnose (Rha), L-fucose (Fuc), D-xylose (Xyl), N-acetyl-D-glucosamine (GlcNAc), N-acetyl-D-galactosamine (GalNAc), N-acetyl-D-mannosamine (ManNAc), N-acetylneuraminic acid (NeuAc) and yeast mannan were from Nakarai Chemical (Kyoto, Japan). Asialo-bovine thyroglobulin, asialo-fetuin, asialo-transferrin, asialo-porcine thyroglobulin, asialo-porcine stomach mucin and asialo-bovine submaxillary mucin was prepared by hydrolysis of the parent sialoglycoprotein with 0.05 M HCl for 1 h at 80°C, followed by dialysis against saline overnight. All other chemicals used in this study were of the highest purity available.

Most lectins from marine algae have no affinity for monosaccharides (Hung, 2009d). This property makes it difficult to isolate the algal lectins by affinity chromatography using

a specific-simple sugar as both a ligand and an eluant. Therefore, lectins from marine algae were isolated by the conventional methods for purifcation of proteins.

Purification of hemagglutinin

The fresh alga was cut into small pieces, homogenized in a blender with 60% cold ethanol to attain a final concentration of 20% and kept at 4^{o}C for 18 h with occasionally stirring. After filtration through a cheese cloth, the filtrate was centrifuged at 6000 rpm for 20 min. To supernatant, cold absolute ethanol (–20^{o}C) was added to attain a final concentration of 80% and the mixture was kept at 4^{o}C overnight. The precipitate was collected by centrifugation at 6000 rpm for 20 min and thoroughly dialyzed against phosphate buffer containing 0.15 M NaCl (pH 7.0). The non-dialyzable fraction was applied to a Superdex R 75 HR 10/30 column equilibrated with the above buffer. The active fractions were pooled, concentrated by ultrafiltration, and dialyzed against 20 mM Tris-HCl buffer (pH 8). The concentrate was applied to ion exchange chromatography on a TSK gel DEAE-5PW column (7.5 x 75 mm) equilibrated with 20mM Tris-HCl buffer (pH 8). The eluate was monitored for absorption at 280 nm and for hemagglutination activity with trypsin-treated rabbit erythrocytes. Active fractions were pooled and dialyzed against distilled water, separately.

Protein contents

Protein contents were determined by the method of Lowry *et al*. (1951) using bovine serum albumin as a standard.

Hemagglutination and hapten-inhibition tests

Hemagglutination activity and hemagglutination-inhibition test were determined with a 2% (v/v) suspension of trypsin-treated rabbit erythrocytes (Hung *et al*., 2009a). Inhibition was observed macroscopically and inhibition activity was expressed as the lowest concentration (mM or μg mL^{-1}) of sugar or glycoprotein, respectively, at which a complete inhibition of 4 hemagglutination units was achieved.

Effect of temperature, pH and metal ions on hemagglutination activity

They were determined according to Hung *et al.* (2009a), using trypsin-treated rabbit erythrocytes.

Molecular weight determination

The molecular masses of purified lectins were determined by both sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrospray-ionization mass spectrometry (ESI-MS). SDS-PAGE was performed using a 10% gel (Laemmli, 1970). The sample was heated at 100 ^{o}C for 5 min with or without 2% 2-mercaptoethanol. After SDS-PAGE, the gel was stained with Coomassie brilliant blue R-250 for proteins. For detection of glycoproteins, proteins on a gel were electrophoretically blotted to a polyvinylidene difluoride (PVDF) membrane and the membrane was subjected to staining for carbohydrate using a G.P. SENSOR kit.

For determination of molecular mass by ESI-MS using a LCQ (Finnigan), intact lectin was purified by reverse-phase high-performance liquid chromatography (HPLC) on a YMC PROTEIN-RP column (6.0 x 250 mm), eluting at a flow rate of 1.0 ml/min with a mixture of 0.1% (v/v) trifluoroacetic acid (TFA) in water (solvent A) and in acetonitrile (solvent B) using the following chromatographic conditions: first, isocratic (20% solvent B) for 10 min, followed by gradients of 20% – 50% solvent B for 30 min. Protein elution was simultaneously monitored at 280 nm. The peak containing lectins was recovered and applied to ESI-MS. The BioMultiview software was used to analyze and deconvolute the raw mass spectrum (LCQ, Finnigan).

