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A New Anti-H Lectin from the Seeds of *Galactia tenuiflora*

JACQUES LE PENDU[1], GILBERT GÉRARD[2], FRANCINE LAMBERT[1], ROSELLA MOLLICONE[1] and RAFAEL ORIOL[1]

[1]*Institut d'Immunobiologie, Hôpital Broussais, 96 rue Didot, 75674 Paris Cedex 14, France*
[2]*Centre de Transfusion sanguine, Hôpital Bellepierre, 97400 St. Denis, Ile de La Réunion, France*

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A new anti-blood group H lectin was isolated from the seeds of *Galactia tenuiflora*. This lectin is mostly specific for the H type 2 trisaccharide but it shows some cross-reactivity with the H type 4 and H type 3 trisaccharides. Differences between this lectin and lectin 1 from *Ulex europaeus* are described. These differences concern the respective abilities of the lectins to recognize erythrocytes from some H deficient phenotypes, the inhibitions by salivas and the tissue distribution of the antigens recognized by the two lectins. The most important differences were noted in the surface epithelium of the stomach. This area is known to express ABH antigens under the control of the *Se* gene as defined by the *Ulex europaeus* lectin 1, yet it is always strongly labelled by the *Galactia tenuiflora* lectin irrespective of the secretor status of the tissue donor.

A number of lectins recognizing the blood group H determinant have been isolated and characterized. These include the lectins from *Lotus tetragonolobus* [1, 2] *Ulex europaeus* [3-5], streptomyces sp. [6] and eel serum (*Anguilla anguilla*) [7, 8]. Some of these lectins have been used to characterize H determinants on various tissues and cells in normal or pathological conditions [9-15]. We now report the isolation and characterization of a new anti-H lectin from the seeds of *Galactia tenuiflora*, a plant found in tropical areas that we collected on Reunion Island, in the Indian Ocean. This search for a new anti-H lectin was stimulated by the high incidence of H deficient individuals that we found on Reunion Island [16]. An anti-H activity has previously been found in the seeds of another species of the same genus, *Galactia filiformis*. However, this last plant, described by Ranadive and Bathia in India [17], is not found on Reunion Island.

We present some preliminary data showing the usefulness of the *Galactia tenuiflora* lectin in histochemistry.

Materials and Methods

Materials

H type 2 Synsorb® prepared by coupling the H type 2 trisaccharide to a solid matrix was obtained from Chembiomed Ltd. (University of Alberta, T6G-2G2, Edmonton, Canada). The artificial antigens, prepared by coupling synthetic oligosaccharides to bovine serum albumin (BSA), were kind gifts from Dr. R.U. Lemieux and Chembiomed Ltd. (15-20 mol hapten are coupled per mol BSA). Biotin *N*-hydroxysuccinimide ester and avidin peroxidase conjugate were obtained from Miles Scientific (Paris, France). Fluorescein isothiocyanate (FITC) and BSA were obtained from Sigma Chemicals (St. Louis, MO, USA). The reagents used for polyacrylamide gel electrophoresis were from Bio-Rad (Richmond, CA, USA). All other chemicals were reagent grade.

Agglutination

Red cells were obtained from normal blood donors who had been typed for ABO and from H deficient individuals from Reunion Island [18]. Titrations were performed in tubes containing 50 μl of serially diluted lectin which were incubated with 50 μl of a 4% suspension of erythrocytes for 30 min at room temperature. All dilutions were performed in 10 mM sodium phosphate, 0.14 M sodium chloride, pH 7.2 (phosphate buffered saline; PBS). The agglutinations were read macroscopically and the results were expressed in terms of cumulative scores [+++ = 10, ++ = 8, + = 5, (+) = 2, - = 0], since we feel that the scores allow an easier comparison of the various phenotypes analysed.

