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The First Bovine β1,4-Galactosyltransferase Reaction with an Acyclic Acceptor Substrate, 3-Acetamido-1,2-propanediol, To Yield a 3-*O*-β-D-Galactopyranosyl-*sn*-glycerol Skeleton

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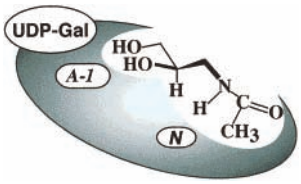
ABSTRACT



Reactivity of bovine β1,4-galactosyltransferase was examined for a series of acyclic acceptor substrates both in the presence and the absence of α-lactalbumin (α-La). It was found that this enzyme could utilize (*R*)-3-acetamido-1,2-propanediol (1) as an acceptor substrate regardless of the cofactor protein. The product was determined to be 1-*O*-β-D-galactopyranosyl-(*R*)-3-acetamido-1,2-propanediol (2). Glycerol without the acetamido group was inactive, indicating that this functional group plays a key role in the enzyme reaction.

The rule of "one enzyme - one glycosidic linkage"[1] expresses extremely high regio- and stereospecificity in glycosylation reactions catalyzed by mammalian glycosyltransferases. Thus, the mammalian enzymes are useful, in particular, for chemoezymic syntheses of natural glycoside linkages.[2] On the other hand, to attempt unusual types of glycosylation reactions by the mammalian enzymes and extend their utility to the syntheses of unnatural glycosides is an attractive challenge.[3] Such studies have been extensively carried out on bovine β1,4-galactosyltransferase (β1,4-GalTase).[4] As is defined by the name, the enzyme catalyzes β-galactosyl transfer to the OH-4 position of D-glucose, *N*-acetyl-D-glucosamine, and the other D-glucose derivatives. However, our previous studies have revealed that this enzyme

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Table 1. ^{1}H NMR Data of (*R*)-**1** and **2** and Conformational Equilibrium at the Acyclic Moiety (500 MHz, D_2O)

	chemical shifts δ (ppm)/J (Hz)[a]							populations of rotamers[b] *gg*:*gt*:*tg*	
compd	H-1*proR*	H-1*proS*	H-2	H-3*proR*	H-3*proS*			C-1/C-2	C-2/C-3
(*R*)-**1**	3.601	3.509	3.799	3.197	3.332			33:51:16	22:60:18
	(4.2,12.0)	(6.5)		(7.5, 14.0)	(4.4)				
2	aglycone moiety								
	3.722	3.886	3.968	3.280	3.363			41:49:10	24:49:27
	(3.7, 11.0)	(6.1)		(6.8, 14.0)	(5.1)				
	β-D-Gal moiety								
	H-1	H-2	H-3	H-4	H-5	H-6*proR*	H-6*proS*	C-5/C-6	
	4.397	3.542	3.648	3.913	3.685	3.787	3.745	21:70:9	
	(7.8)	(10.0)	(3.4)	(0.4)		(8.1, 11.7)	(3.7)		

[a] Assignments of all prochiral methylene protons were based on the NMR data of chrially deuterated *sn*-glycerols and (6*S*)-(6-$^2H_1$)-D-hexoses (refs 10−12). [b] Calculated on the basis of ^{1}H−^{1}H vicinal coupling constants (ref 12).

can yield β1,1-linked (β1,1-transfer)[5] and β1,3-linked disaccharides (β1,3-transfer).[6] In this Letter, we wish to describe another novel reaction for the bovine enzyme that utilizes an acyclic acceptor substrate.

In our previous study,[6] we found that L-glucose and L-xylose carrying an *N*-Ac group at the β-anomeric position are better acceptor substrates of β1,4-GalTase than the corresponding D-series sugar derivatives. Moreover, the enzymatic reaction was regioselective at the position OH-3 of these L-sugar substrates. The finding, which may be called "homochirality switching" in the chiral recognition event, could prove a significant role of the NAc group to be in the enzyme reaction. The possible rationalization for this role led us to propose key enzyme−substrate binding interactions. In the present study, we speculated on the basis of key interactions that this enzyme could utilize even acyclic substrates, although a six-membered ring structure is thought to be essential for the acceptor substrates.[7] At the beginning of the present study, we reinvestigated the role of the ring structure by using acyclic sugar alcohols derived from reducing D-sugar substrates (Figure 1).