RESULTS AND DISSCUSSION

Hemagglutination activity of the extracts

The extracts from *K. striatum* and the color strains of *E. denticulatum* strongly agglutinated trypsin- and papain-treated erythrocytes of sheep and rabbit, and weakly agglutinated native erythrocytes of sheep. The hemagglutination titers of the extract from *K. striatum* showed the same activity between both erythrocytes of sheep and rabbit (Table 1).

The hemagglutination activities of the extract from *K. striatum* was slightly higher than those of the extracts from the two color strains of *E. denticulatum*. However, no agglutination of extracts was observed against chicken and human A, B and O blood types, even when erythrocytes were treated by enzyme. When crude extracts of many algal species were examined for hemagglutination activity toward various native and enzyme-treated erythrocytes, they showed a tendency to agglutinate more strongly non-human animal erythrocytes, especially rabbit erythrocytes.

Table 1. Hemagglutination activity of crude extracts from *K. striatum* and the color strains of *E. denticulatum*. The hemagglutination activity is expressed as a titer that is the reciprocal of the highest two-fold dilution exhibiting positive agglutination.

Species	Titer of crude extracts 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
K. striatum	32	1024	2048	32	1024	2048	-[d]	-	-	-	-	-	-	-	-	-	-	-
E. denticulatum																		
Strain																		
Brown	-	512	1024	8	1024	1024	-	-	-	-	-	-	-	-	-	-	-	-
Green	-	512	1024	8	1024	1024	-	-	-	-	-	-	-	-	-	-	-	-

[a] Native erythrocytes; [b] Trypsin-treated erythrocytes; [c]Papain-treated erythrocytes.
[d] Indicates that the extract showed no hemagglutination.

Isolation of lectins from algal strains of *K. striatum* and *E. denticulatum*

Each algal strain commonly contained the three lectins designated as KSA-1, KSA-2 and KSA-3 for *K. striatum* and EDA-1, EDA-2 and EDA-3 for *E. denticulatum* after the specific names of both algae, respectively. Three lectins from each algal strain were isolated from the 20% ethanol extract, following by precipitation with cold ethanol, gel filtration and ion-exchange chromatography. The three peaks from each algal strain gave a single band with the same mobility in SDS-PAGE (Figure 1a for *K. striatum* and 1b for *E. denticulatum*). From 1,000 g fresh alga, the sum of the yield of lectin was about 80.0 mg for *K. striatum*; 24.5 mg for brown and 8.4 mg for green strains of *E. denticulatum*.

The isolated algal lectins strongly agglutinated rabbit erythrocytes, especially following trypsin-treated erythrocytes. The minimum agglutinating concentration of these lectins was at the level of ng protein mL^{-1} toward trypsin-treated rabbit erythrocytes.