Purification of the Lectin

Mature seeds of *Galactia tenuiflora* were ground to a fine powder. The powder (50 g) was agitated with 300 ml of PBS for 20 h at room temperature. The mixture was centrifuged at 4500 × *g* for 10 min. Ammonium sulphate (20 g) was added to 100 ml of the supernatant and the mixture was allowed to stand overnight at 4°C. It was then centrifuged at 30 000 × *g* for 20 min. To the supernatant, 20 g of ammonium sulphate was added and this new mixture was allowed to stand at 4°C for 2 h. After centrifugation at 30 000 × *g* for 20 min, the precipitate was dissolved in 90 ml of PBS and dialysed against PBS. The agglutinating activity of this fraction was similar to that of the crude extract. The other fractions contained no or very weak agglutinating activity and were discarded. A portion of the active fraction (20 ml) was loaded on a column of H type 2 Synsorb (11 × 1 cm) prepared with 4 g of the immunoadsorbant, equilibrated in PBS. The flow rate was 30 ml/h. The column was then washed with PBS until no adsorbance at 280 nm was recorded. The bound lectin was desorbed with a solution of 2.5 M NH_4OH and immediately neutralized with KH_2PO_4. The flow rate during the washing and elution steps was 150 ml/h. A protein solution (20 ml) with an $O.D._{280}$ of 0.85 was obtained. Since a strong agglutinating activity remained in the non-adsorbed fraction, it was reloaded on the same column and a further 30 ml of desorbed material with an $O.D._{280}$ of 0.35 was obtained. The eluted fractions were then pooled and dialyzed against distilled water. A precipitate formed which could not be resolubilized. The remaining supernatant was freeze-dried and 18 mg of a white powder were obtained.

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out according to the method of Laemmli [19]. Ten percent polyacrylamide was used for protein separation under non-reducing conditions and 80 μg of the purified protein, dissolved in 2% sodium dodecyl sulfate, was loaded on the gel. When β-mercaptoethanol was added to the sample at a final concentration of 5%, 20% polyacrylamide was used and only 20 μg of the lectin in 2% sodium dodecyl sulfate was loaded on the gel.

Labelling of the Lectin

The purified lectin was labelled with biotin by incubating 2 mg of lectin, in 2 ml of 25 mM sodium borate buffer, pH 8.5, with 250 μl of 10 mM biotin-*N*-hydroxy-succinimide ester, for 1 h, at room temperature. The mixture was then dialyzed against 5 mM sodium borate buffer, pH 8.5 and then against PBS.

Labelling of the lectin with fluorescein isothiocynate (FITC) was achieved by incubating 5 mg of the lectin, dissolved in 1 ml of 50 mM sodium carbonate buffer, pH 9.2, with 10 mg of FITC. The incubation was carried out at 37°C for 2 h. The labelled lectin was then separated from the free FITC on a Sephadex G-10 column, 12 × 0.8 cm, equilibrated with PBS.

Inhibitions by Salivas

The purified *Galactia* lectin, diluted to 15 μg/ml in PBS, was coated on polystyrene tubes (L.E.F., Viry-Chatillon, France) by an overnight incubation at room temperature. Salivas (50 μl) serially diluted from 1/10 in 1% BSA/PBS were then incubated for 3 h at room temperature. All salivas had previously been boiled for 10 min and were kept at -20°C until used. After washing the tubes with 1 ml of tap water, 20 000 cpm of the artificial antigen H type 2-BSA, labelled with ^{125}Iodide (10 μCi/μg) by the chloramine T method [20], in 50 μl of 1% BSA/PBS were added. After a final wash with 1 ml of tap water, the tubes were counted in an LKB automatic gamma counter. The concentration of saliva necessary to inhibit 50% of the H type 2-^{125}I-BSA binding was expressed as a $\log_2$ derivative of the saliva dilution (1/10 = 1, 1/20 = 2, 1/40 = 3....).

Binding to Artificial Antigens

Artificial antigens, diluted to 1 μg/ml in PBS, were coated on the plastic of microtitration plates (NUNC, Immunoplate 1, Roskilde, Denmark) by an overnight incubation at room temperature. After adding 100 μl of 3% BSA-PBS and incubating for 1 h in order to prevent non-specific binding of proteins, 100 μl of lectin conjugated to biotin was then added serially diluted in 3% BSA-PBS, and incubated for 3 h. Each plate was then washed four times with tap water, after which 100 μl of avidin-peroxidase conjugate was added, diluted 1:500 in 3% BSA/PBS, and incubated for 3 h at room temperature. After four final washes with tap water, the enzymic reaction was detected with *O*-phenylenediamine. The $O.D._{490}$ nm was read in a Dynatech microelisa autoreader.