The enzyme assay[8] indicated that reduction of D-sugars into acyclic glycitols diminishes their reactivity with β1,4-GalTase to a critical extent. For example, D-xylose lost its reactivity[9] after the reduction regardless of α-La. The initial reaction rate of D-glucose in the presence of α-La was reduced to an extent lower than 0.1% (10 mM) under the same conditions. It was, however, obvious that D-GlcNAc could retain moderate reactivity (ca. 1%) even after the reduction into acyclic alcohol. This result could support our previous idea that the *N*-Ac group plays a key role in the enzyme reaction and even acyclic compounds can become acceptor substrates. It is also notable that the cofactor protein shows little effect on the reactivity of these sugar alcohols.

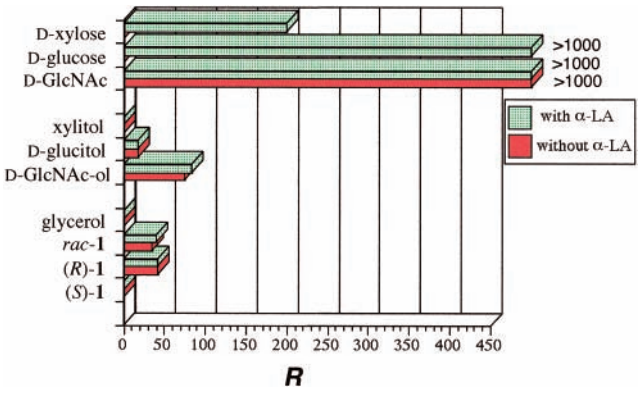
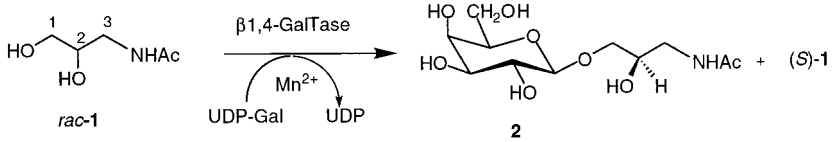


Figure 1. Relative reaction rates of acyclic acceptor substrates (10 mM).

More interesting results were derived for simpler acyclic compounds, glycerol and racemic 3-acetamido-1,2-propanediol (*rac*-**1**). Glycerol without an *N*-Ac group was inactive, whereas the *N*-Ac analogue *rac*-**1** was found to be reactive both in the absence and the presence of α-La. The relative reaction rate at 10 mM was ca. 0.8% of D-GlcNAc (10 mM) in the absence of α-La. Use of the optically resolved isomers could prove that the (*R*)-enantiomer is an active component in the racemate **1**. The reactivity of *rac*-**1** means also that the (*S*)-isomer does not become an acceptor

