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Mammalian galectins bind Galactoseβ1–4Fucose disaccharide, a unique structural component of protostomial *N*-type glycoproteins

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ABSTRACT

Galactoseβ1–4Fucose (Galβ1–4Fuc) is a unique disaccharide exclusively found in *N*-glycans of protostomia, and is recognized by some galectins of *Caenorhabditis elegans* and *Coprinopsis cinerea*. In the present study, we investigated whether mammalian galectins also bind such a disaccharide. We examined sugar-binding ability of human galectin-1 (hGal-1) and found that hGal-1 preferentially binds Galβ1–4Fuc compared to Galβ1–4GlcNAc, which is its endogenous recognition unit. We also tested other human and mouse galectins, i.e., hGal-3, and -9 and mGal-1, 2, 3, 4, 8, and 9. All of them also showed substantial affinity to Galβ1–4Fuc disaccharide. Further, we assessed the inhibitory effect of Galβ1–4Fuc, Galβ1–4Glc, and Gal on the interaction between hGal-1 and its model ligand glycan, and found that Galβ1–4Fuc is the most effective. Although the biological significance of galectin–Galβ1–4Fuc interaction is obscure, it might be possible that Galβ1–4Fuc disaccharide is recognized as a non-self-glycan antigen. Furthermore, Galβ1–4Fuc could be a promising seed compound for the synthesis of novel galectin inhibitors.

1. Introduction

Galectins are a family of carbohydrate-recognition proteins distributed in animals and fungi [1,2]. They are characterized by their evolutionarily conserved carbohydrate-recognition domain (CRD) and the eight amino-acid residues of the CRD important for the binding to β-galactosides. In mammals, over 10 galectins are known and they display functional significance in various cellular events such as cancer, immunity, and inflammation, by binding glycoconjugate containing β-galactoside structure(s) such as Galβ1–4GlcNAc [3,4]. For example, galectin-1 contributes to immune evasion by inducing apoptosis in tumor-directed effector cytolytic T cells [5]. Therefore, recently, galectins have gained much attention as therapeutic targets, and synthesis of galectin(s) inhibitor(s) based on galectin-binding saccharides such as galactose and lactose has been attempted [6–9].

Galactoseβ1–4Fucose is a unique disaccharide exclusively found in *N*-glycans of protostomia [10–15]: however, it might be possible that such a disaccharide unit exists in deuterostomia, since the existences of potential homologues of GALT-1, a galactosyl transferase responsible for the biosynthesis of Galβ1–4Fuc disaccharide in *Caenorhabditis elegans*, have been reported in species except mammals [16]. Several studies and our own reports show that *C. elegans* galectins LEC-6 and LEC-10 and *Coprinopsis cinerea* galectin CGL-2 bind Galβ1–4Fuc disaccharide, which is found as a structural component of *C. elegans* endogenous *N*-glycan, and discuss the biological significance of such interactions [17–21]. In addition, we found that other *C. elegans* galectins bind endogenous Galβ1–4Fuc containing oligosaccharides or chemically synthesized Galβ1–4Fuc disaccharide [18,22–24].

Since Galβ1–4Fuc disaccharide has only been found in protostomia, it could be speculated that Galβ1–4Fuc-binding ability is unique to invertebrate galectins. However, structural analyses of CGL-2–Galβ1–4Fucα1–6GlcNAc and LEC-6–Galβ1–4Fuc crystals reveal that the conserved eight amino-acid residues of galectin are involved in these interactions [19,25]: at least in case of LEC-6, Glu67 is also required for high-affinity binding to Galβ1–4Fuc. Therefore not only invertebrate but also vertebrate galectins might bind Galβ1–4Fuc disaccharide which fulfills the shared

Abbreviations: CRD, carbohydrate-recognition domain; Gal, galactose; Fuc, fucose; Glc, glucose.

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galectin-binding structural requirement; i.e., Galβ-(syn)-gauche configuration [26].

