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On the Preparative Use of Recombinant Pig α(1-3)Galactosyl-Transferase

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ABSTRACT

A series of non-natural N-acyl derivatives of lactosamine is incubated with recombinant α(1-3)galactosyl-transferase and UDP-galactose. The enzyme shows a high promiscuity towards the non-natural acceptors. It selectively transfers a galactose unit onto the 3-OH group of the terminal β-linked galactose in an α-mode to give an array of linear-B trisaccharides.

The biological role of cell surface oligosaccharides in a variety of cell adhesion phenomena is now well established[1,2]. As a result this, class of highly homofunctional molecules is currently examined for potential pharmaceutical applications[3,4]. One recent interest included the immunological properties of oligosaccharides with terminal α-galactosides[5,6]. We recently showed that a number of recombinant glycosyl-transferases turned out to be versatile tools to prepare oligosaccharide libraries on a preparative scale[7]. The investigated transferases surprisingly tolerated a wide range of non-natural acceptors[8] and non-natural donors[9], as well as combinations thereof[10].

Here we wish to report our findings concerning the preparative use of pig α(1-3)galactosyl-transferase[11]. The recombinant enzyme was obtained by expression-cloning from porcine tissue[12]. The recombinant pig enzyme[6] transfers (see scheme) a galactose unit from the activated donor UDP-galactose **2** onto the 3-OH group of a terminal β-linked galactose - like the type-II disaccharide **1** - in an α-mode to give the linear-B trisaccharide structure **3** (R = Ac). This immunologically relevant trisaccharide epitope is recognized by more than 1% of preformed human IgGs[6,14]. In order to investigate the binding properties of these antibodies, which exhibit a high degree of microheterogeneity[15], we prepared a panel of linear-B trisaccharides **3**.

A series of type-II disaccharides **1**, which have the natural N-acetyl group (see scheme, R = Ac) replaced

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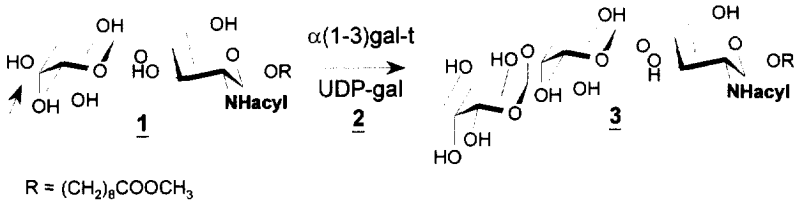
by non-natural acyl moieties have been available from a previous chemo-enzymatic synthesis[7]. The disaccharides **1** (see table for selected examples) are subsequently incubated with UDP-gal and recombinant α(1-3)gal-t[16]. Surprisingly, a wide range of N-acyl derivatives **1** are accepted by the enzyme. The natural N-acetyl group can e.g. be replaced by a carbamate (entry 2) or various protected amino-acid derivatives (entries 3, 4, 6, 7). Also bulky aromatic residues, either lipophilic (entry 8) or hydrophilic (entries 9 - 11), are tolerated and are α-galactosylated in the expected manner in preparatively useful amounts. Some modest yields (entries 2, 3, 10) stem from the low solubility of the corresponding acceptors **1**, which should be optimizable.



Scheme: Enzymatic glycosylation with recombinant α(1-3)galactosyl-transferase.

All structures **3** have been proven by ^{1}H NMR-, ^{13}C NMR- and MS-spectra. Characteristic proton- and carbon-shifts are included in the table and are in good agreement with those of the parent compound (entry 1). Indicative of a second, α-linked galactose unit in the compounds **3** are the proton signals at about 5.10 ppm for H-1 (d, J ~ 3.3 Hz) and the corresponding C-1 signals at about 97 ppm. The (1-3)linkage of the terminal galactose is further substantiated by a down-field shift of the C-3 signal[7] of the penultimate galactose from about 75 ppm in the disaccharides **2** to about 79 ppm in the target structures **3**.

In conclusion, our investigations show a high acceptor promiscuity for recombinant α(1-3)galactosyl-transferase *in vitro*, making this transferase a useful tool for the glycochemist to synthesize rapidly and unambiguously a library of non-natural linear-B trisaccharides[17]. Further evaluations are in progress and will be reported in due course.

