Biochemical and Biophysical Research Communications 373 (2008) 206–212

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Desulfated galactosaminoglycans are potential ligands for galectins: Evidence from frontal affinity chromatography

Jun Iwaki [a], Toshikazu Minamisawa [a,b], Hiroaki Tateno [a], Junko Kominami [a,c], Kiyoshi Suzuki [b], Nozomu Nishi [d,e], Takanori Nakamura [d], Jun Hirabayashi [a,f,*]

[a] Lectin Application and Analysis Team, Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 2, 1-1-1 Umezono, Tsukuba, Ibaraki 305-8568, Japan
[b] Central Research Laboratories, Seikagaku Corporation, 3-1253 Tateno, Higashi-yamato, Tokyo 207-0021, Japan
[c] Fine Chemical and Foods Laboratories, J-Oil Mills Inc., 11 Kagetori-cho, Totsuka-ku, Yokohama, Kanagawa 245-0064, Japan
[d] Department of Endocrinology, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan
[e] GalPharma Co. Ltd., 2217-44 Hayashi-machi, Takamatsu, Kagawa 761-0301, Japan
[f] Department of Functional Glycomics, Life Science Research Center, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

ARTICLE INFO

Article history:
Received 29 May 2008
Available online 13 June 2008

Keywords:
Glycosaminoglycan
Galectin
Frontal affinity chromatography

ABSTRACT

Galectins, a group of β-galactoside-binding lectins, are involved in multiple functions through specific binding to their oligosaccharide ligands. No previous work has focused on their interaction with glycosaminoglycans (GAGs). In the present work, affinities of established members of human galectins toward a series of GAGs were investigated, using frontal affinity chromatography. Structurally-defined keratan sulfate (KS) oligosaccharides showed significant affinity to a wide range of galectins if Gal residue(s) remained unsulfated, while GlcNAc sulfation had relatively little effect. Consistently, galectins showed much higher affinity to corneal type I than cartilageous type II KS. Unexpectedly, galectin-3, -7, and -9 also exerted significant affinity to desulfated, GalNAc-containing GAGs, i.e., chondroitin and dermatan, but not at all to hyaluronan and *N*-acetylheparosan. These observations revealed that the integrity of 6-OH of βGalNAc is important for galectin recognition of these galactosaminoglycans, which were shown, for the first time, to be implicated as potential ligands of galectins.

Galectins form a well-established animal lectin family, occurring extensively in vertebrates, and in invertebrates such as the fly, nematode, and sponge [1–4]. Galectins are defined by their basic specificities to β-galactosides and their conserved amino acid sequences. Fourteen members belonging to the galectin family have been so far identified in mammals. According to their structural architectures, they are classified into three types; proto (galectin-1, -2, -5, -7, -10, -11, -13, and -14), chimera (galectin-3), and tandem-repeat types (galectin-4, -6, -8, -9, and -12) [1]. Galectins are characteristically secreted by a non-classical pathway without signal sequences, and exert various functions through specific binding to their counterpart oligosaccharides expressed on the cell surface and in the extracellular matrix (ECM). Among their functions, galectin-1 inhibits T cell adhesion to the ECM and reduces proinflammatory cytokine secretion [5]. In contrast, galectin-3 facilitates neutrophil adhesion to laminin [6]. Binding of galectin-9 to CD44 blocks interaction of T cells with hyaluronan (HA) [7]. Thus, galectins also serve as modulators of cell adhesion to the ECM.

Proteoglycans are major components of the glycocalyx as well as the ECM on animal cell surfaces. They play important roles in many biological events [8–10]. A large variety of proteoglycans are attributed to not only core proteins but also glycosaminoglycan (GAG) chains, which are attached to the former with great structural diversity. In general, GAG forms a linear polysaccharide chain consisting of a repeated disaccharide unit comprising an amino sugar, such as GlcNAc or GalNAc, and a uronic acid, such as GlcA or IdoA, with the exception Gal in the case of keratan sulfate (KS). On the basis of disaccharide compositions and linkage types, GAGs are classified into heparin/heparan sulfate (HS), HA, chondroitin sulfate (CS)/dermatan sulfate (DS) and KS. Unique structural

