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Identification of lectin isoforms in juvenile freshwater prawns *Macrobrachium rosenbergii* (DeMan, 1879)

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From the serum of juvenile freshwater prawns, we isolated by affinity chromatography on glutaraldehyde-fixed rat erythrocytes stroma, immobilized in Sephadex G-25, a sialic acid specific lectin of 9.6 kDa per subunit. Comparative analysis against adult organisms purified lectin, by chromatofocusing, showed that the lectin from juvenile specimens is composed by four main isoforms with a pI of 4.2, 4.6, 5.1, and 5.6, whereas the lectin from adults is eluted at pH 4.2. The amino acid composition of the lectin obtained from adult and juvenile stages suggest identity, but the compositions are not identical since a higher content of carbohydrates was found in the lectin from younger organisms. The freshwater prawn lectin showed specificity toward N-acetylated amino sugar residues such as GlcNAc, GalNAc, Neu5Ac and Neu5,9Ac; but in juvenile organisms the lectin showed three times less hemagglutinating activity than the lectin from adults. Both lectins agglutinated rat, rabbit and chicken erythrocytes, indicating that Neu5,9Ac in specific O-glycosydically linked glycans seems to be relevant for the interaction of *M. rosenbergii* lectins with their specific cellular receptor. Our results suggest that the physicochemical characteristics of the lectin from the freshwater prawn are regulated through maturation.

***Keywords: Macrobrachium rosenbergii*, sialic acid specific lectin, crustacean lectin**

Introduction

Lectins seem to be ubiquitous proteins in the hemolymph of several invertebrates. They are apparently synthesized by hemocytes [1,2] and can, as occurs in many species, bind to the surface of these cells or circulate in a soluble form in the hemolymph [1–3]. Due to their specificity for sugar residues, in defined sequences or in complex glycoconjugates, it has been assumed that lectins participate as mediators in foreign-substance recognition and ulterior elimination by hemocytes with phagocytic activity [2,3]. Some reports indicate that besides their participation in defense mechanisms, lectins in invertebrates have relevant roles in nutrition, development, symbiosis, tissue reorganization, and cell adhesion [1,2].

The occurrence of lectins in a wide variety of organisms with conserved domains and related functional features supports their important biological roles [2,4]; in recent times, considerable attention has been focused on lectins with capacity to bind specifically sialoglycoconjugates because of their biological relevance and diagnostic value [5–7]. Sialic acid specific lectins have been identified in Arthropoda, Mollusca and Urochordata among other invertebrates [1,2]. In a previous work, we purified and partially characterized a sialic acid-binding lectin from the hemolymph of *Macrobrachium rosenbergii* freshwater prawns [8]. This lectin, which seems to be synthesized by granular hemocytes [9], reacts with sialoglycoconjugates and agglutinates some bacteria through specific interaction with N- and O-acetylated sugar residues present in the bacterial cell wall [10], suggesting its active participation in defense mechanisms of adult organisms.

The freshwater prawn *M. rosenbergii* is a crustacean species of worldwide interest but in contrast to the high farming capacity of this specie in the adult stage, the survival rate of larvae is highly variable from facility to facility [11], suggesting that some defense mechanisms could be modified

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throughout maturation. In this paper we report the isolation and the chemical characterization of the lectin present in the hemolymph of juvenile specimens and compare its physicochemical and biological properties with the lectin obtained from adult organisms, our aim being a better understanding of the biological role and regulation of the activity of these proteins through maturation.

Materials and methods

Lectin extraction and purification

Freshwater *Macrobrachium rosenbergii* prawns were obtained in Jojutla, Morelos, Mexico. Maturation stages were based upon weight and body characteristics, as suggested by Wickins and Beard [12]. Freshwater prawns were separated in two main groups according to their maturation stage adults in intermoult and postlarvae or juvenile. Hemolymph was extracted from the pericardial sinus, and the serum obtained after centrifugation at $16\,000 \times g$ at 4°C for 30 min was dialyzed against tris-buffered saline (TBS 50 mM Tris-HCl, 0.15 M NaCl, 3 mM $CaCl_2$, pH 7.4). The lectin present in the hemolymph of each animal group, was purified by affinity chromatography on a column (1.5×25 cm) containing glutaraldehyde fixed rat erythrocytes stroma entrapped in Sephadex G-25, the column was equilibrated with TBS at 10 ml/h flow rate, 1.5 ml hemolymph (containing 266 mg protein) was deposed onto the column. The unretained fraction was eluted with TBS and the lectin was eluted by addition of 3% acetic acid in water [13]. Fractions of 1.5 ml were collected, dialyzed with Bis-Tris buffer (25 mM Bis-Tris, 25 mM iminodiacetic acid, pH 7.2), before determining the optical density (at A_{280}) of each fraction and the hemagglutinating activity in the presence of rat erythrocytes.

