Lectins from Tropical Sponges

PURIFICATION AND CHARACTERIZATION OF LECTINS FROM GENUS APLYSINA*

Pedro Bonay Miarons‡ and Manuel Fresno
From the Centro de Biologia Molecular “Severo Ochoa,” Universidad Autonoma de Madrid, Cantoblanco, Madrid 28049, Spain

Only a few animal phyla have been screened for the presence and distribution of lectins. Probably the most intensively studied group is the mollusk. In this investigation, 22 species from 12 families of tropical sponges collected in Los Roques National Park (Venezuela) were screened for the presence of lectins. Nine saline extracts exhibited strong hemagglutinating activity against pronase-treated hamster red blood cells; five of these reacted against rabbit red blood cells, four with trypsin-treated bovine red blood cells, and five with human red blood cells regardless of the blood group type. Extracts from the three species studied from genus Aplysina (archeri, lawnosa, and cauliformis) were highly reactive and panagglutinating against the panel of red blood cells tested. The lectins from A. archeri and A. lawnosa were purified to homogeneity by ammonium sulfate fractionation, affinity chromatography on p-aminobenzyl-β-1-thiogalactopyranoside-agarose, and gel filtration chromatography. Both lectins exhibited a native molecular mass of 63 kDa and by SDS-polyacrylamide gel electrophoresis under reducing conditions have an apparent molecular mass of 16 kDa, thus suggesting they occur as homotetramers. The purified lectins contain 3–4 mol of divalent cation per molecule, which are essential for their biological activity. Hemagglutination by hemagglutination was carried out to define the sugar binding specificity of the purified A. archeri lectin. The results indicate a preference of the lectin for nonreducing β-linked p-Gal residues being the best inhibitors of red blood cells binding methyl-β-D-Gal and thiogalactoside (Galβ1-4-thiogalactopyranoside). The behavior of several glycans on immobilized lectin affinity chromatography confirmed and extended the specificity data obtained by hemagglutination inhibition.

In animals, only a few phyla have been screened for the presence and distribution of lectins. In particular, the number of lectins that have isolated from invertebrate organisms is quite small as compared with the great variety of lectins isolated from plant origin and have been limited to those partially characterized from mollusks and crustaceans. Since the discovery of hemagglutinins in sponges by Dodd et al. (1), there have been some reports on the partial characterization (serological and immunoelctrophoretic properties) of lectins from the oldest multicellular animals (2, 3) from the Mediterranean Sea or Japan (4–8). However, their properties and specificities have not been clearly defined. In this report, we present data on an investigation undertaken to search for novel lectins in marine organisms, which could show unique properties. In addition, we describe the purification and characterization of the lectins present in two species of tropical sponges, Aplysina lawnosa and Aplysina archeri. We present information on the nature and specificity of their combining site as examined by hapten inhibition experiments and affinity chromatography.

EXPERIMENTAL PROCEDURES

Materials—The sponges were collected in the Los Roques National Park (Venezuela), in the Caribbean Sea. The sponge tissue was dried at room temperature (about 20 °C for 3 days). The dry material was reduced to powder in a mortar and stored at −70 °C until used. The dry tissue was extracted by stirring 50 g with 1 liter of 0.9% NaCl (containing 1 mM each CaCl2, MgCl2, and MnCl2 plus 0.02% NaN3) overnight at 4 °C. The extract was clarified by centrifugation at 15000 × g for 30 min at 4 °C. The supernatant, filtered through 0.2-μm filters, is referred to as crude extract.

Monosaccharides and Oligosaccharides—All glycoproteins were from Sigma. Monosaccharides and disaccharides were from Sigma and/or Dextra Laboratories. Oligosaccharides other than specified were from Dextra Laboratories. Terminal residues of neuraminic acid were removed by mild acid hydrolysis (10 mM HCl, 70 °C, 1 h) or digestion with Vibrio cholerae neuraminidase (Oxford Glycosystems, Oxford, UK). Oligosaccharides were reductively labeled with NaB3H4 or for oligosaccharides 18, 21, and 23 by galactose oxidase followed by NaB3H4 reduction. Oligosaccharide 6 was obtained from oligosaccharide 4 by digestion with jack bean α-fucosidase (Oxford Glycosystems, UK). Oligosaccharide 17 was obtained from human transferrin by Pronase digestion as described (9); in some instances, we obtained oligosaccharide 16 by hydrazinolysis of oligosaccharide 18. Oligosaccharides 19 and 20 were purified from bovine prothrombin (10), oligosaccharide 21 from BHK cells, and oligosaccharides 22 and 23 from bovine fetuin (11) as described (12). Oligosaccharide 24 was prepared from the tetrasialylated fraction of human α1-acid glycoprotein after hydrazinolysis (13).

