Tetrahedron Letters, Vol. 33, No. 52, pp. 8043-8046, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Transfer of Galactose to the Anomeric Position of N-Acetyl Gentosamine by Galactosyltransferase from Bovine Milk

Yoshihiro Nishida, Torsten Wiemann and Joachim Thiem*

Department of Organic Chemistry, University of Hamburg, Martin-Luther-King Platz 6, D-2000 Hamburg 13, Germany

Summary: Galactosyltransferase was found to transfer ß-galactose to the ß-anomeric position of *N*-acetyl gentosamine to afford a ß,ß(1,1)-linked disaccharide. The reaction was generalized supposing an *N*-acetyl recognition site in the active site.

Galactosyltransferase from bovine milk (GalT, EC 2.4.1.22) catalyzes the transfer of galactose from UDP-galactose to the OH-4 position of *N*-acetyl glucosamine (GlcNAc) or in the presence of α-lactalbumin to glucose. This enzyme has been conveniently used for syntheses of galactose terminated oligosaccharides[1)] since stereo- and regioselectivity of the ßGal transfer allows simple syntheses compared with chemical methods involving laborious steps for protections and deprotections. Although the transfer by GalT from bovine milk has long been known to be regiospecific at the OH-4 position of glucose analogs, we have previously[2)] reported a new type of reaction with 3-acetamido-3-deoxy-D-glucose (Glc3NAc, 1) affording a ß,ß(1,1)-linked disaccharide 3. This reaction, namely ***ßGal1,1 transfer*** was tentatively explained by comparing the stereochemical environments between GlcNAc and Glc3NAc with regard to the reaction sites, OH-4 and OH-1, respectively. Comparison between the two substrates also suggested that other sugar derivatives with 3-acetamido group could be accepted for the ßGal1,1 transfer. In this communication, we investigated as a new substrate 3-acetamido-3-deoxy-D-xylose (Xyl3NAc, **2**[3)]) in order to extend the scope of the new GalT reaction.

The substrate **2** was prepared (20 mg, 37% yield) from 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (100 mg) according to the reported method[4)]. Enzymic assay[5)] showed that **2** was a better substrate than **1**, and in the presence of α-lactalbumin (0.1 mg/mL) the reaction rate was accelerated about six times. In order to identify the product, a mixture of **2** (17 mg, 0.09 mmol), UDP-glucose (50.4 mg, 0.09 mmol), UDP-glucose-4-epimerase (EC 5.1.3.2, 2.5U,), GalT (0.5U) and α-lactalbumin (0.2 mg/1mL) was incubated in Tris-HCl buffer (pH 7.5, 5.5mL). After 24 hrs at 30°C, the reaction mixture was processed in the reported manner[1f)] to afford a crude disaccharide (8 mg, 25%). The structure was identified as 1-*O*-ß-D-galactopyranosyl-3-acetamido-3-deoxy-ß-D-xylopyranoside **4** by ^{1}H-NMR spectroscopy

compared with the spectra of 2 and 3[2]; no α-anomeric proton was observed in 4, and the two ß-anomeric protons observed at 4.83 ppm (J=8.0 Hz, ßXyl3NAc residue) and 4.81 ppm (J=8.0 Hz, ßGal residue) were highly deshielded compared with the ß-anomeric

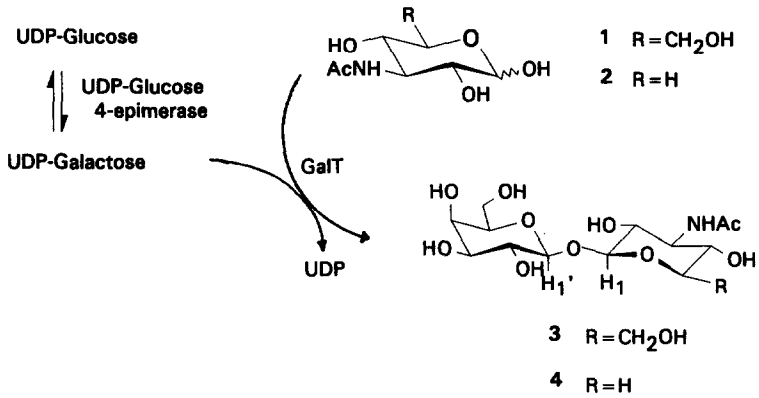


Fig.1 Syntheses of ß1,1-linked disaccharides by galactosyltransferase.

UDP-Galactose was produced in situ from relatively inexpensive UDP-glucose by UDP-glucose epimerase (ref. 6). Xyl3NAc 2 was a better substrate than Glc3NAc 1, but yields were 25-30% for both reactions.

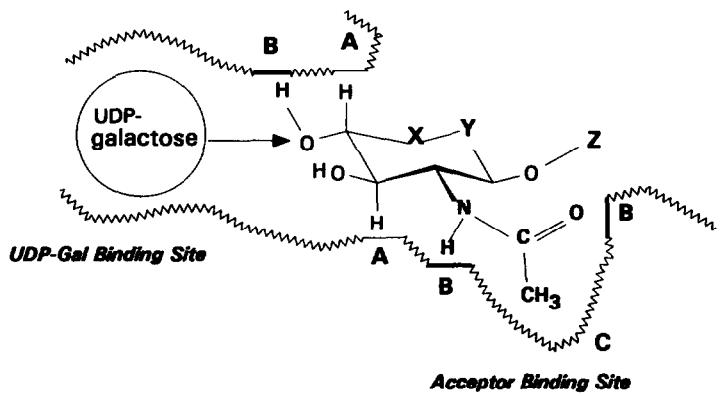
proton of ßGal(1,4)GlcNAc or ßGal(1,4)Glc (*ca.* 4.4 ppm). Comparing the ^{1}H signals between the ß-anomer of 2 and the Xyl3NAc residue of 4 indicated substantial deshielding occurring at H1 and H2 after the glycosidation.

