A SURVEY OF ANTARCTIC ALGAE FOR AGGLUTININS

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ABSTRACT

Since the discovery of haemagglutinating activity in marine algal extracts in 1966, several algal haemagglutinins (lectins) have been detected, isolated and characterized. However, information is slowly emerging, concerning biochemical characteristics of lectins from Antarctic marine algae. Lectins are proteins or glycoproteins which bind, reversibly, to carbohydrates. Eighteen species of Antarctic marine algae have been tested for the presence of haemagglutinins against a several types of erythrocytes (rabbit, chicken, goat, sheep and ABO human blood groups). The protein extracts were prepared in phosphate buffered saline (PBS) pH 7.0. Serial doubling dilutions of extracts were prepared using 0.85% NaCl and an equal volume of 2% suspension of native or enzymatic treated erythrocytes was added to each tube, gentle mixed and left at 25 °C for 60 min before examination for agglutination. Fourteen species, among eighteen, produced haemagglutination of at least one type of blood cell tested. Protein extracts of three species were subjected to haemagglutination inhibition studies performed with a selection of sugars or glycoproteins. The activity of *Adenocystis utriculares* extracts was the only one inhibited by simple sugars. The other two extracts (*Georgiella confluens* and *Gigartina skottsbergii*) were not inhibited by mono- and polysaccharides, but were inhibited by glycoproteins.

Key-words: Agglutinins, Antarctic seaweed, sugar specificity.

RESUMO

AGLUTININAS DE ALGAS ANTÁRTICAS. Desde a descoberta da presença de atividade hemaglutinante em extratos de algas marinhas em 1966, várias hemaglutininas (lectinas) de algas têm sido detectadas, isoladas e caracterizadas. Entretanto, novas informações surgem lentamente, em relação às características bioquímicas de lectinas de algas marinhas Antárticas. Hemaglutininas (lectinas) são proteínas ou glicoproteínas que se ligam, reversivelmente, a carboidratos. Dezoito espécies de algas marinhas Antárticas foram testadas quanto à presença de atividade hemaglutinante contra vários tipos de eritrócitos (coelho, galinha, carneiro, ovelha e humano dos grupos ABO). Os extratos protéicos foram preparados em tampão fosfato salino (PBS) pH 7,0. Diluições seriadas de extratos foram preparadas usando NaCl 0,85% e um volume igual de uma suspensão de eritrócitos a 2%, nativos ou tratados enzimaticamente, foi adicionada em cada tubo e incubada a 25 ºC por 60 minutos antes da análise da hemaglutinação. Quatorze espécies, entre dezoito, foram capazes de produzir hemaglutinação ao menos contra um tipo de células sangüíneas testadas. Extratos protéicos de três espécies foram submetidos a estudos de inibição da atividade hemaglutinante através da utilização de açúcares e glicoproteínas. A atividade do extrato de *Adenocystis utriculares* foi inibida somente por açúcares simples. Os outros dois extratos (*Georgiella confluens* e *Gigartina skottsbergii*) não foram inibidos por mono- e polissacarídeos, sendo inibidos somente por glicoproteínas.

Palavras-chaves: Aglutininas, algas marinhas Antárticas, especificidade a açúcares.

INTRODUCTION

The marine benthic flora of Antarctica is thought to comprise in excess of 120 species (review by Clayton 1994). However, present knowledge of Antarctic benthic marine algae and their distribution is still far from comprehensive in that the sites of algal collections are inevitably concentrated around certain more accessible and more highly frequented locations, many of them close to scientific bases. Phycological studies in Antarctica began last century with the specimens collected by early exploring expeditions. They continued with the work of Skottsberg and various recents expeditions, many including divers (review by Wiencke 1996). The Antarctic seaweed study started in 1817 with the expedition of Gaudichaud, Bory, Montagne, Hooker and Harvey (Papenfuss 1964, Godley 1965), from this date the studies have been intensified in the areas of the taxonomy and biogeography. Some works about Antarctic seaweed have been published, being in its majority related to the occurrence, distribution and taxonomy of different sorts and species. Gain (1912) published a note reporting a new species of *Monostroma* proceeding from Antarctic, later the same author cited the occurrence of three Antarctic macro seaweed species. Zielinski (1981) cited the occurrence of one green seaweed species, five red seaweed species and six brown seaweed species, found in the Admiralty Bay (King George Island, South Shetland Islands). Ramirez (1986) published a systematic list of 46 benthic algal species, being the units catalogued in the Herbarium of the National Museum of Natural History of Santiago, in Chile. Wiencke (1996) published a review about the Antarctic seaweed research reporting the description of the exploration, environment, anatomy, physiology, seasonal development, demand of luminosity, depth and demand of temperature, geographic distribution and the effect of the dehydration, salinity and temperature on the Antarctic seaweed. The haemagglutinins, agglutinins or lectins are carbohydrate-binding proteins having specific binding for some types of mono- or polysaccharides, widely distributed in nature and constituted a group of proteins or glycoprotein present in a wide range of organisms from bacteria to animals (Liener 1986). This carbohydrate-binding specificity has made them interesting tools in a variety of biochemical and biomedical research areas. In basic and medical sciences, lectins are useful for: detection of disease-related alterations of glycan synthesis; blood group typing and definition of secretor status; quantification of aberrations of cell surface glycan presentation, e.g., in malignancy; cell markers for diagnostic purposes including infectious agents (viruses, bacteria, fungi, parasites), and they may also be used to target therapeutic agents (Rudiger & Gabius 2001).

