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SCREENING AND PRELIMINARY CHARACTERIZATION OF HEMAGGLUTININS IN SOUTHERN VIETNAMESE MARINE ALGAE

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Abstract

Forty-two species of marine algae belonging to Chlorophyta, Rhodophyta and Phaeophyta from the Ninh Thuan coast were examined for hemagglutinations activity with native and enzyme –treated different animal and human erythrocytes. All extracts agglutinated at least one type of erythrocytes tested. The strongest activities were found in the rabbit and horse erythrocytes by two Chlorophyta (Caulerpa sertularioides f. longipes, Halimeda discoidea) and one Rhodophyta (Gelidiella acerosa). There was only one species (Halimeda discoidea) which has agglutinated with all the erythrocytes tested. The hemagglutinin-inhibition assays performed with monosaccharides and glycoproteins indicated that none of the hemagglutinins had affinity for monosaccharides but they were inhibited by some glycoprotein tested.

Introduction

Lectin constitute a group of proteins or glycoproteins of non-immune origin, which bind reversibly to carbohydrates and thus combine with the glyco-components on the cell surface [19]. Lectins serve as powerful tools in carbohydrate research for the detection, isolation and characterization of glycoconjugates [26]. Some lectins have properties such as anti-HIV, anti-microbial, anti-fungal and anti-cancer activities [22, 26]. Algal lectins haves also been related to various biological functions such as recognition molecules in cell-cell or cell-matrix interaction, sexual reproduction, regeneration of cell and wound healing processes [18].

The first report of the presence of agglutinins in marine algae by Boyd [7] was followed by report of their presence in marine algae from all over the world, including those from English Japanese, Spanish United States, Brazilian, Pakistani, Chinese, Indian and Antarctic marine algae [5,6,23,16,11,8,4,1,2,12,3,30,19,27]. Hemagglutinins are widely distributed in many marine macroalgae and several algal hemaglutinins have been isolated [17,25]. However, there is only a limited amount of information on purified and characterized algal lectins, compared to those for lectins of higher plants and invertebrates [24].

Algae lectins differ from higher plant lectins in a variety of properties. In general, algal lectins have lower molecular masses than most higher plant lectins and have no affinity for simple sugars but are more specific for complex oligosaccharides, often glycoproteins. Furthermore, most of marine algal lectins do not require divalent cations for their biological

activity [24]. They occur mainly in monomeric forms and have a high proportion of acidic amino acids, with isoelectric points from 4 to 6 [15]. These suggest that algae may be dominant sources of useful lectins for applications in biochemical and biomedical studies.

Vietnam is located in the tropical and subtropical zone with a long coastline of about 3,260km where there is a rich algal flora with about 1,000 estimated and 639 identified species [21]. These algae species may be potential sources of biologically active compounds including lectins. Our previous surveys on Vietnamese marine macroalgae for hemagglutinins have screened 44 species using native and enzyme-treated different animal and human erythrocytes. The present study reports screening for hemagglutination activity in aqueous extracts from 42 macroalgae collected on the Ninhthuan coast of Vietnam. Analyses are included using competitive inhibition experiments of the binding affinity for simple sugars and glycoprotein. The results indicate that hemagglutinin was widely distributed in Southern Vietnamese marine macroalgae and almost all algal species examined contain hemagglutinins that exhibit the interesting properties inherent to many algal lectins.

Material and methods

Collection and extraction of marine algae

All species were collected on the Ninhthuan coast of Vietnam, kept at -20^{0}C until used. Crude extracts were prepared by homogenization with three volumes of 0.02 M phosphate buffer, pH 7.0 containing 0.85% NaCl (phosphate-buffered saline (PBS)) and kept at 4°C for 12 h with occasional stirring. After filtration, the filtrate was centrifuged at 6,000 rpm for 30 min at 4°C, the supernatants were stored at −20°C until used. To the supernatant (extract), solid ammonium sulfate was slowly added to attain a 75% saturation. The mixture was gently stirred and then kept at 4°C for 18 h. The precipitates were recovered by centrifugation at 6,000 rpm for 30 min, dissolved in a small volume of PBS, and thoroughly dialyzed against the same buffer overnight. The nondialyzable fraction was recovered as ammonium sulfate precipitates used for both hemagglutination–inhibition and stability tests.

Erythrocytes

Blood from rabbit, sheep, horse and chicken was obtained from the Institute of Vaccine-Nhatrang, Vietnam and human A, B and O blood from Khanhhoa General Hospital, Vietnam. Blood was maintained at 4^{0}C, a 2% erythrocytes suspension in PBS was prepared from each type of blood (native and treated enzyme) after washing three times with PBS.

