Isolation and characterization of three Ca^{2+} -dependent β -galactoside-specific lectins from snake venoms

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Three lactose-inhibited lectins from the venoms of the snakes *Agkistrodon contortrix contortrix* (southern copperhead), *Ancistrodon piscivorous leuostoma* (western cottonmouth moccasin) and *Crotalus atrox* (western diamondback rattlesnake) have been isolated and newly characterized. The three lectins are similar to thrombolectin, a lectin isolated from the venom of *Bothrops atrox* (fer-de-lance) (Gartner, Stocker & Williams, 1980), with regard to sugar specificity, M_r , Ca^{2+} requirements and sensitivity to reducing agents. Each lectin is a dimer (M_r 28000) consisting of monomers (M_r 14000) indistinguishable on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Haemagglutination activity is dependent on the presence of Ca^{2+} and is inhibited by reducing agents. The lectins are not identical and can be distinguished on the basis of relative affinities for inhibiting sugars, isoelectric points and immunoprecipitation assays using anti-(cottonmouth lectin) serum.

Thrombolectin, the first lectin isolated from snake venom, was discovered in the venom of the fer-de-lance (*Bothrops atrox*) (Gartner *et al.*, 1980). Assays of venom for haemagglutination activity revealed a venom component that cross-linked bovine erythrocytes. Purification and characterization of the agglutinin lead to the identification of a β -galactoside-binding dimeric lectin of M_r 28000. The lectin was similar in certain respects to other β -galactoside-binding lectins of vertebrate origin (Teichberg *et al.*, 1975; DeWaard *et al.*, 1976; Beyer *et al.*, 1980; Franklin *et al.*, 1980; Powell, 1980), especially with regard to sugar specificity, quaternary structure and M_r . However, the snake-venom lectin demonstrated a requirement for Ca^{2+} and sensitivity to reducing agent not found in other lactose-inhibitable lectins purified from vertebrates.

Subsequently, haemagglutinins have been discovered in 15 snake venoms (Ogilvie & Gartner, 1984). In addition to the agglutinins reported here, two of these lectins, one from bushmaster (*Lachesis muta*) venom and one from Jameson's-mamba (*Dendroaspis jamesonii*) venom have been purified and characterized (M. L. Ogilvie & T. K. Gartner,

unpublished work). Both of them are inhibited by lactose, but have characteristics different from those of the four lectins described below.

Experimental

Screening venoms

Snake venoms (Sigma Chemical Co., St. Louis, MO, U.S.A.; Pentapharm, Basel, Switzerland) were screened for haemagglutination activity with microtitre V plates (Dynatech Laboratories, Alexandria, VA, U.S.A.). A 25 mg portion of each venom was dissolved in 4 ml of Tyrode solution (8 g of NaCl, 0.05 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 1 g of NaHCO_3 , 0.02 g of CaCl_2 , 0.1 g of MgCl_2 and 0.2 g of KCl/litre, pH 7.4), and stored at -20°C . Haemagglutination assays were performed as described by Gartner *et al.* (1981). The number of agglutination units for each venom lectin is the reciprocal of the end-point dilution, i.e. the greatest dilution that gives agglutination.

Lectin purification

Pharmacia columns (300 mm $\times$ 9 mm) were packed with Sepharose 4B beads (Pharmacia), equilibrated with Tyrode solution and used at 4°C . A 0.5 g portion of each venom (in 10 ml of Tyrode solution) was applied to a column. Lectins were eluted with 0.1 M-lactose in Tyrode solution. Lectins were evaluated for purity by SDS/poly-

Abbreviations used: SDS, sodium dodecyl sulphate; IgG, immunoglobulin G; CM lectin, cottonmouth lectin; RS lectin, rattlesnake lectin; CuH lectin, copperhead lectin.

acrylamide-gel electrophoresis. Purified lectins were stored in Tyrode solution at -20°C .

Microtitre inhibition assays

Various sugars were dissolved in Tris/saline/ CaCl_2 solution (0.15 M-NaCl/0.01 M-Tris/2 mM- CaCl_2 , pH 7.4), and 25 μl portions of the solutions were placed in the microtitre wells. The first well of each row on the microtitre plate contained twice the concentration of sugar as the other wells in the row. All inhibition assays were performed as described by Gartner *et al.* (1981). Comparisons of results were made with controls lacking inhibitory substances.