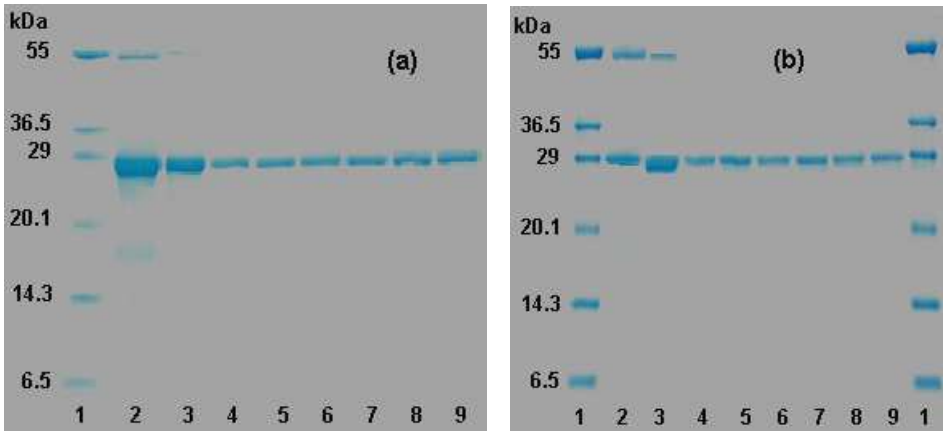

Figure 1. SDS-PAGE of the lectins isolated from *Kappaphycus striatum* (a) and *Eucheuma denticulatum* (b); SDS –PAGE was carried out using a 10% polyacrylamide gel. Protein bands were stained with Coomassie Brilliant blue R-250 reagent; Line 1, Marker of reference proteins; Line 2, 80% ethanol precipitates of *K. striatum* (a) and *E. denticulatum* (b); Line 3, active fraction after gel filtration of *K. striatum* (a) and *E. denticulatum* (b). Lines 4 and 5 for KSA-1 (a) and EDA-1 (b); 6 and 7 for KSA-2 (a) and EDA-2 (b); 8 and 9 for KSA-3 (a) and EDA-3 (b), respectively. In lines of 4 to 9, the samples in the lines of odd number were treated with 2% 2-mercaptoethanol, whereas those in the lines of even number were not.

Table 2. Molecular masses of lectins from *Kappaphycus striatum* and *Eucheuma denticulatum.*

The name of algal species	The name of lectins	Gel filtration (Da)	SDS-PAGE (Da)	ESI-MS (Da)	Subunit structure
K. striatum	KSA-1	25,000	28,000	28,017.0 ± 1.2	Monomer
	KSA-2	25,000	28,000	28,020.0 ± 1.3	Monomer
	KSA-3	25,000	28,000	28,018.0 ± 1.4	Monomer
K. alvarezii (Hung *et al*., 2009c)	KAA-1	25,000	28,000	28,018.0 ± 1.5	Monomer
	KAA-2	25,000	28,000	28,021.0 ± 1.8	Monomer
	KAA-3	25,000	28,000	28,016.0 ± 1.2	Monomer
E. denticulatum	EDA-1	25,000	28,000	27,856.8 ± 2.2	Monomer
	EDA-2	25,000	28,000	27,851.2 ± 2.6	Monomer
	EDA-3	25,000	28,000	27,853.3 ± 1.8	Monomer
E. serra (Kawakubo *et al*., 1997; Hori *et al.,* 2007)	ESA-1	25,000	29,000	ND	Monomer
	ESA-2	25,000	29,000	27949.0	Monomer
E. amakusaensis (Kawakubo *et al*., 1999)	EAA-1	25,000	29,000	ND	Monomer
	EAA-2	25,000	29,000	ND	Monomer
	EAA-3	25,000	29,000	ND	Monomer

ND, not determined.

