Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 161–164

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ENZYMATIC FUCOSYLATIONS OF NON-NATURAL SIALYLATED TYPE-I TRISACCHARIDES WITH RECOMBINANT FUCOSYL-TRANSFERASE-III

Gabi Baisch, Reinhold Öhrlein*, Markus Streiff

NOVARTIS PHARMA AG, Schwarzwaldallee 211, CH-4002 Basle, Switzerland

Received 13 October 1997; accepted 26 November 1997

ABSTRACT

Recombinant fucosyl-transferase-III (Lewis type enzyme) is used to prepare a series of non-natural sialyl-Lewis[a] derivatives on a preparative scale. The enzyme tolerates a wide range of acceptors which have the natural N-acetyl group of the glucosamine moiety replaced by substituted aromatic and hetero-aromatic amides. © 1998 Elsevier Science Ltd. All rights reserved.

Molecular glycobiology has progressed considerably in recent years[1] and glycoconjugates are now found worthwhile targets for drug development in order to treat severe carbohydrate-based disorders[2,3,4]. This class of highly homofunctional compounds is best tackled synthetically by a combined chemo-enzymatic approach[5,6].

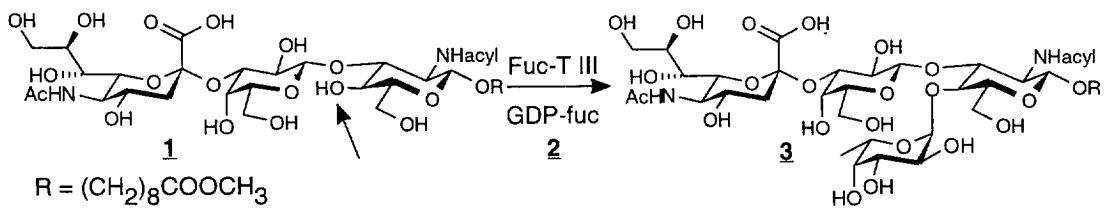
In vivo, glycosyl-transferases transfer a monosaccharide unit from a nucleotide-activated donor to a growing oligosaccharide chain with rigorous stereo- and regioselectivity. We could recently show that β(1-4)galactosyl-transferase[7], recombinant α(2-3)sialyl-transferase[8] and fucosyl-transferase-VI (fuc-T)[9] tolerate a surprisingly wide range of non-natural acceptor substrates and form the desired oligosaccharides with the proper stereochemistry in the presence of their respective donor substrates. Furthermore fuc-T III and fuc-T VI were demonstrated to recognize fucose-donors which had the natural guanosine replaced by various other nucleosides[10]. Also alterations of the fucose part of the donor were tolerated[11] which was first shown with a fuc-T mixture isolated from human milk[12]. In addition both non-natural donors and non-natural acceptors are processed by recombinant fuc-T VI at the same time and are assembled according the parent substrates[13].

Here we want to report our investigations concerning the synthetic scope of recombinant fuc-T III (EMBL accession no. X53578)[14]. *In vivo*, the enzyme transfers a fucose unit from the activated donor guanosine-

E-mail: REINHOLD.OEHRLEIN@pharma.novartis.com.

0960-894X/98/$19.00 © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(97)10202-5