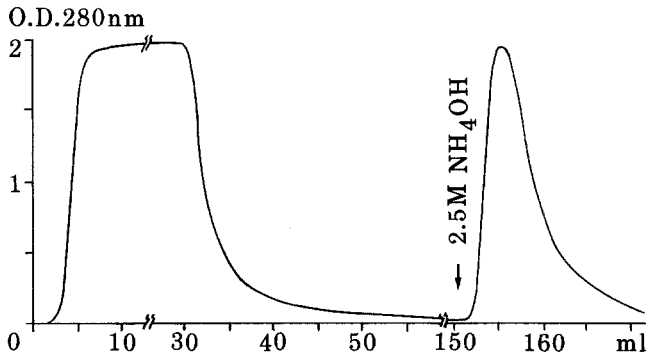


Figure 1. Isolation of the *Galactia* lectin on an H type 2 affinity column (11 × 1 cm). The ammonium sulphate precipitate from the crude extract was applied to the column, and the column was washed with PBS. The bound lectin was eluted with 2.5 M NaOH. The flow rate was 30 ml/h during the loading step and 150 ml/h during the washing and elution steps.

Tissue Samples

Formaldehyde-fixed and paraffin-embedded tissue sections were obtained through the kindness of Dr. C. Krainic (Institut Medicolegal, Paris, France). All tissues, listed in Table 4, except stomach and duodenum samples, had been taken from cadavers examined for forensic medicine purposes. Gastric and duodenal mucosa sections were obtained from Dr. J. Bara (IRSC, Villejuif, France). The digestive mucosa had been fixed in ethanol and paraffin-embedded [21]. ABO and Lewis blood groups of all tissue donors were determined on red cells by conventional agglutination tests. Le(a+b-) donors were considered as ABH non-secretors and Le(a-b+) donors were considered as ABH secretors. The secretor character of Le(a-b-) donors was determined by the reactivity of the anti-H lectin from *Ulex europaeus* either in salivary glands or in the gastroduodenal surface epithelia [21].

Immunofluorescence

The lectin from *Galactia tenuiflora* labelled with FITC diluted in 1% BSA/PBS, was incubated for 30 min in a moist chamber. In a second step, either TRITC-labelled lectin 1 from *Ulex europaeus* (Vector Laboratories, Burlingame, CA, 94010, USA) or TRITC-labelled *Helix pomatia* lectin (IBF, Villeneuve la Garenne, 92390, France) were incubated for 30 min. Optimal dilutions were determined by chessboard titrations. Between each step, washings were performed by incubation for 10 min in PBS. After the last washing, cover slips were mounted with one drop of oxidized *p*-phenylenediamine in glycerol [22]. The excess mounting medium was removed with filter paper and the slides were examined with a Leitz SM-LUX microscope equipped with a Ploemopak 2.3 and a lamp source of 200 W HBO. The I-2 filter-block was used to detect the green fluorescence of FITC-conjugates and the yellow fluorescence of nuclei. The M filterblock was used to detect the red fluorescence of TRITC conjugates. Pictures were taken on a Photoautomat MPS 55 with Fujichrome 400 ASA film.

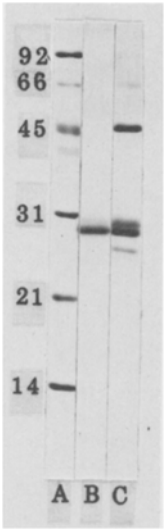


Figure 2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis analysis of the *Galactia tenuiflora* lectin. Lane A, molecular weight standards (kDa). Lane B, affinity purified fraction. Lane C, ammonium sulphate precipitate from the crude extract.

Results

Purification of the Anti-H Lectin

The agglutinating activity from the crude extract was first concentrated by ammonium sulphate precipitation. The anti-H lectin was then purified by affinity chromatography on a column of H type 2 Synsorb. The profile of adsorption and elution of the lectin is shown in Fig. 1. The process had to be repeated twice in order to completely remove the agglutinating activity from the crude material. The electrophoretic pattern of the desorbed protein is shown in Fig. 2. Two bands are observed by SDS-PAGE in the presence of β-mercaptoethanol. These bands have apparent molecular weights of 27 kDa and 29 kDa. Densitometric scanning of the profile indicated that the 29 kDa band represents 25% of the total protein, whilst the 27 kDa band accounts for the remaining 75%. Under non-reducing conditions, the lectin migrates as a broad difuse band with an apparent molecular weight ranging from 50 to 55 kDa (not shown). Fig. 2 also shows the electrophoretic profile of the ammonium sulphate precipitate containing the agglutinating activity. Two major bands comigrate with those of the affinity purified material indicating that the lectin was a major component of the initial ammonium sulphate extract. This explains why the absorbent column was saturated on the first application of the ammonium sulphate precipitate.

