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SYNTHETIC POTENTIAL OF CLONED FUCOSYL-TRANSFERASE III AND VI

Gabi Baisch, Reinhold Öhrlein*, Andreas Katopodis, Markus Streiff, Frank Kolbinger

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

ABSTRACT

Two cloned fucosyl-transferases, fuc-t III and fuc-t VI, are probed with non-natural donor-sugars, in which the natural fucose moiety is replaced by mono-saccharides modified in their 2- or 6-positions. Despite their close sequence homology, the investigated transferases exhibit significant differences in their substrate recognition. © 1997 Elsevier Science Ltd.

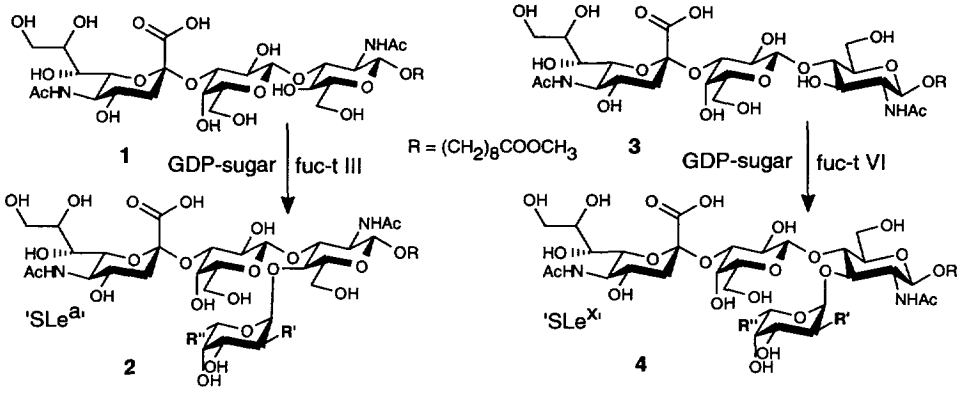
Fucosylated oligosaccharide structures serve as receptor determinants for a variety of cell-adhesion phenomena.[1] Analogs of these sugars are of interest for investigating more closely biologically important carbohydrate-protein interactions.[2,3] Rapid and reliable assemblage of oligosaccharides is therefore desirable. This can be achieved efficiently by a combination of chemical and enzymatic methodologies.[4] The preparative usefulness of mammalian fucosyl-transferases, isolated from natural sources or cloned,[5,6] has been demonstrated. These enzymes use β-guanosine-diphosphate-fucose (GDP-fucose) to transfer a fucose-unit regio- and stereo-specifically to a distinct OH-group of an acceptor saccharide *in vivo* and *in vitro*.

The synthetic versatility of these biocatalysts was recently proven by the finding that cloned fuc-t VI accepts a wide variety of non-natural acceptor-sugars to build up a sialyl-Lewis^x-tetrasaccharide library.[7] The synthetic potential of cloned and overexpressed fucosyl-transferases could be substantially broadened if also non-natural donor-sugars are transfered with the same stereochemical fidelity as the parent fucose. Investigations performed with partially purified fucosyl-transferases (a mixture of two fucosyl-transferases) from human milk showed that such isolates were able to recognize altered fucose-moieties. Thus fucose-residues with large substituents - consisting of oligosaccharides or photolabels - on the 6-position could be transferred in the expected manner.[8,9]

We recently developed a versatile procedure for the synthesis of nucleotide activated donor-sugars[10] which allows an easy access to a number of fucose-donors. Using this procedure to synthesize fucose-donors with

e-mail: REINHOLD.OEHRLEIN@chbs.mhs.ciba.com; FAX ++61 6978975

various nucleotides we found that cloned fuc-t III (Lewis-type enzyme, EMBL accession no. X53578)[11] and cloned fuc-t VI (plasma enzyme, EMBL accession no. L01698)[12] tolerate the replacement of guanosine by a series of purine bases.[13] Here we wish to report our results concerning structural variations of the fucose-part of the donor (see scheme and table 1).



Scheme: Enzymatic fucosylations with fuc-t III and fuc-t VI.

Both fuc-t III and fuc-t VI are incubated[14] with the enzymatically sialylated trisaccharides **1** and **3** [13,16] in the presence of the indicated GDP-sugar donors. As observed with the milk enzymes,[17] both these cloned fucosyltransferases tolerated modifications at the fucose 6-position (**2b,c** and **4b,c**). Fuc-t III transfers the sugar moiety to the 4-OH-group of the N-acetyl-glucosamine-unit in the expected α-mode to give sialyl-Lewis^a-derivatives. Fuc-t VI transfers these sugars to the 3-OH-group of the N-acetyl-glucosamine-unit to give the corresponding sialyl-Lewis^x-congener. The two enzymes differ, however, in respect to modifications at other OH-groups. Whereas fuc-t VI does not tolerate the 4-epi-OH modification (L-glucose), fuc-t III does transfer the 4-epi-isomer (**2d**). Furthermore, the 2-OH-group of fucose in the GDP-'fucose' donor may be replaced by e.g. an amino-group (**2e**) or a fluorine (**2f**). These sugars are transferred in a manner analogous to the parent fucose, resulting in sialyl-Lewis^a-derivatives **2**. In contrast, fuc-t VI did not accept those donor-substrates to form the corresponding sialyl-Lewis^x-derivatives. This surprising result could only be obtained by the separate investigation of the cloned, well characterized proteins, which is interesting because of their close sequence homology.[18]