The first report of the occurrence of lectins in marine algae is relatively recent, since then, several purification studies of lectins from marine algae have been reported (Boyd *et al.* 1966, Rogers *et al.* 1980, Fabregas *et al.* 1985, Hori *et al.* 1988, 1990, 2000, Ainouz & Sampaio 1991, Sampaio *et al.* 1998, 1999, 2002, Kawakubo *et al.* 1999, Nagano *et al.* 2002, Ambrosio *et al.* 2003), but the number of these proteins purified and characterized is small in comparison to lectins from higher plants. Algal lectins differ from higher plant lectins in a variety of properties. In general, algal lectins have lower molecular weight then higher plant lectins and have no affinity for simple sugars but are more specific for complex oligosaccharides, often glycoproteins. In marked contrast to higher land plant lectins, marine algal lectins have been isolated and characterized at a much lower pace since the first report of hemagglutinating activity in these organisms almost 40 years ago (Boyd *et al.* 1966). However, studies related agglutinins from Antarctic seaweeds have not been developed. Further, screening and characterization of algae lectins would be helpful for identification of new lectins and for clarification of the biological significance and molecular evolution of lectins in such organisms (Cardoso *et al.* 2007). The aim of present study was reports the first screening for lectin activity in aqueous extracts from 18 algae collected in King George Island, South Shetland Islands, Antarctic. An1alyses included competitive inhibition experiments of the binding affinity for simple sugars and glycoproteins of the active fractions.

MATERIALS AND METHODS

CHEMICALS

Carbohydrates, proteolytic enzymes and bovine serum albumin (BSA) were purchased from Merck (Darm-stadt, Germany) or Sigma (St. Louis, Missouri, USA) at the highest purity available. All other reagents used were of analytical grade.

MARINE ALGAE

All species were collected during visits to several localities on the South Shetland Islands and the Antarctic Peninsula during the Brazilian expeditions XX and XXI (summers 2001/2002 and 2002/ 2003). Upon collection, the algae were cleaned of epiphytes, rinsed with tap water, and stored at –20 °C before utilisation.

EXTRACTION AND HAEMAGGLUTINATION ASSAYS

The protein extracts were prepared by homogenization with 3 volumes of (0.025M) phosphate buffered saline (PBS) pH 7.0. After centrifugation at 10,000 x g for 30 min at 4 °C the supernatants were stored at –20 °C until used. Haemaglutination tests were carried out with erytrocytes from human and various animals in native state or enzyme-trated with trypsin, bromelain, papain and subtilisin, following standards procedure (Ainouz & Sampaio 1991, Freitas *et al.* 1997). Rabbit red blood cells were obtained by venous puncture from healthy animals reared at the Department of Biochemist and Molecular Biology, UFC. Chicken, goat and sheep blood cells were obtained from healthy animal reared at the Department of Zootecnia, UFC. Human blood samples (ABO system) were obtained from healthy donors at the Hematology Center of the UFC. Serial doubling dilutions of extracts were prepared using 0.85% NaCl. An equal volume of 2% suspension of native or enzymatic treated erythrocytes was added to each dilution. The tubes were gently shaken and left for 60 min, at room temperature, after the degree of macroscopic agglutination was observed. The greatest dilution that could agglutinate the erythrocytes, the haemagglutination titre, was defined as containing one haemagglutinating unit per mL. The activity of the algal extract was recorded as the minimum amount of protein that caused agglutination. Protein concentrations were determined by the method of (Bradford 1976) using BSA as standard.