Chromatofocusing of MrL

The affinity-purified lectin (500 µg) from adult and juvenile organisms was applied into a mono P pre-packed HR column 5/5 mm (Pharmacia, Uppsala, Sweden). The column was equilibrated with Bis-Tris buffer, at a flow rate of 1 ml/min with a maximal pressure of 40 bars using an FPLC system (Pharmacia, Uppsala, Sweden). The lectin isoforms were eluted from the column with a continuous pH gradient, in a 60-min program, using the Pharmalyte pH 7 to 4 polybuffer (Pharmacia) [14]. Fractions of 1 ml were collected and the hemagglutinating activity was determined in the presence of a 2% rat erythrocytes suspension in TBS. For each fraction, the pH and optical density (at A_{280}) were monitored. Each eluted peak was dialyzed against distilled water before lyophilization for further analysis.

Separation of MrL isoforms

The MrL isoforms from juvenile organisms obtained after chromatofocusing were applied into a mono P prepacked HR column 5/5 mm, in anionic form, equilibrated previously with 50 mM Bis-Tris buffer, pH 7.5, at a flow rate of 1 ml/min with a maximal pressure of 40 bars, in a 60 min program using an FPLC system. The MrL isoforms were eluted from the column with a 0–1 M NaCl stepwise gradient in Bis-Tris buffer. Fractions of 1 ml were collected and optical density was monitored at A_{280}. Each eluted peak was dialyzed against distilled water before lyophilization for further analysis. The main fractions separated by ion-exchange chromatography were dialyzed against Bis-Tris buffer, pH 7.5 and re-chromatofocused on the Mono-P column to confirm their pI, in the conditions previously indicated.

Analytical methods

Protein concentration was determined by the method of Bradford [15] using bovine serum albumin as standard. Amino acid composition of 100 µg of purified lectin was determined after hydrolysis for 24 h under vacuum with 2 ml of 6 N HCl at 110°C in sealed containers, in a Beckman 119-CL amino acid analyzer [16]. Carbohydrate content and composition of the lectin, its isoforms, and all glycoproteins used in this study, was determined with the heptafluorobutyrate derivatives of O-methyl-glycosides, obtained after hydrolysis with 0.5 N methanol-HCl for 24 h at 100°C, by gas-chromatography with a capillary column (25×0.32 mm) of 5% Silicone OV 210, (Applied Science Lab, Buffalo, NY), in a Varian 2100 gas chromatograph (Orsay, France); the carrier gas was helium at a pressure of 0.6 bar, and the oven temperature programmed from 100 to 240°C at 2°C per min [17] L-lysine (Sigma Chem., St Louis, MO) was used as internal standard. Identification of each sugar residue was confirmed by mass spectrometry using the Finnigan Automass II mass spectrometer, the HFB derivatives of the O-methyl glucosides of monosaccharides presented relatively high mass: 978 for hexoses, 977 for hexosamines and 1275 for sialic acid [17].

Polyacrylamide gel electrophoresis

The molecular mass and the homogeneity of the purified lectin and its isoforms were evaluated in 10% polyacrylamide gel electrophoresis (PAGE) in the presence of 0.1% sodium dodecyl sulfate (SDS), using the Laemmli buffer system [18], gels were stained with 0.1% Coomassie brilliant blue.

Hemagglutinating activity

Human erythrocytes type A, B, and O from healthy donors were obtained from the Central Blood Bank IMSS, Mexico. Rat, mice, rabbit, sheep, horse, pig and chicken red cells were obtained from the animal facilities at Facultad de Medicina Veterinaria, UNAM, Mexico. The hemagglutinating activity of the *M. rosenbergii* purified lectin and its isoforms was assayed in microtiter U plates (NUNC, Denmark) according to a two-fold serial dilution procedure [8]. The agglutinating activity

was tested with 2% erythrocyte suspension in TBS. Hemagglutinating activity of each purified lectin isoform is reported as the specific activity, which corresponds to the hemagglutinating units/mg lectin Hemagglutination assays were also performed in the presence of erythrocytes previously treated with *Vibrium cholerae* (Sigma) neuraminidase (0.1 U per 500 µl of packed erythrocytes at 37°C for 30 min).

Preparation of glycans and asialoglycoproteins

Fetuin glycosylpeptides were obtained by *Streptomyces griseus* pronase treatment (1 mg of enzyme per 10 mg of glycoprotein incubated for 48 h at 37°C). The O- and N-glycosylpeptides were fractionated by gel filtration on a column (1.6 × 100 cm) containing Bio-Gel P4 equilibrated with 0.05 M pyridine acetate, pH 4.5 [19]. Each O- and N-glycosylpeptide was desalted by filtration on a column (2 × 60 cm) containing Bio-Gel P2, equilibrated with 0.5 M acetic acid in distilled water, lyophilized, and kept at 4°C until use Glycoproteins and O-glycosylpeptides from fetuin were desialylated by incubation at 100°C for 1 h in the presence of 0.02 N sulfuric acid, as described by Spiro and Bhoyroo [20], and desalted on a Biogel P-2 column (2 × 60 cm) equilibrated with 0.5 M acetic acid. The carbohydrate composition of each glycoprotein and glycosylpeptide was determined by gas-liquid chromatography.

