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

Tomoharu Takeuchi [a,b,*], Mayumi Tamura [a], Kazusa Nishiyama [c], Jun Iwaki [d,1], Jun Hirabayashi [d], Hideyo Takahashi [c], Hideaki Natsugari [c], Yoichiro Arata [a], Ken-ichi Kasai [b]

[a] *Laboratory of Molecular Immunology, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan*
[b] *Department of Biological Chemistry, School of Pharmaceutical Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan*
[c] *Laboratory of Synthetic Organic and Medicinal Chemistry, School of Pharmaceutical Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan*
[d] *Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 2, Tsukuba, Japan*

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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 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.

* Corresponding author at: Laboratory of Molecular Immunology, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan. Fax: +81 49 271 8123.
E-mail address: t-take@josai.ac.jp (T. Takeuchi).
[1] Present address: Department of Molecular Medicine and Anatomy, Nippon Medical School, 1-1-5 Sendagi, Tokyo 113-8602, Japan.

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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

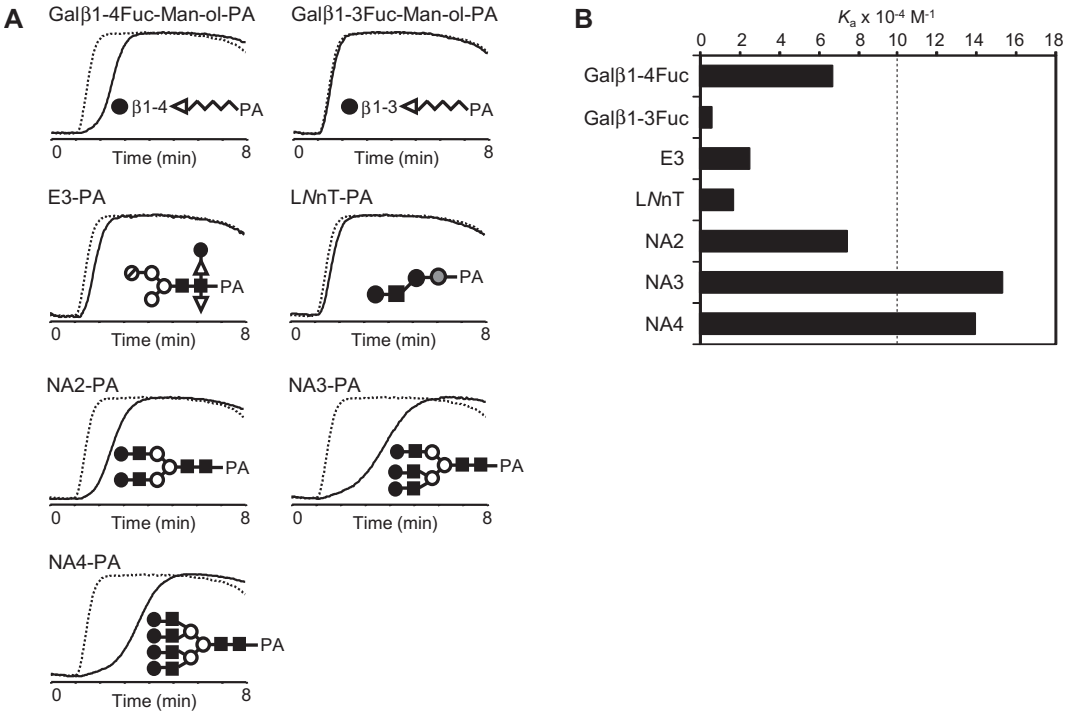

Fig. 1. Frontal affinity chromatography analysis of human galectin-1. (A) Elution profiles of PA-sugars from an immobilized hGal-1 C2S column. The structure of each PA-sugar is depicted in each panel of elution profile. Open circle with diagonal line, hexose; open circle, mannose; grey circle, glucose; 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.

Galβ1–4Fuc-immobilized column [22] basically as described previously [17]. hGal-3 and -9 recombinant proteins were prepared as described previously [26].