Hemagglutination assay

Hemagglutination activity was determined with serial two-fold dilutions as previously reported [11]. Algal extracts were placed in wells (25µl) and to which equal volume of erythrocytes suspension was added. The plates were gently shaken, and left for 2h at room temperature.

Hemagglutination inhibition assay

Hemagglutination inhibition studies were performed using active ammonium sulfate-precipitated fractions from 14 algal samples: *Avrainvillea obscura, Halimenda velasquezii, Halymenia discoidea, Boodlea coacta, Boodlea composita, Valoniopsis pachynema, Dictyosphaeria cavernosa, Dictyosphaeria versluysiiversluysii, Valonia fastigiata, Caulerpa cupressoides, Amansia rhodantha, Rhodymenia anastomosans, Gelidiopsis scoparia* and *Laurencia perforata*. The inhibition assay was carried out following procedures as described by [14], using the following substances: the monosaccharides D-glucose, D-mannose, D-galactose, L-fucose, D-xylose, D-glucosamine, D-gluconic acid, p-nitrophenyl-D-galactoside, p-nitrophenyl-D-glucoside, p-nitrophenyl-D-mannoside; polysaccharide laminaran; and glycoproteins transferrin, asialo-transferrin, fetuin, asialo-fetuin, yeast mannan, porcine thyroglobulin, asialo-porcine thyroglobulin, porcine stomach mucin, and asialo-porcine stomach mucin. The results were expressed as the minimum concentration of simple sugar or glycoprotein

that caused inhibition of the hemagglutinating activity using enzyme-treated rabbit erythrocytes as indicator cells.

Results

The results of the screening are summarized in Table 1.

Chlorophyta

The extracts from 17 Chlorophyta species examined agglutinated at least one type of erythrocyte tested (Table 1). The extract of *Halimeda discoidea* gave positive agglutination for all erythrocytes used. Extracts of *Caulerpa racemosa var. occidentalis, C. racemosa var. lamourouxii, C. racemoza var. clavifera f. macrophysa, Avrainvillea obscura, Valoniopsis pachynema and Boergesenia forbesii* agglutinated only enzyme-treated rabbit erythrocytes. The extract from *H. velasquezii* showed the strongest activity toward horse erythrocytes while that from *C. sertularioides f. longipes* and *H. discoidea* was the strongest activity with enzyme-treated rabbit. The green algal extracts agglutinated all enzyme-treated erythrocytes more strongly than the native cells.

Rhodophyta

All protein extracts procedued from 22 Rhodophyta species agglutinated at least one type of erythrocytes tested. These is only *Rhodymenia anastomosans* could agglutinate with native rabbit erythrocytes. The extracts from *Laurencia papillosa, Amphiroa foliacea, Cheilosporum spectabile, Gelidium sp., Hypnea spinella, Yonagunia formosana, Titanophora pulchra* and *Galaxaura oblongata* did not agglutinate any type of human, sheep, horse and chicken erythrocytes, although they agglutinated enzyme-treated rabbit erythrocytes. The extract from *Gelidiella acerosa* showed the strongest activity toward enzyme-treated and untreated horse erythrocytes. 6 species in 22 Rhodophyta species agglutinated with human erythrocytes but only *Hydropuntia eucheumatoides* agglutinated all of A, B, O human erythrocytes. Thus, the hemagglutination titers of the red algal extracts were higher with animal erythrocytes than with human erythrocytes.

Phaeophyta

The extracts from three Phaeophyta species showed activity with enzyme-treated rabbit, sheep and chicken erythrocytes but no hemagglutination was observed with horse and human erythrocytes.

Hemagglutiation inhibition studies

Table 2 shows the results of agglutinic inhibition assays. The hemagglutination activities of all lectins were not inhibited by any of monosaccharides, but were inhibited by examined glycoproteins. The hemagglutination inhibition profiles with a variety of glycoproteins differed depending on hemagglutinin species. The hemagglutination activities of *A. obscura*, *H. discoidea* and *D. versluysii* were strongly inhibited by asialo-trasferrin (complex N-glycans). The inhibitions of *V. pachynema* and *D. cavernosa* were strongly inhibited by asialo-fetuin bearing both complex N-glycans and O-glycans as well as by porcine thyroglobulin and its asialo derivative bering both complex and high manose type N-glycans, but not inhibited by yeast mannan bearing high mannose N-glycans and porcine stomach mucin bearing O-glycans, suggesting that these two algal species contain, at least, a lectin specific for complex type N-glycans. Similarly, hemagglutinins from *V. fastigiata* and *A. rhodantha* were strongly inhibited by the asialo derivative of porcine thyroglobulin bearing both complex type and high mannose type N-glycans, but were not inhibited by yeast mannan bearing high mannose N-glycans, suggesting that these algal species each contain a lectin specific for complex type N-glycans. With respect to the hemagglutinin of *R. anastomosans*, yeast mannan bearing high mannose N-glycans was most inhibitory and porcine thyroglobulin bearing both high mannose and complex N-glycans was also inhibitory. The result suggests that the algal species contain, at least, a lectin specific for high mannose N-glycans.