HAEMAGGLUTINATION INHIBITION ASSAY

Inhibition studies were performed using three species algal protein extracts: *Georgiella confluens, Gicartina Skottsbergii, Adenocystis utricularis*. The carbohydrate-binding specificity of the purified lectin was assessed by the ability of a series of sugars (L(+)-arabinose, D(-)-cellobiose, D(+)-fructose, D(+)-galactose, D(+)-glucose, D(+)-raffinose, L(+)- and D(+)-rhamnose, D(+)-mannose, D(+)-xylose N-acetyl-D-glucosamine, N-acetyl-D–galactosamine, D(+)-glucosamine, Salicine, lactose, methyl-α-D-galactopyranoside) and glycoproteins (avidin, fetuin, mucin from porcine stomach, mucin from bovine submaxillary gland and mannan from *Saccharomyces cerevisiae*) to inhibit its haemagglutination activity against trypsin treated erythrocytes from chicken. The inhibition assay was carried out by 2-fold serial dilutions of sugars (0.1 M) or glycoprotein (5 mg/mL) solutions in 0.15 M NaCl with a final volume of 100 μL. An equal volume of lectin solution was added to each tube and the mixture allowed interacting for 1 h at room temperature. A 2% suspension of trypsin-treated erythrocytes from chicken was added to each tube for a final volume of 200 μL, and this mixture was incubated at 37 °C for 30 min followed by another 30 min interval at room temperature. The lowest concentration of a specific sugar or glycoprotein that inhibited haemagglutination (minimum inhibitory concentration, MIC) was recorded and used to define inhibitory potency (Ainouz *et al.* 1995).

RESULTS

The results of the screening programmer are summarized in Table I and Table II. The Sheep erythrocytes were the most suitable to detect the haemagglutinating activity followed by rabbit, chicken (Table I). Human B, human A and O (Table II). The goat erythrocytes present the smaller haemaglutination degree (Table I).

RHODOPHYTA

All the seven species showed haemagglutinating activity against at least one type of erythrocytes. Protein extracts of *Palmaria decipiens* and *Gigartina skottsbergii*, exhibited positive agglutination for all the types of erythrocytes used. The extracts of *Georgiella confluens* agglutinated sheep and chicken native and enzyme-treated erythrocytes. *Pantoneura plocamioides*, agglutinated chicken and rabbit erythrocytes and *Gymnogongrus turquetii* agglutinated chicken and sheep treated erythrocytes.

Table I. Haemagglutinating activity of protein extracts from Antarctic algae for different types of animal erythrocytes.

Species	Rabbit					Chicken					Goat					Sheep				
	N	T	B	P	S	N	T	B	P	S	N	T	B	P	S	N	T	B	P	S
Rhodophyta																				
Georgiella confluens	-	-	-	-	-	112.5	4.7	28.2	56.2	56.2	-	-	-	-	-	112.5	112.5	50.0	112.5	28.2
Pantoneura plocamioides	-	-	300	300	-	-	-	112.5	112.5	-	-	-	-	-	-	-	-	-	-	-
Palmaria decipiens	112.5	28.2	56.2	56.2	28.2	-	450	450	225	28.1	-	-	-	7.1	112.5	112.5	112.5	112.5	56.2	28.1
Gigartina skottsbergii	-	912.5	-	-	-	-	-	-	-	14.2	-	-	1.8	57	14.2	1.8	912.5	1.8	1.8	456.2
Gymnogongrus turquetii	-	-	-	-	-	-	-	-	-	450	-	-	-	-	-	-	-	-	-	225
Halimenia sp	-	239.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Porphyra sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phaeophyta																				
Desmarestia antarctica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chordaria linearis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Durvillaea antarctica	-	-	-	2.1	135.5	-	-	-	-	-	-	-	-	-	-	-	-	135.6	-	-
Phaeurus antarcticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adenocystis sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	228.8	-	114.4	-	-
Adenocystis utricularis	-	-	-	3.7	15	-	-	-	-	-	-	-	-	-	-	240.4	120.2	30	120.2	240.4
Cystosphaera jacquinotii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorophyta																				
Eteromorpha lingulata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	61.5	-	-
Monostroma hariotii	-	-	-	-	-	-	-	-	164.6	-	-	-	-	-	-	-	-	-	-	-
Prasiola cladophyla	-	-	-	-	39.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prasiola crispa	-	451.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The values represents the minimum concentration (μg/mL) of total protein which produced agglutination; (-) No detectable activity; N: non treated erythrocytes; T: trypsin treated erythrocytes; B: bromelain treated erythrocytes; P: papain treated erythrocytes; S: subtilisin treated erythrocytes.