2.4. Frontal affinity chromatography analysis

Immobilization of recombinant galectins on HiTrap NHS-activated Sepharose (GE Healthcare, St. Giles, UK) and frontal affinity chromatography analysis were performed basically as described previously [17]. In brief, each PA-sugar at a concentration of 5 nM was applied to an immobilized galectin column at a flow rate of 0.25 mL/min at 20 °C, and the elution profile was monitored by a fluorescence detector. For examining the effect of sugars on the interaction between hGal-1C2S and NA2-PA, 5 nM NA2-PA was applied to the column with various sugars. The K_d value for the interaction between galectin and PA-sugar was determined according to the following basic equation of frontal affinity chromatography: $K_d = B_t/(V–V_0) – [A]_0$. In this equation, B_t is the effective ligand content, V is the volume of the elution front, V_0 is the V of rhamnose-PA that is not bound by galectins, and $[A]_0$ is the initial concentration of the PA-sugar. If the $[A]_0$ is negligibly smaller than K_d, the equation can be simplified as $K_d = B_t/(V_f–V_0)$. In this study, the B_t value of each of immobilized mouse galectin columns was calculated from the data obtained by concentration-dependent analysis with various concentrations of Galβ1–4Fuc, and that of each of human galectin columns was calculated from the reported K_d value for the interaction between each of them and PA041 [30]. The K_a values were calculated on the basis on the equation $K_a = 1/K_d$.

3. Results and discussion

3.1. Human galectin-1 has higher binding affinity to Galβ1–4Fuc than toward Galβ1–4GlcNAc

To clarify that vertebrate galectins bind the Galβ1–4Fuc disaccharide, we first examined the binding ability of human