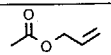
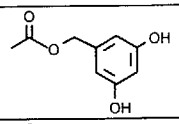
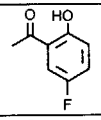
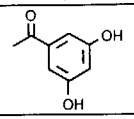
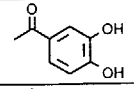
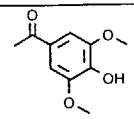
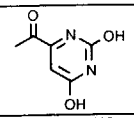
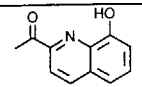
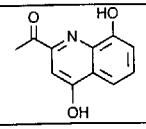
diphosphofucose (GDP-fuc) onto the 4-OH group of the N-acetylglucosamine moiety of a type I sugar chain in an α-mode to form Lewis[a] or sialyl-Lewis[a] structures, respectively (see scheme and table). A number of sialylated type-I sugars **1** have been prepared enzymatically (see preceding paper). They have the natural N-acetyl group of the GlcNAc-unit replaced by large, relatively unpolar (entries 4,7), highly polar aromatic (entries 3,5,6) or heterocyclic amides (entries 8,9,10). Although a few very little alterations at the N-acyl position had been reported for the milk enzyme previously[15], surprisingly, all the trisaccharides **1** have been accepted by the recombinant, homofunctional transferase. All the trisaccharides **1** are fucosylated by fuc-T III at the 4-OH group of the N-acyl glucosamine subunits in the expected α-mode despite the great steric bulk of the amino substituent. This is proven by the MS-, ^{1}H NMR- and ^{13}C NMR-data obtained from the new tetrasaccharides **3**. These data are in good agreement with the data of the parent compound (entry 1) and literature reports[15]. The shifts of selected nuclei have been included in the table. The shift and coupling constant of the H-1 of fucose is especially indicative for an α-linkage of that sugar. The (1-4)linkage of fucose to the glucosamide is further corroborated by a significant upfield shift of the neighbouring C-3 of the GlcN-acyl unit far below 80 ppm when compared with the shift of the respective C-3 atom of the starting material (see preceding paper).



Scheme: Enzymatic α(1-4)fucosylations.

In conclusion our findings demonstrate the high promiscuity of recombinant fuc-T III with regard to the acceptor substrate. This renders the enzyme a valuable biocatalyst for the unambiguous and efficient preparation of sialyl-Lewis[a] libraries for high through-put-screens. Thus another valuable tool is added to the synthetic arsenal of the glycobiologist. In combination with the results given previously for β(1-4)galactosyl-transferase[8], α(2-3)sialyl-transferase[9] and fuc-T VI[10,13], thus numerous non-natural oligosaccharides may now be prepared very efficiently via biocatalysts. Evaluations concerning the combined use of non-natural donors and non-natural acceptors are in progress and will be reported in due course.

Table: All measurements in D_2O-CD_3OD (ref. D_2O: 4.80 ppm and $\underline{C}D_3OD$: 49.00 ppm); a) d J ~ 8.5 Hz; b) dd J ~ 11.5 Hz and ~ 3 Hz; c) doublet J ~ 3 - 4 Hz; d) quartet J ~ 6.8 Hz; e) not resolved.

entry	acyl	% (mg)	GlcNacyl: H-1 [a]; C-1, C-2	Gal: H-1 [a]; C-1	Sia: H-2e [b]; C-3	Fuc: H-1 [c]; H-6 [d]; C-1, C-6	C-others: OCH3, NHR
1		97 (8.4)	4.38; 102.37; 57.48	4.35; 104.63	2.79; 42.49	4.96; 1.12; 99.61; 16.60	51.98; 23.63
2		78 (8.5)	4.42; 103.06; 59.81	4.42; 104.69	2.78; 42.42	4.98; 1.11; 99.48; 16.64	51.97; 134.79
3		52 (7.3)	4.62; 101.91; 59.91	4.38; 103.83	2.83; 42.12	4.97; 1.13; 99.53; 16.73	52.06; 159.66
4		100 (6.4)	4.69; 102.29; 58.34	4.42; 103.67	2.70; 41.88	5.01; e); 99.50; 16.70	51.96; 120.07
5		87 (14.8)	4.46; 102.05; 58.41	4.22; 103.69	2.78; 42.16	5.01; 1.13; 99.51; 16.72	51.96; 159.87
6		56 (6.8)	4.44; 102.17; 58.80	4.31; 103.72	2.74; 41.93	4.99; e); 99.51; 16.71	51.96; 127.55
7		70 (12.1)	4.69; 102.46; 58.54	4.48; 103.75	2.69; 41.67	5.01; 1.10; 99.59; 16.72	51.94; 148.99
8		80 (13.4)	4.56; 101.66; 58.26	4.43; 104.12	2.75; 41.51	5.03; 1.13; 99.55; 16.67	51.98; 176.12
9		52 (9.0)	4.64; 102.78; 57.98	4.49; 103.49	2.61; 41.56	5.03; e) 99.96; 16.72	51.93; 120.55
10		76 (19.7)	4.60; 102.69; 58.14	4.49 103.64	2.67; 41.70	5.02; e); 99.59; 16.71	51.91; 114.84