Agglutination

The purified lectin was tested for its agglutination capacity on erythrocytes of various ABH phenotypes. The lectin strongly agglutinates red cells from A_1, A_2, B and O donors.

Table 1. Agglutination scores given by the purified lectin from *Galactia tenuiflora* on erythrocytes of various ABO phenotypes.

Red cell phenotype	Number tested	Average score[a] ± S.D.
A_1	3	101 ± 4
A_2	4	116 ± 5
B	3	113 ± 3
O	4	117 ± 5
Oi	3	114 ± 4
Ai	2	85 and 32
O_h Bombay	3	2 ± 2
O_h Reunion	3	4± 1
A_h Reunion	4	3 ± 3
B_h Reunion	5	1 ± 1

[a] Agglutinations were performed in saliva with two fold serial dilutions of the lectin. Cumulative scores were calculated as follows: + + + = 10, + + = 8, + = 5, (+) = 2, — = 0.

The scores obtained with red cells from A_1 and B donors were only slightly weaker than those obtained with red cells from A_2 and O donors. These agglutination patterns were all similar. Cord erythrocytes of blood group O (Oi) were also strongly agglutinated, but cord erythrocytes from blood group A newborns (Ai) were considerably less strongly agglutinated by the lectin. Erythrocytes from two groups of H deficient individuals were also tested, and it appeared that the lectin from *Galactia tenuiflora* does not allow differentiation between these subgroups as a faint agglutination of all H deficient red cells was observed (Table 1).

Inhibitions by Salivas

Salivas from both secretor (OLe^b) and non-secretor (OLe^a) individuals could inhibit the binding of the *Galactia tenuiflora* lectin to the H type 2 BSA artificial antigen (Table 2). Nevertheless, salivas from secretors were about 10 times more potent than salivas from non-secretors. In a similar assay, the anti-H lectin from *Ulex europaeus* was inhibited about 10 times more effectively by salivas from secretors and was not inhibited at all by salivas from non-secretors [23].

Table 2. Inhibition of binding of H type 2-^{125}I-BSA to the purified lectin from *Galactia tenuiflora* by salivas.

Salivary phenotype	Number tested	Dilution of saliva for 50% inhibition ± S.D.[a]
O Le^a	14	3.8 ± 1.7
O Le^b	14	7.3 ± 1.1

[a] Logarithmic scale, see Materials and Methods.

Binding of Synthetic Oligosaccharides

In order to determine the carbohydrate specificity of the *Galactia tenuiflora* lectin, it was tested for its capacity to bind to artificial antigens prepared by coupling synthetic oligosaccharides to BSA. All the compounds listed in Table 3 were tested and the results are shown in Fig. 3. Only four antigens out of twenty were recognized by the lectin in this assay. The strongest was H type 2. H type 4, H type 3 and *N*-acetyllactosamine were also positive, but showed considerably weaker binding. It is noteworthy that the A type 2 antigen was not recognized by the lectin, nor were the H type 1, Le^b and Y antigens.