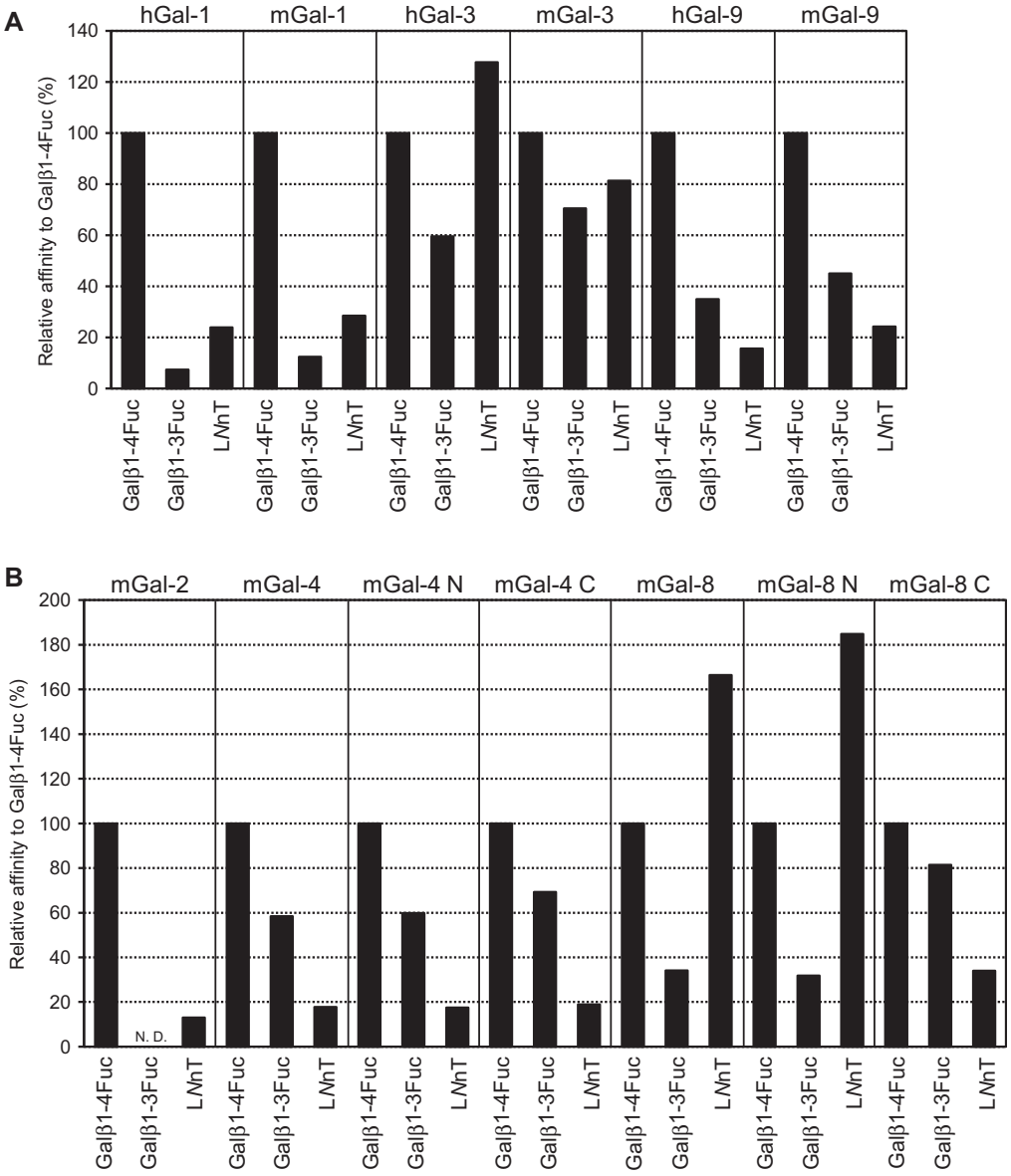

Fig. 2. Frontal affinity chromatography analysis of major human and mouse galectins. (A) Interaction between human and mouse Gal-1, -3, and -9 and PA-sugars and relative affinity values toward Galβ1–4Fuc. (B) Interaction between mouse Gal-2, -4, and -8 and PA-sugars and relative affinity values toward Galβ1–4Fuc. The relative affinity values were based on K_d values for the interaction between galectins and PA-sugars shown in Supplementary Table II. These experiments were performed at least two times and showed the representative results. N.D. means not determined.

galectin-1 (hGal-1), a well-studied major galectin, toward various sugars by using an immobilized hGal-1 column and frontal affinity chromatography analysis (Fig. 1). hGal-1 showed stronger affinity toward Galβ1–4Fuc-Man-ol-PA than Galβ1–3Fuc-Man-ol-PA, indicating that it prefers the 1–4 linkage to the 1–3 linkage. hGal-1 also showed affinity for E3-PA, an oligosaccharide obtained from endogenous *N*-glycan of *C. elegans* containing the Galβ1–4Fuc disaccharide unit. Affinities for Galβ1–4Fuc-Man-ol-PA and E3-PA were higher than that for L*N*nT-PA, which contains a Galβ1–4GlcNAc disaccharide unit, the endogenous recognition unit of vertebrate galectins [3], although they were lower than those for NA2-PA, NA3-PA, and NA4-PA, which contain 2, 3, and 4 Galβ1–4GlcNAc units, respectively.

hGal-1 has lower affinity for E3-PA than for Galβ1–4Fuc-Man-ol-PA, although both compounds contain the Galβ1–4Fuc disaccharide unit. In *C. elegans* LEC-6, the presence of Glu67 was found to be important for binding to the Fuc residue of the Galβ1–4Fuc disaccharide, especially in the natural glycan such as E3 [25]: point mutation of the Glu67 of LEC-6 severely weakens the affinity for E3-PA, while exerting a relatively mild effect on the affinity for Galβ1–4Fuc-Man-ol-PA. Therefore, it is likely that the lack of the Glu residue in hGal-1 renders lower affinity towards E3-PA. Overall, these results indicate that hGal-1 prefers Galβ1–4Fuc–Galβ1–4GlcNAc, although the recognition mechanism remains to be elucidated.

3.2. Other mammalian galectins also bind the Galβ1–4Fuc disaccharide

Next, we asked whether other well-studied galectins also prefer Galβ1–4Fuc over Galβ1–4GlcNAc (Fig. 2A and Supplementary Table II). hGal-3 showed substantial affinity for Galβ1–4Fuc-Man-ol-PA, although slightly lower than that for L*N*nT-PA. hGal-9 showed preferential binding to Galβ1–4Fuc-Man-ol-PA over L*N*nT-PA. A similar tendency was observed in case of mouse Gal-1, -3, and -9. These results show that not only hGal-1 but also other major human and mouse galectins have Galβ1–4Fuc-binding ability. We also examined sugar-binding abilities of other mouse galectins and observed that these galectins, except mGal-8 and mGal-8 N-CRD, also preferentially bind the Galβ1–4Fuc disaccharide unit (Fig. 2B and Supplementary Table II).