In this study, we report that human galectin-1 and other mammalian galectins also bind Galβ1–4Fuc disaccharide, although the biological significance of such interactions remains obscure. We also found the chemically synthesized Galβ1–4Fuc derivative Galβ1–4Fuc-OMe inhibit the interaction between hGal-1 and its glycan-ligand more effectively than lactose and galactose.

2. Materials and methods

2.1. Materials

Galβ1–4Fuc-Man-ol-PA and Galβ1–3Fuc-Man-ol-PA, sugars labeled with pyridylamine via a spacer derived from mannitol, were chemically synthesized [27]. NA2-PA (PA001; Galβ1–4GlcNAcβ1–2Manα1–3 (Galβ1–4GlcNAcβ1–2Manα1–6) Manβ1–4GlcNAcβ1–4GlcAc-PA), NA3-PA (PA002; Galβ1–4GlcNAcβ1–2 (Galβ1–4GlcNAcβ1–4) Manα1–3 (Galβ1–4GlcNAcβ1–2Manα1–6) Manβ1–4GlcNAcβ1–4GlcNAc-PA), NA4-PA (PA004; Galβ1–4GlcNAcβ1–2 (Galβ1–4GlcNAcβ1–4) Manα1–3 (Galβ1–4GlcNAcβ1–2 (Galβ1–4GlcNAcβ1–6) Manα1–6) Manβ1–4GlcNAcβ1–4GlcNAc-PA), LNnT-PA (PA041; Galβ1–4GlcNAcβ1–3Galβ1–4Glc-PA), and rhamnose-PA were purchased from Takara Bio (Shiga, Japan). E3-PA, a PA derivative of natural *N*-glycan, which contains the Galβ1–4Fuc unit isolated from *C. elegans* (structure shown in Fig. 1A), was prepared as reported previously [17]. Lactose monohydrate (lactose) and D-glucose (glucose) were purchased from Wako (Osaka, Japan). Methyl-β-D-galactopyranoside (Gal-β-OMe) was purchased from Sigma–Aldrich (St. Louis, MO). 4-D-Galactosyl-β-L-methyl fucopyranoside (Galβ1–4Fuc-β-OMe) was chemically synthesized, and its structure was confirmed by ^{1}H-NMR analysis (details regarding the synthesis will appear elsewhere).

2.2. Plasmids construction

All of the open-reading frames of mouse Galectin-1 (NM_008495), Galectin-2 (NM_025622), Galectin-3 (NM_001145953), Galectin-4 (NM_010706), Galectin-8 (EF524570), and Galectin-9 (NM_001159301) with restriction digestion sites were amplified by PCR (primers used in this study are shown in Supplementary Table I) from the cDNA mixture prepared from ddY mouse embryo, which was kindly provided by Dr. Riyo Morimoto (Teikyo University) or from the mouse (BL/6) stomach first-strand cDNA (Genostaff, Tokyo, Japan). The amplified PCR fragments were cloned into pCRII (Life Technologies, Carlsbad, CA) or pGEM-T (Promega, Madison, WI) cloning vector. Each insert DNA fragment was digested with appropriate digestion enzymes and subcloned into pET21a (Merck Millipore, Billerica, MA) or pET-FLAG vector [28], to generate pET-mGal-1, pET-mGal-2, pET-mGal-3, pET-FLAG-mGal-4, pET-FLAG-mGal-8, and pET-FLAG-mGal-9 *Escherichia coli* expression plasmids. For the construction of pET-mGal-1C2S plasmid, Cys2Ser point mutation which inhibit oxidation of Cys2 and subsequent inactivation of Gal-1 [29] was introduced by PCR using pGEM-mGal-1 plasmid, and the insert was subcloned into pET21a vector. For the constructions of pET-FLAG-mGal-4 N-CRD, C-CRD, -mGal-8 N-CRD, and C-CRD, the region corresponding to each of N- and C-terminal CRD of mGal-4 and mGal-8 predicted by SMART (http://smart.embl-heidelberg.de/) with digestion sites was amplified by PCR, and then subcloned into pET-FLAG vector.