Tissue Distribution of the Determinants Recognized by the Lectin

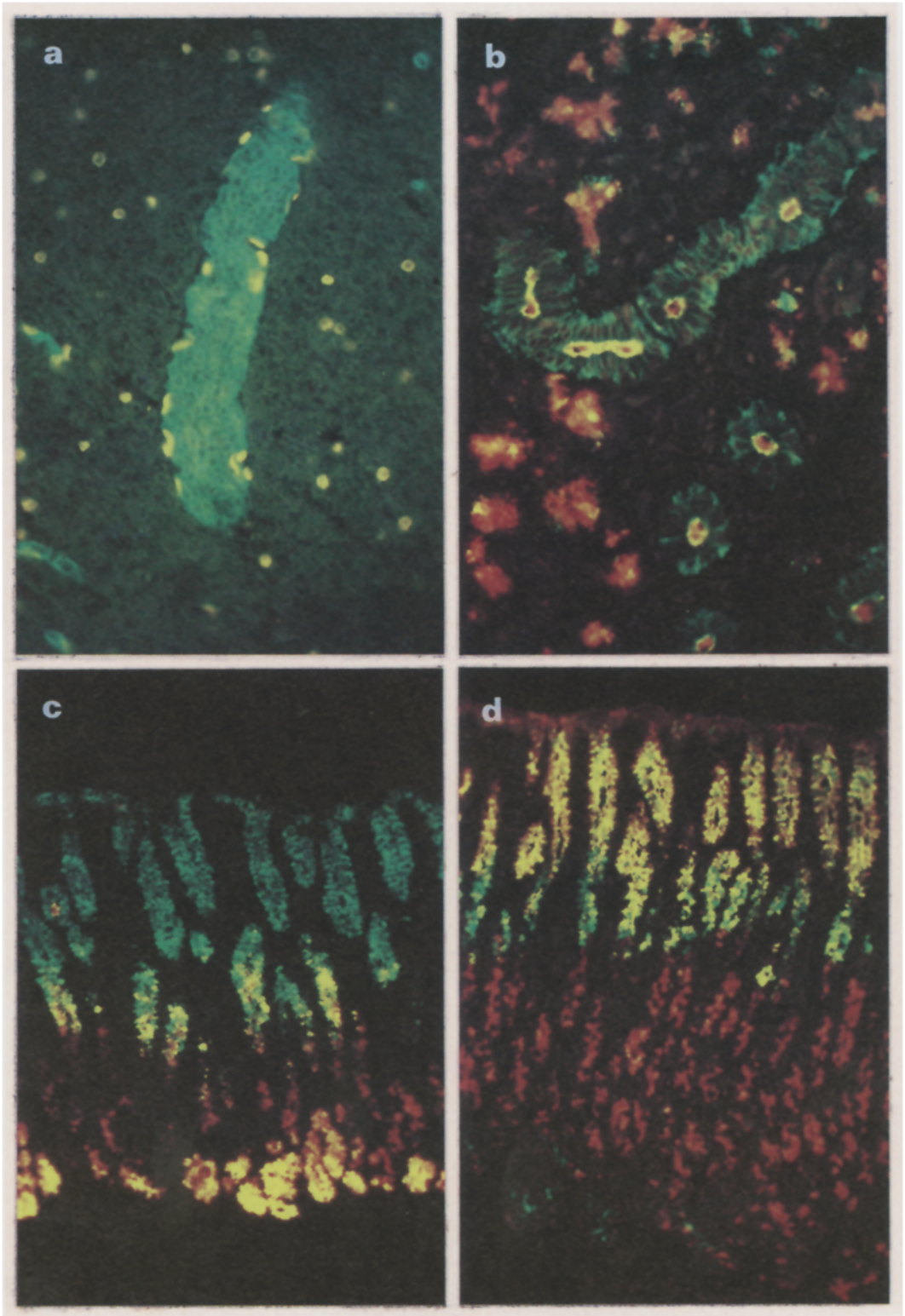
In all tissues tested, the lectin from *Galactia* strongly labelled the red cells and vascular endothelium (Fig. 4a). This labelling was absent from the skin of a B_h Reunion phenotype person. In the skin of H (normal) individuals, the sweat glands were labelled irrespective of the secretory status of the tissue donors. The sweat glands of the B_h person were, however, negative. In addition, it appeared that all other tissues that can express ABH determinants could be labelled by the lectin conjugated to FITC (Table 4). However, double staining with the lectin 1 from *Ulex europaeus* revealed some important differences between the two lectins.

In the salivary glands from secretor individuals, the lectin from *Galactia tenuiflora* labelled mainly the salivary duct cells, the labelling being especially strong on the apical membrane of these cells, and it did not label most acinar cells. The labelling given by the lectin 1 from *Ulex europaeus* was notably different, since this lectin labelled strongly the mucous of the mucous acinar cells and weakly the membranes and the cytoplasm of the serous acinar cells, but it was negative on the duct cells (Fig. 4b). In the salivary glands from non-secretor individuals, only a weak staining of the duct cells could be observed with the lectin from *Galactia tenuiflora* (not shown).