Solutions (2 mM) of dithiothreitol (Sigma) and EDTA, (Sigma) were prepared in TCS and TS (0.15 M-NaCl/0.01 M-Tris, pH 7.4) respectively. Portions (25 μl) of either of these solutions were placed in every well except the first well of each row, which contained either 4 mM-EDTA or dithiothreitol. Red blood cells were suspended in TS rather than TCS for all EDTA-containing microtitre assays.

Electrophoresis

SDS/5–20% (w/v)-polyacrylamide-gradient gels were run with the buffer system of Laemmli (1970). A 10 μg portion of protein was boiled for 3 min in non-reducing sample buffer (2%, w/v, SDS) or 10 min in reducing sample buffer [2% SDS/10% (v/v) 2-mercaptoethanol]. Gels were run at pH 8.3 and then stained with Coomassie Brilliant Blue-R (Sigma).

Non-SDS/exponential-polyacrylamide (5–20%)-gradient gels were prepared with 50 mM-lactose. Lectins (15 μg) were diluted 1:1 in sample buffer containing 100 mM-lactose but no SDS. Lactose-containing sample buffer with 4 mM-EDTA was added to other lectin samples. Samples were not boiled. Non-SDS gels were run with SDS only in the tray buffer.

Isoelectric focusing was performed with Bio-Rad carrier ampholytes (pH 4–9) in 5% polyacrylamide (Bio-Rad Laboratories, 1979). Proteins (10 μg) were applied with Whatman no. 1 filter-paper discs to gels under non-reducing conditions and run 2 h to achieve a final voltage of 1200 V. Proteins in gels were fixed with Coomassie Brilliant Blue R. Lectin isoelectric points were determined by comparison with control gels from which the pH of gel slices was measured at 1 cm intervals.

Determination of covalently bound sugar

Total-hexose assays were performed as described by Dubois *et al.* (1956) with the modifications suggested by Levi & Teichberg (1981). A 50 μl portion of a 1:1 dilution of 90% liquefied

phenol (Sigma) and water were added to 0.4 μl of Tyrode solution containing 20 or 40 μg of lectin; 1 ml of H_2SO_4 was added and immediately vortex-mixed. Incubation mixtures were left for 10 min at room temperature and 20 min at 30°C before determining A_{480} with a Bauch and Lomb Spectronic 100 spectrophotometer. Results were compared with those produced by Tyrode solution containing 2.5–20 μg of glucose.

Spectrophotometry

Absorbance spectra for the four lectins were obtained using an Aminco absorbance spectrophotometer. Lectins were diluted into Tyrode solution and readings were made in the absorbance range 250–350 nm. Difference spectra were determined by comparing lectin samples in 4 mM-EDTA or 10 mM-lactose with samples without EDTA or lactose.

The same samples were read on a Perkin-Elmer MFP-44B fluorescence spectrophotometer in the 300–400 nm range. Difference spectra were obtained as described above.

Atomic-absorption spectroscopy was performed by using lectin dialysed extensively (48 h at 4°C) against double-glass-distilled water. Lectin samples were atomized in a Varian model-875 spectrophotometer. Standard samples of Ca^{2+} in water were analysed during the same trial. Samples and controls were read at 211.6 nm. The number of Ca^{2+} ions bound per lectin dimer was determined by dividing the concentration of Ca^{2+} in each lectin sample by the concentration of atomized lectin.

Antiserum

Rabbit anti-(CM lectin) immune serum was obtained by intramuscular injection of 2 mg of CM lectin (in Freund's complete adjuvant) followed by injection of 1 mg (in Freund's incomplete adjuvant) of lectin 4 weeks later. Anti-(bushmaster lectin) serum was obtained in a similar way. All inocula contained Freund's adjuvant, emulsified 1:1 with lectin solution. Serum collected 2 weeks after the final inoculum was precipitated with 100%-satd. $(\text{NH}_4)_2\text{SO}_4$ and dialysed in phosphate-buffered saline (8.77 g of NaCl, 1.15 g of Na_2HPO_4 , 0.2 g of KH_2PO_4 /litre, pH 7.0). Preimmune serum (obtained from the same rabbit before immunization) was precipitated with $(\text{NH}_4)_2\text{SO}_4$ in the same manner as the anti-(CM lectin) antiserum.