Hemagglutination Assay—Red blood cells from the following species were used: bovine, rat, hamster, rabbit and human blood group A, B, and O. The blood was collected in 3.8% sodium citrate and washed three times with 0.9% NaCl by centrifugation at 3000 × g for 5 min. The final pellet was resuspended with 0.9% NaCl to give a 4% cell suspension. The hemagglutination was performed at room temperature by serial dilution (1:2 with 0.9% NaCl) with a Takatsy microtitrator (Dynatech Laboratories Inc., Chantilly, VA) using 0.025-m1 loops and 4% suspension of erythrocytes. A Coulter counter was used to count the remaining cells in suspension after a 1-h incubation; 1 hemagglutinating unit is defined as the amount of lectin able to agglutinate and hence precipitate 75% of the red blood cells in suspension after 60 min. For semiquantitative results (as during the purification), the hemagglutinating activity was defined as the reciprocal of the end point dilution giving a clear macroscopic agglutination at 60 min. Where indicated, the 4% red
blood cell suspension was treated with Pronase (1 mg/ml; Sigma) or trypsin (250 μg/ml; Sigma) in 20 mM sodium acetate, pH 5.8, 150 mM NaCl containing 10 mM CaCl2 or in 100 mM Tris, pH 8.1, 100 mM NaCl, respectively, for 1 h at 37°C. The red blood cells were washed three times before being resuspended at 4% in phosphate-buffered saline.

Purification of Aplysina lectin — The clear, dialyzed 15–30% ammonium sulfate fraction (250 ml) was passed through a p-amino benzyl-β-D-thiogalactopyranoside-agarose (Sigma) column (2 × 8 cm) equilibrated in Buffer A (Hepes 50 mM, NaCl 100 mM, pH 7.6, containing CaCl2, MgCl2, and MnCl2, each at 1 mM) and washed with the same buffer until the optical density at 280 nm was below 0.020. Specific elution of the lectin was observed by 0.5 mM thiogalactoside in Buffer A. Fractions (2 ml) were collected and analyzed for protein content by the BCA method and for hemagglutinating activity against pronase-treated hamster erythrocytes. This purification scheme was used for both A. archeri and A. lawnosa extracts.

Binding Specificity of the Carbohydrate-binding Proteins — This was done by quantitative hapten inhibition using immobilized asialo α1-acid glycoprotein as a model glycoprotein. Briefly, asialo α1-acid glycoprotein dissolved in 50 mM sodium carbonate buffer, pH 9.6, containing 0.02% sodium azide (Buffer A) at 4 μg/ml was applied (50 μl) to each well of a 96-well microtiter plate and incubated for more than 2 h at 4°C. The plate was then rinsed three times with 50 mM sodium phosphate buffer, pH 7.4, containing 0.05% Tween 20 (Buffer B). The remaining sites on the plate were coated by incubation with 350 μl of Buffer A containing 1% bovine serum albumin at 25°C for 1 h. A 50-μl aliquot of biotinylated purified A. archeri lectin at about 50 pmol/ml in 50 mM Tris buffer, pH 7.4, containing 150 mM NaCl was mixed with aliquots of the different glycans at 4°C for 2 h and then applied to each well and incubated at room temperature for 4 h. The plate was washed three times with Buffer A and streptavidin-horseradish peroxidase conjugate was added to each well. Finally, the plate was washed four times with Buffer B, and 100 μl of the substrate solution (ABTS, 0.3 mg/ml, dissolved in 50 mM citrate buffer, pH 5.0, containing 0.012% H2O2) was added and incubated at 25°C for 10–20 min. The reaction was terminated by the addition of 100 μl of 5% SDS and the absorbance read at 405 nm.