In the intestine, the two anti-H lectins gave essentially the same pattern. The surface mucosa and Brünner's glands of the duodenum from secretor individuals was labelled by both lectins. In non-secretors, only the Brünner's glands were labelled. In the proximal colon of secretor individuals, the epithelium was labelled by both lectins, but the distal colon was always negative.

The most surprising result was obtained in the stomach. In secretor individuals a strong staining of the surface epithelium and of the deep glands was obtained with the two anti-H lectins. However, in non-secretors, the *Ulex* lectin binds only to the deep glands and not at all to the surface mucosa, whilst the *Galactia* lectin binds mainly to this surface mucosa but also to some deep gastric glands (Fig. 4c). This pattern, first noticed in the pyloric region, has also been demonstrated in the cardia and the fundus. In the oesophagus, the determinants recognized by the *Galactia* lectin were under the control of the secretor gene as in the surface mucosa of the duodenum and in the proximal colon.

Finally, the fine tissue specificity of lectins is exemplified by the use of double labelling. Fig. 4d shows the labelling of the fundus from an OLe^a donor with the lectin from *Galactia tenuiflora* (green) and the lectin from *Helix pomatia* (red). Four distinct areas of the mucosa are evident. The surface pits are labelled in yellow since all cells are positive


a
b
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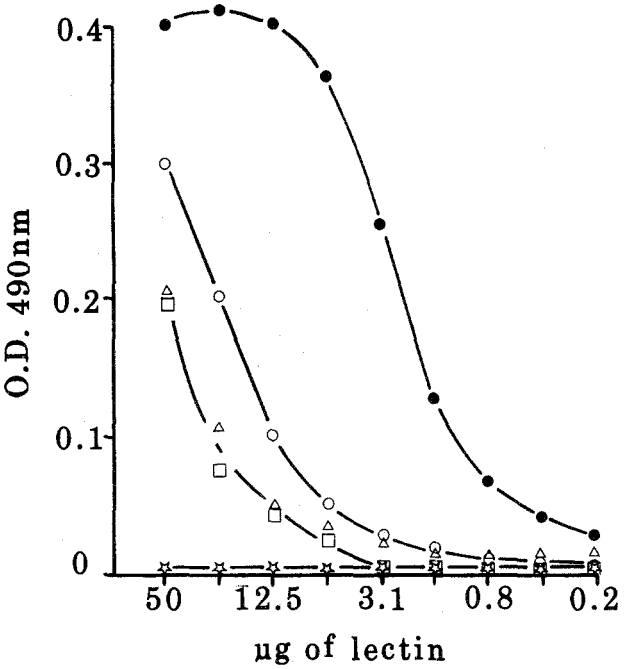

Figure 3. Binding of the *Galactia tenuiflora* lectin to artificial antigens. All antigens listed in Table 3 were coated on the plastic of microtitre wells at 1 µg/ml. The lectin labelled with biotin was then added and the reaction revealed with avidin conjugated to horseradish peroxidase using *O*-phenylenediamine as substrate. (●) H type 2-BSA; (○) H type 3-BSA; (△) H type 4-BSA; (□) type 2 precursor-BSA; (*) Average of all other antigens tested.

Figure 4. Immunofluorescent staining of paraffin-embedded normal human tissues. FITC-labelled *Galactia tenuiflora* lectin was observed with the I-2 filterblock. TRITC-labelled *Ulex europaeus* and *Helix pomatia* lectins were observed with the M filterblock. Double photographic exposures of the same field with the two filterblocks were performed to obtain both the green and the red fluorescence on the same picture.

a. Olfactory bulf of an OLe^b donor stained with *Galactia tenuiflora*. Vascular endothelium of all vessels is positive. Erythrocytes inside the blood vessels are also positive. The cell nuclei are stained brown-yellow with the oxdized *p*-phenylenediamine used as mounting medium. × 300).

b. Parotid gland of an OLe^b donor. Double staining with *Galactia tenuiflora* and *Ulex europaeus* lectin 1. Striated and excretory ducts and some serous and mucous acinar cells are positive with *Galactia* (green). Most acinar cells are positive with *Ulex* (red). Some acinar cells and the saliva in the lumen of the ducts show the superimposition of both fluorescence stains (bright yellow). Nuclei are stained brown by the *p*-phenylenediamine.(× 300).

