Isolation and characterization of multiple-lectins from serum of the desert locust Schistocerca gregaria (Orthoptera: Acrididae)

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Abstract

Three lectins, designated as Sg1, Sg2 and Sg3, were identified in the serum of the desert locust Schistocerca gregaria. With the use of \((\text{NH}_4)_2\text{SO}_4\) fractionation, ion-exchange chromatography on DEAE-cellulose and affinity chromatography on CNBr-activated Sepharose 4B, three pools, each containing one of the putative lectins, were obtained. IEF, native PAGE and SDS/PAGE revealed that the three pools contained Sg1, Sg2 and Sg3, respectively, with \(pI = 6.39, 8.11\) and 6.8; native PAGE \(R_m = 0.44, 0.32\) and 0.32; SDS/PAGE subunits approximate molecular weights = 21.5, 44.5, and 43.9 kDa, respectively. Under reducing conditions, SDS/PAGE has separated each of Sg1 and Sg2 into two bands that indicate the presence of covalent interactions between their subunits, which are not present in Sg3. The three lectins are more highly specific for rabbit RBCs than for those of other vertebrates. The HA of lectins are \(\text{Ca}^{2+}\)-dependent, heat-labile, and are inhibited strongly by \(\alpha\)-linked-D-galactosides, followed by L-\((\pm)\)-rhamnose.

Keywords

multiple lectins, isolation, serum, hemagglutination, sugar inhibition, \(\alpha\)-linked-D-galactosides, rhamnose, \(S.\) gregaria

Abbreviations

HA, hemagglutinating activity
IEF, isoelectric focusing
PAGE, polyacrylamide gel electrophoresis
pl, isoelectric point
RM, relative mobility
SDS, Sodium dodecyl sulfate

Introduction

Lectins can be defined as proteins that recognize specific carbohydrate structures and thereby agglutinate cells by binding to cell-surface sugars, glycoproteins, and other glycoconjugates (Lis & Sharon 1998). They are usually structurally complex molecules with one or more carbohydrate-recognition domains and therefore with possible multiple binding sites (Gillespie et al. 1997, Dodd & Drickamer 2001). Among these lectins, those that require \(\text{Ca}^{2+}\) for their activity are called C-type lectins (Drickamer 1988). Animal lectins have been found in various invertebrates as well as vertebrates (Barondes 1984), either in soluble or in membrane-bound form. These lectins can play a variety of physiological roles: in particular, they are crucial in the innate immune system where they bind, as referred to above, to the carbohydrates present on the surface of potential pathogens (Rudd et al. 2001).

The most extensively studied functions of hemolymph lectins have been their roles in insect immune defense systems (Ratcliffe & Rowley 1980, Vasta et al. 2007). Furthermore, induced or endogenous hemolymph lectins and/or hemocyte-associated lectins have been shown to be involved in phagocytosis (Kawasaki et al. 1993, Wheeler et al. 1993, Wilson et al. 1999), encapsulation (Konomo & Natori 1985), nodule formation (Kyriakides et al. 1993), activation of the phenoloxidase system (Chen et al. 1995) and hemolymph coagulation (Minnick et al. 1986).

Relatively few insect lectins have been purified and characterized. The majority of insect lectins characterized to date have been detected in the hemolymph in most cases they are thought to be synthesized in the fat body (Kubo et al. 1984, Amanai et al. 1991) and/or the hemocytes (Amiranti 1976, Stiles et al. 1988, Amanai et al. 1991, Boucias & Pendland 1993). Lectins have been isolated and characterized from some insects, e.g., dipterans (Konomo et al. 1980, Stynen et al. 1985, Ingram & Molyneux 1990, McKenzie & Preston 1992, Haq et al. 1996, Chen & Billingsley 1999, Volf et al. 2002), lepidopterans (Pendland & Boucias 1986, Castro et al. 1987, Qu et al. 1987, Amanai et al. 1990, Gűl & Ayvallı 2002, Ourch et al. 2005, Chai et al. 2008), orthopterans (Stebbins & Hapner 1985, Drif & Breheulin 1994, Ayaa 2004), dictyopterans (Kubo & Natori 1987, Chen et al. 1993), phasmids (Richards et al. 1988), and hemipterans (Gomes et al. 1991). Their structure, function, and carbohydrate-binding properties still need further investigation to clarify their role in the innate immune system of insects.