Sugar Content — The carbohydrate content was measured by the phenol-H2SO4 method with glucose as the standard.

Metal Content and Effect of Metal Cations in Lectin Activity — Samples of purified lectins were analyzed by atomic absorption spectroscopy, either directly or after dialysis against 10 mM EDTA, pH 7.2, followed by dialysis against 150 mM NaCl in the presence of Chelex 100 resin (Bio-Rad). The metal-free lectins were tested for hemagglutinating activity described above before incubation for 1 h in the absence or presence of different divalent cations.

Equilibrium Dialysis — Solutions of purified A. archeri or A. lawnosa lectins (2.8 mg/ml) and lactose containing [14C]lactose were made in 100 mM Tris-HCl, pH 6.5. Each chamber of the dialysis cells received 100 μl of the protein or monosaccharide solution. After equilibration for 50 h at 4°C, aliquots (50 μl) were removed from each chamber and counted on a Beckman scintillation counter after being mixed with 10 ml of aqueous scintillation fluid.

Affinity Chromatography — Purified Aplysina archeri lectin (AAL)3 was coupled to AffiGel 10 (Bio-Rad) as suggested by the manufacturer in the presence of 100 mM lactate. The resin containing 4.5 mg of lectin/ml of resin was packed into a column (3-mm bed volume). The packed column was washed with 25 ml of Buffer A (50 mM Hepes, pH 6.8, 100 mM NaCl, and CaCl2 and MgCl2, each at 10 mM). Labeled glycopeptides were applied to the column in volumes of 100 μl or less and allowed to interact for at least 1 h at 4°C. The columns were eluted with Buffer A followed by 100 μl and 100 μl thiogalactoside in Buffer A. Fractions (0.5 ml) were collected and assayed for radioactivity. The recovery of labeled material was always higher than 92% of the amount applied to the column.

RESULTS

In an effort to identify lectins with novel affinity properties, we studied 22 species from 12 families of tropical sponges for the presence of hemagglutinating activity against a panel of normal or protease-treated red blood cells from different species. The results are summarized in Table I. A total of 10 species from 8 families showed some sort of agglutinating activity against some of the red blood cells tested. Three of those species also showed lytic activity against some of the red blood cells used in this system. The remaining 12 sponge species analyzed showed only lytic activity against all or some of the red blood cells tested. No species selectivity was noticed with the exception of Niphates erecta extract that showed hemagglutinating activity only against red blood cells from rat. In addition, there was no evidence of red blood cells susceptible to hemagglutination that could be used as a model system in the search for lectin activities.

Interestingly, all the members of the Aplysina sp. showed the highest titers of hemagglutinating activities in addition to being able to agglutinate red blood cells from all of the species tested. This finding prompted us to purify and characterize the lectin activity(ies) of A. archeri and A. lawnosa, from which a sufficient amount of biological material could be collected.

The dark brown–violet crude saline extract from A. archeri was first fractionated by precipitation with (NH4)2SO4 with more than 75% of the total hemagglutinating activity found in the cut between 15 and 30% saturation (NH4)2SO4 that contains less than 20% of the total protein (Fig. 1A), giving a purification of almost 4-fold above the crude extract. The clear, dialyzed solution (15–30% cut) was then applied to the affinity chromatography as described under "Experimental Procedures." After extensively washing the nonadsorbed proteins, the hemagglutinating activity was specifically eluted with 200 mM thiogalactoside. Eluting the column with 1 M NaCl or 1 M MgCl2 did not release more hemagglutinating activity or material absorbing at 280 nm (Fig. 1B). In another experiment, the column was eluted with 500 mM EDTA releasing a sharp peak containing the hemagglutinating activity; further elution with thiogalactoside did not release any more hemagglutinating activity from the column (data not shown). It is important to mention that the specific activity of the material eluted with EDTA was consistently lower than the material eluted specifically with thiogalactoside. When A. lawnosa extracts where

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1 The abbreviations used are: AAL, Aplysina archeri lectin; PAGE, polyacrylamide gel electrophoresis.
applied to the affinity column, the pattern of elution was identical (data not shown) to that of *A. archeri*.

