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Differences in *N*-acetyllactosamine synthesis between β-1,4-galactosyltransferases I and V

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Unlike classical β-1,4-galactosyltransferase (β-1,4-GalT I), β-1,4-GalT V (formerly IV) has little activity towards 1 mM *N*-acetylglucosamine [Sato *et al.* (1998) *Proc Natl Acad Sci USA 95:* 472-477]. The human β-1,4-GalTs I and V were expressed individually in Sf-9 cells by transfection of the full coding sequences, and their *N*-acetyllactosamine synthetase activities were determined towards different *N*-acetylglucosamine concentrations. Kinetic studies using the cell homogenates as an enzyme source revealed that β-1,4-GalTs I and V possess Km values of 0.6 mM and 33 mM towards *N*-acetylglucosamine, and of 48 μM and 41 μM towards UDP-Gal, respectively. No significant inhibition of *N*-acetyllactosamine synthesis with α-lactalbumin was observed for β-1,4-GalT V but the significant inhibition with α-lactalbumin was observed for β-1,4-GalT I.**

Keywords: **recombinant β-1,4-GalTs I and V/Km values/*N*-acetyllactosamine synthesis**

Introduction

In the past decades the occurrence of multiple β-1,4-galactosyltransferases (β-1,4-GalTs) has been implicated by differences in their sensitivities to α-lactalbumin or in their degrees of inhibition with higher acceptor *N*-acetylglucosamine concentrations [1–3]. In support of this observation, there are five isolated human cDNAs which encode proteins sharing 52, 44, 41, 37 and 33% identities to the classical β-1,4-GalT (β-1,4-GalT I) and being named β-1,4-GalTs II, III, IV, V and VI, respectively, according to their homology distances [4–7]. Previously we isolated a novel β-1,4-GalT* which was named β-1,4-GalT IV according to the order of discovery [5, 8] but is now referred to as β-1,4-GalT V in the paper based on its homology distance from the classical transferase. Like β-1,4-GalT I, β-1,4-GalTs II, III and IV have been shown to transfer galactose towards *N*-acetylglucosamine [4, 7]. Quite interestingly, however, effect of α-lactalbumin on *N*-acetyllactosamine synthetase activities of β-1,4-GalTs II, III and IV was different [4, 7]. The *N*-acetyllactosamine synthesis by β-1,4-GalT II was inhibited with α-lactalbumin like β-1,4-GalT I but by β-1,4-GalT IV was stimulated with α-lactalbumin. Only slight inhibition was observed for *N*-acety-lactosamine synthesis by β-1,4-GalT III. Little or no characterization of β-1,4-GalTs V and VI towards *N*-acetyllactosamine synthesis has been described although β-1,4-GalTV can transfer galactose to *para*-nitrophenyl-thio-β-D-GlcNAc(GlcNAcβ-S-*p*NP) quite effectively [5, 8] and β-1,4-GalT VI appears to be a UDP-Gal:glucosylceramide β-1,4-GalT [9]. In the present study, whether or not β-1,4-GalT V can transfer galactose to *N*-acetylglucosamine was investigated with the different acceptor concentrations in the absence or presence of α-lactalbumin using recombinant β-1,4-GalT I as a control.

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**DDBJ/GenBank/EMBL database, acc. no. AB004550.

Materials and methods

Chemicals and plasmid

Disaccharides, Galβ1→3GlcNAc and Galβ1→4GlcNAc were purchased from Sigma Chemical Co. (St. Louis, MO). Plasmid, pGalT1, containing human β-1,4-GalT I cDNA ligated into the *Eco*R I site of pYES2 (Invitrogen), was kindly provided by Drs. S. Takamatsu and M. Takeuchi at Kirin Brewery Co. Ltd. (Yokohama, Japan).