The present work reports on isolation and on the biological, physicochemical and molecular characterization of multiple lectins from serum of the 5th-instar desert locust \(S.\) gregaria.
and at -20°C over a period of 1 week to 3 months, were tested for their
effect on HA (Ayaad et al. 2009 this issue).

Determination of total protein concentration.—The total protein con-
centration was determined according to the method of Bradford
(1976), using Coomassie Brilliant Blue (COBB). Bovine serum al-
bumin (BSA) fraction V (Sigma-Aldrich), dissolved in 0.15 M NaCl,
was used as a protein standard.

Isolation of lectins [(NH₄)₂SO₄ – salting out of serum proteins].— Insect
serum (25 ml) was diluted with an equal volume of 0.02 M TBS pH
7.0 containing 2 mM CaCl₂ and 1 mM MgCl₂ (TBS/Ca²⁺−Mg²⁺). Pro-
teins were precipitated by addition of (NH₄)₂SO₄ (Sigma-Aldrich) at a
concentration of 70%, with gentle stirring at 4°C. The resulting
precipitate was collected by centrifugation and dialyzed in Visking-
dialysis tubing (mol. wt cut-off [MWCO]: 12000-14000 Da) (Serva
Electrophoresis GmbH, Germany) against distilled water for 24 h at
4°C with three changes. The dialyzed solution was then centrifuged
at 8000 rpm for 5 min at 4°C to remove insoluble materials.

Ion-exchange chromatography of lectins.—Twenty grams of DEAE-cellu-
lose (Amersham Pharmacia Biotech) were washed in 0.5 M NaOH
for 10 min, then rinsed in distilled water, followed by 0.8 M HCl,
and finally 0.5 M NaOH, to remove contaminants. The washed
matrix was thoroughly rinsed free of NaOH with distilled water
and mixed with a sufficient quantity of starting buffer (0.01 M TBS
containing 0.01 M CaCl₂ and 0.01 M MgCl₂, pH 7.0) to produce a
thin suspension. The treated matrix was packed into a column (20
cm × 1.0 cm i.d.) and thoroughly washed with the starting buffer.
A sample from the last step of (NH₄)₂SO₄ fractionation (approximately
20 ml of 2.85 mg protein/ml) was applied to the DEAE-cellulose
column. The column was then washed with the starting buffer until
the baseline of absorption at 280 nm was achieved, and then elu-
on with a linear gradient of 0-0.25 M NaCl in 200 ml of the starting
buffer. The flow rate was adjusted to 10 ml/h. Fractions (tubes)
constituting the separated peaks were tested separately for HA against
rabbit RBCs; all fractions displaying HA were then pooled for use in
affinity chromatography.

Affinity chromatography of lectins.—Lectins of the same peak were
isolated from the other proteins contained therein, by use of CNBr-
activated Sepharose 4B (Sigma-Aldrich) affinity chromatography
as described by Korman et al. (1980) with slight modifications. CNBr-
activated Sepharose 4B matrix was packed into a column (10X1 cm
i.d.) and extensively washed with about 500 ml of TBS/Ca²⁺−Mg²⁺,
PH 7.0 containing 0.02% Na₃A (Sigma-Aldrich). Each separate
pool of fractions, having HA from the DEAE-cellulose column, was
separately applied (slowly) to the CNBr-Sepharose 4B column at 4
°C. This latter column was washed successively with TBS, to which
0.5 M NaCl was added, until no further protein was detected in the
elu-highlighting the absorbance at 280 nm. Lectins (the bound
material) were then eluted with 0.3 M raffinose solution in TBS/
Ca²⁺−Mg²⁺ (Ayaad 2004, Ayaad et al. 2009). The collected fractions
of each peak were pooled and each pool separately dialyzed extensively
against TBS/Ca²⁺−Mg²⁺, then against de-ionized distilled water to
remove raffinose; then HA of each pool was assayed against rabbit
RBCs. Each pool of fractions of the resulting peaks was lyophilized
and stored at -20°C until use.

IEF of lectins.—The isoelectric points of lectins, contained in pools
collected from affinity chromatography columns, were estimated
on a pH 3.6 to 9.3 gradient polyacrylamide gel, according to the
method of O’Farrell (1975), in slab gels (140 × 120 × 0.75 mm).
Electrophoresis ran at 350 V for 17-18 h, yielding approximately
6000 Vh at 22°C. The pi values of the isolated lectins were deter-
mined by running a mixture of standard proteins of known isoelec-
tric points on the same gel. We obtained a number of mixtures of
proteins with different pi values (IEF MIX-Sigma, USA, pH 3.6-9.3)
containing amyloglucosidase (pi 3.6), trypsin inhibitor (pi 4.6),
β-lactoglobulin A (pi 5.1), carboxy anhydrase II (bovine, pi 5.9),
carbonic anhydrase I (human, pi 6.6), myoglobin (pi 6.8, 7.2),
lecin from Lens culinaris (pi 8.2, 8.6, 8.8), and trypsinogen (pi
9.3): identifications of the different pi values were made using an
image densitometer G 700 (Bio-Rad, USA).