Further purification of the lectins was accomplished by gel filtration on a calibrated Sephacryl S200 column (1.5 × 90 cm). The hemagglutinating activity from either species eluted from the column as a sharp symmetric peak at a volume corresponding to an apparent molecular mass of 63,000 Da (Fig. 2), with no additional peaks exhibiting hemagglutinating activity. The fractions with the highest specific activity were pooled and concentrated by ultrafiltration.

The purified lectins were analyzed by SDS-PAGE as shown in Fig. 3. Each purified lectin appeared as a single band corresponding to an apparent molecular mass of 16,000 Da, regardless of the presence or absence of reducing agents, which according to the native molecular mass, suggests that the lectins are organized in the native state as homotetramers.

By chromatofocusing of the purified lectins, both of them eluted as sharp bands at pH 4.1 and 4.5 for *A. archeri* and *A. lawnosa*, respectively (Fig. 4). The carbohydrate content of the purified lectins amounted to 3.5 and 5% for *A. archeri* and *A. lawnosa* agglutinins, respectively.

The hemagglutinating activity of both lectins was abolished by demetalization; this effect was reversible, as addition of CaCl₂, MgCl₂ to the metal-free lectins fully restored the activity. By contrast, manganese was less effective in restoring the hemagglutinating activity (about 45%), and zinc was without effect. Analysis by atomic spectroscopy of the metal content of the purified native lectins revealed that they contain significant amounts of calcium and magnesium. The contents were as follows: 0.5–0.8 atoms of Ca²⁺ and 0.3–0.4 atoms of Mg²⁺/subunit. As the total of calcium and magnesium is close to 1 mol/mol subunit of molecular mass 16 kDa, it suggests that these metals may be in the same site. These metal contents were diminished by 80–90% by prior dialysis against EDTA.

The number of carbohydrate binding sites on the purified lectins was determined by equilibrium dialysis, using [¹⁴C]lactose (which is also able to displace the lectins from the affinity support used for the purification). The data are shown in Fig. 5, plotted according to Scatchard. The number of binding sites obtained from that curve was found to be 3.8 ± 0.4 and 3.6 ± 0.3 for *A. archeri* and *A. lawnosa* lectins, respectively. As both
second best inhibitor (50% inhibition at 12 nmol, 30 times more potent than D-Gal). With the more bulky substituent 4-methylumbelliferyl aglycon, the differences were less significant, as shown in Table II.

Those results indicate a preference for β-linked D-Gal. Of the disaccharides, those with terminal nonreducing β-linked D-Gal were more active than those with terminal nonreducing α-linked D-Gal. Melibiose, raffinose, stachyose, Galα1–3Gal, and 3-fucosyllactose were about 2 times less active than D-Gal. Lactose and other disaccharides with Galβ1–3 and β1–4 linked were slightly more active than D-Gal. There appears to be a significant preference for a GlcNAc subterminal to galactose, because lactose is a somewhat weaker inhibitor than lactosamine. Confirming the requirement for a terminal nonreducing β-linked D-Gal was the fact that Glcβ1–4Gal and Glcα1–2Gal were inactive. However 3-fucosyllactose was just 2 times less effective than lactose as inhibitor. The best disaccharide inhibitor was thiogalactoside, 180 times more potent than D-Gal.

To gain further insight into the carbohydrate binding specificity of purified A. archeri lectin, hapten inhibition experiments were performed. An analysis of the results shown in Fig. 6 and summarized in Table II reveals that D-galactose and D-fucose were almost equal inhibitors on a molar basis; D-glucose and D-mannose were inactive up to 5000 nmol. D-GalNAc and D-GalNH2 inhibited 50% at 2500 and 1200 nmol, respectively, being 3.2 and 6.7 times less active than D-Gal. 2-Deoxy-D-Gal was three times more potent than D-Gal. Thus the site is specific for a terminal nonreducing D-Gal with the modifications at C-2 being important and that the OH at C-6 not essential. Introduction of a nonpolar substituent (methyl, p-nitrophenyl or 4-methylumbelliferyl) in either anomic configuration proved a significant modification in increasing the affinity for the lectin. However, in all cases, the β-configuration was preferred. Thus, the most potent monosaccharide, methyl-β-D-Gal is 37 times more potent than D-Gal compared with only 3.7 times for methyl-α-D-Gal; and p-nitrophenyl-β-D-Gal is almost 7 times more effective than D-Gal compared with only 2 times for the α conformer. Methyl-β-D-thiogalactose was the