Scheme 1. Enantioselective Galactosylation of *rac*-**1**



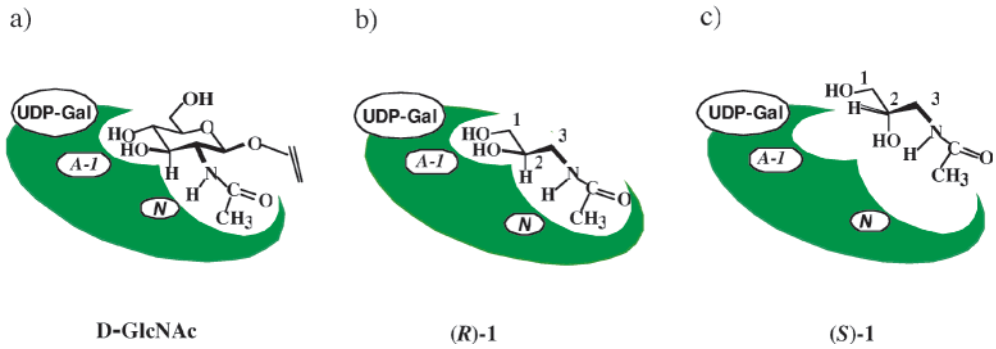


Figure 2. Key enzyme−substrate binding interactions in the absence of α -lactalbumin. Natural substrate D-GlcNAc is assumed to utilize two types of key binding interactions at *A-1* (reaction site) and *N* (*N*-Ac binding site) (a). The acyclic (*R*)-**1** substrate taking a *gt-gt* conformer can satisfy these key binding interactions and permit galactosylation at the primary OH-1group (b). The antipodal (*S*)-**1** cannot adopt these binding interactions by any conformers (c).

substrate nor an inhibitor. To verify this finding, the enzyme reaction was performed for *rac*-**1** in a preparative scale by applying a conventional UDP-glucose/UDP-glucose-4-epimerase/β1,4-GalTase system.[9] The reaction was carried out using excess amounts of the acceptor substrate (ca. 10 equiv for UDP-glucose) to improve the reaction rates. After incubation for 48 h followed by purification with gel permeation and silica gel column chromatography, the galactosylated product **2** was isolated in 25% yield on the basis of the amounts of UDP-glucose. The excess acceptor substrate **1** could also be recovered (90% theoretical yield) after the same chromatographic procedure. The ^{1}H NMR spectrum of product **2** indicated that the (*R*)-isomer reacted selectively to give a single β-galactosyl product (Table 1). No diastereomer could be detected in the NMR spectrum, supporting the enantioselective reaction as expected from the preceding enzyme assay. Otherwise, a nonenantioselective or a nonenzymatic reaction pathway might have given a pair of diastereomeric isomers. On the basis of ^{1}H NMR rules to discriminate prochiral methylene protons, H*proR* and H*proS* in acyclic 1,2-diols[10] and (1−6)-linked disaccharides,[11] it could be determined that the H-1*proS* signals of (*R*)-**1** shifted to lower field more significantly than H-1*proR* and H-2 after galactosylation. This shift means that the primary hydroxyl group was galactosylated to yield 3-*O*-β-D-galactopyranosyl-(*R*)-3-acetamido-1,2-propanediol, **2** (Scheme 1).

The above finding could be rationalized by applying our cartoon models for the acceptor binding site[6] (Figure 2). There it is assumed that the acyclic compound **1** in aqueous media takes the equilibrium of three staggered conformers, i.e., *gauche*−*gauche* (*gg*), *gauche*−*trans* (*gt*), and *trans*-*gauche* (*tg*), along each of the C−C single bonds. The (*R*)-isomer can take nine different conformers arising from the C1/C-2 and C-2/C-3 bonds. Here, it is obvious that the *gt-gt* conformation matches with the stereochemistry required for the key binding interactions with this enzyme (Figures 2a and 2b), while in the case of the (*S*)-**1** neither the same *gt-gt* conformation nor the other staggered conformation can fit to the binding interactions (Figures 2c). Calculation of the conformational equilibrium for (*R*)-**1** based on its ^{1}H NMR data[12] revealed that the *gt-gt* conformation is the most favored one in aqueous media (ca. 30%) and may be responsible for the enzyme reaction.

In conclusion, it has been demonstrated for the first time that the bovine β1,4-GalTase can utilize acyclic acceptor substrates in an enantio- and regiostereospecific way to afford a 3-*O*-β-galactopyranosyl-*sn*-glyceride skeleton. The study could add new observations to the structural requirements of acceptor substrates as follows: (1) a ring structure is not essential and (2) a primary OH group can become the reaction site. The results strongly imply that any cyclic or acyclic compound possessing an (*R*)-3-acetamido-1,2-propanediol as the partial structure may become a good substrate candidate of the bovine β1,4-GalTase.

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