Native PAGE of lectins.—The identity of the destined lectins con-
tained in the pools collected from affinity chromatography columns
(rafinose-eluents) was confirmed by native PAGE. Electrophoresis
was carried out on 10% polyacrylamide gel under nondenaturating
conditions, according to the method of Schägger and von Jagow
(1991), using a Tris-glycine running buffer without SDS.

After isolation of protein bands by the nondenaturing PAGE,
the characteristic bands were detected on the gel by staining and
matching procedures, then were sliced away from the remainder
of the gel with a sharp razor blade. Each of these specific gel areas
was cut into pieces, approximately 1 mm² for each, and the pieces
of each band soaked in 2 ml of distilled water in a separate tube
overnight at room temperature. Each tube was centrifuged at 6000
rpm for 10 min; the eluate (supernatant) was transferred into a new
tube. The eluted proteins were concentrated by speed vacuum for
30 min and then stored at -20°C until used.

SDS/PAGE of lectins (under nonreducing and reducing conditions).—
SDS/PAGE of isolated lectins was carried out by the discontinuous
buffer system of Laemmli (Laemmli 1970). Some samples
were denatured with 2% SDS containing 5% β-mercaptoethanol
by boiling for 3 min. Treated samples were centrifuged at 10000
for 5 min before being loaded onto the gels. Electrophoresis
was carried out at a constant voltage of 200 V for 90 min. The gels
were calibrated with standard molecular weight proteins [New England
Biolabs Ltd. (low range: 116, 91, 46.4, 34.3, 28.7, and 21 kDa) and/or
Titan Biotech Ltd. (high and low range: 200, 116, 97, 66, 45, and
21 kDa)] and the quantifications of the different molecular weights
were made using an image densitometer G 700 (Bio-Rad).

Results and Discussion

The isolated lectins

Isolation of lectins.—The methods and procedures used for isolation
of the 5th-instar S. gregaria lectins are presented in Figure 1.

After (NH₄)₂SO₄ fractionation and DEAE-cellulose chromatog-
raphy, the HA was found to be present in fractions of peak I (18
ml), peak II (24 ml), and peak III (18 ml), but not in peaks IV
and V (Fig. 2). Therefore, fraction pools of each of peaks I, II,
and III were isolated to contain lectins. These pools were separately,
and slowly applied to the prepared CNBr-activated Sepharose 4B
column for affinity chromatography.

On washing each column successively with TBS to which 0.5 M
NaCl was added until no further protein was detected, it was shown
that most proteins were not adsorbed to the column, appeared in
the through-flow fractions, and had no HA (Fig. 3a, b, c). When

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Fig. 1. Summary of methods and procedures used to isolate serum lectins of 5th instar S. gregaria.

Table 1. Purification schedule of the 5th instar S. gregaria serum lectins.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Hemagglutinating activity (HA)</th>
<th>Specific activity (HA/mg protein)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A- Crude lectins and salting out</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole serum (crude lectins)</td>
<td>25</td>
<td>2.28</td>
<td>512</td>
<td>224.5</td>
<td>100</td>
</tr>
<tr>
<td>70% saturated (NH₄)₂SO₄</td>
<td>150</td>
<td>0.62</td>
<td>256</td>
<td>412.9</td>
<td>50</td>
</tr>
<tr>
<td>B- DEAE cellulose chromatography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak I</td>
<td>18</td>
<td>1.99</td>
<td>8</td>
<td>4.02</td>
<td>1.562</td>
</tr>
<tr>
<td>Peak II</td>
<td>24</td>
<td>1.73</td>
<td>16</td>
<td>9.24</td>
<td>3.125</td>
</tr>
<tr>
<td>Peak III</td>
<td>18</td>
<td>2.04</td>
<td>16</td>
<td>7.84</td>
<td>3.125</td>
</tr>
<tr>
<td>C- Affinity chromatography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pool 1</td>
<td>7.5</td>
<td>0.85</td>
<td>128</td>
<td>150</td>
<td>25</td>
</tr>
<tr>
<td>Pool 2</td>
<td>7.5</td>
<td>0.66</td>
<td>256</td>
<td>387.8</td>
<td>50</td>
</tr>
<tr>
<td>Pool 3</td>
<td>6</td>
<td>0.55</td>
<td>256</td>
<td>465.4</td>
<td>50</td>
</tr>
</tbody>
</table>
Table 2. Hemagglutinating activity (HA) of the 5th instar *S. gregaria* serum lectins contained in the collected pools from affinity chromatography, against a group of vertebrate RBCs.

