CHARACTERIZATION OF THE C-TYPE LECTIN FROM THE MARINE SPONGE (*STYLISSA FLEXIBILIS*)

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SUMMARY

A lectin from the marine sponge *Stylissa flexibilis*, designated as SFL, was purified by cold ethanol precipitation followed by ion exchange chromatography on DEAE Sepharose column and Sephacryl S-200 gel filtration. SFL is a dimeric glycoprotein of 32 kDa subunits linked by a disulfide bridge with a molecular mass of 64 kDa by SDS-PAGE and 65 kDa by Sephacryl S-200 gel filtration chromatography. The lectin preferentially agglutinated enzyme treated human A erythrocytes, whereas it did not agglutinate any type of rabbit, human B and O erythrocytes, irrespective of treatment with enzymes. The hemagglutination activity of lectin was strongly inhibited by monosaccharide, D-galactose and glycoproteins, asialo-porcine stomach mucin and asialo-fetuin, indicating that lectin is specific for O-glycans. Activity of SFL was stable over a range of pH from 5 to 8, up to 60 °C for 30 min and its activity was Ca^{2+} dependent, indicating that SFL was belonged to the C-type lectin family and requires metal for biological activity. SFL caused agglutination of *Vibrio alginolyticus* and *V. parahaemolyticus* in a dose dependent manner and inhibited the growth rate of these bacterial strains, suggesting that the lectin caused the agglutination through binding to the target receptor(s) on the surface of Vibrios. Thus, SFL can be considered as a good source of lectin(s) being useful as carbohydrate probe and antibacterial reagent.

Keywords: *Stylissa flexibilis, antibacterial activity, carbohydrate binding specificity, lectin, marine sponge*

INTRODUCTION

Lectins, or carbohydrate-binding proteins, are present in various organisms from viruses to mammals 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 also are promising candidates for medicinal and clinical application (Sharon, Lis, 2003).

Recently, a large number of lectins from marine sponges possessing various biochemical characteristics have been purified and identified including: galectins, C-type, tachylectin-like and F-type lectins (Gardères *et al*., 2015). Like other natural products isolated from marine organisms, lectins from marine sponges have shown great potential as candidates for new drugs, due to their wide range of biological activities, such as pro-

inflammatory and antitumoral (Kawsar *et al*., 2011; Queiroz *et al*., 2009), mitogenic (Atta *et al*., 1989; Bretting *et al*., 1981a; Xiong *et al*., 2006; Dresch *et al*., 2012), chemotactic (Dresch *et al*., 2008; Queiroz *et al*., 2008), cytotoxic properties (Pajic *et al*., 2002), antibacterial activities (Schröder *et al*., 2003) and antiprotozoan effects against *Leishmania chagasi* (Moura *et al*., 2006; Medeiros *et al*., 2010). Thus, marine sponges may be dominant sources of useful lectins for basic research and applications.

Furthermore, antibacterial activities have been reported for lectins from various biological sources (Santi-Gadelha *et al*., 2006; Charungchitrak *et al*., 2011; Schröder *et al*., 2003; Moura *et al*., 2006; Kawsar *et al*., 2011). However, little is known about the effects of lectins from marine organisms towards marine vibrios, except the species-specific activities against vibrios have been reported for the lectins from the red algae, such as ESAs from *Eucheuma serra* and GMA from *Galaxaura marginata* (Liao *et al*., 2003), EDAs from *Eucheuma denticulatum* (Le

Dinh Hung *et al*., 2015a), KSAs from *Kappaphycus striatum* (Le Dinh Hung *et al*., 2015b) and GPE from *Gracilaria fisheri* (Boonsri *et al*., 2017). Marine vibrios are halophilic Gram-negative proteobacteria, which occupy a diverse range of ecological niches including sediments, water column, and in association with organisms either as symbionts or pathogens (Tracy *et al*., 2007). It is an economically important disease of fish, marine invertebrates and is responsible for high mortality rates in aquaculture worldwide (Marhual *et al*., 2010). Among the Vibrios, *Vibrio alginolyticus* and *V. parahaemolyticus* are quite important, since it causes serious episode to marine fish and shellfish including shrimp (Zorrilla *et al*., 2003; Marhual *et al*., 2010). In Vietnam, *V. alginolyticus, V. parahaemolyticus* and *V. harveyi* reduced greatly yields of farmed shrimps (FAO, 2013). Thus, the objective of this research was to report on the isolation, biochemical properties, carbohydrate-binding specificity and biological effect including the antibacterial activities against the shrimp pathogenic vibrios of the lectin from marine sponge *S. flexibilis* for future applications.