Table II. Haemagglutinating activity of protein extracts from Antarctic algae for different types of human erythrocytes.

Species	A					B					O				
	N	T	B	P	S	N	T	B	P	S	N	T	B	P	S
Rhodophyta															
Georgiella confluens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pantoneura plocamioides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palmaria decipiens	-	450	450	112.5	225	-	-	-	112.5	112.5	-	225	112.5	56.25	112.5
Gigartina skottsbergii	-	912.5	1.8	228.1	456.2	-	-	-	228.1	228.1	-	456.2	114.0	-	456.2
Gymnogongrus turquetii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Halimenia sp	-	-	-	-	-	59.8	478.8	-	-	-	-	-	-	-	-
Porphyra sp	-	-	-	-	-	3.6	-	-	-	-	-	-	-	-	-
Phaeophyta															
Desmarestia antarctica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chordaria linearis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Durvillaea antarctica	-	-	-	-	-	135.6	-	-	-	-	-	-	-	-	-
Phaeurus antarcticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adenocystis sp	-	-	-	-	-	14.3	-	-	-	-	-	-	-	-	-
Adenocystis utricularis	-	-	-	-	-	120.2	-	-	-	-	-	-	-	-	-
Cystosphaera jacquinotii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorophyta															
Eteromorpha lingulata	-	-	-	-	-	129.3	-	-	-	-	-	-	-	-	-
Monostroma hariotii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prasiola cladophyla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prasiola crispa	-	-	-	-	-	9.8	-	-	-	-	-	-	-	-	-

The values represents the minimum concentration (μg/mL) of protein which produced agglutination; (-) No detectable activity; N: non treated erythrocytes; T: trypsin treated erythrocytes; B: bromelain treated erythrocytes; P: papain treated erythrocytes; S: subtilisin treated erythrocytes.

Protein extracts of *Halimenia* sp., agglutinated human blood-group B and rabbit erythrocytes. *Porphyra* sp. showed the lesser haemaglutination degree, it was only specifies for human blood-group B enzyme-treated. The minimum amount of protein required to produce agglutination was observed by *Gigartina skottsbergii* against goat, human blood-group A and sheep enzyme-treated erythrocytes. The protein extracts agglutinated enzyme-treated erythrocytes more strongly than the native cells.

CHLOROPHYTA

All protein extracts produced by the green algae agglutinated at least one kind of erythrocytes. The strongest activity was observed with *Prasiola crispa* using human B erythrocytes, although it showed a low agglutination against trypsin treated rabbit erythrocytes. *Enteromorpha lingulata* was capable to agglutinating sheep erythrocytes bromelain treated and exhibited human blood group B specificity. The extracts from *Monostroma hariotii* agglutinated only chicken papain treated erythrocytes and the *Prasiola cladophila* exhibited agglutination activity against rabbit blood cells subtilisin treated.

PHAEOPHYTA

Three out of seven species showed haemagglutinating activity against several type of erythrocyte. Protein extract of *Durvillaea antarctica* showed the strongest activity against rabbit papain treated erythrocytes. The extract from *A. utricularis* agglutinated preferentially rabbit, sheep and human blood group B erythrocytes. The protein extracts from *Desmarestia antarctica*, *Chordaria linearis*, *Phaeurus antarcticus*, *Cystosphaera jaquinotii*, failed to exhibit agglutination activity with all types of erythrocyte.

HAEMAGGLUTINATION INHIBITION STUDIES

The three extracts showing strong haemagglutination titres were used for sugar inhibition studies with several simple sugars and glycoproteins (Table III). Only the extract from *A. utricularis* showed inhibition by simple sugars, although the glycoproteins failed to inhibit the haemagglutinating activity. The haemagglutinating activity of the *Georgiella confluens* and *Gigartina skottsbergii* were inhibited only by glycoproteins. The *G. skottsbergii* extracts were inhibited more strongly by mucin from bovine submaxillary gland followed by fetuin. *G. confluens* extracts were inhibited equally by fetuin and mucin from porcine stomach. Avidin and mannan from *Saccharomyces cerevisiae* failed to inhibit the agglutination activity of the three species tested.