<table>
<thead>
<tr>
<th></th>
<th>P₁</th>
<th>P₂</th>
<th>P₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>128</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>Sheep</td>
<td>4</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>Human:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A⁺</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>B⁻</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>O⁻</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Rat</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Horse</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* measured in a sample pool of 20 insects for each RBC type.

the bound material (lectins) was then eluted with 0.3 M raffinose solution in TBS/Ca-Mg. Small protein peaks containing HA were realized (Fig. 3a, b, c). Fractions constituting each peak were considered as different pools [pool 1 (P₁), pool 2 (P₂), and pool 3 (P₃)], each containing different lectins. After dialysis to remove raffinose, the three resulting pools exhibited prominent HA; therefore, they seemed to contain different lectins. The specific activity of lectins recovered from serum by use of these methods and procedures showed a 1.5 to 4-fold increase in the specific activity (Table 1), indicating prominent reproducibility.

**Homogeneity and molecular characteristics of lectins**

Homogeneity and molecular characteristics of the isolated lectins contained in the pools P₁, P₂, and P₃ were monitored and confirmed by use of PAGE and SDS/PAGE (under nonreducing and reducing conditions).

**Isoelectric focusing (IEF).** —The obtained results (Fig. 4) reveal some characteristics of these lectins. P₁ and P₃ both focused as single bands at pH 8.11 and pH 6.84, respectively. However, P₂ showed a major band (~84%) focused at pH 6.39 and a minor band (16%) focused at pH 6.53 and realized in association to the major one. Therefore, each of the P₁ and P₃ seems to be formed of a single and separate lectin. The nature of the two-band components of P₂ were confirmed by native PAGE, accompanied with HA determination to each separated band (see below).

The pl values of P₂ protein appear to lie in the slightly alkaline

**Fig. 2.** Ion-exchange chromatography on DEAE-cellulose, of the 5th instar *S. gregaria* serum proteins, resulting from salting out by 70% (NH₄)₂SO₄. Elution with a linear gradient of 0–0.25 M NaCl. Fractions of peaks I, II, III have HA against rabbit RBCs; but those of peaks IV, V have none.
Fig. 3. Affinity chromatography of lectins, contained in peaks I, II, III (a, b, c, respectively) of proteins separated from serum of 5th instar S. gregaria by DEAE-cellulose column, on CNBr-activated Sepharose 4B. Lectins (bound components) were eluted with 0.3 M raffinose solution in TBS/ Ca²⁺-Mg²⁺ (indicated by arrows).
Appearance of native PAGE patterns of Sg$_4$ (P$_{4m}$), Sg$_3$ and Sg$_2$ as a single band with high molecular weight range, indicates that each band may be formed of aggregates (oligomers) of subunits. This assumed oligomeric native form of lectins of the 5th instar S. gregaria serum is reported before in other insects, e.g., the orthopterans M. sanguinipes (Stebbins & Hapner 1985), L. migratoria (Drf & Brehelin 1994), and adult S. gregaria (Ayad 2004), and in the dipteran G. fuscipes (Ingram & Molyneux 1990).

SDS/PAGE.—When the lectins Sg$_4$ (P$_{4m}$), Sg$_3$ and Sg$_2$ were sliced away from the native PAGE, eluted, then electrophoresed by SDS/PAGE, under both nonreducing (Fig. 6), and reducing (Fig. 7) conditions, using 10% separating gel and 5% stacking gel, additional characteristics were revealed. Under the nonreducing conditions, each of the Sg$_4$, Sg$_3$, and Sg$_2$ was separated by SDS/PAGE as a single band with approximate molecular weights of 21.5, 44.5, and 43.9 kDa respectively (Fig. 6). This may indicate that subunits of each lectin are of approximately the same molecular weight and are held together by noncovalent interactions. Noncovalent linking of protein subunits was reported in leucins of other insects, e.g., the dipteran S. peregrina (Komano et al. 1980), lepidopterans H. cecropia and S. exigua (Castro et al. 1987, Boucias & Pendland 1993), and the phasmid E. tiaratum (Richards et al. 1988).