MATERIALS AND METHODS

Materials

Marine sponge *Stylissa flexibilis* (Lévi, 1961) was collected at the Vinh Hy bay (108°59′27″E, 11°34′39″N), Ninh Thuan Province, Vietnam, in April, 2016, brought to the laboratory, and kept at -20 °C until used. The marine sponge samples were identified by MSc. Thai Minh Quang – Institute of Oceanography, Vietnam. Prepacked columns used were purchased; Sephacryl S-200 (1.6×60 cm) and DEAE Sepharose fast flow ion exchange chromatographic column (1.6×20 cm) from GE Healthcare (Sweden). Blood from rabbit was obtained from the Institute of Vaccine Nha Trang, Vietnam and human A, B, and O bloods from Khanh Hoa General Hospital, Vietnam. L-fucose, D-glucose, D-mannose, D-galactose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, N-acetyl-D-galactosamine, transferrin, fetuin, porcine thyroglobulin, and porcine stomach mucin were purchased from Sigma Chemical Co. Yeast mannan and N-acetyl-neuraminic acid was from Nakarai Chemical Co. Three species of shrimp pathogenic Vibrios, *Vibrio alginolyticus, V. harveyi* and *V. parahaemolyticus* were obtained from Research Institute for Aquaculture No. 3, Vietnam.

Extraction and purification of lectin

Specimens were ground to a fine powder and extracted with 50 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl (TBS) for 6 h at 4°C. After filtration through a cheese cloth, the filtrate was centrifuged at 3500×g for 30 min at 4°C. To the supernatant, cold absolute ethanol (-20°C) was added to attain a final concentration of 80% and the mixture was kept at 4°C overnight. The resulting precipitates were collected by centrifugation at 3500×g for 30 min at 4°C and thoroughly dialyzed against 20 mM Tris–HCl buffer (TB), pH 7.5. The non-dialyzable fraction was applied to a DEAE Sepharose fast flow ion exchange chromatographic column (1.6×20 cm), equilibrated with the above buffer. After elution of unbound proteins and pigments in TB, column was eluted with 0.5 M NaCl in 20 mM Tris–HCl buffer, pH 7.5; the active fractions were pooled, concentrated by ultrafiltration, and dialyzed against 50 mM Tris–HCl, 150 mM NaCl buffer (pH 7.5). The concentrate was subjected to gel filtration on a Sephacryl S-200 column (1.6×60 cm) and eluted with the same buffer. The eluate was monitored at absorbance of 280 nm for protein and for hemagglutination activity with trypsin-treated human A erythrocytes. Active fractions were pooled for further analysis (Moura *et al*., 2006).

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

Each blood sample was washed three to five times with 50 volumes of 150 mM NaCl. After washing, a 2% erythrocyte suspension (v/v) was prepared in 150 mM NaCl 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 in saline (Le Dinh Hung *et al*., 2009).

Hemagglutination assay

Hemagglutination assays were carried out using a microtiter method in a 96-well microtiter V-plate (Le Dinh Hung *et al*., 2009). First, 25 µL amounts of serially two-fold dilutions of a test solution were prepared in 50 mM Tris–HCl, 150 mM NaCl buffer (pH 7.5) containing 20 mM CaCl$_2$ on a microtiter V-

plate and incubated at room temperature for 1 h. To each well, 25 μL of a 2% erythrocyte suspension was added and the mixtures gently shaken and incubated at room temperature for 2h. 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 duplicate for each test solution.

Hemagglutination-inhibition test

Hemagglutination-inhibition tests were carried according to the method previously described (Le Dinh Hung *et al*., 2009) with minor modification. Briefly, first, 25 μL amounts of serially two-fold dilutions of sugar or glycoprotein were prepared in TBS containing 20 mM $CaCl_2$. To each well, an equal volume of a lectin solution (4 doses of agglutination) prepared in TBS containing 20 mM $CaCl_2$ was added, and the plate was mixed gently and allowed to stand at room temperature for 1h. Finally, 25 μL of a 2% suspension of trypsin-treated human A erythrocytes was added to each well, and the plate was gently shaken and incubated for a further 1h. Inhibition was observed macroscopically and inhibition activity was expressed as the lowest concentration of sugar or glycoprotein. The assay was performed in duplicate per sugar and glycoprotein.