Abbreviations: CH, chondroitin; CRD, carbohydrate recognition domain; CS, chondroitin sulfate; DN, dermatan; DS, dermatan sulfate; ECM, extracellular matrix; FAC, frontal affinity chromatography; GAG, glycosaminoglycan; Gal, galactose; galectin-*C, C-terminal domain of galectin-*; galectin-*N, N-terminal domain of galectin-*; GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; HA, hyaluronan; HS, heparan sulfate; IdoA, iduronic acid; IPTG, isopropyl-β-D-thiogalactopyranoside; KS, keratan sulfate; LN, *N*-acetyllactosamine; NAH, *N*-acetylheparosan; PA-, pyridylaminated; *p*NP-, *p*-nitrophenyl; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Sia, sialic acid; Xyl, xylose.

* Corresponding author. Address: Lectin Application and Analysis Team, Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 2, 1-1-1 Umezono, Tsukuba, Ibaraki 305-8568, Japan. Fax: +81 29 861 3125.
E-mail address: jun-hirabayashi@aist.go.jp (J. Hirabayashi).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.05.190

features as well as specific expressions of GAGs are closely related to their physiological functions. However, due to the highly heterogeneous features of GAGs, many mechanism-based studies remain to be carried out.

Galectin functions are well correlated with specific cell–cell and cell–ECM interactions based on the principle of β-galactoside recognition. To date there have been no systematic studies of galectin–GAG interactions, although GAGs are considered to be potential ligands for galectins. To assess this potential, we adopted frontal affinity chromatography (FAC), an established method for quantitative glycan–protein interaction analysis [11–13], to characterize galectin binding to a series of GAG polysaccharides, and to some structurally-defined oligosaccharides. Most of the human galectins studied were found to bind to βGal/GalNAc-containing GAGs, provided the latter preserve critical recognition sites for galectins. The GAGs include partially-sulfated KS, desulfated CS (chondroitin, CH) and DS (dermatan, DN), but not HA and a biosynthetic precursor of HS without sulfates (*N*-acetylheparosan, NAH).

Materials and methods

Materials. Lactosyl-Sepharose was prepared as described [14]. NHS-activated Sepharose 4FF was purchased from GE Healthcare (Little Chalfont, Buckinghamshire, UK). A PA- and *p*NP-oligosaccharide library was prepared as described previously [12,13]. When necessary, PA- and *p*NP-oligosaccharides were purified to apparent homogeneity (>95%) by HPLC with a PALPAK type R column (Takara, Tokyo, Japan).

Preparation of PA-glycosaminoglycans. Bovine corneal KS(KS-I), shark cartilageous KS(KS-II), HA, NAH, CH, DN, and HA_{2-5} oligomers were prepared by Seikagaku Corp. (Tokyo, Japan) and National Institute of Advanced Industrial Science and Technology (AIST, Ibaraki, Japan). Six KS-related oligosaccharides, G2K1, G1L1, G4L4, L2L4, L4L4, and SL2L4, were prepared by enzymatic and chemical treatments followed by separation as described [15]. CH and DN were chemically desulfated from CS and DS, respectively. Each weight-average molecular weight of GAG polymers used in this study was as follows, KS-I, 10 kDa; KS-II, 20 kDa; HA, 10 kDa; NAH, 38 kDa; CH, 5 kDa; DN, 5 kDa. CH_{2-5} oligomers were a generous gift from Dr. Sugiura (Seikagaku Corp., Tokyo, Japan) and Dr. Kimata (Institute for Molecular Science of Medicine, Aichi Medical University, Japan). All the above-mentioned GAGs and GAG oligomers were pyridylaminated with GlycoTag (Takara, Tokyo, Japan) and purified by HPLC as described previously [12].

Preparation of human galectins. All genes of human galectins (CRDs) were cloned downstream of the T7 promoter of the appropriate expression vector as described [16]. For galectin-1, the stable mutant, in which Ser residues substitute for all oxidation-sensitive Cys residues of wild-type galectin-1, was used in this study. This stable mutant shows identical binding-specificities to those of intact galectin-1 (unpublished data). Galectins were over-expressed in *Escherichia coli* BL21-CodonPlus (DE3)-RIL strain (Stratagene, La Jolla, CA, USA) under the control of isopropyl-β-D-thiogalactopyranoside (IPTG, Fermentas Hanover, MD, USA) at appropriate temperatures. Each cell lysate was subjected to affinity chromatography with lactosyl-Sepharose as described [17]. The purity was checked by SDS–PAGE and the final protein concentration was determined by Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