Plasmid construction and expression in Sf-9 cells

The cDNA encoding the full length of human β-1,4-GalT I was prepared by digestion of pGalT1 with *Bam*H I and *Xba* I. The cDNA fragment (1.3 kb) containing a *Bam*H I-restriction site at the 5′-end and an *Xba* I-restriction site at the 3′-end was ligated into the *Bam*H I-*Xba* I site of the baculovirus transfer vector pVL1393 (Pharmingen). The resultant plasmid, pVL1393/β-1,4-GalT I, pVL 1393/β-1,4-GalT V which was described previously [8] or pVL1393 as a control was co-transfected with BaculoGold™ Linear-

ized Baculovirus DNA into Sf-9 cells (9×10^6 cells) by the method described in BaculoGold™ system's protocol (Pharmingen).

Galactosyltransferase assay

The harvested cells were washed with phosphate-buffered saline (pH 7.4) (PBS) three times and suspended in 0.1 M 2-(*N*-morpholino)ethane sulfonic acid (MES) buffer (pH 7.0) containing 1.25% Triton X-100 by sonication. The cell homogenates were used for β-1,4-GalT assay as an enzyme source according to the method described previously [5]. In all cases the reaction mixture contained 0.1 M MES buffer (pH 7.0) containing 4mM 5′-AMP, 250 μM UDP-[^{3}H]Gal (48.8 Ci/mmol), 1 mM GlcNAcβ-S-*p*NP or *N*-acetylglucosamine of different concentrations as an acceptor, 20 mM $MnCl_2$ and enzyme preparation in a total volume of 50 μl.

Characterization of Galβ1→4GlcNAc linkage

In order to determine the galactosyl linkage formed by β-1,4-GalTs I and V, the product included in the β-1,4-GalT assay mixture was analyzed by Dionex BioLc system (Dionex) using Galβ1→3GlcNAc and Galβ1→4GlcNAc as standards [10]. In brief, the desalted product was applied to a Carbopak PA1 (4 mm × 250 mm) and eluted with a gradient (0-40%) of 1 M sodium acetate in 0.1 M NaOH at a flow rate of 1 ml/min. Elution position of the product was monitored with a pulsed amperometric detector.

Results and discussion

N-Acetyllactosamine synthesis by β-1,4-GalT V

In our previous study, β-1,4-GalT V failed to transfer galactose effectively to 1 mM *N*-acetylglucosamine [5]. In contrast, other newly discovered β-1,4-GalTs II and III have been shown to galactosylate *N*-acetylglucosamine quite effectively like β-1,4-GalT I [4]. By transfection of the β-1,4-GalT V cDNA into Sf-9 cells, β-1,4-GalT activity towards GlcNAcβ-S-*p*NP was detected, which was in proportion to the amounts of the cell homogenates included in the reaction mixture, while no significant β-1,4-GalT activity towards GlcNAcβ-S-*p*NP was detected in the homogenates of Sf-9 cells and of its mock-transfected cells (data not shown). When ^{3}H-galactosylated GlcNAcβ-S-*p*NP was digested with diplococcal β-galactosidase, which specifically cleaves the Galβ1→4GlcNAc linkage but not the Galβ1→3GlcNAc linkage [11], followed by Sep-Pak C_{18} column chromatography, all of the radioactivity was recovered in the pass-through fraction of the column (data not shown), indicating that galactose is transferred to the acceptor in a β-1,4-linkage. When *N*-acetyllactosamine synthetase activity was tested with the transferase using different *N*-acetylglucosamine concentrations, effective transfer of galactose was observed only towards the acceptor of higher than 3 mM. The maximum synthesis of *N*-acetyllactosamine by β-1,4-GalT V was achieved at 200 mM *N*-acetylglucosamine (closed circles in Fig. 1). In contrast, under the same conditions, the cell homogenates containing recombinant β-1,4-GalT I showed effective galactosylation of even 0.78 mM *N*-acetylglucosamine and maximum galactosylation of 12.5 mM *N*-acetylglucosamine (open circles in Fig. 1). At the concentrations of higher than 25 mM, *N*-acetyllactosamine synthesis by β-1,4-GalT I was inhibited. Since the products formed by β-1,4-GalTs V and I were eluted at the same position as standard Galβ1→4GlcNAc from a Carbopak PA1 column, *N*-acetyllactosamine products formed by β-1,4-GalTs V and I should have a type 2 structure. Due to the limited solubility of *N*-acetylglucosamine in the assay system, galactose transfer to the acceptor of higher than 250 mM was not determined.