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**Fig. 4.** Chromatofocusing of purified A. archeri lectin. 168 μg of purified AAL were applied to a MonoP column (Amersham Pharmacia Biotech) and eluted according to the manufacturer's instructions with a pH gradient from 6.5 to 3. Fractions of 1 ml were collected and assayed for protein by A_280nm (OD280nm; ■), for pH (□) and for hemagglutinating activity (HU; hemagglutinating unit) against Pronase-treated hamster red blood cells (□) as described previously.

**Fig. 5.** Equilibrium dialysis data for the binding of [14C]lactose to purified AAL at 4 °C. Experiments were performed at 10 °C, pH 6.8, and at a final lectin concentration of 2.8 mg/ml. L is the free concentration of ligand ([14C]lactose) at equilibrium, and r is the ratio of bound ligand to total lectin (Mr, 63,000).

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**Fig. 6.** Hapten inhibition of biotinylated AAL binding to immobilized asialo-α1 acid glycoprotein. Aliquots of biotinylated purified AAL (50 nM) were preincubated with various concentrations of hapten at 4 °C for 2 h before adding to microtiter wells coated with asialo-α1 acid glycoprotein. Bound lectin was measured after incubation with streptavidin-horseradish peroxidase conjugate as described under “Experimental Procedures.”
with a fucose residue (oligosaccharides 7–8) or a sialic acid residue (oligosaccharide 10) did not affect the differential recognition but reduced the overall affinity as observed by comparing to the binding profile of compounds 3 and 4. Substitution of the reduced glucitol end group with Fuc\(\alpha\)1–3Gal and Fuc\(\alpha\)1–3GalNAc branches that were also distinguished by the binding profile to the immobilized lectin column; this suggests that the exact linkage pattern determines the binding, contrary to what has been found for the tetra-antennary structure 24 was eluted from the column with 10 mM thiodigalactoside.

When analyzing the binding profile of branched oligosaccharides, it was noticed that they bind to the column more tightly than linear oligosaccharides, as shown for oligosaccharides 19 and 20, containing different proportions of Gal\(\beta\)1–3GlcN\(\alpha\)c and Gal\(\beta\)1–4GlcN\(\alpha\)c, which are distinguished on linear oligosaccharides (compounds 3 and 4) were also distinguished by the binding profile to the immobilized lectin column; this suggests that the exact linkage pattern determines the binding, contrary to what has been found for Tetracarpidium conophorum lectin (12) but quite similar to the strict specificity for Gal\(\beta\)1–4GlcN\(\alpha\)c exhibited by the marine sponge Halichondria okadai lectin (14). Glycopeptides bearing three terminal Gal\(\beta\)1–4GlcN\(\alpha\)c sequences (structures 22 and 23) showed high affinity for binding to AAL. By contrast, the tetra-antennary structure 24 was eluted from the column with 10 mM thiodigalactoside.

Regarding the inhibition by glycopeptides, it was found that consistent with the specificity against terminal nonreducing \(\beta\)-linked \(n\)-gal residues, the best inhibitors of all the glycopeptides tested were the asialo forms, as shown in Table II. No inhibitory effect was shown by the sialylated glycopeptides, confirming an absolute requirement for terminal \(\beta\)-galactosyl residues for interaction with AAL. Porcine thyroglobulin, which contains complex-type glycans terminated predominantly with exposed \(\beta\)-galactosyl residues (15), was a very effective inhibitor. The poor activity of asialofetuin could be related to the poor accessibility of its oligosaccharide chains. Further confirmation of the specificity is shown by the fact that treatment with \(\beta\)-galactosidase of the asialo form of thyroglobulin and \(\alpha\)-1acid glycoprotein almost abolished the inhibitory activity to the native form of the glycopeptides.