Table 1 Hemagglutination activity of extracts from marine algae for different types of erythrocytes

	Hemagglutination titer of algal extracts[a]																				
	Rabbit			Sheep			Horse			Chicken			Human A			Human B			Human O		
	N[b]	T[c]	P[d]	N	T	P	N	T	P	N	T	P	N	T	P	N	T	P	N	T	P
Chlorophyta																					
Caulerpa racemosa var. occidentalis	-[e]	32	128	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. racemosa var. lamourouxii	-	-	64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. racemoza var. clavifera f. macrophysa	-	-	1024	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. serulata var. boryana	-	16	1024	2	8	4	4	4	4	-	4	-	8	16	16	-	-	2	4	4	8
C. sertularioides f. longipes	-	32	2048	16	32	8	-	-	-	-	-	-	16	16	64	-	-	-	-	-	-
C. cupressoides	-	16	256	2	4	4	8	8	8	-	2	2	8	16	16	-	4	8	8	16	8
Avrainvillea obscura	-	32	128	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Halimeda velasquezii	-	8	8	2	8	4	1024	1024	1024	-	2	4	4	8	8	4	4	8	-	8	8
H. discoidea Decaisne	64	2048	2048	16	64	32	256	512	512	8	64	128	16	64	32	16	32	32	16	32	32
Chaetomorpha crassa	-	32	128	4	8	4	8	16	8	8	8	16	8	4	8	8	16	8	4	8	16
Boodlea coacta	-	4	64	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. composita	-	16	128	2	8	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Valoniopsis pachynema	-	64	512	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Boergesenia forbesii	-	8	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dictyosphaeria cavernosa	-	32	128	-	-	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-
D. versluysii	-	32	64	4	4	8	-	-	-	4	2	4	-	-	8	-	-	16	64	4	32
Valonia fastigiata	-	16	256	8	8	16	-	-	-	8	4	4	-	8	64	4	16	64	16	16	32
Rhodophyta																					
Amansia rhodantha	-	32	128	2	4	8	-	-	-	-	-	2	-	-	2	-	-	-	-	-	-
Laurencia papillosa	-	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. flexilis	-	16	512	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-
L. corymbosa	-	32	128	-	-	-	-	-	-	-	-	-	-	-	4	-	-	4	-	-	-
L. dinhii	-	2	32	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
L. perforata	-	16	128	-	2	2	-	-	-	-	2	2	-	-	4	-	-	4	-	-	-
L. concreta	-	2	256	-	-	-	-	-	-	-	2	4	-	-	-	-	-	-	-	-	-

Amphiroa foliacea	-	16	128	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cheilosporum spectabile	-	32	64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mastophora rosea	-	8	32	2	4	8	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Gelidiella acerosa	-	-	128	-	-	-	2048	2048	2048	-	4	4	-	-	-	-	-	-	-	-	-
Gelidium sp.	-	-	512	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hypnea spinella	-	-	512	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Halymenia dilatata	-	4	4	2	8	2	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-
Yonagunia formosana	-	8	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Titanophora pulchra	-	256	1024	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydropuntia eucheumatoides	-	256	128	8	16	4	-	-	-	-	-	-	8	8	8	4	16	16	8	16	16
H. edulis	-	16	16	-	-	2	128	64	32	-	-	-	-	-	-	-	-	-	-	-	-
Galaxaura oblongata	-	-	512	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelidiopsis scoparia	-	256	256	-	-	64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceratodictyon spongiosum	-	16	16	-	-	-	-	-	-	4	2	4	-	-	-	-	-	-	-	-	-
Rhodymenia anastomosans	8	128	128	8	32	64	-	256	256	64	64	64	-	-	-	-	-	-	-	-	-
Phaeophyta																					
Dictyota divaricata Lamouroux	-	2	128	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D. dichotoma (Hudson) Lamouroux	-	4	128	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroclathrus tenuis C.K.Tseng & Lu	-	-	32	-	-	-	-	-	-	-	16	16	-	-	-	-	-	-	-	-	-

[a] Hemagglutination activity was expressed as a titer, the reciprocal of the highest dilution showing positive hemagglutination, as described in "Materials and methods"
[b] Native erythrocytes
[c] Trypsin-treated erythrocytes
[d] Papain-treated erythrocytes
[e] No detectable activity

Table 2 Hemagglutination-inhibition test of algal agglutinins with sugars and glycoproteins.