Sugar specificity

The lectin's sugar specificity was confirmed by comparing the inhibitory activity of various sugars glycoproteins, glycosylpeptides and their desialylated derivatives, on the hemagglutination induced by these lectins against rat erythrocytes as follows: the lectin was diluted in TBS to four hemagglutinating units (HAU, titer = 4), 25 µl of this lectin dilution was placed in microtiter U plates and incubated 1 h at room temperature with 25 µl of different concentrations of sugars, glycoproteins and glycosylpeptides, then 25 µl of a 2% rat erythrocytes suspension in TBS was added. Results are expressed as the minimal concentration required to inhibit four hemagglutinating units [10]. The molar concentration of glycoproteins and glycopeptides was calculated on the basis of their oligosaccharide content as determined by gas chromatography.

Results

Purification of the lectin from adult and juvenile organisms

The maturation stage of freshwater prawns was determined based mainly on weight and some morphological characteristics. The organisms considered juvenile or postlarval were specimens with an average weight of 2.33 ± 0.5 g (n = 25), translucent body and undeveloped chelae. Adults were specimens with an average weight of 13.2 ± 1.5 g (n = 35), developed chelae tips, and a hard exoskeleton. From the hemolymph of juvenile and adult freshwater prawns, we purified a lectin by affinity chromatography using glutaraldehyde-treated stroma from rat erythrocytes. In both cases, the lectin was eluted from the affinity chromatography column by decreasing the pH of the chromatography medium to 2.8 by addition of 3% acetic acid. The protein yield in the purification process indicated that the purified lectin represents 1.4 and 0.7% of the protein content of the hemolymph of juvenile and adult organisms, respectively.

Chromatofocusing of the lectin from adult and juvenile organisms

As indicated in Figure 1, the chromatofocusing analysis revealed that the lectin from adult freshwater prawns is constituted by a homogeneous group of proteins (termed A-I), since 90% of the hemagglutinating activity is eluted at pH 4.2 in a single peak (Figure 1a), whereas the elution profile of the lectin purified from juvenile organisms is constituted by four main isoforms (termed as J-I to IV, Figure 1b). Further purification of the lectin isoforms from juvenile organisms was performed by ion exchange chromatography. Figure 2, shows the purification profile for isolectins J-I and J-IV; the elution of J-II and J-III lectin isoforms was similar to the profile observed with J-IV isoform. The main fractions obtained in such chromatographic procedure were rechromatofocused on the Mono-P column, and our results indicated that the isoforms eluted at pH 5.6, 5.1, 4.6 and 4.2, corresponded to isoforms J-I to J-IV, respectively. The J-IV isoform, with a pI of 4.2, contained 51% of the hemagglutinating activity loaded on the column Isoforms J-II and J-III eluted at pH 5.1 and 4.6, contained 18 and 20% of the initial hemagglutinating activity, respectively. The lowest hemagglutinating activity was observed in the J-I isoform, which contained 11% of the hemagglutinating activity loaded onto the column. The J-IV isoform seems to correspond to the AI lectin of adult organisms, since it is eluted at the same pH from the column (pH 4.2) and showed the highest hemagglutinating activity (Figure 1a, b).

Chemical characterization

The purified lectin from adult or juvenile organisms, as well as its purified isoforms, is a homogeneous glycoprotein of 9.6 kDa as determined by SDS-PAGE analysis (Figure 3). The amino acid composition of both, juvenile and adult purified lectins suggest identity, but the compositions are not identical (Table 1). Both lectins are constituted mainly by glycine, serine, glutamic and aspartic acid residues and, in minor proportion, by cystein and trytophan residues but lower amounts of amino acids such as isoleucine, phenylalanine, arginine, and threonine were observed in the lectin from juvenile organisms than in the lectin from adults (Table 1). The saccharidic part of both lectins showed quantitative and qualitative differences, the lectin obtained from juvenile organisms is more glycosylated than the lectin from adults, it contained 15% of sugars per weight as