Double-reciprocal plot analysis revealed that the recombinant β-1,4-GalT V has Km values of 33 mM for *N*-acetylglucosamine and of 41 μM for UDP-Gal, and that the recombinant β-1,4-GalT I has Km values of 0.6 mM for *N*-acetylglucosamine and of 48 μM for UDP-Gal (Table 1). Using GlcNAcβ-S-*p*NP as an acceptor, Km values of 128 μM for the acceptor and of 42 μM for UDP-Gal were obtained with β-1,4-GalT V, and Km values of 81 μM for the acceptor and of 47 μM for UDP-Gal were obtained

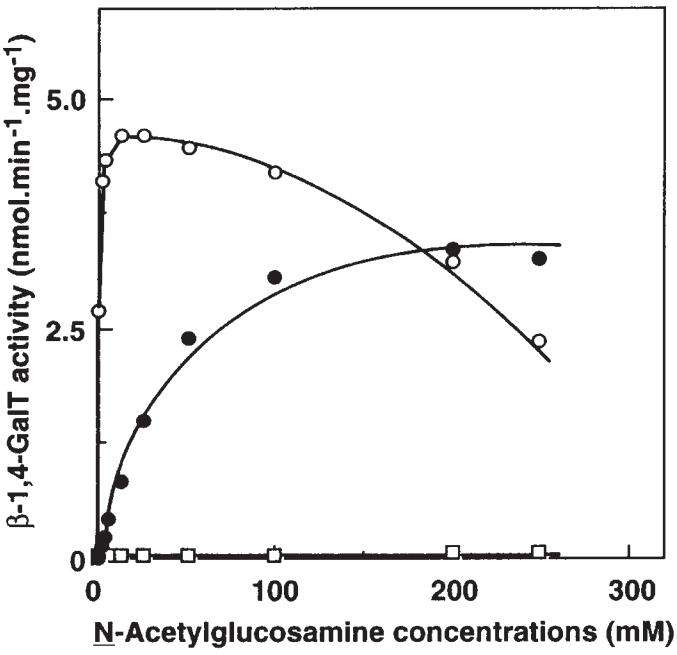

Figure 1. Effect of *N*-acetylglucosamine concentrations on the rates of recombinant human β-1,4-GalT V and I activities. Both activities of β-1,4-GalTs V (closed circles) and I (open circles) were determined as a function of increasing *N*-acetylglucosamine concentrations using the homogenates of Sf-9 cells transfected with the respective β-1,4-GalT cDNA. As a control, β-1,4-GalT activities were determined using the homogenates of the mock-transfected cells (open squares).

Table 1 Michaelis constants (Km) of recombinant β-1,4-GalTs I and V.

	Km		*Km*	
Transferase	*UDP-Gal (μM)*	*GlcNAc (mM)*	*UDP-Gal (μM)*	*GlcNAcβ-S-pNP (μM)*
β-1,4-GalT I	48 ± 1.1	0.6 ± 0.05	47 ± 1.2	81 ± 2.4
β-1,4-GalT V	41 ± 0.9	33 ± 1.4	42 ± 1.1	128 ± 3.2

Values are means of three independent experiments with standard errors.

with β-1,4-GalT I (Table I). These results indicate that the Km values of β-1,4-GalTs V and I towards UDP-Gal are relatively constant but are variable towards acceptor molecules, and support that β-1,4-GalT V can galactosylate *N*-acetylglucosamine only of higher concentrations. It is of quite interest that both β-1,4-GalTs V and I possess similar Km values towards UDP-Gal even though they share only 37% identity in their primary structures [5].