**DISCUSSION**

The results presented in this report show that the presence of lectins in marine sponges is not as widespread as in other marine organisms (mollusks and crustaceans) or in plants. Interest in marine sponges as sources of novel reagents for cell biology has flourished due to the recent report on the isolation and characterization of the anti-HIV (human immunodeficiency virus) compound niphatevirin from *N. erecta* (16), a calcium channel blocker from *Ciona celata* (17), and ilimaquine (18–20) from another marine sponge. Of the 22 species from 12 families analyzed in this report, only 10 species were found to exhibit some degree of hemagglutination activity against one or several of the red blood cells tested. None of the species tested from the genuses *Agelas* or *Ircinia* showed hem-
agglutinating activity in our system, but they exhibited strong lytic activity against some of the red blood cells tested. By contrast, the three members of the genus *Aplysina* that we examined showed the strongest hemagglutinating activity against all of the red blood cells tested. For that reason, purification and biochemical characterization of the hemagglutinins from two species (*archeri* and *lawnosa*) of that genus were accomplished. The lectins were purified to homogeneity by affinity chromatography on \(p\)-aminobenzyl-\(\beta\)-1-thiogalactopyranoside-agarose and gel filtration.

The purified lectins from both species were shown as a single band in SDS-PAGE regardless of the presence or the absence of reducing agents with an apparent subunit molecular weight of 16,000. The native molecular weight of both lectins was 63,000, thus suggesting that both lectins in the native state exhibit a homotetrameric structure. That is a common structural feature from some of the sponge lectins characterized to date, along with the acidic pI (4, 5, 21–25). The tetrameric structure was further confirmed by the Scatchard analysis, which indicated the presence of four homogeneous carbohydrate binding sites per native molecule of lectin. The hemagglutinating activity of the lectins was significantly affected by demetalization and was restored after remetalization by dialysis against buffers containing calcium or magnesium ions but not by manganese. This result clearly indicates that the metal content of the purified lectins (~1 mol of divalent cation/mol of 16,000-Da subunit) is necessary for exhibiting the carbohydrate binding properties of the proteins.

Because of the growing number of applications in biochemistry and cell biology, the isolation and characterization of a new lectin requires a detailed study of its carbohydrate binding specificity. For that reason, we carried out a detailed study with the *A. archeri* purified lectin. We have obtained consistent data from both hapten inhibition experiments and affinity chromatography (as modified from Sato et al. (12)). The results can be summarized as follows: AAL exhibits high specificity for terminal \(\beta\)-linked galactosyl residues, common for several sponge lectins characterized to date (4, 5, 7, 8, 14, 22–25). Modifications that include the substitution of the terminal galactose with \(\alpha\)2–3 sialic acid drastically reduced the binding activity, as seen with other galactose-specific lectins such as *Erythrina* species lectins (26) or *Ricinus communis* agglutinin, which does not bind \(\alpha\)2–3 sialylated galactosyl sequences but retains binding ability for \(\alpha\)2–6 sialylated (27, 28). An interesting aspect of the binding specificity of AAL is that even when substitution of the subterminal GlcNAc with \(\alpha\)1–2 fucose (as seen in structures 7 and 8 compared with oligosaccharides 3 and 4) in linear oligosaccharides reduces the binding affinity, it still retains the preference for Gal\(\beta\)1–4GlcNAc versus Gal\(\beta\)1–3GlcNAc, which could be resolved by affinity chromatography. The fact that the inhibitory potency of galactose is the same as lactose does suggest the existence of an extended binding site. However, the substitution of glucose for GlcNAc, as in lactosamine, or the inclusion of a bulky hydrophobic group, as in \(p\)-nitrophenyl- or 4-methylumbelliferyl-\(\beta\)-D-galactoside, increased the inhibitory potency between 3- and 6-fold compared with galactose. The preceding result is a clear indication of an extended binding site or at least of the existence of a hydrophobic subsite that potentiates the binding when occupied. Furthermore, analysis of the requirements for binding also points to the notion that the lectin interacts with extended sugar sequences, because residues located sub terminally determine the binding affinity, as shown before for the structures 7 and 8. However, substitution of sugar residues downstream of the nonreducing terminal galactose (compare oligosaccharides 4 and 9) did not reduce the binding affinity, thus delimiting the extension of the binding site. In the same sense, a bisecting GlcNAc\(\beta\)1–4 residue just reduces the binding affinity of a bi-antennary N-glycan but is still retained by the immobilized lectin. By comparing the behavior of oligosaccharides 15 and 16, which displayed differently the two lactosamine units but exhibited the same binding profile, it is clear that the constraints imposed on the lactosamine units by the more rigid Man\(\alpha\)1–3 arm (29) are not determinant. A characteristic of the AAL is the fact that it exhibits better affinity for bi- and tri-antennary Gal\(\beta\)1–4GlcNAc- and Gal\(\beta\)1–3GlcNAc-carrying