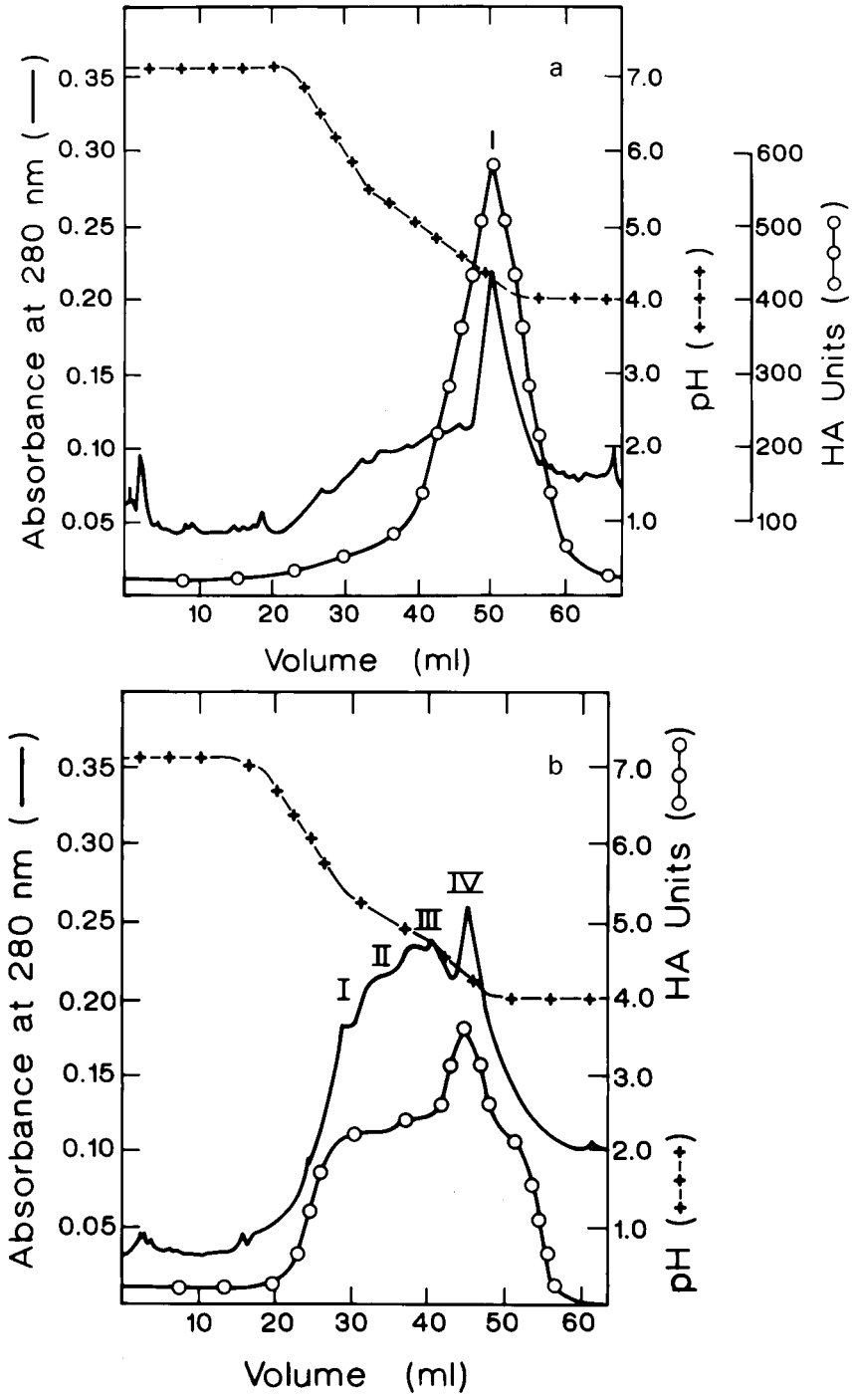

Figure 1. Isolation of the isoforms of the lectin from adult (a) and juvenile (b) *Macrobrachium rosenbergii* freshwater prawns by chromatofocusing. Each lectin (500 μg) was applied into a Mono P column in an FPLC system. The protein was eluted using a pH gradient with a pH 7.2 to 4 polybuffer (+ - +). Detection of the optical density of each 1.5 ml fraction was at A_{280} (continuous line). Hemagglutinating activity was determined in the presence of rat erythrocytes (○-○), results represent the total hemagglutinating units (HAU) in each collected fraction.

opposed to 11% in adult organisms (Table 2) as determined by gas-chromatography. As shown in Table 2, juvenile isolectins contained Gal, Man, and GlcNAc residues and, in minor proportion, GalNAc; juvenile J-IV isoform also contained N-acetyl-neuraminic acid. A similar composition of carbohydrates was observed in the lectin from adult organisms. These results indicate that proportionally the adult lectin is more sialylated.