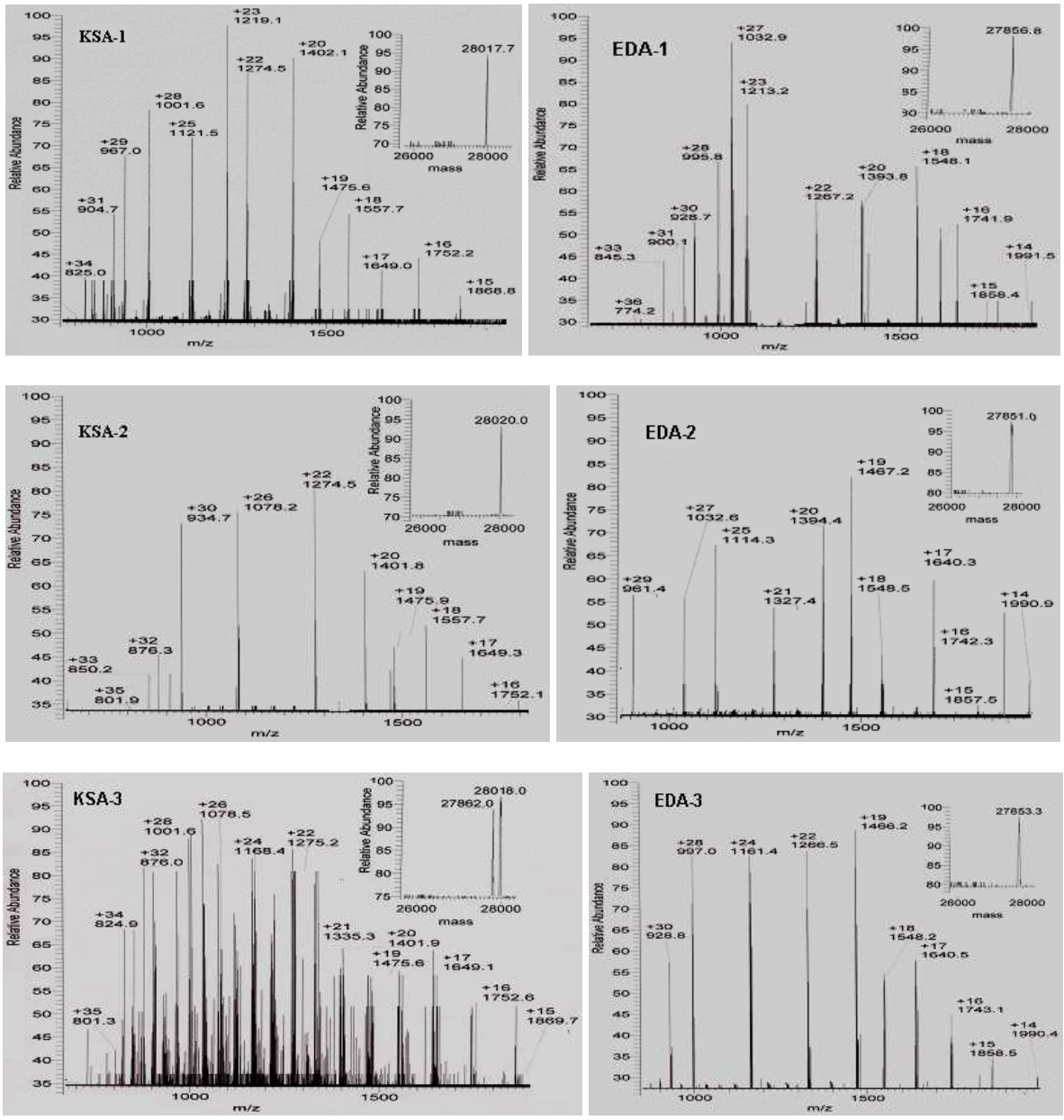

Figure 2. ESI-MS of the lectins isolated from *K. striatum* (KSA-1, KSA-2 and KSA-3) and *E. denticulatum* (EDA-1, EDA-2 and EDA-3)**.**

Molecular mass of purified lectins

The molecular masses of purified lectins were determined by both sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrospray-ionization mass spectrometry (ESI-MS) (Table 2). The relative molecular masses of lectins from *K.*

striatum (KSA1-3) and *E. denticulatum* (EDA1-3) were estimated to be 25,000 Da by gel filtration and 28,000 Da by both non-reducing and reducing SDS-PAGE, respectively. The bands of 28,000 Da were negative for carbohydrate staining on a PVDF membrane transferred after running SDS-PAGE. By ESI-MS, the molecular masses of intact KSA-1, KSA-2 and KSA-3 were determined to be 28,017 ± 1.2, 28,020 ± 1.3 and 28,018 ± 1.4 Da, respectively, and those of of intact EDA-1, EDA-2, and EDA-3 were 27,856.8 ± 2.2, 27,851.2 ± 2.6 and 27,853.3 ± 1.8 Da, respectively (Figure 2). These indicate that all the lectins were monomeric proteins without carbohydrate.

Effect of temperature, pH and metal ions on hemagglutination activity

Hemagglutination activities of lectins from *K. striatum* and *E. denticulatum* were not affected by either the presence of EDTA or addition of divalent cations such as Ca^{2+} and Mg^{2+}, thus indicating that these lectins are not a metalloprotein. The requirement for metals is not a general characteristic of most algal lectins (Hori *et al*., 1990; Rogers, Hori, 1993), although this feature was observed for the lectins from the red algae *Enantiocladia duperreyi*, *Ptilota plumosa* and *P. filicina* (Benevides *et al*., 1998; Rogers *et al*., 1977; Sampaio *et al*., 1998a) and the green algae *Ulva lactevirens* and *U. lactuca* (Sampaio *et al*., 1996; 1998b).

Activities of isolated lectins from both algae above were stable in a wide range of pH from 3 to 10, and were not changed by incubation at 60°C for 30 min, whereas they gradually decreased as the incubation temperature exceeded 60°C (Figure 3).

