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Note

Binding of hydroxylysine-linked saccharides by galaptin, a galactoside-binding animal tissue lectin*,†

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Galaptins[1] are a group of D-galactoside-binding lectins present in animal tissues. Many galaptins were characterized as being thiol-dependent, cation-independent, developmentally-regulated, and lactose-inhibitable[2], and they belong to the S-type lectins classified by Drickamer[3]. The distribution and localization of galaptin has been well investigated for rabbit[4,5] and chick[6–8] tissues. One of the major sites of galaptin localization was in the extracellular matrix associated with collagen. Catt and Harrison[5] proposed that galaptin plays a major role in the synthesis and integrity of the extracellular matrix. Gabius *et al.*[9] reported the localization of galaptin(s) in the extracellular matrix of human breast tissue. Galaptin, defined here as that lectin which is usually isolated as a native 30-kDa dimer composed of identical 14.5-kDa subunits, was found to be present in the extracellular matrix of human lung[10], and of cutaneous and colonic tissue[11].Galaptin was present in the basement membrane and associated with collagen fibrils in the stroma. Galaptin was also detected in matrix synthesized *in vitro* by bovine corneal endothelial cells[12]. About 27% of the total galaptin could be extracted from this matrix with 0.1M lactose.

Lotan and Raz[13] suggested that β-D-galactoside-binding lectins play a role in neoplasia as mediators of cell adhesion *via* their surface localization on neoplastic cells. The surface-localized lectins(s) presumably would interact with receptors in the extracellular matrix.

The nature of the interactions between matrix and the specific lectin that we have defined as galaptin is unknown, but it apparently involves more than one type of binding. The study reported herein was carried out to determine whether α-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→5)-L-Hyl (**1**) and β-D-Gal*p*-(1→5)-L-Hyl (**2**) residues, which are present in various collagens[14], might play a role in anchoring galaptin to the matrix *via* the galaptin carbohydrate-binding site.

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EXPERIMENTAL

Materials. — Sponges were obtained from Thomas Scientific, (Swedesboro, NJ) and Immulon I micro ELISA plates from Dynatech Labs. Inc. (Chantilly, VA). L-Hydroxylysine was obtained from Sigma Chemical Co. (St. Louis, MO) and horseradish peroxidase from Boehringer Mannheim Corp. (Indianapolis, IN). Galaptin was isolated from human spleen by affinity chromatography on lactose-Sepharose[15], as previously described[16]. Galaptin was alkylated with iodoacetamide[12] to eliminate the thiol requirement for retention of carbohydrate-binding activity. Alkylated galaptin was conjugated to horseradish peroxidase by coupling with glutaraldehyde as follows. Galaptin (1 mg) was added to peroxidase (3 mg) and 1% glutaraldehyde (120 μL) in 200mM NaCl–8mM phosphate–100mM lactose, pH 6.8 (1 mL). Conjugation proceeded for 15 h at 4°. The conjugate was diluted 9-fold with 10mM Tris, pH 7.3, and it was adsorbed onto DEAE-Sephacel (1 mL). After washing with 8mM phosphate, pH 7.3, to remove lactose, the conjugate was eluted with 200mM NaCl–8mM phosphate, pH 7.3, and purified by affinity chromatography on lactose-Sepharose. The purified conjugate was stored in 1% bovine serum albumin–50% glycerol at −20°.

α-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→5)-L-Hyl (**1**) was prepared from natural sponges according to the method of Smith[17], and it was hydrolyzed in 0.1M HCl according to Butler[18] to liberate β-D-Gal*p*-(1→5)-L-Hyl (**2**). After rotary evaporation of the hydrolyzate and of added methanol to remove HCl, the sample was chromatographed on Dowex 50 (H^+) cation-exchange resin to give pure β-D-Gal*p*-(1→5)-L-Hyl[19]. The identity of the sugars present in the compounds was investigated, before and after hydrolysis in 4M HCl for 2 h at 100°, by t.l.c. on silica gel with 5:4:1 chloroform–methanol–water as developing solvent.

Methods. — Inhibition of galaptin binding by ligands was determined with a modified ELISA. Asialofetuin (2 μg) was adsorbed onto the wells of microtiter plates and the plates were fixed with 2% formaldehyde[20]. Galaptin-peroxidase conjugate (12 ng) in 120mM NaCl–10mM phosphate–0.05% Tween 20–0.01% thimerosal, pH 7.3 (60 μL), was mixed with an equal volume of ligand of varying concentrations. After incubation for 60 min at 4°, the conjugate–ligand (100 μL) was added to the ELISA-plate wells and the plates were incubated for 60 min at 4°. The plates were washed with the aforementioned ice-cold Tween 20 buffer, and bound peroxidase was assayed with diammonium 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) as described[20].

