Protein Expression and Purification 111 (2015) 82–86

Contents lists available at ScienceDirect

Protein Expression and Purification

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

Purification of galectin-1 mutants using an immobilized Galactoseβ1–4Fucose affinity adsorbent

Tomoharu Takeuchi [a,*], Mayumi Tamura [a], Nobuaki Ishii [a], Hiroki Ishikida [a], Saori Sugimoto [a], Daichi Suzuki [a], Kazusa Nishiyama [b], Hideyo Takahashi [b], Hideaki Natsugari [b], Yoichiro Arata [a]

[a] Laboratory of Biochemistry, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan
[b] Laboratory of Synthetic Organic and Medicinal Chemistry, School of Pharmaceutical Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan

ARTICLE INFO

Article history:
Received 9 February 2015
and in revised form 31 March 2015
Available online 6 April 2015

Keywords:
Galectin
Galactoseβ1–4Fucose
Affinity purification
Frontal affinity chromatography
Galectin-1

ABSTRACT

Galectins are a family of lectins characterized by their carbohydrate recognition domains containing eight conserved amino acid residues, which allows the binding of galectin to β-galactoside sugars such as Galβ1–4GlcNAc. Since galectin–glycan interactions occur extracellularly, recombinant galectins are often used for the functional analysis of these interactions. Although it is relatively easy to purify galectins via affinity to Galβ1–4GlcNAc using affinity adsorbents such as asialofetuin–Sepharose, it could be difficult to do so with mutated galectins, which may have reduced affinity towards their endogenous ligands. However, this is not the case with *Caenorhabditis elegans* galectin LEC-6; binding to its endogenous recognition unit Galβ1–4Fuc, a unique disaccharide found only in invertebrates, is not necessarily affected by point mutations of the eight well-conserved amino acids. In this study, we constructed mutants of mouse galectin-1 carrying substitutions of each of the eight conserved amino acid residues (H44F, N46D, R48H, V59A, N61D, W68F, E71Q, and R73H) and examined their affinity for Galβ1–4GlcNAc and Galβ1–4Fuc. These mutants, except W68F, had very low affinity for asialofetuin–Sepharose; however, most of them (with the exception of H44F and R48H) could be purified using Galβ1–4Fuc–Sepharose. The affinity of the purified mutant galectins for glycans containing Galβ1–4Fuc or Galβ1–4GlcNAc moieties was quantitatively examined by frontal affinity chromatography, and the results indicated that the mutants retained the affinity only for Galβ1–4Fuc. Given that other mammalian galectins are known to bind Galβ1–4Fuc, our data suggest that immobilized Galβ1–4Fuc ligands could be generally used for easy one-step affinity purification of mutant galectins.

Introduction

Galectins are a family of animal lectins that, through their binding to glycans, play important roles in various biological processes. Galectins are characterized by the evolutionarily conserved carbohydrate-recognition domain (CRD)[1] containing eight well conserved amino acid residues responsible for galectin binding to glycans with β-galactoside structures such as Galβ1–4GlcNAc [1,2]. Since galectin–glycan interactions occur in the extracellular compartments, a simple and direct approach to elucidate functional properties of galectins is to analyze the effect of recombinant galectins on cultured cells in vitro [3–6]. In such an approach, galectin mutants that could not bind to their endogenous ligand glycans could be useful for obtaining definitive results.

The preference of galectins to β-galactosides can be exploited for one-step purification of recombinant proteins by affinity chromatography using affinity adsorbents with immobilized-β-galactoside glycans or glycoconjugates such as lactosyl–Sepharose and asialofetuin–Sepharose [7]. However, such adsorbents could not be used for the purification of mutant galectins, which have low or no affinity to endogenous glycans such as Galβ1–4GlcNAc; mutants of each of the aforementioned well conserved amino acids in the CRD of human galectin-1 have little affinity for asialofetuin–Sepharose [8,9]. In such cases, the purification of galectin mutants defective in β-galactoside-binding requires the addition of an epitope tag [10] or the combination of several types of chromatographic techniques [11]. However, the introduction of epitope tags could affect protein conformation and/or functional activity and multi-step chromatographic

* Corresponding author. Tel./fax: +81 49 271 8123.
E-mail address: t-take@josai.ac.jp (T. Takeuchi).