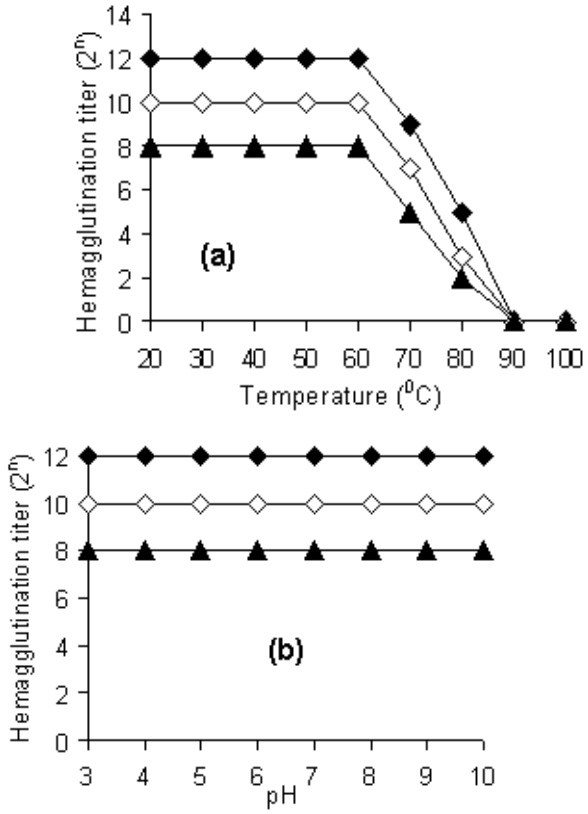

Figure 3. The effects of temperature (a) and pH (b) on hemagglutination activities of lectins KSAs and EDAs. KSA-1 and EDA-1 (◇); KSA-2 and EDA-2 (◆); and KSA-3 and EDA-3 (▲).

Carbohydrate-binding specificity

The hemagglutination activities of all lectins were not inhibited by any of monosaccharides, lactose and fucoidan, but were inhibited by examined glycoproteins, except transferrin and porcine stomach mucin (Table 3). This phenomenon has been seen in many other algal agglutinins (Hori *et al.*, 1990; Rogers, Hori, 1993) and appears to be a common feature of many algal lectins. In contrast, most terrestrial plant lectins are inhibited by simple sugars (Goldstein, Poretz, 1986).

The strong inhibition was detected with porcine- and bovine thyroglobulins, their asialo-derivatives and yeast mannan, all of which have high-mannose type N-glycans in the molecules although both thyroglobulins contain complex type N-glycans, too. Porcine thyroglobulins contain both high-mannose type (unit A-type) bearing at least 9 different

structures (Tsuji *et al*., 1981) and complex type (unit B-type) bearing at least 8 different structures (Yamamoto *et al*., 1981). Among the unit-A types, the common structure of high-mannose type N-glycans is $Man_5GlcNAc_2Asn$ with (α1-6) and (α1-3)Man residues branched from Man(α1-6) arm of the core trimannose. Moreover, yeast mannan, which is a high-mannose N-glycan with the (α1-6) linkage in its backbone and (α1-3) linkage in this side chains, was relatively good inhibitor of all lectins (Figure 4). Bovine thyroglobulin was relatively a good inhibitor. This glycoprotein contains the polymannose (unit A) and complex type (unit B). Among the unit A, carbohydrate units consist of an average of 8 to 9 residues of mannose and 2 residues of N-acetylglucosamine (Arima *et al*., 1972).