Preparation of trypsin-treated porcine stomach mucin

Porcine stomach mucin (10 mg) was dissolved in 5 mL of 50 mM TBS (pH 7.5). Trypsin (5 mg) was added to the sample and the solution obtained was incubated at 37°C for 24h. Treated PSM was heated to 100°C for 30 min then cooled (final reaction volumes were 10 mL) and further used as inhibitor.

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*., 2009) with minor modification. 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 TBS, pH 7.5. The hemagglutination activity was determined in the absence or presence of $CaCl_2$. To examine the effect of temperature, each the lectin solution was treated at various temperatures (30 – 100°C) for 30 min, then immediately cooled on ice, and hemagglutination activity was determined as above. To examine the effect of pH, each the lectin solution was dialyzed at 4°C overnight against 100 mL of 50 mM buffers of various pH from 3 to 10 and then dialyzed against 150 mM NaCl solution containing 100 mM $CaCl_2$ to eliminate the pH effect. Hemagglutination activity was determined with trypsin-treated human A 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 fractions of 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 for proteins. The molecular mass of the native lectin was measured by passing it through the Sephacryl S-200 (1.6×60 cm) column in TBS buffer, pH 7.5. The standard proteins used were phosphorylase B (94 kDa), BSA (67 kDa), ovalbumine (45 kDa) and carbonic anhydrase (30 kDa).

Antibacterial activity

Antibacterial activity was determined according to the method of Charungchitrak *et al*. (2011). Bacteria, *Vibrio alginolyticus, V. harveyi* and *V. parahaemolyticus* obtained from Research Institute for Aquaculture No. 3, VietNam, were grown in LB broth (10 g bacto-tryptone, 5 g bacto-yeast extract, 10 g NaCl, 1 L H_2O, pH 7·0) and shaken at 200 rpm for 16 - 18 h at 37°C. Cells were collected, washed three times with 0.15 M NaCl solution and converted to colony forming units (10^5–10^6 CFU mL^{-1}). Serial two-fold dilutions of an original solution of SFL (100 μg mL^{-1}) were prepared in TBS containing 20

mM CaCl$_2$ in test tubes. To each dilution, a 100 μL of each bacterial suspension (10^5–10^6 CFU mL^{-1}) was added, and the mixtures were gently shaken and incubated at 37°C for 24h. As a positive reference using ampicillin solution (1000 μg mL^{-1}) and negative control without both lectin and ampicillin using TBS containing 20 mM CaCl$_2$, all was examined in the same way. After incubation, the turbidity of reaction solutions was measured at 600 nm as the index of bacterial growth. The bacterial growth in the presence of SFL and ampicillin was compared to that in a control. The inhibition activities of SFL at various concentrations were determined as the ratio (%) to that of ampicillin (1000 μg mL^{-1}) and expressed as an efficient concentration (EC$_{50}$) relative to the inhibition activity of ampicillin at 1000 μg mL^{-1}. All assays were carried out in triplicate.

RESULTS AND DISCUSSION

Extraction and purification of lectin

The lectin from crude extract of the marine sponge *S. flexibilis* was recovered as a precipitate with 80% cold ethanol and showed strong hemagglutination activity towards trypsin- and papain-treated human A erythrocytes in TBS buffer containing 20 mM CaCl$_2$, whereas it did not agglutinate erythrocytes of native or enzyme treated rabbit and human B and O blood types (Table 1).

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) exhibited strong hemagglutination activity, whereas the second peak (II) showed no activity (Figure 1b). Thus, the purified lectin was designated as SFL. The results of purification are summarized in Table 2.

Physical and chemical properties of SFL

SFL gave a band of 64 kDa in the absence of a reducing agent (β-mercaptoethanol) and consisted of two identical subunits of 32 kDa in reducing condition (Figure 2a). The native molecular mass of SFL was estimated to be 65 kDa by gel filtration (Figure 2b). Thus, the lectin was a dimeric rotein composed of two identical subunits of 32 kDa, which is linked by a disulfide bond.