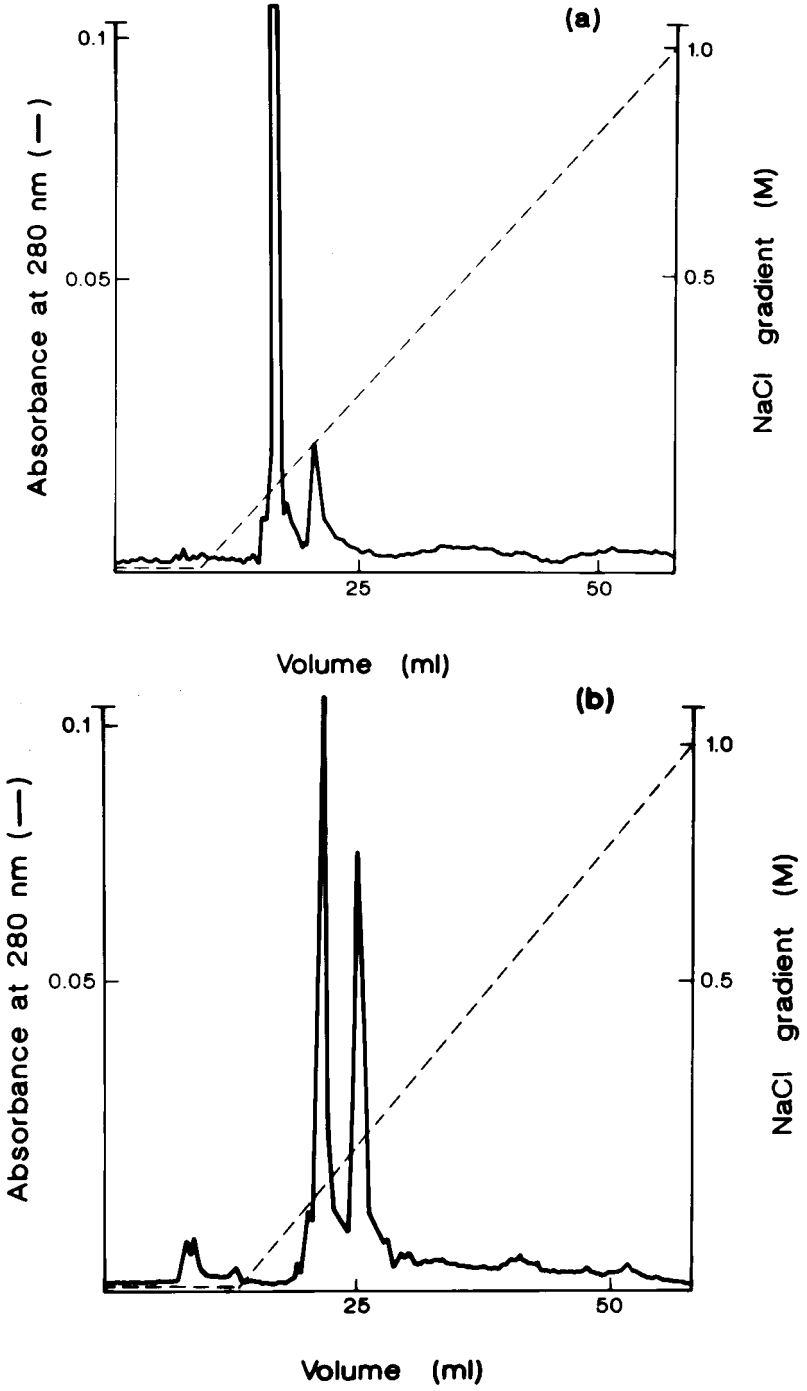

Figure 2. Purification of *M. rosenbergii* lectin-isoforms from juvenile organisms by ion exchange chromatography on a mono P column (anionic form) in an FPLC system. The lectin fractions previously obtained by chromatofocusing (500 μg) were applied to the column equilibrated previously with 25 mM Bis-Tris/iminodiacetic acid buffer, pH 7.2. Isoforms were eluted by a stepwise NaCl gradient (dotted line). Detection of the optical density of each 1 ml fraction was at A_{280} (continuous line). Representative experiments for fractions: J-I (a) and J-IV (b) MrL isoforms is presented (See Figure 1 for nomenclature).

Hemagglutinating activity and sugar specificity

The lectin from adult and juvenile organisms as well as its purified isoforms showed the same specificity for erythrocytes and sugar residues. Although the lectin purified from juvenile organisms showed three times less hemagglutinating activity than the lectin from adults, both lectins recognized only rat, rabbit, and chicken erythrocytes, and failed to agglutinate erythrocytes from other animal species such as human (A, B, and O), horse, pig, or sheep. Removing sialic acid from

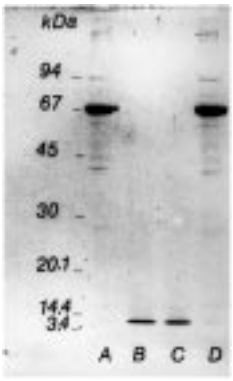

Figure 3. SDS-polyacrylamide gel electrophoresis of the lectin purified from the hemolymph of juvenile and adult *Macrobrachium rosenbergii* freshwater prawns by affinity chromatography A) Hemolymph (35 μg) and B) purified lectin (5 μg) from adult organisms, C) purified lectin (5 μg) and D) hemolymph from juvenile organisms (35 μg). Molecular weight markers are Phosphorylase b (94 kDa), bovine serum albumin (67 kDa), egg albumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), lactalbumin (14.4 kDa) and bovine insulin chain B (3.4 kDa).

Table 1. Amino acid composition (Res/Mol*) of the lectin purified from juvenile and adult *Macrobrachium rosenbergii* freshwater prawns.

Residue	*Juvenile*	*Adult*
Asx	8.1	7.9
Glx	8.6	8.2
Ser	6.1	6.2
Gly	6.6	6.7
His	2.1	2.2
Arg	2.9	3.3
Thr	3.7	4.2
Ala	5.6	5.7
Pro	3.6	3.7
Tyr	1.2	1.1
Val	4.8	4.8
Met	1.3	1.4
Cys	0.5	0.5
Ile	3.5	4.2
Leu	6.0	6.6
Phe	4.1	4.5
Lys	5.8	6.0
Total	74.5	77.2

*Residues/mol, considering a molecular weight of 9.6 kDa per subunit and a 15 and 11% sugar per weight content in juvenile and adult lectin, respectively.