DISCUSSION

Marine algae from only a few parts of the world have been examined for agglutinins and the number of active species was found to be very limited in comparasion with that of higher plants. Lectin activity in 78% of these previsouly unexamined species was detected and exhibited positive results with at least against one of the different types of erythrocytes tested. From the results of this screening we observed that sheep and rabbit enzyme-treated erythrocytes were more sensitive to detect the haemagglutinating activity on the algal extracts examined. Similar observations with Brazilian marine algae have been reported by (Ainouz *et al.* 1992, Freitas *et al.* 1997, Zheng & Lu 2002). Due to the weak agglutination against human erythrocytes produced by seaweed extracts, (Rogers *et al.* 1980) used 2% erythrocytes suspensions contend albumin or erythrocytes enzymed-treated, the objective was to display the carbohydrate and glycoconjugates that exists on the surface of erythrocytes membrane, facilitating the agglutination. In this work, 49 green, red and brown marine algae species had been used, however only 14 extracts produced agglutination. The addition of the albumin or the enzymatic treatment caused increment in the haemagglutination activity of the majority of extracts. Furthermore, animal erythrocytes have been reported to be more suitable for lectin detection in marine algae than human cells (Fabregas *et al.* 1985, Hori *et al.* 1988, Chiles & Bird, 1989, Hori *et al.* 1990, Dalton *et al.* 1995). Only one algae extract (*Porphyra* sp.) tested showed specific agglutination towards human blood types. However, eight protein extracts were capable to agglutinating human erythrocytes, beyond animals erythrocytes. (Zheng & Lu 2002) related that thirty-three species of marine algae belonging to Rhodophyta, Phaeophyta and Chlorophyta from the Fujian cost were examined for agglutinins with different animal and human erythrocytes. Protein extracts from 26 species were active against at least one type of the erythrocytes

Table III. Inhibition of erythrocyte agglutination activity.

Simple sugar	Extracts		
	A. u	*G.c*	*G.s*
N-acetyl - D-Glucosamine	-	-	-
N-acetyl - D-Galactosamine	-	-	-
D(+)glucosamine	6.25	-	-
L(+) rhamnose	6.25	-	-
D(+) rhamnose	0.4	-	-
Salicine	0.4	-	-
D(+) galactose	-	-	-
D(+) glucose	-	-	-
Lactose	-	-	-
L(+) arabinose	-	-	-
methyl-α-D-galactopyranoside	-	-	-
D(+) mannose	-	-	-
D(-)cellobiose	-	-	-
D(+) raffinose	-	-	-
D(+) xilose	-	-	-
D(+) fructose	-	-	-
Glycoproteins			
Avidin	-	-	-
Fetuin	-	2.5	2.5
Mucin from bovine submaxillary gland	-	-	0.5
Mucin from porcine stomach	-	2.5	-
Mannan from *Saccharomyces cerevisiae*	-	-	-

(-) No detectable inhibitory activity

A.u = *Adenocystis utricularis*; G.c =*Georgiella confluens* ; G.s = *Gigartina skottsbergii*, Minimum concentration (mM or mg/mL for simple sugars or glycoproteins, respectively) of the substance which produced inhibition.

tested and 16 were capable to agglutinating human erythrocytes. Haemagglutination inhibition studies of three algal species were carried out using simple sugars and glycoproteins. The agglutination activity of *A. utricularis* was inhibited only by simple sugars while the *Georgiella confluens* and *Gigartina scottsbergii* were inhibited only by glycoproteins. The lack of specificity for simple sugars by these algal preparations is in agreement with several works already reported in the literature (Hori *et al.* 1990, Rogers & Hori 1993, Dalton *et al.* 1995) and appears to be a common feature of many algal lectins. It is generally considered that algal agglutinins lack specificity for monosaccharides. This consideration is supported by the fact that agglutinic activity of many purified algal agglutinins was not inhibited by monosaccharides (Ferreiros & Criado 1983, Rogers & Topliss 1983, Hori *et al.* 1986, Kanoh *et al.* 1992, Kakita *et al.* 1997). However, lectins from the genus *Codium* have been reported to be inhibited by *N*-acetylgalactosamine (Bird *et al.* 1993). (Alvarez-Hernandez *et al.* 1999) also showed that this monosaccharide was specific for an agglutinin purified from the same species. The red marine algae *Ptilota plumosa* (Rogers *et al.*1977) and *Ptilota serrata* (Rogers *et al.* 1990) exhibited strong inhibition by galactose and their derivatives. Activity present in *Caulerpa cupressoides* (Freitas *et al.* 1997) exhibited inhibition by simple sugars galactose, lactose, raffinose and fructose. These results suggests that haemagglutinins in Antarctic marine algae are a new survey to the isolation and characterization of these class of proteins.

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