Table 1. Hemagglutination activity of the precipitated fraction from the crude extract of the marine sponge *S. flexibilis*. The hemagglutination activity is expressed as a titer that is the reciprocal of the highest two-fold dilution exhibiting positive agglutination.

Hemagglutination titer with erythrocytes (H.U./mL)																							
Rabbit						Human A						Human B						Human O					
TBS			TBS+Ca			TBS			TBS+Ca			TBS			TBS+Ca			TBS			TBS+Ca		
N[a]	T[b]	P[c]	N	T	P	N	T	P	N	T	P	N	T	P	N	T	P	N	T	P	N	T	P
-[d]	-	-	-	-	-	2	2	2	4	128	128	-	-	-	-	-	-	-	-	-	-	-	-

[a] Native erythrocytes. [b]Trypsin-treated erythrocytes. [c]Papain-treated erythrocytes. [d]No hemagglutination. - TBS: 20 mM Tris-HCl, 0.15 M NaCl, pH 7.5 buffer. - TBS+Ca: 20 mM Tris-HCl, 0.15 M NaCl, pH 7.5 buffer containing 20 mM CaCl$_2$.

Table 2. Summary of purification of lectin from the marine sponge *S. flexibilis*.

| Extraction and purification step	Protein (mg)	Total activity[a] H.U./mL	Specific activity[b] H.U/mg	Yield (%)	Purification factor
Extraction	381.9	3040	7.9	100	1
Ethanol precipitation	46.8	2816	60.2	92.6	7.6
Ion exchange	10.4	896	86.2	29.5	10.9
Gel filtration	2.9	332	114.4	10.9	14.5

[a]Inverse of the highest dilution still causing agglutination of trypsin-treated human A erythrocytes. [b]Hemagglutination units per mg of protein.
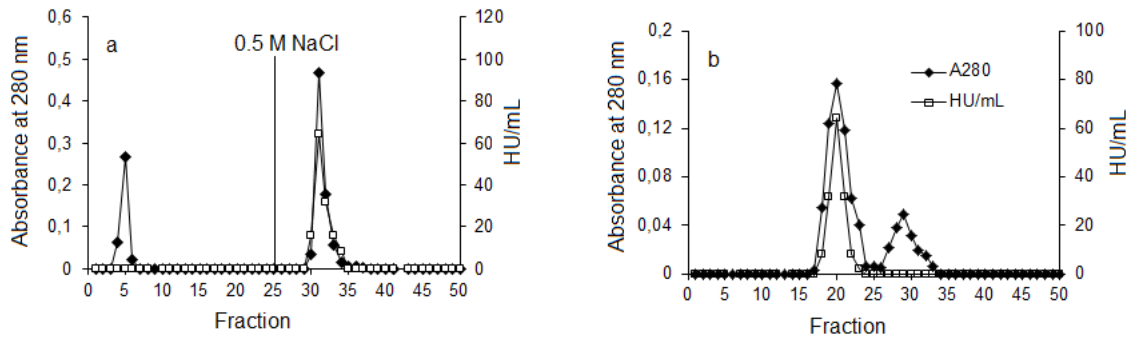
Figure 1. (a) Ion-exchange chromatography of the precipitate fraction obtained from crude extract of the marine sponge *Stylissa flexibilis* on a DEAE Sepharose fast flow column. The column (1.6×20 cm) was equilibrated with 50 mM Tris– HCl buffer, pH 7.5, lectin was eluted with 0.5 M NaCl in the equilibration buffer (lectin-containing fractions are indicated). **(b)** Gel chromatography on a Sephacryl S-200 column (1.6 ×60 cm) of the active peak obtained by ion-exchange chromatography. The column was eluted with 50 mM Tris– HCl, 150 mM NaCl buffer, pH 7.5 at a flow rate of 0.8 mL min^{-1}. Fractions were collected and measured at absorbance of 280 nm (——◆——) for protein and for hemagglutination activity with trypsin-treated type A erythrocytes (——☐——). HU hemagglutination unit.
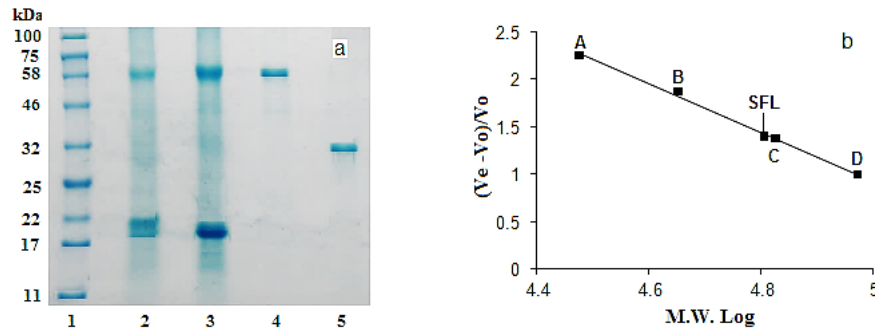
Figure 2. (a) SDS-PAGE of the lectin isolated from the marine sponge *S. flexibilis*. SDS –PAGE was carried using a 10% polyacrylamide gel. Protein bands were stained with Coomassie Brilliant blue R-250 reagent. Lane 1 protein marker (New England BioLabs Inc); lane 2, a 80% ethanol precipitate; lane 3, active fractions obtained from ion-exchange chromatography; lane 4, active fractions obtained by gel filtration in non-reducing condition; lane 5, active fractions obtained by gel filtration in reducing condition by β-mercaptoethanol. (b) Determination of molecular weight of SFL by gel filtration on Sephacryl S-200 column. Protein molecular weight standards were: (A) carbonic anhydrase (30 kDa), (B) ovalbumine (45 kDa), (C) bovine serum albumin (67 kDa) and (D) phosphorylase B (94 kDa).
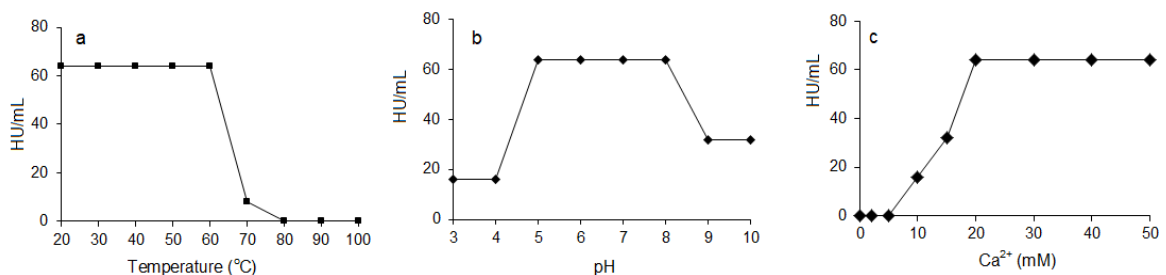
Figure 3. (a) The effects of temperature, (b) pH and (c) CaCl$_2$ on hemagglutination activities of lectin.