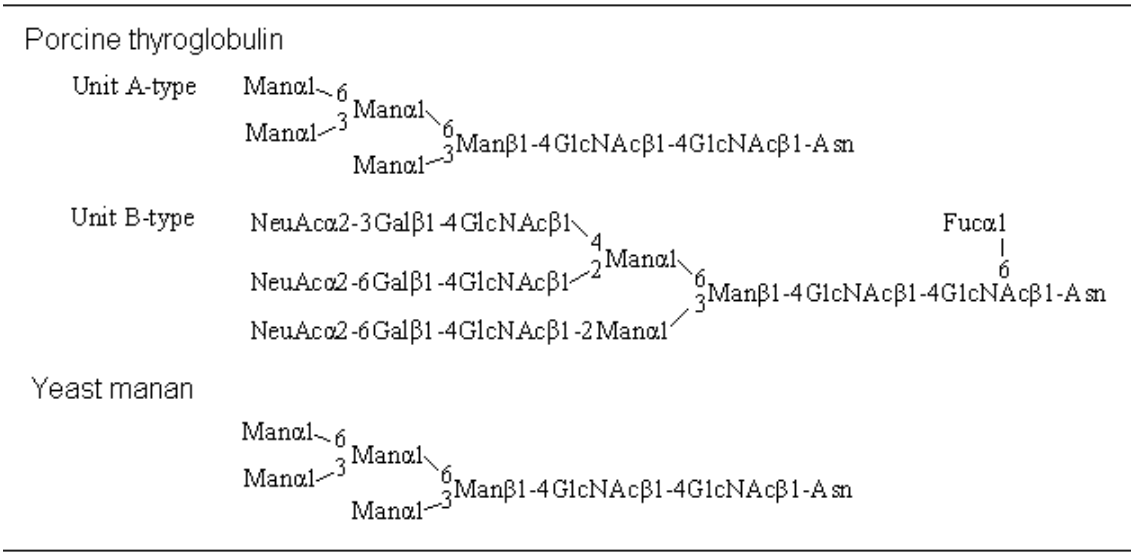

Figure 4. Major saccharide determinants of N-glycoproteins that inhibited the hemagglutination activity of the lectins from *Kappaphycus striatum* and *Eucheuma denticulatum*.

Among the unit B-type of porcine thyroglobulin, the major N-glycans are mono- and disialylated (α1–6) fucosylated biantennary structures terminated with (α2–6)-linked sialic acid (NeuAc) on the (α1–3)Man antennae. The fact that lactotransferrin, which possesses two (α1–6) fucosylated diantennary N-acetyllactosamine-type glycans per molecule (Spik *et al*., 1982), did not show the agglutination activity even at concentration of 2 mg mL^{-1}

(Table 3), implying that these lectins could not recognize the Fuc(α1–6)GlcNAc core sequence of the di- and triantennary glycans in porcine thyroglobulin.

Fetuin and its asialo form were also inhibitory. This glycoprotein contains six oligosaccharide chains, namely three disaccharides (T antigen) O-linked to Threonine or Serine residues, and three complex glycans N-linked to Asparagine residues (Baenziger *et al.*, 1979).

The bovine submaxillary mucin bearing at least 16 different structures was also relatively a good inhibitor (Savage *et al*., 1990; 1991). Of its oligosaccharides, 85% are acidic O-linked oligosaccharide chains, including a high density of sialyl Tn antigens and sialyl core3 saccharide sequences. The neutral O-linked glycans of bovine submaxillary mucin include the human blood groups A and H, the core1, core2, core3 and core4 (Chai *et al*., 1992). The fact that porcine stomach mucin bearing only O-glycans did not show any inhibitory activity even at concentration of 2 mg mL^{-1}, the carbohydrate structures of this glycoprotein have in common the Gal(β1–3)GalNAc(α1- core unit, which bears one or more N-acetyllactosamine branches. The latter are terminated either by Fuc in (α1–2) linkage or by GlcNAc in (α1–4) linkage to Gal (Karlsson *et al*., 1997).

The inhibition with O-linked glycoproteins were observed for many lectins including the *Eucheuma* and *Kappaphycus* lectins (Kawakubo *et al*., 1997; 1999; Hung *et al*., 2009c) that exhibit the binding specificity for high-mannose N-glycans in the oligosaccharide-binding experiments (Hori *et al*., 2007). It may be possible that such inhibition is caused by some non-specific interaction between lectins and O-linked glycoproteins. However, transferrin bearing only complex type N-glycans and porcine stomach mucin bearing only O-glycans were no inhibitory.

As a result, the data suggest that the (α1-6), (α1-3)-linked or at both terminal mannose residues in a common sequence ($Man_5GlcNAc_2Asn$) of bovine-/ porcine thyroglobulin or yeast mannan could be ligands recognized by lectins from both carrageenophytes, indicating that each colour strain of both algae contains lectins specific for high-mannose-type N-glycans. High-mannose type N-glycan binding specificity has also been reported for

lectins from other marine algae such as *Kappaphycus alvarezii* (Hung *et al.*, 2009c), *Eucheuma amakusaensis, E. serra* (Kawakubo *et al.*, 1997; 1999), *Boodlea coacta* (Hori *et al.*, 1986), *Carpopeltis flabellata* (Carnin) (Hori *et al.*, 1987), *Solieria robusta* (Hori *et al.*, 1988) and *Gracilaria bursa-pastoris* (Okamono *et al.*, 1990). All of them could recognize the structure of (α1-6) linked polymannose and/or trimannose at the core in N-glycosidic sugar chain of yeast mannan.