Under the reducing conditions, SDS/PAGE separation (Fig. 7) showed a single band only from Sg$_4$ (21.7 kDa), whereas each of Sg$_3$ and Sg$_2$ were separated into two bands. Those resulting from Sg$_4$ have approximate molecular weight of 32.6 and 31.7 kDa, and those of Sg$_3$ are of 32.9 and 31.5 kDa. The separation of two different bands with two different molecular weights from each of the Sg$_4$ and Sg$_3$ (Fig. 8) indicates that each of these latter is formed of two different types of subunits linked with disulphide bonds. This character was reported before for some insect leucins, for example, the orthopterans T. commodus (Hapner & Jermy 1981), and M. sanguinipes (Stebbins & Hapner 1985), BD1, and BD12 of the dipteran B. discoidalis (Chen et al. 1993), and allo A-I and -II of the coleopteran A. dactylana (Umetu et al. 1984). The low molecular-weight range of the single-banded Sg$_4$ separated by the nonreducing and reducing SDS/PAGE (21.5 to 21.7 kDa) is near to those of other insects, for instance, 30-kDa lectin of P. americana (Kubo & Natori, 1987), 20-kDa lectin of S. peregrina (Fujita et al. 1998).

**Physicochemical properties of the isolated lectins**

**Stability.**—The obtained data revealed that HA was completely abolished after 25 min exposure to 100°C, but reduced to 75% only upon exposure to 60°C for the same time. On the other hand, it was observed that HA was stable when exposed to 25°C (room temperature) for the same period of time. These observations indicate that lectins of the 5th instar S. gregaria are heat-labile in nature. Heat instability is characteristic for lectins of some other insects, e.g., the orthopterans T. commodus, (Hapner & Jermy 1981), and M. sanguinipes (Stebbins & Hapner 1985), the phasmid E. tiaratum (Richards et al. 1988), and the dipteran G. fuscipes (Ingram & Molyneux 1990). However, in contrast to these cases, in the coleopteran L. decemlineata (Minnick et al. 1986), and the orthopteran L. migratoria (Drf & Brehelin 1994) the lectins were reported to be heat resistant when subjected to elevated temperatures of 70-100°C.

For the storage temperature and period, the HA of lectins of 5th instar S. gregaria were stable when stored at -20°C, with an extremely slow insignificant decline observed on prolonged storage (3 mo).
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OF GALACTOSEANDIN 4TIARATUM INSTABILITYWASOBSERVEDWHENETHETLECTINWASNOTSTOREDINLACTOSE.
ICHARDS ETAL.$IVALENTCATIONREQUIREMENTFOR (!ˆ 4HEPRESENTDATASHOWTHAT
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(!3OMECATIONSSUCHAS-GOR:NCANONLYPARTLYREPLACE#A
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(LSOTHELECTINSOF 4COMMODUS 3SANGUINIPES 3EXIGUA 3TIARATUMANDADULT 3GREGARIA WEREALSO
LOSTINTHEPRESENCEOF%$4!0ENDLAND"OUCIAS 3TEBBINS
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LECTINSDENOTEDAS#
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NAMEDTOREMECTTHESPECIALIMPORTANCEOFCARBOHYDRATEBINDING:
ELENSKY'READY%ACHOFTHE
KNOWN#
TYPELECTINSHASCARBOHYDRATE
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BINDINGSITEANDMAYALSOCONTAIN
ASECONDA
SUGARSPECIFICITYANDINHIBITIONTO(! OFLECTINSISOLATEDFROMSERUMOFTHINSTAR 3GREGARIA AGAINST RABBIT RBCS SHOWS CERTAIN CHARACTERISTICS. THE INHIBITION OF SUGARS AND OF GLYCOCONJUGATES TO HA OF SG, SG, AND SG, SHOWED GREAT
RESEMBLANCETOONEACHOTHER (Table 4). THE HA OF THESE LECTINS WAS PREFERENTIALLY INHIBITED (IC$_{50}$ = 6.25 mM) BY RAFFINOSE, α-M-NITROPHENYL-D-GALACTOSE, α-P-NITROPHENYL-D-GALACTOSE, AND α-METHYL-D-
GALACTOSE, AND THE MONOSACCHARIDE L-(+)-RHAMNOSIDE. NONE OF THESE
LECTINS WERE INHIBITED BY D-(+)-MANNOSE, TRELHOSIDE AND N-ACETYLGALACTOSAMINE (200 mM), OR LAMINARIN (> 1%). LOW SENSITIVITIES (IC$_{50}$ = 50-100 mM) WERE OBSERVED IN THE CASE OF THE FREE SUGARS D-GALACTOSE, D-GLUCOSE, SUCCOSE, LACTOSE, AND ALSO BY N-ACETYL-D-GALACTOSAMINE. THESE RESULTS ALLOW SPECULATION THAT THE CARBOHYDRATE-BINDING SITE OF THE CARBOHYDRATE RECOGNITION DOMAIN OF THESE LECTINS PREFERENCES (WITHIN THE RANGE OF THE TESTED SUGARS) α-LINKED-D-GALACTOSIDES OVER