3.3. The interaction between hGal-1 and one of its glycan ligand is inhibited by synthetic Galβ1–4Fuc-OMe more effectively than lactose

The finding that mammalian galectins, especially hGal-1, preferentially bind Galβ1–4Fuc–Galβ1–4GlcNAc led us to the hypothesis that Galβ1–4Fuc might inhibit galectin-glycan interaction more effectively than lactose and galactose. To clarify this, we chemically synthesized a Galβ1–4Fuc derivative, Galβ1–4Fuc-OMe, in which the hydroxyl group at the C^1 of the Fuc residue is methylated, and consequently, the fucopyranoside ring is kept closed. We compared the effects of Galβ1–4Fuc-OMe, lactose (Galβ1–4Glc), galactose-β-OMe, a galactose derivative, and glucose on the interaction between hGal-1 and NA2-PA as a model of its endogenous glycan ligand (Fig. 3). Galβ1–4Fuc-OMe inhibited the interaction more effectively than lactose and Gal-β-OMe. This result suggests potential importance of the Galβ1–4Fuc disaccharide as a seed compound for the production of hGal-1 inhibitors.

In the present study, we found that vertebrate galectins bind the unique Galβ1–4Fuc disaccharide and suggested its possible use for a seed compound for the production of galectin inhibitors. The Galβ1–4Fuc disaccharide unit has been found only in invertebrate species [10–15]. Potential homologue of GALT-1, which is responsible for the production of this disaccharide, has not been found in mammalian species [16]. Therefore, it is not clear why

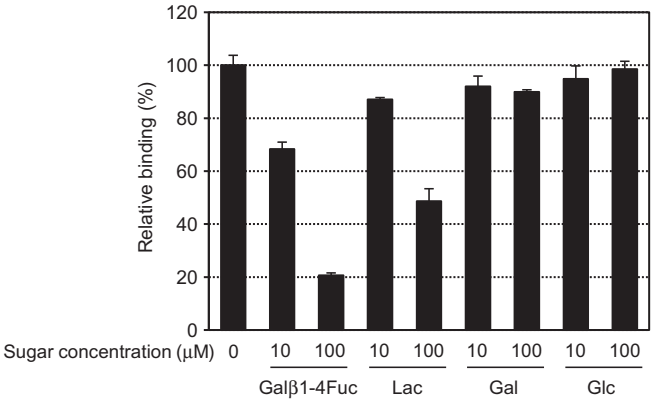

Fig. 3. Effects of sugars on the interaction between hGal-1 and its model ligand glycan. Relative binding values were calculated on the basis of the effect of sugars on the binding (V-V_0 values) between hGal-1 and NA2-PA as measured by FAC analysis. Galβ1–4Fuc-OMe, Galβ1–4Fuc; lactose, lac; Gal-β-OMe, gal; glucose, Glc. Data are expressed as mean ± S.D. (n = 3).

most of the mammalian galectins used in this study showed preferential binding to the Galβ1–4Fuc disaccharide over Galβ1–4GlcNAc, which is their endogenous recognition unit. However, galectins are known to recognize non-self glycans [31], and the Galβ1–4Fuc disaccharide has been found in parasitic nematodes *Ascaris suum* and *Oesophagostomum dentatum* [15]. Therefore, it is possible that the binding ability of the vertebrate galectins toward Galβ1–4Fuc, a non-self structural unit, has been conserved during evolution, and this possibility implies the potential importance of the recognition of the Galβ1–4Fuc disaccharide by galectins in host-pathogen interaction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.05.135.

References

[1] K. Kasai, J. Hirabayashi, Galectins: a family of animal lectins that decipher glycocodes, J. Biochem. 119 (1996) 1–8.
[2] D.N. Cooper, Galectinomics: finding themes in complexity, Biochim. Biophys. Acta 1572 (2002) 209–231.
[3] C. Boscher, J.W. Dennis, I.R. Nabi, Glycosylation, galectins and cellular signaling, Curr. Opin. Cell Biol. 23 (2011) 383–392.
[4] S. Di Lella, V. Sundblad, J.P. Cerliani, C.M. Guardia, D.A. Estrin, G.R. Vasta, G.A. Rabinovich, When galectins recognize glycans: from biochemistry to physiology and back again, Biochemistry 50 (2011) 7842–7857.
[5] N. Rubinstein, M. Alvarez, N.W. Zwirner, M.A. Toscano, J.M. Ilarregui, A. Bravo, J. Mordoh, L. Fainboim, O.L. Podhajcer, G.A. Rabinovich, Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection; a potential mechanism of tumor-immune privilege, Cancer Cell 5 (2004) 241–251.
[6] L. Ingrassia, I. Camby, F. Lefranc, V. Mathieu, P. Nshimyumukiza, F. Darro, R. Kiss, Anti-galectin compounds as potential anti-cancer drugs, Curr. Med. Chem. 13 (2006) 3513–3527.
[7] R.Y. Yang, G.A. Rabinovich, F.T. Liu, Galectins: structure, function and therapeutic potential, Expert Rev. Mol. Med. 10 (2008) e17.