Unlike lectins from other sources, such as C-type lectin (Ng *et al*., 2002), legume lectin ConA (Mega *et al*., 1992; Naismith, Field, 1996) or monocot mannose-binding lectins (Barre *et al*., 1996). These lectins possess affinity for monosaccharides (mannose or glucose), and consist of two or four subunits (Hester, Wright, 1996), whereas lectins from carrageenophytes had no binding affinity for monosaccharide and existed in a monomeric form.

Table 3. Hemagglutination-inhibition test of the lectins isolated from the colour strains (brown and green) of *Kappaphycus striatum* and *Eucheuma denticulatum*. The value indicates the lowest concentration of sugar (mM) and glycoprotein (μg mL^{-1}) at which complete inhibition of hemagglutination (titer 4) was achieved.

Sugars & glycoprotein	***K. alvarezii***		***K. striatum***		***E. denticulatum***		***E. serra***	***E. amakusaensis***
	Brown	**Green**	**Brown**	**Green**	**Brown**	**Green**	**Brown**	**Brown**
Sugar (mM)								
Monosaccharides[a]	-[b]	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-
Fucoidan	-	-	-	-	-	-	-	-
Glycoprotein (μg mL^{-1})								
Transferrin	-	-	-	-	-	-	-	-
Fetuin	31.2	31.2	31.2	31.2	62.5	62.5	62.5	62.5
Yeast mannan	1.9	1.9	3.9	3.9	3.9	3.9	3.9	3.9
Porcine thyroglobulin	0.9	0.9	1.9	1.9	1.9	1.9	ND	ND
Asialo-porcine thyroglobulin	0.9	0.9	1.9	1.9	1.9	1.9	ND	ND
Bovine thyroglobulin	7.8	7.8	7.8	7.8	7.8	7.8	3.9	3.9
Asialo-bovine thyroglobulin	7.8	7.8	7.8	7.8	7.8	7.8	ND	ND
Bovine submaxillary mucin	31.2	31.2	31.2	31.2	31.2	31.2	250.0	250.0
Asialo-bovine submaxillary mucin	31.2	31.2	31.2	31.2	31.2	31.2	ND	ND
Porcine stomach mucin	-	-	-	-	-	-	ND	ND

The value indicates the lowest concentration of sugar (mM) and glycoprotein (μg ml^{-1}) at which complete inhibition of hemagglutination (titer 4) was achieved. [a] The monosaccharides examined are described in Materials and methods. [b] No inhibition at 100 mM for monosaccharides and 2,000 μg ml^{-1} for glycoproteins. ND, not determined. K. alvarezii (Hung *et al.,* 2009c); E. serra (Kawakubo *et al.,* 1997); E. amakusaensis (Kawakubo *et al.,* 1999). Monosaccharides are listed in Materials and Methods.

Monomeric lectins also have been reported from marine algae (Hori *et al*., 1990; Rogers, Hori, 1993) as well as some bacteria (Fujita *et al*., 1975). Regarding cell agglutination by lectins, it generally has been explained that lectins have one binding site per one or two subunit(s), and by association of the subunits, they possess at least two binding sites, which enable them to agglutinate cells. Therefore, it remains to be determined how such a monomeric form causes the agglutination of cells. It is possible that each monomeric lectin from marine algae has at least two binding sites per molecule, as seen in a subunit of a wheat germ agglutinin (Goldstein, Poretz, 1986), a *Streptomyces. sp* lectin (Fujita *et al*., 1982), and lectins from cyanobacteria Cyanovirin-N from *Nostoc ellipsosporum* (CV-N) (Bewley *et al*., 2001; 2002), SVN from *Scytonema varium* (Bokesch *et al*., 2003), and *Oscillatoria agardhii* OAA (Sato *et al*., 2007) or that a lectin aggregates by itself on the cell surface to bring about cell agglutination.