Preparation of galectin miniature column for frontal affinity chromatography. Galectins were immobilized on NHS-activated Sepharose 4FF according to the manufacturer's instructions. In brief, galectins dissolved in 350 μl of coupling buffer (0.2 M $NaHCO_3$, 0.5 M NaCl, pH 8.3) were coupled with 100 μl of NHS-activated Sepharose. After coupling at room temperature for 3 h with continuous rotation, the resultant resin was incubated with 1 ml of blocking buffer (0.5 M monoethanolamine, 0.5 M NaCl, pH 8.3) for 30 min with rotation, and packed into a miniature column (2.0 × 10 mm). Specifications of the galectin-immobilized column used for finding B_t value are listed in Table S1.

Frontal affinity chromatography. FAC analysis was performed as described previously [12,13]. Four galectin columns were concomitantly set into an automated FAC-T system (Shimadzu, Kyoto, Japan) and were equilibrated extensively with TBS. Analysis of both PA- and *p*NP-oligosaccharides was performed at a constant flow rate of 0.125 ml/min at 25 °C. To determine effective ligand content (B_t), for each galectin column, concentration-dependence analysis was carried out with various concentrations of appropriate *p*NP-oligosaccharides. B_t and K_d values were obtained by Woolf–Hofstee plot using experimental data, $V - V_0$. In order to determine $V - V_0$ values for PA-oligosaccharides, PA-M3B (Takara, Tokyo, Japan) was used as a negative control throughout this study. The resulting B_t values were used for calculation of a series of K_d values with the FAC analyzer software under the basic equation of FAC as follows.

$$K_d = B_t/(V - V_0) - [A]_0 \quad (1)$$

Under the given conditions ($[A]_0 << K_d$), Eq. (1) is simplified as in Eq. (2).

$$K_d = B_t/(V - V_0) \quad (2)$$

Results

Galectins bind to desulfated Gal of KS-related oligosaccharides

KS consisting of the repeating unit [-3Galβ1-4GlcNAcβ1-] as a backbone is located abundantly in cartilage, cornea, and brain, and in many other tissues in small amounts as a glycan constituent of proteoglycans [18]. Distinct from other GAGs, KS is attached to core regions of either *N*- or *O*-linked oligosaccharides, but lacks the consensus tetrasaccharide GlcAβ1-3Galβ1-3Galβ1-4Xylβ1- to which other GAGs are attached. Moreover, KS is exceptional among GAGs in that galactose occupies the position of a uronic acid in the repeating-disaccharide unit. Sulfation frequency of KS is diversified in terms of location and disease [19,20]. Since KS has the same backbone structure as poly-*N*-acetyllactosamine (poly-LN), it is intriguing to see the effect of 6-*O*-sulfation of the individual components, i.e., Gal and GlcNAc residues. For this purpose, we prepared six structurally-defined KS-related oligosaccharides (Fig. 1A). As galectins, we produced recombinant proteins for 10 carbohydrate-recognition domains (CRDs) of previously-established human galectins. These include proto-type galectin-1, -2 and -7, and chimera-type galectin-3, and both N- and C-terminal CRDs of tandem-repeat type galectin-4, -8, and -9 (4N and 4C, 8N and 8C, 9N and 9C, respectively). Interaction analysis between these recombinant CRDs and KS oligosaccharides was carried out by means of FAC.

To determine which sulfation position in KS influences the galectin binding, we first compared the affinities of non-sulfated (i) G2K1, partially-sulfated (ii) L2L4 and completely-sulfated (iii) G4L4, and (iv) L4L4 (Fig. 1A-i–iv). Galectin-1, -2, -3, -7, -9N, and -9C were found to bind to both non-sulfated and partially-sulfated oligosaccharides, but they showed no affinity to completely-sulfated oligosaccharides (Fig. 1B). No substantial binding was observed for galectin-4 and -8 CRDs. Notably, 6-*O*-sulfation of terminal and internal Gal completely abolished the binding of all galectins (Fig. 1B-iii and iv), while that of GlcNAc linked to terminal Gal was not crucial (Fig. 1B-ii). Thus, the intact 6-OH of Gal is