SFL was relatively thermostable because their activities were unchanged after incubation at 60°C for 30 min; however, the activities gradually decreased as incubation temperature exceeded 60°C (Figure 3a). The activities of SFL were stable in a range of pH from 5 to 8 (Figure 3b) and was dependent on the

presence of CaCl$_2$ as evidenced by complete activity loss in the presence of EDTA, and the addition of Ca^{2+} ions regains the activity of lectin (Figure 3c), indicating that SFL should be included in the C-type lectin family. The dependence on presence of divalent cations for biological activity has also been reported for many lectins from marine sponges, such as AaL from *Aplysina archeri* and AlL from *Aplysina lacunose (*Miarons, Fresno, 2000), AvL from *Aphrocallistes vastus (*Gundacker *et al*., 2001), CvL from *Cliona varians (*Moura *et al*., 2006) and PsL from *Pellina semitubulosa* (Engel *et al*., 1992).

Carbohydrate-binding specificity

Carbohydrate-binding specificity of SFL was examined by hemagglutination-inhibition test. Hemagglutination activity of SFL was inhibited by monosacc

Inhibition by D-galactose has been indicated for lectins from marine sponges, such as lectins from *A. vastus* (Gundacker *et al*., 2001), *Axinella dissimilis* (Bretting *et al*., 1981b), *Cinachyrella alloclada* (Atta *et al*., 1989), *C. varians* (Moura *et al*., 2006), *Desmapsama anchorata* (Atta *et al*., 1990), *Halichondria okadai* (Kawsar *et al*., 2008), *H. panicea* (Kamiya *et al*., 1990), *Haliclona cratera*