erythrocytes, by treatment with *V. cholerae* neuraminidase, abolished the capacity of both lectins to agglutinate the erythrocytes from rat, rabbit, and chicken. The hemagglutinating activity of juvenile and adult lectins was inhibited only by N-acetylated amino sugars such as Neu5Ac, Neu5,9Ac, GlcNAc, GalNAc and, in minor proportion, Neu5Gc, α- or β-methyl glycosides from sialic acid and Neu5Ac (α2,3 and α2,6) N-acetylactosamine showed an eight- to sixteen-fold higher capacity to inhibit the hemagglutinating activity of the lectin obtained in both maturation stages, than the N-acetylated amino sugars. The amount of N-acetylated amino sugars required to inhibit the hemagglutinating activity of the juvenile lectin isoforms J-I to J-III was fourfold higher than the amount of sugars required to inhibit the hemagglutinating activity of the isoform J-IV or the adult lectin Sialylated glycoproteins, such as fetuin, and bovine and ovine submandibular gland mucin were more powerful inhibitors of adult and juvenile lectins than simple sugars. Hen ovalbumin, human transferrin, IgG, α_1-glycoprotein acid, desialylated fetuin, and desialylated ovine and bovine mucins failed to inhibit the hemagglutinating activity of the lectins (Table 3). Fetuin O-glycosylpeptides isolated after pronase digestion and gel-filtration chromatography inhibited the hemagglutinating activity of all the freshwater lectin isoforms; whereas, N-glycosylpeptides or asialo-O-glycosylpeptides were not able to inhibit the lectins activity (Table 3).

Table 2. Carbohydrate composition (Res/Mol*) of the purified isolectins from *Macrobrachium rosenbergii* juvenile and adult freshwater prawns.

	Juvenile				*Adult*
Carbohydrate	*J-I*	*J-II*	*J-III*	*J-IV*	*A-I*
Mannose	2.9	2.9	2.4	2.1	1.4
Galactose	2.4	2.7	2.2	2.4	1.3
N-acetyl-D-galactosamine	0.8	0.8	0.8	0.7	0.3
N-acetyl-D-glucosamine	1.5	0.9	0.9	1.1	0.8
N-acetyl-neuraminic acid	0.0	0.0	0.2	0.5	0.9
Sugar content (%)**	15.2	15.0	14.5	14.5	11.0

*Residues/mol, considering a molecular weight of 9.6 kDa per subunit. The carbohydrate content and composition was determined with the heptafluorobutyrate derivatives of O-methyl-glycosides of each lectin and its isoforms by gas chromatography.
**The reported percentage represents the relation between protein and carbohydrate concentration in the freeze-dried lectin.

Table 3. Effect of sugars and glycoproteins (mM) on the hemagglutinating activity of the lectins from *Macrobrachium rosenbergii* juvenile and adult freshwater prawns.

	Juvenile isoforms				
Compound	*J-I*	*J-II*	*J-III*	*J-IV*	*Adult*
Neu5Gc	200	200	200	200	100
GalNAc	200	200	100	100	50
GlcNAc	200	200	200	100	50
Neu5Ac	50	50	25	25	25
Neu5,9Ac	25	25	25	25	12.5
Neu5Ac α-methylglycoside	25	12.5	7.5	7.5	7.5
Neu5Ac β-methylglycoside	25	12.5	12.5	7.5	7.5
Neu5Ac (α2,3) N-acetylactosamine	25	25	12.5	12.5	12.5
Neu5Ac (α2,6) N-acetylactosamine	25	25	12.5	12.5	12.5
Colominic acid (Neu5Acα2,8)**	25	25	15	10	10
Fetuin	0.006	0.003	0.003	0.001	0.001
Fetuin O-glycosylpeptides***	0.1	0.1	0.05	0.05	0.05
Bovine submandibular mucin	0.01	0.01	0.005	0.005	0.005
Ovine submandibular mucin	0.1	0.1	0.05	0.05	0.05

*Minimal concentration needed to inhibit 4 HAU (Titer = 4) of lectin from juvenile (7 μg/ml) and adult (2 μg/ml) specimens. Sugars without inhibitory activity at 200 mM were L- and D-fucose, D-mannose, D-glucose, D-galactose, D-galactosamine, D-glucosamine, D-mannosamine, melezitose, lactose, and raffinose. Hen ovalbumin, human transferrin, IgG, α_1-glycoprotein acid, desialylated fetuin, ovine and bovine glycoproteins, as well as fetuin N-glycosylpeptides and asialo-O-glycosylpeptides were not inhibitory at a 10 mM concentration.
**Values are presented in mg/ml since the molecular weight is not known.
***Glycosylpeptides obtained from fetuin after pronase digestion and gel filtration. The molar concentration of glycoproteins and glycoslypeptides was calculated on the basis of their oligosaccharide content as determined by gas chromatography.

Discussion

In invertebrates, the most likely candidates for recognizing foreign material, due to their high specificity for carbohydrates, are the lectins [1–3,21]. These proteins have been shown to play important roles also in the innate immunity of mammals [2]. Previous work indicate that the innate immune system of the freshwater prawn may recognize several bacteria species through the specific interaction of the lectin, present in the hemolymph, with the acetyl groups of reducing sugars exposed on the bacterial cell wall [10].