c. Pyloric mucosa of an OLe^a donor. Double staining with *Galactia* and *Ulex* show four different patterns. From the surface epithelium down to the neck area only *Galactia* is positive (green). Some mucous secreting cells in the neck and deep gastric glands show superimposition of both stains (bright yellow), whereas other mucous secreting cells located in an intermediate area of gastric glands are mainly stained with *Ulex* (red). (× 120).

d. Fundus of an OLe^a donor. Double staining with *Galactia tenuiflora* and *Helix pomatia* lectins. The mucous secreting cells of the gastric pits are positive with both lectins (bright yellow). The *Galactia* stain predominates in the neck mucous cells (green). *Helix pomatia* stains strongly certain cells along the body of the glands (red) and *Galactia* stains faintly the lumen of the deepest area of the gastric glands (green). (× 120).

Table 3. Structures of the synthetic oligosaccharides, coupled to BSA, used to determine the carbohydrate specificity of the lectin from *Galactia tenuiflora*.

Trivial name	Structure
Type 1 precursor or Le^c	Galβ1-3GlcNAcβ-O-R^a
Type 2 precursor or I	Galβ1-4GlcNAcβ-O-R
Lewis disaccharide	Fucα1-4GlcNAcβ-O-R
Le^a (type 1)	Galβ1-3GlcNAcβ-O-R 4 Fucα1
X or Le^x (type 2)	Galβ1-4GlcNAcβ-O-R 3 Fucα1
Le^b (type 1)	Galβ1-3GlcNAcβ-O-R 2 4 Fucα1 Fucα1
Y or Le^y (type 2)	Galβ1-4GlcNAcβ-O-R 2 3 Fucα1 Fucα1
H type 1 or Le^d	Galβ1-3GlcNAcβ-O-R 2 Fucα1
H type 2	Galβ1-4GlcNAcβ-O-R 2 Fucα1
H type 3	Galβ1-3GalNAcα-O-R 2 Fucα1
H type 4	Galβ1-3GalNAcβ-O-R 2 Fucα1
A disaccharide	GalNAcα1-3Galβ-O-R
A trisaccharide	GalNAcα1-3Galβ-O-R 2 Fucα1
A type 1	GalNAcα1-3Galβ1-3GlcNAcβ-O-R 2 Fucα1
A type 2	GalNAcα1-3Galβ1-4GlcNAcβ-O-R 2 Fucα1
A type 3	GalNAcα1-3Galβ1-3GalNAcα-O-R 2 Fucα1
A type 4	GalNAcα1-3Galβ1-3GalNAcβ-O-R 2 Fucα1

A Le^b (type 1)	GalNAcα1-3Galβ1-3GlcNAcβ-O-R 2 4 Fucα1 Fucα1
B disaccharide	Galα1-3Galβ-O-R
B trisaccharide	Galα1-3Galβ-O-R 2 Fucα1

[a] R = $(CH_2)_8$-CO-NH—BSA

with both lectins. The isthmus area is labelled in green since only the *Galactia* lectin is positive. The neck and the body of gastric glands are labelled in red by the *Helix pomatia* lectin and finally, the base area of the gastric glands is only labelled in green by the *Galactia* lectin.

Discussion

We have shown that one of the major proteins from the crude extract of the seeds of *Galactia tenuiflora* is an anti-blood group H lectin since it strongly agglutinates red cells from all donors except those from H deficient individuals. The affinity purified lectin gave two bands on SDS-PAGE gels under reducing conditions. Its behaviour under non-reducing conditions suggests that the native lectin occurs as dimers of these two subunits, although it is not certain whether the 27 kDa subunit is a degradation product of the 29 kDa subunit or if the two subunits are completely different.