Sugars and glycoproteins										
Samples	Sugars	T	A-T	F	A-F	PTG	A-PTG	PSM	A-PSM	YM
Chlorophyta										
Avrainvillea obscura	-[a]	-	3.9	1000	31.2	31.2	15.6	-	-	-
Halimeda velasquezii	-	500	31.2	125	31.2	62.5	31.2	-	500	-
Halimeda discoidea	-	250	3.9	1000	15.6	15.6	7.8	500	500	-
Boodlea coacta	-	1000	62.5	62.5	62.5	125	62.5	1000	500	500
Boodlea composita	-	-	250	500	15.6	125	125	-	-	500
Valoniopsis pachynema	-	500	31.2	15.6	7.8	62.5	62.5	-	1000	-
Distyosphaeria cavernosa	-	250	15.6	500	7.8	31.2	15.6	-	500	-
Distyosphaeria versluysii	-	125	1.9	250	7.8	31.2	3.9	250.0	125	-
Valonia fastigiata	-	-	62.5	500	250	250	3.9	-	-	-
Caulerpa cupresoides	-	-	62.5	31.2	62.5	500	250	-	31.2	-
Rhodophyta										
Amansia rhodantha	-	-	62.5	250	62.5	31.2	31.2	-	-	-
Rhodymenia anstomosan	-	-	-	1000	1000	250	125	-	-	125
Gelidiopsis scoparia	-	-	1000	-	125	-	125	-	-	-
Laurencia perforata	-	-	1000	-	1000	-	500	-	-	-

Hemagglutination-inhibition test was carried out as described in Materials and methods. Each value indicates the lowest concentration of sugar (mM) and glycoprotein (μg/ml) at which a complete inhibition of hemagglutination (titer, 4) was achieved.

T, transferrin; A-T, asialo-transferrin; F, fetuin; A-F, asialo-fetuin; PTG, porcine thyroglobulin; A-PTG, asialo-porcine thyroglobulin; PSM, porcine stomach mucin; A-PSM, asialo-porcine stomach mucin; YM, yeast mannan.

[a] Indicate no inhibition at the concentration of 100 mM for monosaccharide and

2,000 μg mL^{-1} for glycoprotein

Discussion

The 42 species surveyed, 17 Chlorophyta, 22 Rhodophyta and 3 Phaeophyta species were found to contain hemagglutinins active toward at least one type of erythrocyte tested. All of algal species surveyed were active hemagglutinins. Of the algal species surveyed in this study, 13 Chlorophyta, 18 Rhodophyta and 2 Phaeophyta species were newly found to contain hemagglutinins. From the results of this screening we observed that enzyme-treated sheep and rabbit erythrocytes were more sensitive to detect the hemagglutinating activity on the algal extracts examined. Similar observations with other Brazilian marine algae have been reported by Ainouz [2]. Furthermore, animal erythrocytes have been reported to be more suitable for lectin detection in marine algae than human cells [16, 8, 11, 15, 9].

Hemagglutination–inhibition studies on 14 algal species were carried out using simple sugars and glycoproteins. As for most algal lectins, the glycoproteins showed to be good inhibitors of hemagglutination activity present in marine algae. The lack of specificity for simple sugars from these algal preparations in the survey is in agreement with several works already reported that many algal lectins have affinity for glycoproteins, but not for monosaccharides [15,13,24] and appears to be a common feature of many algal lectins. The 14 algal hemagglutinins examined, activities of all the hemagglutinins were inhibited by glycoproteins bearing high mannose N-glycans, complex N-glycans, or O-glycans. Among the inhibitory glycoproteins tested, asialo-transferrin, asialo-fetuin, and asialoporcine thyroglobulin were the best inhibitors against almost all hemagglutinins examined, indicating that elimination of sialic acid residues from parental glycoproteins enhanced greatly inhibitory potential of parental glycoproteins. Strong inhibition by asialo-glycoproteins bearing complex type N-glycans has also been reported for several marine algal hemagglutinins such as those from *Hypnea japonica* [13], *Hypnea cervicornis, Hypnea musciformis* [20] and *Ptilota serrata* [25]. Based on the present preliminary results, lectins specific for high mannose N-glycans, complex N-glycans or O-glycans are expected to be present in Vietnamese marine macroalgae.

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