[8] K.A. Stannard, P.M. Collins, K. Ito, E.M. Sullivan, S.A. Scott, E. Gabutero, I. Darren Grice, P. Low, U.J. Nilsson, H. Leffler, H. Blanchard, S.J. Ralph, Galectin inhibitory disaccharides promote tumour immunity in a breast cancer model, Cancer Lett. 299 (2010) 95–110.
[9] D. Giguere, S. Andre, M.A. Bonin, M.A. Bellefleur, A. Provencal, P. Cloutier, B. Pucci, R. Roy, H.J. Gabius, Inhibitory potential of chemical substitutions at bioinspired sites of beta-D-galactopyranose on neoglycoprotein/cell surface binding of two classes of medically relevant lectins, Bioorg. Med. Chem. 19 (2011) 3280–3287.
[10] Y. Zhang, T. Iwasa, M. Tsuda, A. Kobata, S. Takasaki, A novel monoantennary complex-type sugar chain found in octopus rhodopsin: occurrence of the Galβ1-4Fuc group linked to the proximal N-acetylglucosamine residue of the trimannosyl core, Glycobiology 7 (1997) 1153–1158.
[11] N. Takahashi, K. Masuda, K. Hiraki, K. Yoshihara, H.H. Huang, K.H. Khoo, K. Kato, *N*-Glycan structures of squid rhodopsin, Eur. J. Biochem. 270 (2003) 2627–2632.
[12] M. Wuhrer, M.L. Robijn, C.A. Koeleman, C.I. Balog, R. Geyer, A.M. Deelder, C.H. Hokke, A novel Gal(β1-4)Gal(β1-4)Fuc(α1-6)-core modification attached to the proximal N-acetylglucosamine of keyhole limpet haemocyanin (KLH) *N*-glycans, Biochem. J. 378 (2004) 625–632.
[13] A.J. Hanneman, J.C. Rosa, D. Ashline, V.N. Reinhold, Isomer and glycomer complexities of core GlcNAcs in *Caenorhabditis elegans*, Glycobiology 16 (2006) 874–890.
[14] K. Paschinger, E. Razzazi-Fazeli, K. Furukawa, I.B. Wilson, Presence of galactosylated core fucose on *N*-glycans in the planaria Dugesia japonica, J. Mass Spectrom. 46 (2011) 561–567.
[15] S. Yan, S. Bleuler-Martinez, D.F. Plaza, M. Kunzler, M. Aebi, A. Joachim, E. Razzazi-Fazeli, V. Jantsch, R. Geyer, I.B. Wilson, K. Paschinger, Galactosylated fucose epitopes in nematodes: increased expression in a caenorhabditis mutant associated with altered lectin sensitivity and occurrence in parasitic spECIES, J. Biol. Chem. 287 (2012) 28276–28290.
[16] A. Titz, A. Butschi, B. Henrissat, Y.Y. Fan, T. Hennet, E. Razzazi-Fazeli, M.O. Hengartner, I.B. Wilson, M. Kunzler, M. Aebi, Molecular basis for galactosylation of core fucose residues in invertebrates: identification of *Caenorhabditis elegans N*-glycan core α1,6-fucoside β1,4-galactosyltransferase GALT-1 as a member of a novel glycosyltransferase family, J. Biol. Chem. 284 (2009) 36223–36233.
[17] T. Takeuchi, K. Hayama, J. Hirabayashi, K. Kasai, *Caenorhabditis elegans N*-glycans containing a Gal-Fuc disaccharide unit linked to the innermost GlcNAc residue are recognized by *C. elegans* galectin LEC-6, Glycobiology 18 (2008) 882–890.
[18] T. Takeuchi, K. Nishiyama, K. Sugiura, M. Takahashi, A. Yamada, S. Kobayashi, H. Takahashi, H. Natsugari, K. Kasai, *Caenorhabditis elegans* galectins LEC-6 and LEC-1 recognize a chemically synthesized Galβ1-4Fuc disaccharide unit which is present in protostomia glycoconjugates, Glycobiology 19 (2009) 1503–1510.
[19] A. Butschi, A. Titz, M.A. Walti, V. Olieric, K. Paschinger, K. Nobauer, X. Guo, P.H. Seeberger, I.B. Wilson, M. Aebi, M.O. Hengartner, M. Kunzler, *Caenorhabditis elegans N*-glycan core β-galactoside confers sensitivity towards nematotoxic fungal galectin CGL2, PLoS Pathog. 6 (2010) e1000717.