Immunodiffusion slides contained 100 mM-lactose. Immune and preimmune sera were adjusted to similar protein concentrations before use in Ouchterlony assays. Immune serum was tested against the venom lectins electrolectin (kindly provided by Dr. T. R. Podleski, Cornell University, Ithaca, NY, U.S.A.) and chicken lactose

lectin I (kindly provided by Dr. S. H. Barondes, University of California, San Diego, CA, U.S.A.).

Antiserum was added to lectin-containing microtitre assays in the following way: 25 μl of antiserum and 25 μl of lectin were placed in the first well of each row, then serially diluted across the plate. As controls, preimmune serum was substituted for antiserum.

Immunofluorescence

A 100 μl portion of a 2.5% suspension of trypsin-treated formaldehyde-fixed human erythrocytes were incubated for 1 h with 10 μg of CM lectin in Tris/saline containing either 5 mM-dithiothreitol, 10 mM-EDTA or 20 mM-lactose. Excess lectin was removed by washing in Tris/ CaCl_2 /saline. Anti-(CM lectin) serum was then incubated with the erythrocytes. Finally, tetramethylrhodamine-labelled goat anti-(rabbit IgG) (American Qualex, La Mirada, CA, U.S.A.) was added to the erythrocytes, followed by tetramethylrhodamine-labelled rabbit anti-(goat IgG) (American Qualex). Erythrocytes were washed in Tris/ CaCl_2 /saline after each incubation. Samples were examined for fluorescence with an Olympus Vanox microscope especially equipped for fluorescence microscopy.

pH ranges for lectin-erythrocyte binding were established in the following way: human erythrocytes were incubated with each lectin for 1 h in buffers at pH 2.3 (0.1 M-glycine/HCl), pH 8.5 (TS), pH 9.5 (0.01 M-sodium borate) and pH 10.5 (0.05 M- Na_2CO_3). Cells were washed, adjusted to pH 7.4, and then exposed to anti-(CM lectin) serum. Finally the erythrocytes were incubated with rhodamine-labelled, anti-(rabbit IgG) (each incubation was followed by extensive washing in Tris/saline). Cells were examined with the microscope described above.

Results

Screening crude venoms

Trypsin-treated formaldehyde-fixed bovine, horse, human and rabbit erythrocytes were agglutinated by all four venom solutions. Venom agglutinins were particularly potent in tests using bovine erythrocytes. Titres for fer-de-lance, cottonmouth, rattlesnake and copperhead venoms were 64, 1024, 64 and 1024 respectively. All venom-induced haemagglutination activities were inhibited by lactose.

Purification, yield and specific activity

Maximum yields of lectin per 0.5 g of venom were as follows: thrombolectin, 1.22 mg; CM lectin, 4.9 mg; RS lectin, 1.92 mg; CuH lectin, 1.9 mg.

Specific activities (total units of agglutination per mg of lectin) of the proteins tested with bovine

erythrocytes were: thrombolectin, 5.6×10^7 ; CM lectin, 4.7×10^8 ; RS lectin 4.3×10^7 ; CuH lectin, 6.2×10^7 .

Microtitre inhibition assays

The concentration of a sugar blocking one well of haemagglutination activity, i.e., the concentration inhibiting half-maximum lectin activity, was determined for 11 sugars. Thiodigalactoside was the most potent carbohydrate inhibitor of the four lectins (Table 1). The affinity of RS lectin for inhibiting sugars was approximately twice that for the other lectins.

The haemagglutination activity of each lectin was dependent on Ca^{2+} and inhibited by reducing agents. EDTA blocked all lectin activity. The inhibitory effect of EDTA was overcome by 2 mM- Ca^{2+} . Dithiothreitol (5 mM) in the assay solution inhibited more than 90% of lectin activity.

Electrophoresis

Under non-reducing conditions, all four lectins migrated as 28 kDa bands (Fig. 1). Only RS lectin was distinguishable from the other lectins; it produced a doublet containing a darkly staining 28 kDa band and a less-densely-stained band located just above it. Since the doublet disappeared on reduction, it is likely that its appearance was due to intrachain disulphide bonds. The lectins appeared virtually identical under reducing condi-

Fig. 1. SDS/polyacrylamide-gel electrophoresis of lectins under reducing (tracks 1–5) and non-reducing (tracks 6–9) conditions

Reducing conditions: track 1, Bio-Rad low- M_r standards (phosphorylase b , M_r 92500; albumin, 66200; ovalbumin, 45000; carbonic anhydrase, 31000; soya-bean trypsin inhibitor, 21500; lysozyme, 14400); track 2, CM lectin; track 3, thrombolectin; track 4, RS lectin; track 5, CuH lectin. Non-reducing conditions: track 6, CM lectin; track 7, thrombolectin; track 8, RS lectin; track 9, CuH lectin.