Representative experimental procedure: To a mixture of 600 µl of bidistilled water, 450 µl of Na-cacodylate buffer (250 mM, pH = 6.5) and 150 µl of a 250 mM $MnCl_2$-solution are added 14.8 mg (15.8 µmol) of trisaccharide **1** (entry 5), 13.8 mg (21.7 µmol) GDP-fuc[16] and 2.1 mg of bovine serum albumine (Boehringer). The clear mixture is incubated at 37°C in a plastic tube with 120 µl (720 mU) fuc-T III and 3 µl (51 U) of calf intestine alkaline phosphatase (Boehringer no. 108146, 7500 U/ 498 µl). After a TLC (CH_2Cl_2 - Methanol - water mixtures) shows the consumption of the starting acceptor **1**, the turbid solution is centrifuged and the clear supernatant passed over a C-18 reversed-phase column, washed with water, eluted with methanol and finally purified over silica-gel (CH_2Cl_2 - Methanol - water mixtures). Lyophilization from dioxane-water yields 14.8 mg (87%) of compound **3** (entry 5) as a white powder which is pure according MS-, ^1H- and ^{13}C NMR-analysis.

References:

1) Molecular Glycobiology, ed. by M. Fukuda, O. Hindsgaul, Oxford University Press **1994**.
2) Z. J. Witczak, Curr. Med. Chem. **1995**, *1*, 392.
3) R. A. Dwek, Chem. Rev. **1996**, *96*, 683.
4) J. C. McAuliffe, O. Hindsgaul, Chem. & Ind. **1997**, *3*, 170.
5) C.-H. Wong, R. L. Halcomb, Y. Ichikawa, T. Kajimoto, Angew. Chem. Int. Ed. Engl. **1995**, *34*, 521.
6) M. M. Palcic, O. Hindsgaul, TIGG **1996**, *8*, 37.
7) G. Baisch, R. Öhrlein, B. Ernst, Bioorg. Med. Chem. Lett. **1996**, *6*, 749.
8) G. Baisch, R. Öhrlein, M. Streiff, B. Ernst, Bioorg. Med. Chem. Lett. **1996**, *6*, 755.
9) G. Baisch, R. Öhrlein, A. Katopodis, B. Ernst, Bioorg. Med. Chem. Lett. **1996**, *6*, 759.
10) G. Baisch, R. Öhrlein, A. Katopodis, Bioorg. Med. Chem. Lett. **1996**, *24*, 2953.
11) G. Baisch, R. Öhrlein, A. Katopodis, M. Streiff, F. Kolbinger, Biorg. Med. Chem. Lett. in press
12) G. Srivastava, K. J. Kaur, O. Hindsgaul, M. M. Palcic, J. Biol. Chem. **1992**, *267*, 22356.
13) G. Baisch, R. Öhrlein, A. Katopodis, Biorg. Med. Chem. Lett. in press.
14) K. Sasaki, K. Kurata, K. Funayama, M. Nagata, E. Watanabe, S. Ohta, N. Hanai, T. Nishi, J. Biol. Chem. **1994**, *269*, 14730.
15) P. V. Nikrad, M. A. Kashem, K. B. Wlasichuk, G. Alton, A. P. Venot, Carbohydr. Res. **1993**, *250*, 145.
16) G. Baisch, R. Öhrlein, Biorg. Med. Chem. **1997**, *5*, 383.
17) M. M. Palcic, Methods Enzymol. **1994**, *230*, 300.