We purified the lectins present in the hemolymph of adult of juvenile freshwater prawns, and compared their physicochemical characteristics and specificity for sugars and erythrocytes. The lectin isolated from adult organisms had stronger hemagglutinating activity than the lectin from juvenile organisms; however, both lectins and their purified isoforms agglutinated rat, rabbit, and chicken erythrocytes. The hemagglutinating activity of both types of lectins was inhibited by N-acetylated amino sugar residues such as Neu5Ac, Neu5,9Ac, GlcNAc, and GalNAc; α or β-methyl glycosides from sialic acid, but α2,3- or 2,6-linked sialic acid were better inhibitors than simple sugars. This result suggests that the anomeric position of C2 from Neu5Ac acid does not seem to be relevant for *M. rosenbergii* lectin recognition. Sialylated glycoproteins, such as fetuin, and ovine and bovine submandibular mucin, which contain O-glycosydically, linked glycans [22,23] were more powerful inhibitors of the hemagglutinating activity of the lectin on both maturation stages, than monosaccharides. The specificity of both lectins for sialylated O-glycosydically linked glycans, was confirmed by the fact that the isolated O-glycosydically linked glycans from fetuin inhibited the lectins activity [20]. Sialylated N-glycosydically linked peptides from fetuin, or glycoproteins containing N-glycosydically linked glycans such as hen ovalbumin, human transferrin, IgG, or α_1-glycoprotein acid, lacked capacity to inhibit the lectins hemagglutinating activity. The relevance of the sialic acid for the interaction of *M. rosenbergii* lectin with these glycoproteins and glycopeptides as well as with cellular receptors, was confirmed when we eliminated the sialic acid from those glycoproteins and found that their inhibitory capacity was abolished.

While Neu5Ac is the major type of sialic acid found in human and sheep erythrocytes, and Neu4,5Ac or Neu5Gc is the most predominant sialic acid derivative present in horse and pig erythrocytes, respectively [24,25], the erythrocytes from rat, rabbit and chicken possess great amounts of Neu5,9Ac, accounting for 25, 29 and 30% of total sialic acid respectively [23–25]. These data suggest that Neu5,9Ac linked to specific glycan structures, is a receptor determinant for the *M. rosenbergii* lectin in both studied maturation stages.

The lectin from adult or juvenile freshwater prawns is a dimeric glycoprotein of 20 kDa conformed by 9.6 kDa subunits [8,13]. The lectin from juvenile organisms contained four isoforms with different pI (5.6 to 4.2), in contrast to the lectin from adults which contained only one isoform at pI 4.2,

suggesting that the subunits conforming the lectin in juvenile prawns have different physicochemical properties. The lectins constituted by subunits at pI 5.6 showed lower hemagglutinating activity, which explains, in part, why the lectin from juvenile prawns showed the lower hemagglutinating activity than the lectin from adults. The lectin purified from either adults or juvenile organisms showed identical molecular weight, and similar amino acid composition, however, they showed quantitative and qualitative differences in their sugar content and in the amount of Neu5Ac residues, the lectin from adults is less glycosylated than the juvenile, and showed higher concentrations of sialic acid. It is not known how this influence the three-dimensional conformation of the lectin, its functional properties or its biological role.

Research is in progress to define whether the expression of each lectin isoform could be the result of allelomorphic forms of the same protein [26], or if their glycosylation pattern, is regulated throughout maturation by environmental [27], neuroendocrine [28], or hormonal factors such as ecdisteroids [29,30]. It is highly probable that the maturation process which might modulate the lectin isoforms induces a diminution in the defense mechanisms and therefore increases the lability of juvenile organisms to infective diseases.

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References

1 Vasta GR, Ahmed H, Fink NE, Elola MT, Marsh AG, Snowden A, Odom EW, Animals lectins as self/non-self recognition molecules. Biochemical and genetic approaches to understanding their biological roles and evolution, *Proc Ann N Y Acad Sci* **712**, 55–73 (1994).

2 Olafsen JA, Lectins: models of natural and induced molecules in invertebrates. In *Advances in Comparative and Environmental Physiology,* Vol 24, (Springer Verlag, Berlin Heidelberg, 1996) pp. 49–76.

3 Vazquez L, Sierra C, Agundis C, Juarez S, Zavala A, Zenteno E, Mecanisms de defensa en crustáceos, *InterCiencia* **23**, 344–8 (1998).

4 Kawabata S, Iwanaga S, Role of lectins in the innate immunity of horseshoe crab, *Dev Comp Immunol* **23**, 391–400 (1999).

5 Slifkin M, Doyle RJ, Lectins and their application to clinical microbiology, *Clin Microbiol Rev* **3**, 197–218 (1990).

6 Alvarez G, Lascurain R, Perez A, Martínez S, Montaño L, Degand P, Zenteno E, Relevance of sialoglycoconjugates in murine thymocytes during maturation and selection in the thymus, *Immunol Invest* **24**, 9–18 (1999).

7 Guevara J, Espinosa B, Zenteno E, Vazquez L, Luna J, Perry G, Mena R, Altered glycosylation pattern of proteins in alzheimer's disease, *J Neuropathol Exp Neurol* **57**, 905–14 (1998).

8 Vazquez L, Masso F, Rosas P, Mantaño L, Zenteno E, Purification and characterization of a lectin from *Macrobrachium rosenbergii*, *Comp Biochem Physiol* **105B**, 617–23 (1993).