Table 1. Concentration of sugars causing inhibition of half-maximum haemagglutination activity

Trypsin-treated formaldehyde-fixed human erythrocytes were used in these assays.

Sugars	Lectin ...	[Sugar][mM, except for mucin (mg/ml)]			
		CuH	CM	RS	Thrombolectin
Thiodigalactoside		0.25	0.12	0.1	0.2
Lactose		0.75	0.25	0.12	0.35
Methyl β -D-galactopyranoside		0.3	0.3	0.25	0.5
D-Fucose		0.3	0.3	0.25	1
Methyl α -D-galactopyranoside		1	1	0.75	1.5
L-Rhamnose		1.5	0.75	0.3	2
D-Galactose		1.5	0.75	0.25	2.5
L-Glucose		10	5	2.5	10
Sucrose		30	30	10	30
Glucosamine		>30	>30	>30	>30
Mucin		5	5	1	5

Fig. 2. Non-SDS/50 mM-lactose/polyacrylamide-gel electrophoresis of lectins in the presence or absence of 4 mM-EDTA

EDTA present: track 1, CM lectin; track 2, thrombolectin; track 3, RS lectin; track 4, CuH lectin. Track 5, Bio-Rad low- M_r standards (see Fig. 1). EDTA absent: track 6, CM lectin; track 7, thrombolectin; track 8, RS lectin; track 9, CuH lectin.

tions on SDS/polyacrylamide-gel electrophoresis (Fig. 1). Each migrated as a single band with an apparent M_r of 14000.

Three of the lectins (thrombolectin, CM lectin and CuH lectin) demonstrated a 'lactose effect' on lactose-containing gels (Fig. 2). The 28 kDa band of thrombolectin was significantly diminished in intensity as a consequence of lactose in the gel. A high- M_r smear also appeared, possibly representing aggregates of the protein. CM and CuH lectins migrated as three discrete, but closely associated, bands (triplets) in lactose-containing gels. These triplets had M_r values of approx. 28000. Only RS lectin appeared unaffected by lactose in non-SDS gels. In all cases, the 'lactose effect' disappeared in

the presence of EDTA or SDS. Levi & Teichberg (1981) reported a 'lactose effect' on electrolectin. However, in contrast with the situation with snake-venom lectins, SDS or EDTA did not negate this effect.

Isoelectric focusing (under non-reducing conditions) revealed multiple bands for all the lectins. Isoelectric points of these bands were as follows: thrombolectin, a single band of pI 6.4 and a triplet of pI 9.5–9.7; CM lectin, a triplet of pI 7.2–7.3; RS lectin, a smear of more than ten bands of pI 7.0–7.6; CuH lectin, a triplet of pI 8.3–8.4. As mentioned above, with the exception of RS lectin, these lectins migrate as single bands on SDS-containing gels (under reducing conditions).

Determination of covalently bound sugar

No carbohydrate was detected using the Dubois *et al.* (1956) assay. Thus the lectins do not appear to be glycoproteins.

Spectrophotometry

Absorbance spectra (Fig. 3) for the four lectins were identical, as were fluorescence spectra (results not shown). The absorbance spectra for each lectin demonstrated maximum absorbance at 280 nm, with a shoulder at 290 nm. Fluorescence spectra of the four proteins was maximal at 335 nm.

Difference spectroscopy (absorbance and fluorescence spectra) of the lectins was performed in the presence and absence of either EDTA or lactose. Neither substance produced a change in the original spectra. Thus little perturbation of exposed aromatic amino acids took place on lectin–lactose interaction or chelation of Ca^{2+} from the lectin molecules. Other lectins, namely electrolectin (Levi & Teichberg, 1981) and peanut agglutinin (Jimbo & Matsumoto, 1982), have

Fig. 3. *Lectin absorbance spectra* —, CM lectin; ----, thrombolectin; ···, RS lectin; - · -, CuH lectin. All lectins showed a major peak at about 280 nm and a shoulder at about 290 nm.

demonstrated changes in fluorescence spectra after interaction with lactose.