Consequently, GalT was found to transfer ß-galactose to the ß-anomeric position of Xyl3NAc as well as Glc3NAc (Fig. 1). In order to explain the new reaction, a pictorial map of the acceptor binding site of GalT proposed by Berliner *et al.*[7] could be successfully used (Fig. 2). Here, the map was modified to generalize both the ßGal1,4 transfer to GlcNAc and the ßGal1,1 transfer to 1 and 2 as depicted in Fig. 2. The new map shows that for sugar derivatives with ring oxygen (or another hetereoatom such as *S* or *N*[1g]) at the Y-position, the reaction will result in the ßGal1,4 transfer. On the other hand, the reaction will become ßGal1,1 transfer for sugars with a heteroatom at the X-position. Such a mechanism could be valid among *N*-acetamido sugars for which site directed recognition may be governed by an *N*-acetyl binding locus in the active site.

The pictorial map in Fig. 2 can be used also to design new substrates for both types of the GalT reactions or inhibitors[8]. For example, it is obvious to

predict that a variety of di- or oligosaccharides with a ß,ß-trehalose skeleton can be prepared in the same way by replacing the Z-moiety by other substituents or sugars.

In summary, Xyl3NAc was found to be acceptable in the ßGal1,1 transfer reaction of GalT similarly to Glc3NAc, and this new type of reaction was generalized by proposing a new structural map for the acceptor binding site. Since amino sugars are key components of many antibiotic oligosaccharides, the chemoenzymic approach using GalT reactions summarized in Figs. 1 and 2 will be conveniently used as simple and environmentally friendly methods for developing new antibiotic oligosaccharides.



1:	(Glc3NAC)	X=O,	Y=CH(CH_2OH)eq.,	Z=H
2:	(Xyl3NAc)	X=O,	Y=CH_2,	Z=H
	GlcNAc	X=CH(CH_2OH)eq,	Y=O,	Z=H

Fig. 2 Postulated structural map of the substrate binding site of GalT.

The "A" site is defined as sterically hindered area where only a C-H group is accepted, while "B" is defined as binding area where a particular functional group, i.e, hydrogen of OH or NH, oxygen of carbonyl is bound, "C" is a hydrophobic region where an alkyl group is accepted (Ref. 7).

Acknowledgment: Support of these studies by the Alexander von Humboldt Stiftung through a fellowship to Y. N., the Deutsche Forschungsgemeinschaft, the Bundesministerium für Forschung und Technologie, and the Fonds der Chemischen Industrie is gratefully acknowledged.

References and Notes

1) H. A. Nunez and R. Barker, *Biochemistry*, 1980, *19*, 495.
For reviews see,
a) E. J. Toone, E. S. Simon, M. D. Bednarski and G. M. Whitesides, *Tetrahedron*, 1989, *45*, 5365.
b) S. David, C. Augé and C. Gautheron, *Adv. Carbohydr. Chem. & Biochem.*, 1991, *49*, 175.
c) P. Stangier and J. Thiem, *ACS Symposium Ser.*, 1991, *466*, 63.
d) C. H. Wong, K. K. C. Lie, T. Kajihara, L. Shen, Z. Zhong, D. P. Dumas, J. L. C. Liu, Y. Ichikawa and G. J. Shen, *Pure & Appl. Chem.*, 1992, *64*, 1197.
For recent results see:
e) C. Augé, C. Gautheron and H. Pora, *Carbohydr. Res.*, 1989, *193*, 288.
f) J. Thiem and T. Wiemann, *Angew. Chem. Int. Ed. Eng.*, 1990, *29*, 80; J. Thiem and T. Wiemann, *Angew. Chem., Int. Ed. Eng.*, 1991, *30*, 1163; J. Thiem and T. Wiemann, *Synthesis*, 1992, 141.
g) C. H. Wong, Y. Ichikawa, T. Krach, C. Gautheron, D. Dumas, and G. C. Look, *J. Am. Chem. Soc.*, 1991, *113*, 8137.
h) M. M. Palcic and O. Hindsgaul, *Glycobiology*, 1991, *1*, 205.
2) Y. Nishida, T. Wiemann, V. Sinnwell and J. Thiem, submitted.
3) R. E. Schaub and M. J. Wiss, *J. Am. Chem. Soc.*, 1958, *80*, 4683.
4) J. S. Brimacombe, J. G. H. Bryan, A. Husain, M. Stacey, and M. S. Tolley, *Carbohydr. Res.*, 1967, *3*, 318.
5) D. K. Fitzgerald, B. Colvin, R. Marval, K. E. Ebner, *Anal. Biochem.*, 1970, *36*, 43.
6) a) C. H. Wong, S. H. Haynie and G. M. Whitesides, *J. Org. Chem.*, 1982, *47*, 5416.
b) S. Sabesan and J. C. Paulsen, *J. Am. Chem. Soc.*, 1986, *108*, 2068.
7) L. J. Berliner, M. E. Davis, K. E. Ebner, T. A. Beyer and J. E. Bell, *Mol. & Cell Biochem.*, 1984, *62*, 37.
8) Y. Kajihara, H. Hashimoto and H. Kodama, *Carbohydr. Res.*, 1992, *229*, C5 and related references therein.

(Received in Germany 28 August 1992)