Sugar specificity and inhibition to HA. — The present data show that the isolated lectins SG, SG, and SG require Ca$^{2+}$ to express their
HA. SOME cATIONS SUCH AS Mg$^{2+}$ OR Zn$^{2+}$ CAN ONLY PARTLY REPLACE Ca$^{2+}$; however other divalent cations, such as Mn$^{2+}$, HAVE NO EFFECT (Table 3). AS EXPECTED, ADDITION OF EDTA CAUSED COMPLETE INHIBITION TO
HA. ALSO REMOVAL OF Ca$^{2+}$ BY DIALYZING THE LEKTIN SOLUTIONS AGAINST
EITHER TBS ALONE OR TBS WITH 10 mM EDTA, SIMILARLY RESULTED IN
A COMPLETE LOSS OF HA OF THESE LECTINS. EDTA PRESUMABLY EXERTED ITS EFFECT THROUGH CHELATION OF DIVALENT CATIONS THAT STRUCTURALLY
ASSOCIATED WITH THE LEKTINS. THE HA OF C-TYPE LEKTINS OF T. COMMODOUS, M. SANGUINIPES, S. EXIGUA, E. TIARATUM AND ADULT S. GREGARIA WERE ALSO
LOST IN THE PRESENCE OF EDTA (PENDLAND & BOUCIAS 1986, STEBBINS &

LEKTINS ISOLATED FROM SERUM OF THE 5TH INSTAR S. GREGARIA, THE OBSERVED
REQUIREMENT OF Ca$^{2+}$ TO ENHANCE AND MAINTAIN HA OF THE THREE LEKTINS SG, SG, AND SG, PROMINENTLY INDICATES THAT THESE LEKTINS ARE Ca$^{2+}$-DEPENDENT. THIS PROPERTY IS A GENERAL CHARACTERISTIC OF A GROUP OF
LEKTINS DENOTED AS C-TYPE LEKTINS. THIS TYPE OF LEKTIN WAS ORIGINALLY
NAMED TO REFLECT THE SPECIAL IMPORTANCE OF Ca$^{2+}$ IN THE MECHANISM
OF CARBOHYDRATE BINDING (ZELENSKY & GREADY 2005). EACH OF THE
KNOWN C-TYPE LEKTINS HAS CARBOHYDRATE-RECOGNITION DOMAIN
STRUCTURES CONTAINING A CONSERVED Ca$^{2+}$-BINDING SITE; AND MAY ALSO
CONTAIN A SECOND SITE (DRICKAMER 1999).

Fig. 5. Native PAGE of the 5th instar S. gregaria serum proteins (lane 1), and the lectins contained in the pools P$_{c}$, P$_{v}$, and P$_{l}$ (lanes 2, 3, and 4 respectively) collected from Sepharose 4B affinity chromatography columns. The gel was stained with COBB R-250.

Since the storage medium of the isolated lectins under investigation was without any additive-sugars, it seems that these lectins do not need the presence of sugars for their stability. This situation is analogous to that reported in S. peregrina (KOMANO ET AL. 1980).

However, in contrast to this case, isolated lectins from other insects require addition of sugar to the storage medium to maintain their activity. For example, in T. commodus (HAPNER & JERMYN 1981), the lectin required the addition of galactose. Also the lectins of M. sanguinipes (STEBBINS & HAPNER 1985), once isolated, were so unstable that disaggregation of the multimer occurred readily in the absence of D-galactose; and in E. tiaratum instability was observed when the lectin was not stored in lactose (RICHARDS ET AL. 1988).