In the cases of β-1,4-GalTs II and III, *N*-acetylglucosamine of higher concentrations also caused inhibition of *N*-acetyllactosamine synthesis [4]. Therefore, β-1,4-GalT V is quite different from β-1,4-GalTs I, II and III as to *N*-acetyllactosamine synthesis. Requirement of higher *N*-acetylglucosamine concentrations for *N*-acetyllactosamine synthesis has been observed for β-1,4-GalT in porcine trachea [1]. It is, therefore, of interest to clarify whether or not this activity is identical to that of β-1,4-GalT V. The property of which β-1,4-GalT V can galactosylate *N*-acetylglucosamine of higher concentrations could be important in part for galactosylation of O-linked oligosaccharides with core 2 or core 4 structure which are expressed in a cluster on peptides (reviewed in [12]).

Effect of α-lactalbumin on *N*-acetyllactosamine synthetase activities of β-1,4-GalT V

It is well known that α-lactalbumin modulates the acceptor specificity of β-1,4-GalT I and inhibits the *N*-acetyllactosamine synthesis [13, 14]. When effect of α-lactalbumin on *N*-acetyllactosamine synthetase activities of β-1,4-GalT V was examined using 100 mM *N*-acetylglucosamine, its activity was inhibited at most by 6% with 2% α-lactalbumin (closed circles in Fig. 2). In contrast, more than 90% of β-1,4-GalT I activities towards 1 mM *N*-acetylglucosamine was inhibited with 2% α-lactalbumin (open circles in Fig. 2). Quite interestingly, *N*-acetyllactosamine synthesis by β-1,4-GalT II but not by β-1,4-GalT III has been also shown to be inhibited strongly with α-lactalbumin [4]. In contrast, *N*-acetyllactosamine synthetase activities of β-1,4-GalT IV were stimulated with α-lactalbumin [7]. These results indicate that optimal concentration of *N*-acetylglucosamine for *N*-acetyllactosamine synthesis and α-lactalbumin sensitivity are quite different among novel β-1,4-GalTs, which may also reflect their unique acceptor specificities currently being investigated.

If individual β-1,4-GalTs are to be shown to have fine acceptor specificities which are different from that of β-1,4-GalT I, then the presence of multiple β-1,4-GalTs could explain why some biological systems recognize only a certain group of cell surface galactose residues attached to the N-linked sugar chains of contactinhibin and CD45 in the regulation of cell growth and apoptosis, respectively, rather than those expressed on neighboring molecules [15, 16], because these biologically active galactose residues may be brought about by novel β-1,4-GalTs.

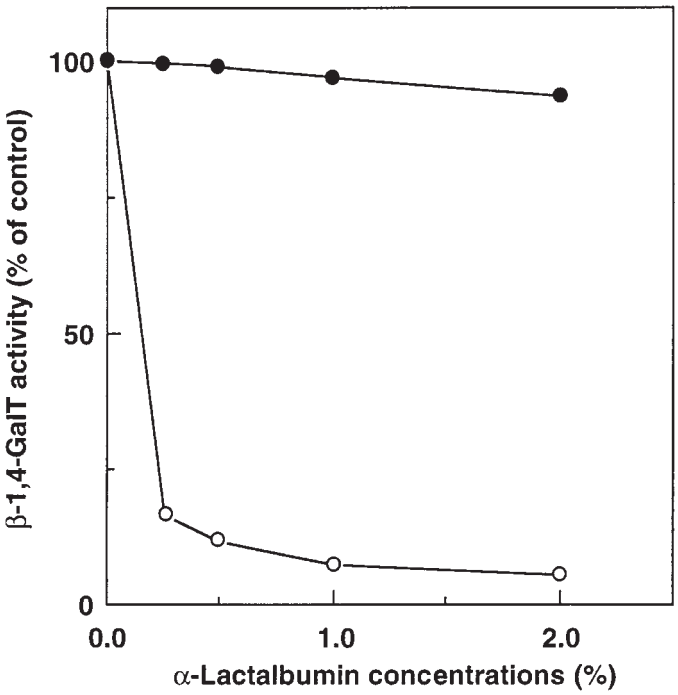

Figure 2. Effect of α-lactalbumin on recombinant human β-1,4-GalT I and V activities. The *N*-acetyllactosamine synthetase activities of β-1,4-GalT V (closed circles) and by β-1,4-GalT I (open circles) were determined in the presence of increasing α-lactalbumin concentrations.

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