Atomic-absorption spectroscopy of the four lectins demonstrated a high affinity for Ca^{2+} . The following values are expressed as molecules of Ca^{2+} /lectin dimer: thrombolectin, 13; CM lectin, 11; RS lectin, 15; CuH lectin, 33. As mentioned above, these lectins were extensively dialysed against double-glass-distilled water before determination of bound Ca^{2+} . The Ca^{2+} concentration of this water was $2.5 \mu\text{M}$.

Antiserum

The snake-venom lectins cross-reacted with both anti-(CM lectin) and anti-(bushmaster lectin) sera (Figs. 4 and 5). Thrombolectin, bushmaster, CM, RS and CuH lectins were arranged on Ouchterlony slides so that each lectin was adjacent to the other four lectins for testing against anti-(CM lectin) serum or anti-(bushmaster lectin) serum. Each lectin precipitated in the presence of anti-(CM lectin) serum. A spur was produced between each lectin pair (partial identity), except CM and CuH lectins. Anti-(bushmaster lectin) antiserum also demonstrated cross-reactivity with the lectins mentioned above. Control assays using preimmune serum produced no precipitin lines.

Anti-(CM lectin) serum inhibited the haemagglutination activity of all four lectins. Relative inhibitory effect of serum on the haemagglutination activity of the lectins in decreasing order is: CM lectin > CuH lectin > thrombolectin > RS lectin. Preimmune serum controls did not affect the haemagglutination activity of any of the lectins.

No precipitin lines were produced in tests using anti-(CM lectin) serum versus electrolectin and chicken lactose lectin I. However, others have reported that immune serum raised against electro-

Fig. 4. *Ouchterlony assays (in 1% agar) against anti-(CM lectin) serum*

For both assays (a and b) anti-(CM lectin) serum was contained in the centre wells (C). (a): 1, CM lectin; 2, thrombolectin; 3, CuH lectin; 4, CM lectin; 5, bushmaster lectin; 6, RS lectin. (b): 1, thrombolectin; 2, RS lectin; 3, CuH lectin; 4, bushmaster lectin.

Fig. 5. *Ouchterlony assays (in 1% agar) against anti-(bushmaster lectin) serum*

The centre well (C) contained anti-(bushmaster lectin) serum. 1, Bushmaster lectin; 2, RS lectin; 3, CuH lectin; 4, bushmaster lectin; 5, thrombolectin; 6, CM lectin.

lectin showed cross-reactivity with lectins from chick pectoral muscle and mouse thymus (Levi & Teichberg, 1982). Both of these are β -galactoside-binding lectins, but neither require Ca^{2+} for activity.

Immunofluorescence

Erythrocytes exposed to CM lectin in the presence of Tris/ CaCl_2 /saline were intensely fluorescent. However, erythrocytes treated with CM lectin in the presence of EDTA or lactose were not fluorescent. Therefore Ca^{2+} is required for both univalent and bivalent binding of lectin to erythrocytes. Also, lactose prevents binding. In contrast, dithiothreitol diminished, but did not eliminate, binding of lectin to erythrocytes. Thus it appears that reduction of disulphide bonds significantly decreased the affinity of the lectin for its receptors,

rather than simply abolishing polyvalency by producing monomers with normal affinity. This effect may have been caused by breaking intra-chain disulphide bonds. (Immunoprecipitation studies revealed no loss of lectin antigenicity as a consequence of reduction, chelation of Ca^{2+} or ligand binding.)

Immunofluorescence studies revealed that each lectin bound to erythrocytes over a broad pH range. All four lectins remained bound after incubation at pH 2.3, as evidenced by brightly fluorescent erythrocytes. Cells were also brightly fluorescent after exposure to the lectins at pH 8.5. At pH 9.5, however, all but thrombolectin-treated cells demonstrated barely discernible fluorescence. Thrombolectin apparently bound tenaciously at pH 9.5, but much less tenaciously at pH 10.5 (only weakly fluorescent erythrocytes). This assay did not distinguish between monovalent or polyvalent binding of lectin to erythrocytes. Therefore it is not known if the lectins cross-link erythrocytes at the extremes of pH tested by this method.

Discussion

The present results reveal similarities and differences between the lectins purified and characterized in this study. The structural and functional similarities of these lectins are listed below.