[1] *Abbreviations used:* CRD, carbohydrate-recognition domain; EDTA, ethylenediamine tetraacetic acid; Fuc, fucose; Gal, galactose; Glc, glucose, GlcNAc, *N*-acetylglucosamine; PBS, phosphate-buffered saline; ME, mercaptoethanol; PA, pyridylamine.

http://dx.doi.org/10.1016/j.pep.2015.04.001
1046-5928/

purification can be difficult and time-consuming and in most cases results in product loss.

Galactoseβ1–4Fucose (Galβ1–4Fuc) is a unique disaccharide unit found only in *N*-glycans of invertebrates [12,13]. As evidenced by species distribution of potential orthologs of *GALT-1*, the galactosyltransferase gene responsible for the synthesis of Galβ1–4Fuc in *Caenorhabditis elegans*, is detected in invertebrates but not in mammals [14].

Galβ1–4Fuc has been identified as an endogenous recognition unit of *C. elegans* galectin LEC-6 [15,16]. The eight amino acid residues important for the recognition of Galβ1–4GlcNAc are also conserved in LEC-6, which is able to bind glycans containing Galβ1–4GlcNAc [17]. X-ray crystallographic analyses have revealed that almost all of the eight amino acid residues in the CRD of LEC-6 and CGL-2, a fungal galectin that interacts with Galβ1–4Fuc-containing *N*-glycans of *C. elegans*, are also important for the binding to Galβ1–4Fuc [18,19]. However, as evidenced by site-directed mutagenesis, each of the eight amino acid residues in LEC-6 had a relatively small impact on the interaction with Galβ1–4Fuc compared to that with Galβ1–4GlcNAc [16]. Furthermore, experiments with immobilized-Galβ1–4Fuc have revealed that various *C. elegans* galectins have the affinity for Galβ1–4Fuc disaccharide [20], although the eight amino acids are relatively less conserved in *C. elegans* galectins [21], for example, LEC-9, which has two substitutions among the 8 amino acid residues, could bind immobilized-Galβ1–4Fuc. These results indicate that immobilized Galβ1–4Fuc adsorbent may be useful for affinity purification of mutant galectins that cannot bind vertebrate-type glycans. Therefore, in the present study, we examined the effect of site-directed mutagenesis of each of the eight amino acid residues in mouse galectin-1 (mGal-1) on its affinity for Galβ1–4GlcNAc and Galβ1–4Fuc. Our results show that immobilized Galβ1–4Fuc could be applied for the purification of galectins with the mutations in the conserved eight amino-acid residues.

Materials and methods

Materials

Galβ1–4Fuc–Man-ol–PA and Galβ1–3Fuc–Man-ol–PA, sugars labeled with pyridylamine (PA) via a spacer derived from mannitol, were synthesized as described previously [22]. NA2–PA (PA–sugar chain 001; Galβ1–4GlcNAcβ1–2Manα1–3 (Galβ1–4GlcNAcβ1–2Manα1–6) Manβ1–4GlcNAcβ1–4GlcAc–PA), NA3–PA (PA–sugar chain 002; Galβ1–4GlcNAcβ1–2 (Galβ1–4GlcNAcβ1–4) Manα1–3 (Galβ1–4GlcNAcβ1–2Manα1–6) Manβ1–4GlcNAcβ1–4GlcNAc–PA), NA4–PA (PA–sugar chain 004; Galβ1–4GlcNAcβ1–2 (Galβ1–4GlcNAcβ1–4) Manα1–3 (Galβ1–4GlcNAcβ1–2 (Galβ1–4GlcNAcβ1–6) Manα1–6) Manβ1–4GlcNAcβ1–4GlcNAc–PA), LNnT–PA (PA–sugar chain 041; Galβ1–4GlcNAcβ1–3Galβ1–4Glc–PA), and rhamnose–PA were purchased from Takara Bio (Shiga, Japan).