RESULTS AND DISCUSSION

We recently analyzed the binding-site specificity of human splenic galaptin[20,21]. The conclusions derived were in general agreement with those presented by Sparrow *et al.*[22] for an apparently identical lectin (HL-14) isolated from human lung. Those studies showed that galaptin had a pronounced specificity for the β-D-Gal*p*-(1→3)- and β-D-Gal*p*-(1→4)-D-GlcNAc sequences with no apparent affinity for D-GalNAc nonreducing groups. However, the nature of the aglycon linked to the potentially reducing terminus

sugar of mono- and higher-order-glycosides has a significant effect on the anomeric specificity and binding affinity for lectin–ligand interaction[20,21,23]. Of interest was the observation that substitution of the OH-2 of β-D-galactopyranosyl residues by methyl or L-fucosyl groups had little effect on lectin-ligand interaction[20,22]. Also, substitution of OH-3 of D-galactosyl residues by *N*-acetylneuraminyl or 2-acetamido-2-deoxy-D-glucosyl groups did not appear to influence lectin–ligand binding[20,22]. It has also been observed that the 15-kDa galactoside-binding lectin synthesized by human buffy coat cells could be eluted from affinity columns with amine compounds[23]. The identity of that lectin has not been demonstrated, although it is suspected to be the monomeric form of galaptin. Therefore, it seemed plausible that α-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→5)-L-Hyl (1) and β-D-Gal*p*-(1→5)-L-Hyl might bind to galaptin with high affinity and be able to play a significant role in anchoring galaptin to the extracellular matrix.

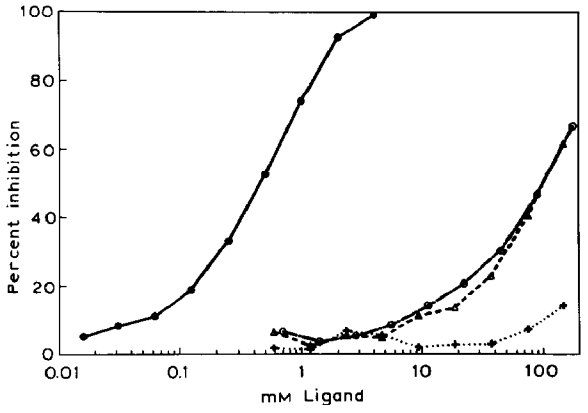
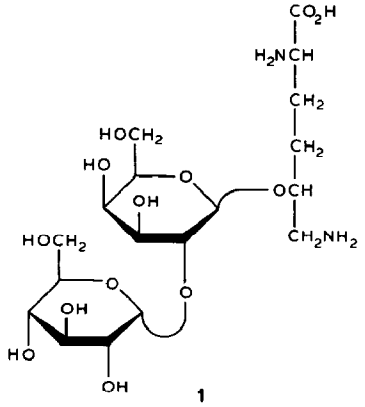
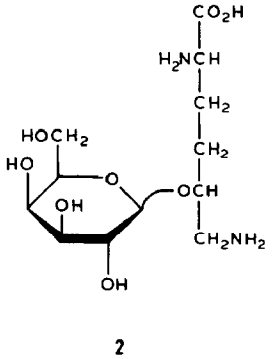


Fig. 1. Inhibition of galaptin binding to asialofetuin. A modified ELISA using galaptin-peroxidase conjugate was carried out as described in the Experimental section: (-●-●-) Lactose, (--△--△--) compound **1**, (-.-○-.-○-.-) compound **2**, and (... + ... + ...) L-hydroxylysine.



1



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The results of galaptin-binding inhibition assays are shown in Fig. 1. Relative to lactose, the collagen-derived saccharides glycosidically-linked to hydroxylysine have very low affinity for binding to galaptin. The I_{50} values are 0.45, 90, and 88mM for lactose, **1**, and **2**, respectively. The I_{50} values for D-galactose were 35mM[20] and 63mM[21] in other assays; hence, the presence of the hydroxylysine residue as an aglycon did not appear to increase the binding affinity of D-galactosides for galaptin. In agreement with binding data on other compounds having O-2- and O-3-substituted D-galactosyl residues[20], **1** and **2** gave identical inhibition curves. L-Hydroxylysine was ineffective as a galaptin inhibitor.

It is unlikely that the compounds studied here play a role in binding galaptin to the extracellular matrix. Other possibilities exist, however. These include the presence of disulfide bonds between galaptin and collagen, and interaction of galaptin with *N*-linked oligosaccharides present in laminin[24] and some collagens[25].

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