Divalent cation requirement for HA. — The present data show that the isolated lectins SG, SG, and SG require Ca$^{2+}$ to express their
HA. SOME cATIONS SUCH AS Mg$^{2+}$ OR Zn$^{2+}$ CAN ONLY PARTLY REPLACE Ca$^{2+}$; however other divalent cations, such as Mn$^{2+}$, HAVE NO EFFECT (Table 3). AS EXPECTED, ADDITION OF EDTA CAUSED COMPLETE INHIBITION TO
HA. ALSO REMOVAL OF Ca$^{2+}$ BY DIALYZING THE LEKTIN SOLUTIONS AGAINST
EITHER TBS ALONE OR TBS WITH 10 mM EDTA, SIMILARLY RESULTED IN
A COMPLETE LOSS OF HA OF THESE LECTINS. EDTA PRESUMABLY EXERTED ITS EFFECT THROUGH CHELATION OF DIVALENT CATIONS THAT STRUCTURALLY
ASSOCIATED WITH THE LEKTINS. THE HA OF C-TYPE LEKTINS OF T. COMMODOUS, M. SANGUINIPES, S. EXIGUA, E. TIARATUM AND ADULT S. GREGARIA WERE ALSO
LOST IN THE PRESENCE OF EDTA (PENDLAND & BOUCIAS 1986, STEBBINS &

LEKTINS ISOLATED FROM SERUM OF THE 5TH INSTAR S. GREGARIA, THE OBSERVED
REQUIREMENT OF Ca$^{2+}$ TO ENHANCE AND MAINTAIN HA OF THE THREE LEKTINS SG, SG, AND SG, PROMINENTLY INDICATES THAT THESE LEKTINS ARE Ca$^{2+}$-DEPENDENT. THIS PROPERTY IS A GENERAL CHARACTERISTIC OF A GROUP OF
LEKTINS DENOTED AS C-TYPE LEKTINS. THIS TYPE OF LEKTIN WAS ORIGINALLY
NAMED TO REFLECT THE SPECIAL IMPORTANCE OF Ca$^{2+}$ IN THE MECHANISM
OF CARBOHYDRATE BINDING (ZELENSKY & GREADY 2005). EACH OF THE
KNOWN C-TYPE LEKTINS HAS CARBOHYDRATE-RECOGNITION DOMAIN
STRUCTURES CONTAINING A CONSERVED Ca$^{2+}$-BINDING SITE; AND MAY ALSO
CONTAIN A SECOND SITE (DRICKAMER 1999).

Fig. 6. SDS-PAGE analysis (under nonreducing conditions) of lectins SG, SG, and SG (lanes 1, 2, and 3 respectively) isolated from serum of the 5th instar S. gregaria. The gel was stained with COBB R-250. Molecular weights (kDa) of protein standards (lane M) are shown on the left.

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Table 3. Effects of divalent cations and EDTA (cation chelator) on hemagglutinating activity (HA) of the lectins Sg₁, Sg₂, and Sg₃ isolated from serum of 5th instar S. gregaria.

<table>
<thead>
<tr>
<th></th>
<th>Sg₁</th>
<th>Sg₂</th>
<th>Sg₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺ - free medium</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>20 mM Ca²⁺</td>
<td>128</td>
<td>256-512</td>
<td>256</td>
</tr>
<tr>
<td>20 mM Mg²⁺</td>
<td>64-128</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>20 mM Zn²⁺</td>
<td>128</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>20 mM Mn²⁺</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>10 mM EDTA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 mM EDTA</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

*Standard assay condition; i.e., reference value of HA.

Table 4. Inhibition by sugars and glycoconjugates to hemagglutinating activity (HA) of lectins Sg₁, Sg₂, and Sg₃, isolated from serum of the 5th instar S. gregaria, against rabbit RBCs.

| Minimum concentration* (mM/or %) required for 50 % inhibition (IC₅₀) to HA |
|-----------------|-----------------|
| Sg₁ | Sg₂ | Sg₃ |
| Monosaccharides | | | |
| D-(-)-galactose | 100 | 100 | 100 |
| D-(-)-glucose | >100 | 50 | >100 |
| L-(-)-rhamnose | 25 | ≥ 6 | 25 |
| Oligosaccharides | | | |
| Lactose | 100 | 100 | 100 |
| Raffinose | 12 | 6 | 6 |
| Sucrose | 100 | 100 | >100 |
| N-acetylated sugars | | | |
| N-acetyl-D-galactosamine | 100 | 50 | >50 |
| Others | | | |
| α-methyl-D-galactose | 12 | 12 | 25 |
| α- m-nitrophenyl-D-galactose | 6 | 6 | 6 |
| α-p-nitrophenyl-D-galactose | 6 | < 6 | < 6 |

*Data presented are from experiments repeated three times. Values > 200 mM [(D-(-)-mannose, trehalose, and N-acetyl-D-glucosamine) or >1 % (zymosan and laminarin) indicate that no inhibition of agglutination was observed. All inhibitions were of two wells unless otherwise indicated.