(1) Evidence for structural similarities resides in the observation that all four lectins are virtually identical on SDS-containing gels. All are apparently 28 kDa dimers that can be converted by reduction into monomers of apparently indistinguishable M_r values.

(2) All four lectins tenaciously bind Ca^{2+} (as evidenced by atomic-absorption spectrophotometry). In addition, the lectins require Ca^{2+} for haemagglutination activity. Consistent with this observation is the disappearance of the 'lactose effect' in non-SDS gels on addition of EDTA to lectin solutions. Thus Ca^{2+} -binding sites are found on all the lectin molecules. This is not unique to the snake-venom lectins, since sites for cation-binding have been previously identified for concanavalin A (Obata *et al.*, 1981).

(3) The function of each of the proteins is dependent, in part, on inter- or/and intra-chain disulphide bonds, since reducing agents destroy activity. The lectins may be bivalent or polyvalent only in a dimeric configuration, thus relying on interchain disulphide bonds for maintenance of haemagglutination activity. Conversely, their monomeric subunits may be bivalent and if so, dependent on intrachain disulphide bonds for maintenance of an active configuration.

(4) Thrombolectin and CM, RS and CuH lectins are all precipitated by antisera made

against bushmaster or CM lectin. Thus the lectins are immunologically related, i.e. they possess similar or identical epitopes.

(5) Spectrophotometric analyses of the lectins produced absorbance and fluorescence spectra which were virtually superimposable. Since these spectra are produced by aromatic amino acids, the distribution and number of these amino acids are apparently very similar within the proteins.

Differences between the four lectins are stated below.

(1) The lectins behave differently on non-SDS, lactose-containing gels. Whereas the migration of RS lectin is unaffected by the presence of lactose, the migration of the other three lectins is significantly affected by the ligand. Thrombolectin responds to lactose in a manner different from that shown by CM and CuH lectins. The different behaviour of the lectins in lactose-containing gels is further evidence for structural differences between the lectins. It is not understood why the lectins do not migrate identically on gels containing lactose and Ca^{2+} . Lack of a lactose effect for the RS lectin cannot be attributed to an insufficient affinity for the sugar, since RS lectin apparently has the greatest affinity for inhibiting ligands (Table 1).

(2) All four lectins have strikingly different isoelectric points. The lectins migrate as multiple bands on iso-focusing gels despite the fact that thrombolectin, CM and CuH appear as single bands on SDS-containing gels. The basis of this behaviour is not known. Heterogeneity of very similar lectins was also revealed by isoelectric focusing of lectins purified from the seeds of *Bandeira simplicifolia* (Murphy & Goldstein, 1977). Those authors attributed this heterogeneity to the presence of isolectins. This may be the case with the snake-venom lectins.

(3) Each of the lectins cross-react with anti-(CM lectin) serum. However, the formation of spurs between all lectin combinations except CM and CuH indicates antigenic differences. In other words, only partial immunological identity exists between the lectins.

(4) The fact that CuH lectin bound the most Ca^{2+} molecules/dimer indicates that this lectin may have significantly more Ca^{2+} -binding sites than the other lectins.

The function of the β -galactoside-binding lectins described here is not known. Although soluble, β -galactoside-binding lectins are widely distributed in Nature (Barondes, 1984), in no case has a function been unequivocally demonstrated for one of these lectins. Only in one case has a function been convincingly demonstrated for a β -galactoside-binding lectin, but this is for a membrane-bound lectin (Ashwell & Harford, 1982). It has

been suggested that the soluble β -galactoside-binding lectins have different functions in different tissues (Beyer *et al.*, 1979) and different functions in the same tissue at different times during development (Phillips & Gartner, 1980). However, the matter remains unresolved.

Beyer *et al.* (1979) provided data consistent with the suggestion that a β -galactoside-binding lectin secreted by intestinal goblet cells may attach mucin to the intestinal wall and thereby provide protection of the intestine from degradation by digestive enzymes. The snake-venom lectins described here also interact with mucin; that is, mucin inhibits their haemagglutination activities. Since the venom gland, but not the venom, contains mucin (Tu, 1977), the snake-venom lectins might facilitate formation and attachment of mucin sheets to the epithelial cells of the venom gland and thereby protect the gland from proteolytic damage due to enzymes in the venom. This matter requires further investigation.

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