[20] L.L. Maduzia, E. Yu, Y. Zhang, *Caenorhabditis elegans* galectins LEC-6 and LEC-10 interact with similar glycoconjugates in the intestine, J. Biol. Chem. 286 (2011) 4371–4381.
[21] T. Takeuchi, Y. Nemoto-Sasaki, Y. Arata, K. Kasai, Galectin LEC-6 interacts with glycoprotein F57F4.4 to cooperatively regulate the growth of *Caenorhabditis elegans*, Biol. Pharm. Bull. 34 (2011) 1139–1142.
[22] T. Takeuchi, K. Nishiyama, A. Yamada, M. Tamura, H. Takahashi, H. Natsugari, J. Aikawa, K. Kojima-Aikawa, Y. Arata, K. Kasai, *Caenorhabditis elegans* proteins captured by immobilized Galβ1-4Fuc disaccharide units: assignment of 3 annexins, Carbohydr. Res. 346 (2011) 1837–1841.
[23] T. Takeuchi, K. Sugiura, K. Nishiyama, H. Takahashi, H. Natsugari, Y. Arata, K. Kasai, Sugar-binding properties of the two lectin domains of LEC-1 with respect to the Galβ1-4Fuc disaccharide unit present in protostomia glycoconjugates, Biol. Pharm. Bull. 34 (2011) 1134–1138.
[24] Y. Nemoto-Sasaki, S. Takai, T. Takeuchi, Y. Arata, K. Nishiyama, A. Yamada, H. Takahashi, H. Natsugari, K. Kasai, The DC2.3 Gene in *Caenorhabditis elegans* encodes a galectin that recognizes the Galactoseβ1-4Fucose disaccharide unit, Biol. Pharm. Bull. 34 (2011) 1635–1639.
[25] H. Makyio, T. Takeuchi, M. Tamura, K. Nishiyama, H. Takahashi, H. Natsugari, Y. Arata, K.I. Kasai, Y. Yamada, S. Wakatsuki, R. Kato, Structural basis of preferential binding of fucose-containing saccharide by the *Caenorhabditis elegans* galectin LEC-6, Glycobiology 23 (2013) 797–805.
[26] J. Iwaki, H. Tateno, N. Nishi, T. Minamisawa, S. Nakamura-Tsuruta, Y. Itakura, J. Kominami, T. Urashima, T. Nakamura, J. Hirabayashi, The Galβ-(syn)-gauche configuration is required for galectin-recognition disaccharides, Biochim. Biophys. Acta 2011 (1810) 643–651.
[27] K. Nishiyama, A. Yamada, M. Takahashi, T. Takeuchi, K. Kasai, S. Kobayashi, H. Natsugari, H. Takahashi, Synthesis of fluorescence-labeled Galβ1-3Fuc and Galβ1-4Fuc as probes for the endogenous glyco-epitope recognized by galectins in *Caenorhabditis elegans*, Chem. Pharm. Bull. 58 (2010) 495–500.
[28] T. Takeuchi, R. Sennari, K. Sugiura, H. Tateno, J. Hirabayashi, K. Kasai, A C-type lectin of *Caenorhabditis elegans*: its sugar-binding property revealed by glycoconjugate microarray analysis, Biochem. Biophys. Res. Commun. 377 (2008) 303–306.
[29] J. Hirabayashi, K. Kasai, Effect of amino acid substitution by sited-directed mutagenesis on the carbohydrate recognition and stability of human 14-kDa β-galactoside-binding lectin, J. Biol. Chem. 266 (1991) 23648–23653.
[30] J. Hirabayashi, T. Hashidate, Y. Arata, N. Nishi, T. Nakamura, M. Hirashima, T. Urashima, T. Oka, M. Futai, W.E. Muller, F. Yagi, K. Kasai, Oligosaccharide specificity of galectins: a search by frontal affinity chromatography, Biochim. Biophys. Acta 1572 (2002) 232–254.
[31] G.R. Vasta, H. Ahmed, M. Nita-Lazar, A. Banerjee, M. Pasek, S. Shridhar, P. Guha, J.A. Fernandez-Robledo, Galectins as self/non-self recognition receptors in innate and adaptive immunity: an unresolved paradox, Front. Immunol. 3 (2012) 199.