Both the β-form and also the free D-galactose.

The affinity of the 5th instar isolated S. gregaria serum lectins toward α-linked galactosides is a feature that has also been reported in other acidides, such as L. migratoria (Drif & Brehein 1994) and adult S. gregaria (Ayaad 2004). On the other hand, affinity of lectins to β-linked-D-galactosides, such as lactose and lactulose, was recorded in the coleopteran A. dichotoma (Umetzu et al. 1984), its lectins being inhibited by this group of carbohydrates. The latter lectin specificity is also known in vertebrates, where the best ligands are α-galactosides (Baronides 1984). Some lectins purified from other insect species, especially from orthopterans (Stebbins & Happner 1985, Lackie 1981, Jurenka et al. 1982, Happner 1983, Drif & Brehein 1989) show an affinity for a broad spectrum of carbohydrates. Another orthopteran T. commodus (Happner & Jermy 1981), and the dipteran P. duboscqi (Voll et al. 2002) possess lectins with amino sugar-binding affinity. In numerous other insect species, mainly lepidopterans, hemolymph lectins show affinity for galactose and lactose (Pendland & Boucias 1986) or to glucosides (Minnick et al. 1986, Qu et al. 1987). Lectins from another group of insects have binding affinity to mannose, e.g., the dictyopteran B. discoidalis (Chen et al. 1993), and the lepidopteran H. virens (Ourt et al. 2005), and to mannose, e.g., the dipteran A. stephensi (Chen & Billingsley 1999).

In this context, C-type lectins are known to have a common carbohydrate recognition domain (Weis et al. 1991, Drickamer 1992), or two domains (Yu & Kanost 2000), that may increase their binding affinity to carbohydrates. This domain contains a conserved Ca²⁺-binding site, and may contain a second one (Drickamer 1999). The binding mechanism involves interaction with carbohydrate and the conserved Ca²⁺, which may form hydrogen bonding with acid and amide side groups (Weis & Drickamer 1996).

The ligand specificity of the carbohydrate recognition domain is largely dependent on the position of hydroxy groups on the free or conjugated sugar, particularly the 3-OH and 4-OH (Ng et al. 1996, Kolatkar & Weis 1996). In addition to these interactions further specificity may be achieved by hydrogen bonds and electrostatic interactions with the surface of the protein (Ng & Weis 1997, Kolatkar et al. 1998). In the present work, agglutination of rabbit RBCs by C-type lectins Sg₁, Sg₂, and Sg₃ of the serum of the 5th instar S. gregaria was inhibited most efficiently by the α-linked-D-galactosides followed by L-(-)-rhamnose. However, the free monosaccharide D-(-)-galactose and the β-linked-D-galactosides (as in lactose) were of very low efficiency. In the latter cases, the orientation of 3-OH and 4-OH are the same for the same sugar. D-(-)-galactose; and the same was observed for 2-OH and 4-OH in D-(-)-galactose and L-(-)-rhamnose. Therefore, not only the orientation of 2-OH, 3-OH and 4-OH, but also the configuration (α or β) and the nature of the substituents at C₆ or the functional group at C₃, seem to affect the binding specificity and affinity of the 5th instar S. gregaria lectins to carbohydrates.

Fig. 7. SDS-PAGE analysis (under reducing conditions) of lectins Sg₁, Sg₂, and Sg₃ (lanes 1, 2, and 3, respectively) isolated from serum of the 5th instar S. gregaria. The gel was stained with COBB R-250. Molecular weights (kDa) of protein standards (lane M) are shown on the left.
According to these parameters, carbohydrate-binding specificity is crucial in recognition of these cell-surface carbohydrates, including those of the invading pathogens (McGreal et al. 2004). Therefore, animal C-type lectins are among the important proteins in pathogen recognition and cellular interaction (Rudd et al. 2001, Weis et al. 1998) by binding to the carbohydrate component of the surface molecular patterns of these targets (Janeway 1989).

References