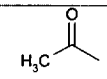
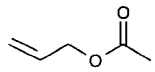
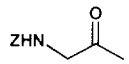
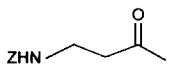
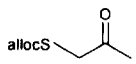
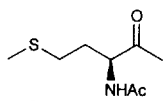
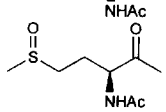
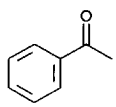
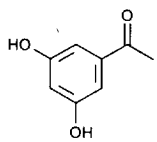
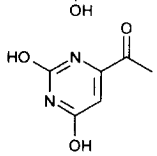
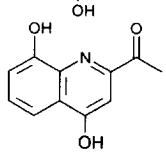
entry	acyl	%	α-gal		β-gal		glcNAc	other
		(mg)	C-1	H-1*	C-1	C-3	C-1	C
1		38 (12.4)	97.05	5.10	104.33	79.02	102.39	34.73
2		44 (13.1)	97.48	5.08	104.76	79.57	103.01	117.55
3		42 (21.8)	97.36	5.11	104.65	79.43	102.38	45.04
4		49 (8.5)	97.23	5.11	104.37	79.49	101.90	129.00
5		61 (21.7)	97.00	5.11	104.22	79.18	101.87	119.36
6		75 (13.8)	97.33	5.08	102.38	79.39	100.65	15.25
7		84 (23.9)	96.85	5.08	104.16	78.77	101.91	37.85
8		64 (7.2)	97.35	5.10	104.68	79.43	102.67	129.56
9		69 (17.1)	97.76	5.05	105.13	80.01	102.84	138.31
10		27 (6.6)	97.32	5.06	104.55	79.44	102.14	34.74
11		64 (10.4)	97.38	5.10	104.72	79.46	102.61	127.12

Table: Yields and NMR-data of the α-galactosides **3**; all measurements in CD_3OD (400 MHz resp. 62.9 MHz with internal ref. 3.31 ppm CD_3OD and 49.00 ppm CD_3OD); * doublet ($J \approx 3.3$ Hz).

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11) Gustafsson, K. et al. *Immunol. Rev.*, **1994**, *141*, 59.

12) The coding region for pig α(1-3) galactosyltransferase (accession number L36535) was amplified from porcine tissue and sequenced. A DNA fragment encoding for amino acids 24-359 fusion of pig α(1-3) gal-t was then isolated by PCR and cloned into expression vector sPROTA2.neoI to create an exported protein A fusion of the enzyme. The methodology used was essentially the same as described in[13]. For large scale expression the fusion protein was recloned into Baculovirus. The secreted ProteinA-α(1-3)gal-t fusion protein was harvested 3 days post-infection from the medium of insect cells and purified using SP-and Q-Sepharose followed by affinity chromatography on UDP-hexanolamine-Sepharose.

13) Kolbinger, F.; Streiff, M.; Katopodis, A. *J. Biol. Chem.*, **1998**, *273(1)*, 433.

14) Galili U. in: Evolution and Pathophysiology of the Human Natural Anti-α-Galactosyl IgG Antibody, *Springer Semin. Immunopathol.*, **1993**, *15*, 155.

15) Cooper, K. D. C.; Karen, E.; Oriol, R. *Immunol. Rev.*, **1994**, *141*, 151.

16) **Representative experimental procedure**: 9.0 mg (14.6 μmol) of disaccharide **1** (entry 8), 12.8 mg (20.9 μmol) UDP-galactose (YAMASA Corp. Jpn.) and 1.7 mg bovine serum albumine (Boehringer) are added to a mixture of 1150 μl bidistilled water, 200 μl of DMSO and 400 μl sodium cacodylate-buffer (0.5 M, pH = 6.52) containing 18.5 mg (93.4 μmol) $MnCl_2$. This mixture is briefly vortexed and then incubated at 37°C with 125 μl of a galactosyl-transferase solution (3U/ml) and 3 μl calf intestine alkaline phosphatase (Boehringer no. 108146, 7500 U/498 μl) for 48 h. The turbid mixture is then passed over a short C-18 reversed phase column, washed with water and eluted with methanol. The organic phase is evaporated and the residue purified on silica gel (eluent: dichloro-methane - methanol - water / 10 - 2 - 0.2) to give 7.2 mg (64 %) of the title compound as a white powder after a final lyophilization from dioxane-water.

17) Recently the parent compound (R = Ac) has been prepared in a one-pot reaction with both β(1-4)- and α(1-3)galactosyl-transferase on a microscale: Hokke, C. H.; Zervosen, A.; Elling, L.; Joziasse, D. H.; van den Eijnden, D. H. *Glycoconjugate J.*, **1996**, *13*, 687.