(Pajic *et al*., 2002), *P. semitubulosa* (Engel *et al*., 1992), *Axinella polypoides* (Bretting, Königsmann, 1979), *Chondrilla nucula* (Schröder *et al*., 1990), *A. archeri* and *A. lacunose* (Miarons, Fresno, 2000), *Axinella corrugate* (Dresch *et al*., 2012) and *H.okadai* (Kawsar *et al*., 2011). Lectins with affinityfor galactose appear to have important roles in modulating immune responses in marine animals (Yousif *et al*., 1994; Mistry *et al*., 2001; Kurata, Hatai, 2002). Activities of SFL were strongly inhibited by asialo-fetuin and asialo-porcine stomach mucin. Inhibition by asialo-fetuin has been evidenced for lectins from marine sponges, such as HOL-30 from *Halichondria okadai* (Kawsar *et al*., 2008) and HcL from *Haliclona cratera* (Pajic *et al*., 2002). On the other hand, inhibition by porcine stomach mucin and their asialo derivatives bearing O-glycans that is related to galactose binding specificity reported for many lectins from marine sponges, such as CauL from *Craniella australiensis (*Xiong *et al*., 2006), Halilectin 2 and Halilectin 3 from *Haliclona caerulea* (Carneiro *et al*., 2013a, 2013b), HpL from *H. panicea* (Kamiya *et al*., 1990), ACL-II from *A. corrugata* (Dresch *et al*., 2012), HcL from *Haliclona crater* (Pajic *et al*., 2002) and AaL from *A. archeri* (Miarons, Fresno, 2000).

Table 3. Hemagglutination-inhibition test of SFL with carbohydrates and glycoproteins.

| Carbohydrates	Minimum inhibitory concentration (mM)	Glycoproteins	Minimum inhibitory concentration (µg mL^{-1})
D-Mannose	[a]-	Porcine stomach mucin	500
D-Glucose	-	Asialo-Porcine stomach mucin	62.5
L-Fucose	-	Trypsin-treated Porcine stomach mucin	500
D-Galactose	6.25	Fetuin	500
N-Acety-D-Mannosamine	-	Asialo-Fetuin	62.5
N-Acetyl-D-Glucosamine	-	Transferin	-
N-Acetyl-D-Galatosamine	-	Asialo-Transferin	-
N-Acetyl neuraminic acid	-	Yeast Mannan	-

Trypsin-treated A blood group human erythrocytes were used. Inhibitory activity is expressed as the minimum inhibitory concentration of sugars that is required to inhibit completely the hemagglutination activity (4 doses of agglutination). Dashes indicate no inhibitory activity at the concentration for 100 mM for monosaccharide and 2000 µg mL^{-1} for glycoproteins.

Antibacterial activity

SFL obtained from marine sponge *S. flexibilis* significantly inhibited the growth at more than 1.56 µg mL^{-1} for both *V. parahaemolyticus* and *V. alginolyticus*, whereas it did not affect the growth of *V. harveyi* even at 100 µg mL^{-1}. The highest concentration of SFL (100 µg mL^{-1}) suppressed the

growth of *V. alginolyticus* to 78% and of *V. parahaemolyticus* to 74% in comparison to that in the control without lectin solution. The degrees of inhibition at various concentrations of SFL to that of ampicillin at 1000 µg mL^{-1} were plotted as the inhibition ratios relative to ampicillin. The antibacterial activity of SFL was comparable to that

of ampicillin with a relative EC_{50} of 6.45 µg mL^{-1} for *V. parahaemolyticus* and 5.95 µg mL^{-1} for *V. alginolyticus* (Figure 4).
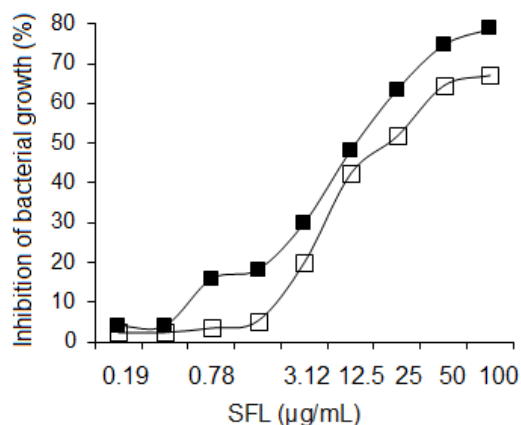
Figure 4. Antibacterial activity and inhibition of antibacterial activity. The growth of *V. alginolyticus* and *V. parahaemolyticus* after incubation at 37°C for 24h was measured at absorbance of 600 nm. The inhibitory effect of SFL against the bacterial growth was expressed in percentage where the inhibition effect of 1000 µg mL^{-1} ampicillin (a positive control) was hundred percent and that of non-lectin or -ampicillin supplemented solution (a negative control) was zero percent. SFL treatments with *V. alginolyticus* (▪) and *V. parahaemolyticus* (▫).