2.3. Preparation of recombinant proteins

hGal-1C2S, mGal-1C2S, mGal-2, mGal-3, mGal-4, mGal-4 N-CRD, mGal-4 C-CRD, mGal-8, mGal-8 N-CRD, mGal-8 C-CRD, and mGal-9 recombinant proteins were expressed in *E. coli* by using pET-hGal-1C2S plasmid [29] and the plasmids described above and affinity purified by using asialofetuin-Sepharose column or

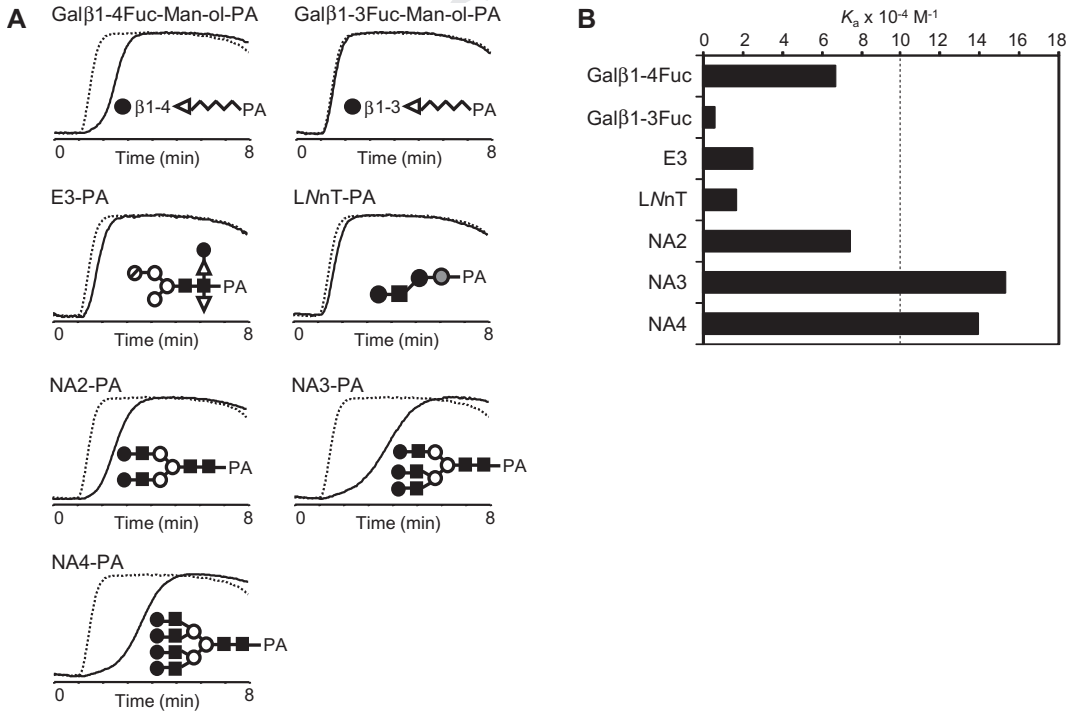

Fig. 1. Frontal affinity chromatography analysis of human galectin-1. (A) Elution profiles of PA-sugars from an immobilized hGal-1 C2S column. The structure of each PA-sugar is depicted in each panel of elution profile. Open circle with diagonal line, hexose; open circle, mannose; grey circle, glucose; filled circle, galactose; filled square, *N*-acetylglucosamine; open triangle, fucose. The elution profile of each PA-sugar (solid line) was superimposed on that of rhamnose-PA (broken line) which has no affinity for hGal-1. (B) Bar graph representation of K_a values for the interaction between hGal-1 and PA-sugars. The K_a values for the interaction between hGal-1 and PA-sugars were calculated as described in Section 2. These experiments were performed at least two times and showed the representative results.

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