| No. | Structure | Profile |
|-----|-----------|---------|
| 1   | Gal\(\beta\)1–4GlcNAc | B       |
| 2   | Gal\(\beta\)1–4GlcNAc | C       |
| 3   | Gal\(\beta\)1–3GlcNAc–1–3Gal\(\beta\)1–4GlcNAc | C       |
| 4   | Gal\(\beta\)1–3GlcNAc–1–4GlcNAc | B       |
| 5   | Gal\(\beta\)1–3GlcNAc–1–6GlcNAc | A       |
| 6   | Gal\(\beta\)1–3Fuc\(\alpha\)1–4GlcNAc–1–3Gal\(\beta\)1–4GlcNAc | A       |
| 7   | Gal\(\beta\)1–4Fuc\(\alpha\)1–3GlcNAc–1–4GlcNAc | B       |
| 8   | Gal\(\beta\)1–3NeuNAc–6Gal\(\beta\)1–3GlcNAc–1–4GlcNAc | B       |
| 9   | Gal\(\beta\)1–3NeuNAc–6Gal\(\beta\)1–3GlcNAc–1–4GlcNAc | B       |
| 10  | Gal\(\beta\)1–3NeuNAc–6Gal\(\beta\)1–3GlcNAc–1–4GlcNAc | B       |
| 11  | NeuNAc–2Gal\(\beta\)1–4GlcNAc | A       |
| 12  | Gal\(\beta\)1–4GlcNAc–1–3Gal\(\beta\)1–3Gal\(\beta\)1–4GlcNAc–1–6GlcNAc | D       |
| 13  | Gal\(\beta\)1–3Fuc\(\alpha\)1–4GlcNAc–1–3Gal\(\beta\)1–4GlcNAc–1–6GlcNAc | C       |
| 14  | Gal\(\beta\)1–4GlcNAc–1–2Man\(\alpha\)1–3Man\(\alpha\)1–4GlcNAc–1–6GlcNAc–1–3Gal\(\beta\)1–4GlcNAc | C       |

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glycans than does another sponge lectin characterized recently (30); the reduced affinity shown for the tetra-antennary glycan 24 could be due to lack of accessibility to the binding site of a small subunit.

The immunobilized lectin purified from A. archeri was able to resolve bi-antennary glycans containing different proportions of Galβ1–3GlcNAc and Galβ1–4GlcNAc sequences. The two isomers tested by tri-antennary glycans (oligosaccharides 22 and 23) exhibited the same binding profile, and the lectin was not able to discriminate both forms, in contrast to lectins such as Phaseolus vulgaris leucoagglutinin (31) and Datura stramonium (32), which exhibit some preference for tri-antennary glycans carrying the Galβ1–4GlcNAc sequences on the C-2 and C-6 of the Manα1–6 and on the C-2 of Manα1–3 of the core sequence. The tetra-antennary glycan tested in this report exhibited a reduced affinity for the lectin, which could reflect lack of accessibility to the binding site of the lectin.

The function of the lectins characterized in this report is unknown, even when they share some properties with other sponge lectins, most notably binding specificity for galactosides (4, 5, 14, 23, 33–35) and, in most cases, calcium dependence. In past years, attempts to compare the sponge lectins to the aggregation factor to cells (39).

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