It has also been reported that several lectins isolated from marine sponges can inhibit the growth of various bacterial pathogens and fungi (Schröder *et al*., 2003; Moura *et al*., 2006; Kawsar *et al*., 2011). However, there seems to be not reported yet for the activities of lectin from marine sponges against shrimp and fish pathogens. SFL inhibited the growth of shrimp pathogenic bacteria, *V. alginolyticus* and *V. parahaemolyticus*, suggesting that these Vibrios have asialo-PSM like structure on the cells, which might respond as a receptor(s) for SFL, while *V. harveyi* do not have such structure on their cell surfaces. Almost all microorganisms express surface-exposed carbohydrates, which might respond as a receptor(s) for lectin. Thus, the mode of interaction between lectins with bacteria varies significantly among lectins from various organisms, mainly depending on their carbohydrate binding specificity.

CONCLUSION

SFL, a lectin from marine sponge *S. flexibilis*, showed novel properties, including a dimeric protein composed of two identical subunits linked by a disulfide bond. Hemagglutination activity of SFL was dependent on the presence of CaCl$_2$, indicating that SFL should be included in the C-type lectin family. SFL preferably bond to asialo derivatives of O-glycans that is related to galactose binding specificity, indicating that the terminal D-galactose residues in O-glycans were critical for lectin binding. SFL inhibited the growth of *V. parahaemolyticus* and *V. alginolyticus*. These results indicate that the marine sponge *S. flexibilis* may be a good source of a lectin that may be useful as an antibacterial agents.

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ĐẶC TÍNH CỦA LECTIN DẠNG C TỪ HẢI MIÊN (*STYLISSA FLEXIBILIS*)

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TÓM TẮT

Một lectin từ hải miên *Stylissa flexibilis*, được đặt tên là SFL, đã được tinh chế qua các bước kết tủa bằng ethanol, sắc ký trao đổi ion trên cột DEAE Sepharose và sắc ký lọc gel Sephacryl S-200. SFL là một glycoprotein dạng dimer 2 tiểu đơn vị 32 kDa gắn cầu nối disulfide, có khối lượng phân tử là 64 kDa với SDS-PAGE và là 65 kDa với sắc ký lọc gel trên cột Sephacryl S-200. Lectin này có đặc tính ưu tiên gây ngưng kết hồng cầu người nhóm máu A được xử lý enzyme, trái lại nó không ngưng kết với bất kỳ dạng hồng cầu thỏ, hồng cầu nhóm máu người B và O, ngay cả khi hồng cầu được xử lý enzyme. Hoạt tính của lectin bị ức chế mạnh bởi đường đơn, D-galactose và glycoproteins, asialo-porcine stomach mucin và asialo-fetuin, chỉ ra rằng lectin đặc hiệu cho O-glycan. SFL bền trong khoảng pH từ 5 đến 8, chịu được 60 °C trong 30 phút, SFL có hoạt tính phụ thuộc vào cation hóa trị hai Ca^{2+} nên thuộc họ lectin dạng C (C-type). SFL có khả năng gây ngưng kết có tính phụ thuộc vào liều lượng và ức chế sự tăng trưởng vi khuẩn *Vibrio alginolyticus,V. Parahaemolyticus* gợi ý rằng lectin này gây ra sự dính kết các tế bào thông qua việc gắn lên các thụ thể đích trên bề mặt của vi khuẩn Vibrio. Như vậy, SFL có thể được xem là một nguồn lectin tốt để dùng làm mẫu dò đối với carbohydrate và làm chất kháng vi khuẩn.

Từ khóa*: Stylissa flexibilis, đặc tính liên kết carbohydrat, Hải miên, hoạt tính kháng khuẩn, lectin*