The lectins from *K. striatum* and *E. denticulatum* shared similar biochemical properties to those from *Eucheuma amakusaensis* (EAAs), *E. serra* (ESAs) (Kawakubo *et al*., 1997; 1999) and *K. alvarezii* (KAAs) (Hung *et al*., 2009c), including affinity for glycoproteins bearing high-mannose type N-glycans, identical molecular mass, and stability at relatively high temperatures and over a wide pH range. The similarity in biochemical properties between lectins from *K. striatum*, *E. denticulatum* and *E. serra* (ESA-2) (Kawakubo *et al*., 1997), *K. alvarezii* (KAAs) (Hung *et al*., 2009c), suggests that lectins KSAs and EDAs are strictly specific for high mannose type N-glycans like ESA-2 (Hori *et al*., 2007). Recently, studies on oligosaccharide binding specificity of lectins that the high-mannose binding nature was critical for their antiviral activities (Ziółkowska, Wlodawer, 2006) have also been reported for lectins from higher plant such as legume lectin ConA (Mega *et al*., 1992; Naismith, Field, 1996) or monocot mannose-binding lectins (Barre *et al*., 1996), from animal C-type lectin (Ng *et al*., 2002), from red algae *Griffithsia* spp (GRFT) (Mori *et al*., 2005), *E. serra* ESA-2 (Hori *et al*., 2007), and from cyanobacteria *Oscillatoria agardhii* OAA (Sato *et al*., 2007), *Nostoc ellipsosporum* (CV-N) (Bewley *et al*., 2001; 2002), *Scytonema varium* (SVN) (Bokesch *et al*., 2003), *Microcystis viridis* lectin (MVL) (Bewley *et al*., 2004), and from an antibody 2G12, which is one of the few broadly

neutralizing monoclonal antibodies directed against HIV-1 (Calarese *et al*., 2005). All of them preferentially recognizes the different branched oligomannoside structures of N-glycans and have strict specificity for high-mannose type N-glycans.

The high mannose N-glycans are generally detected at the early stage in the N-glycan synthesis of glycoproteins, because high-mannose type is first synthesized in endoplasmic reticulum, and then modified and converted to complex N-glycans in Golgi apparatus before secreting of mature glycoproteins outside cells. This mean that the high-mannose N-glycans may be abundant in some undifferentiated cells and/or cells of lower organisms that defects in N-glycan synthesis lead to a variety of diseases. Practically, the lectins (KAAs, KSAs and EDAs) from the carrageenophytes mentioned above, showed the novelty of carbohydrate-binding specificity for high-mannose type N-glycans, distinct from other high-mannose binding lectins, promising their uses as new sugar-probes. However, at present molecular structures and biological activities of the lectins still remain to be elucidated, although those lectins are predicted to have some unique and important features shown in the strict binding specificity for some definite carbohydrate structures.

CONCLUSION

The lectins isolated from *K. striatum* and *E. denticulatum* shared similar biochemical characteristics, including hemagglutination-inhibition test and molecular mass. They have common preferential affinity for glycoproteins bearing high-mannose type N-glycans and identical molecular weights, and are active at relatively high temperatures and over a wide pH range. The similarity of chemical properties of lectins among the algae belonging to the genera *Kappaphycus* and *Eucheuma* suggests that these algae will draw considerable attention not only as the source of carrageenan but also as a potential source of novel lectins.

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LECTIN ĐẶC HIỆU N-GLYCAN DẠNG GIÀU MANNOSE TỪ RONG ĐỎ, CARRAGEENOPHYTE

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TÓM TẮT

Rong đỏ *Kappaphycus striatum* và *Eucheuma denticulatum* là các mẫu rong kinh tế quan trọng cho sản xuất carrageenan. Các lectin được cô lập từ các mẫu rong này cho thấy các tính chất giống nhau bao gồm hoạt tính ngưng kết máu, đặc tính liên kết carbohydrate và khối lượng phân tử tương đương nhau của một protein monome (khoảng 28.000 Da). Hoạt tính ngưng kết máu của các lectin đã bị ức chế mạnh bởi các glycoprotein mang dạng giàu mannose như porcine thyroglobulin và mannan từ nấm men. Hoạt tính của chúng không bị ảnh hưởng bởi sự có mặt của EDTA hoặc khi thêm cation hóa trị hai, bên trong một phạm vi rộng của pH từ 3 đến 10 và không bị thay đổi khi được gia nhiệt ở 60^{o}C trong 30 phút. Vì vậy, các mẫu rong này có thể là một nguồn lectin có giá trị để phát triển chúng thành thuốc thử thăm dò N-glycan dạng giàu mannose.

Từ khóa: *Đặc tính liên kết carbohydrate, Eucheuma denticulatum, Hemagglutinin, Kappaphycus striatum, Khối lượng phân tử, lectin*

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