9 Sierra C, Perez A, Agundis C, Zenteno E, Vazquez L, Subcellur organization of the seric lectin in haemocytes from the freshwater prawn *Macrobrachium rosenbergii* (Decapoda, Nanantia). In *Crustaceans and the Biodiversity Crises*, edited by Schram FR, von Vaupel Klein JC, (Brill, Leiden Boston Koln, 1999), Vol 1, pp. 961–70.

10 Vazquez L, Jaramillo L, Rosas P, Lascurain R, Cooper EL, Zenteno E, Bacterial agglutination by the sialic acid specific lectin from the freshwater prawn *Macrobrachium rosenbergii*, *Comp Biochem Physiol* **113B**, 355–9 (1996).

11 Limpandanai D, Tansakul R, Culture of giant freshwater prawn (Macrobrachium rosenbergii) in a small resevoir, *Aquaculture* **20**, 257–60 (1980).

12 Wickins JF, Beard TW, Observations on the breeding and growth of the giant freshwater prawn *Macrobrachium rosenbergii* De Man in the laboratory, *Aquaculture* **3**, 159–64 (1974).

13 Vazquez L, Lanz H, Montaño LF, Zenteno E, Biological activity of the lectin from *Macrobrachium rosenbergii* hemolymph. In *Lectins: Biol Biochem Clin Chem*, edited by Van Driessche E, Bog Hansen TC, (Textop Hellerup, Dinamarca 1994), Vol. 10, pp. 261–5.

14 Zenteno E, Qin Z, Ortega M, Montreuil J, Debray H, Fast purification of *Phaseolus vulgaris* isolectins, *Prep Biochem* **24**, 175–83 (1994).

15 Bradford M, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal Biochem* **72**, 248–254 (1976).

16 Bidlingmeyer BA, Cohen SA, Tarvin TL, Rapid analysis of amino acids using pre-column derivatization, *J Chromatogr* **33**, 93–104 (1984).

17 Zanetta JP, Timmerman T, Leroy Y, Determination of constituents of sulfated proteoglycans using a methanolysis procedure and gas chromatography/mass spectrometry of heptafluorobutyrate derivatives, *Glycobiology* **9**, 255–66 (1999).

18 Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* **227**, 680–683 (1970).

19 Zenteno E, Lascurain R, Vazquez L, Montaño L, Debray H, Montreuil J, The sugar specificity of *Amaranthus leucocarpus* lectin, *Glycocon J* **9**, 204–08 (1992).

20 Spiro RG, Bhoyroo VD, Structure of the O-glycosidically linked carbohydrate units of fetuin, *J Biol Chem* **249**, 704–11 (1974).

21 Vazquez L, Maldonado G, Agundis C, Perez A, Cooper EL, Zenteno E, Morphology of hemocytes from fresh water prawn *Macrobrachium rosenbergii*, *Exp Zoology* **279**, 265–72 (1997).

22 Schauer R, Chemistry, metabolism, and biological functions of sialic acids, *Adv Carb Chem Biochem* **40**, 131–235 (1982).

23 Schauer R, Casalsst J, Corfield AP, Veh RW, Subcellular site of the biosynthesis of O-acetylated sialic acids in bovine submandibular gland, *Glycocon J* **5**, 257–70 (1988).

24 Sarris AH, Palade GE, The sialoglycoproteins of murine erythrocyte ghosts. A modified periodic acid-Schiff stain procedure staining nonsubstituted and 0-acetylated sialyl residues on glycopeptides, *J Biol Chem* **254**, 6724–31 (1979).

25 Herrler G, Reuter G, Rott R, Klent HD, Schauer R, N-acetyl-9-O-acetylneuraminic acid, the receptor determinant for influenza C virus, is a differentiation marker on chicken erythrocytes, *Biol Chem Hoppe Seyler* **368**, 451–4 (1987).
26 Theopold U, Rissler M, Fabbri M, Schmidt O, Natori S, Insect glycobiology: a lectin multigene family in Drosophilia melanogaster, *Biochem Biophys Res Commun* **261**, 923–7 (1999).
27 Bucke D, Aquatic pollution: effects on the health of fish and shellfish, *Parasitology* **106**, S25–S37 (1993).
28 Wilder MN, Okumura T, Aida K, Hanyu I, Ecdysteroid fluctuations during embryogenesis in the giant freshwater prawn, *Macrobrachium rosenbergii*, *Gen Comp Endocrinol* **80**, 93–100 (1990).
29 Freire CA, McNamara JC, Rosa JC, Greene LJ, Neuroendocrine control of osmotic regulation in the freshwater shrimp *Macrobrachium olfersii* (Wiegmann) (Crustacea, decapoda): free amino acid concentrations in the hemolymph, *Gen Comp Endocrinol* **100**, 83–91 (1995).
30 Chan SM, Rankin SM, Kelley LL, Characterization of the molt stages in *Penaeus vannamei*: setogenesis and hemolymh levels of total protein, ecdysteroids and glucose, *Biol Bull* **175**, 185–92 (1988).

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