The binding to synthetic oligosaccharides revealed that the lectin strongly recognized the H type 2 trisaccharide, weakly recognized the H type 3 and 4 trisaccharides and *N*-acetyllactosamine but recognized neither the H type 1 trisaccharide nor the Y and Le^b tetrasaccharides. This might suggest either an unexpected cross-reactivity or heterogeneity of the lectin. Studies on the conformation of the oligosaccharides recognized by the lectin are required to fully understand this point. Substitution of the galactosyl residue of the H type 2 trisaccharide by an *N*-acetylgalactosamine also abolished the reactivity of the lectin. Thus a major difference between the anti-H lectin from *Galactia* and those from *Ulex europaeus* or *Lotus tetragonolobus* is that these two last lectins recognize the Y determinant [2, 5, 24].

The evidence that the *Galactia* lectin also binds to determinants not recognized by the lectin 1 from *Ulex europaeus* was provided by the immunohistochemical studies. The first example is given by the salivary glands where the *Galactia* lectin labels mainly the salivary ducts whereas the *Ulex* lectin labels mainly the acinar cells and the mucous of secretor individuals. In non-secretor individuals, the lectin from *Galactia* gave a weak staining of the duct cells but the lectin from *Ulex* was negative. These observations are in accordance with the saliva inhibitory patterns as it appeared that the *Galactia* lectin was not very strongly inhibited by the salivas from secretor individuals when compared

Table 4. Overview of the immunofluorescence staining given by the lectin from *Galactia* on paraffin embedded tissues[a].

Tissue	Staining
Salivary glands	+
Oesophagus mucosa	+
Stomach mucosa	+
Small intestine mucosa	+
Large intestine mucosa	
proximal	+
distal	—
Pancreas	
exocrine	+
islets of Langerhans	—
Liver	
hepatocytes	—
biliary ducts	—
Skin	
epidermis	—
sweat glands	+
Lung	
alveolar epithelium	—
bronchial epithelium	+
Kidney	
glomeruli	+
proximal convoluted tubules	—
distal convoluted tubules	+
collecting tubules	+
Nervous system	
brain	—
spinal cord	—
posterior root ganglia	+
Heart	—
Skeletal muscle	—
Connective tissue	—
Spleen and lymph nodes	—
Vascular endothelium	+
Erythrocytes	+

[a] For some tissues of endodermal origin, the expression is under control of the secretor status of the tissue donor (see text).

to the *Ulex europaeus* lectin 1 [23]. Furthermore, the salivas from non-secretor individuals weakly inhibited the *Galactia* lectin but did not inhibit *Ulex* lectin at all [23].

Another major difference between these two lectins was observed in the stomach. The expression of ABH in the surface epithelium of the stomach, is under the control of the secretor gene, whereas it is independent of the secretor gene in the gastric glands [21]. This was clearly demonstrated by the corresponding reactivity of the *Ulex europaeus* lectin 1. However, the lectin from *Galactia tenuiflora* was strongly positive on the stomach surface epithelium of both secretor and non-secretor individuals. Some anti-A and anti-B antibodies have been found which react in a similar way in this part of the

stomach [25]. It is thus possible that a particular type of ABH determinant is expressed on the surface epithelium of the stomach independently of the *Se* gene and possibly independently of the *H* gene. However, the chemical structure of these determinants remains to be determined.

We have shown that the vascular endothelium from a B_h person was not stained by the anti-H lectins from *Galactia* and from *Ulex europaeus*, thus suggesting that the expression of ABH antigens on the vascular endothelium is, indeed, under the control of the *H* gene.

A final difference between the *Galactia* lectin and the *Ulex europaeus* lectin 1 was shown by agglutination. The lectin from *Galactia* gave a weak but constant agglutination of the red cells of all H deficient phenotypes. This could be due to its weak cross-reactivity with the type 2 precursor *N*-acetyllactosamine. The anti-H lectin from *Ulex europaeus* is quite different, since it completely fails to agglutinate the erythrocytes from "Bombay" individuals, but it reacts quite strongly with the erythrocytes from O_h "Reunion" individuals, who where thus defined as having a partial H deficiency [18]. The reason why the H determinants of O_h "Reunion" erythrocytes are not recognized by the *Galactia* lectin is still obscure. These determinants have not been characterized as yet but since the lectin strongly recognizes the H type 2 trisaccharide, the possibility exists that O_h "Reunion" individuals do not have this structure on their erythrocytes. We therefore expect the lectin from *Galactia* to be a very useful reagent in the characterization of the H active determinants from the salivary glands, the stomach epithelium and the "Reunion" O_h red cells.

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