Plasmid construction

In this study, we used mGal-1 carrying C2S substitution, which is thought to inhibit oxidation-dependent inactivation of its carbohydrate affinity without affecting carbohydrate-binding specificity [8]. Site-directed mutagenesis of mGal-1 was performed by PCR using the pET-mGal-1 C2S plasmid [23] and the following primers (substitutions are underlined): H44F, 5′-TTCTTCAATC-CTCGCTTCAATG-3′ and 5′-TAGGCACAGGTTGTTGCTG-3′; N46D, 5′-GATCCTCGCTTCAATGCCC-3′ and 5′-GAAGTGTAGGCACAGGTTG-TTGC-3′; R48H, 5′-CACTTCAATGCCCATGGAG-3′ and 5′-AGGATTG-AAGTGTAGGCACAG-3′; V59A, 5′-GCGTGTAACACCAAGGAAG-3′ and 5′-AATGGTGTTGGCGTCTCC-3′; N61D, 5′-GACACCAAGGA-AGATGGGAC-3′ and 5′-ACACACAATGGTGTTGGC-3′; W68F, 5′-TTTGGAACCGAACACCGGGAAC-3′ and 5′-GGTCCCATCTTCCT-

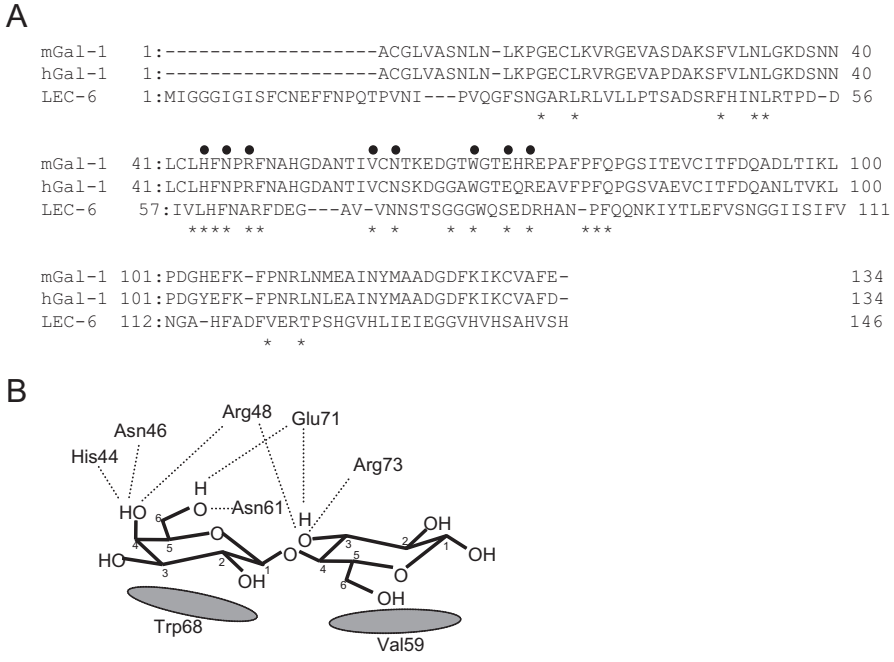

Fig. 1. Multiple sequence alignment of mouse and human galectin-1 and *C. elegans* galectin LEC-6, and a model of interaction between Galβ1–4Glc and galectin-1. (A) Amino acid sequence alignment of mouse galectin-1 (mGal-1; NP_032521.1), human galectin-1 (hGal-1; NP_002296.1), and LEC-6 (NP_497215.1). The initiating methionine is not indicated in mGal-1 and hGal-1, because it is removed during processing to mature hGal-1 [30]. The eight amino acids (H, N, R, V, N, W, E, and R) conserved among the galectin family proteins are denoted by closed circles; other conserved amino acids are denoted by asterisks. (B) A model of interaction between Galβ1–4Glc and the binding site of mGal-1 based on the crystal structure of human galectin-2 in complex with Galβ1–4Glc [31]. Hydrogen bonds are denoted by broken lines; van der Waals interactions are denoted by gray circles.