The structural identity of the discussed, new sialyl-Lewis^a- and sialyl-Lewis^x compounds has been proven by MS- ^{1}H and ^{13}C NMR data. The chemical shifts of some of the reporter groups are compiled in table 2.

Besides these data, extensive NMR measurements, COSY, HETCOR or HMQC and ROESY, confirmed the connectivity of the 'fucose' residues, which are all α-linked to the N-acetylglucosamine-units.

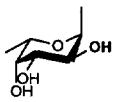
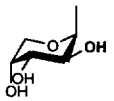
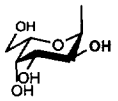
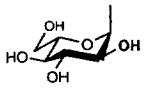
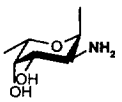
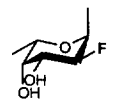
sugar	2	%	[mg]	4	%	[mg]
	**a**	97	51.0	**a**	82	7.7
	**b**	75	9.2	**b**	80	9.2
	**c**	83	9.9	**c**	58	6.9
	**d**	57	7.1	**d**	0	n.t.
	**e**	48	7.6	**e**	0	n.t.
	**f**	88	12.0	**f**	0	n.t.

Table 1: Fucosylations with GDP-fucose analogs; n.t. not transferred.

compound	glc-NAc: C-1, 2	sia: C-2, 3	gal: C-1	'fuc': C-1, 6	'fuc': H-1[a], 6
2a	102.4; 57.5	101.3; 42.5	104.6	99.6; 16.6	5.00; 1.14
2b	102.3; 57.4	100.9; 42.5	104.4	100.3; 65.3[b]	5.02; -
2c	102.2; 57.6	101.0; 42.5	104.8	99.9; 62.4	5.03; -
2d	102.5; 57.3	101.0; 42.3	105.1	98.9; 62.5	5.06; -
2e	101.8; 58.2	100.9; 42.3	104.4	100.9; 16.6	5.18; 1.11
2f	102.4; 57.5	100.9; 42.5	104.7	97.4[c];16.4	5.15[d]; 1.05
4a	102.4; 56.5	100.6; 42.6	103.7	99.5; 16.3	4.96; 1.08
4b	102.5; 57.2	100.8; 42.3	103.8	100.3; 65.2	5.07; -
4c	102.4; 57.3	100.9; 42.4	104.0	100.4; 62.6	5.03; -

Table 2: Confirmative data of 'fucosylated' tetrasaccharides: a) all doubletts, J ~ 4 – 5 Hz, b) C - 5; c) J = 20.2 Hz; d) 19 F NMR: δ = 208.9 ppm (J = 50.8, 11.6 Hz).

In conclusion, this work shows the broad preparative versatility of fuc-t III with regard to GDP-'fuc' donors. Non-natural fucose-moieties, both modified in the 6- or 2-position, are readily accepted and transferred like the

parent fucose *in vitro*. In contrast, fuc-t VI does not exhibit such a broad substrate tolerance. Only fucose analogs, modified in the 6-position, are accepted by this enzyme. A recent study dealing with fuc-t V[19] another member of this closely related group of enzymes, showed that 2-F-fucose acts as an inhibitor on this enzyme! These combined findings are very important for the preparative applications of the different fucosyl-transferases, respectively.

Investigations concerning the combined use of non-natural donor sugars and non-natural acceptors to create oligosaccharide libraries for ligand screenings are to be submitted elsewhere in due course.

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14) **Representative incubation procedure**: 10.1 mg (12.0 µmol) of trisaccharide **1**, 14.0 mg (21.6 µmol) of GDP-galactose[10] and 1.3 mg bovine serum albumine (Boehringer) are added to a mixture of 450 µl of a 250 mM cacodylate buffer solution (pH = 6.5), 150 µl of a 250 mM manganese-dichloride solution and 600 µl of bidistilled water.[15] The clear solution is treated with 166 µl (6U/ml) of a fucosyl-transferase III (or fucosyl-transferase VI, correspondingly) solution and 2 µl (~34 U) calf intestine alkaline phosphatase (Boehringer no. 108146, 7500 U/498 ml). The mixture is incubated at 37°C with stirring for 4 days. The resulting turbid solution is then centrifuged and the supernatant passed over a short C-18 reversed phase column, lyophilized and subsequently purified on silica gel (eluent: methylene chloride - methanol - water / 10 - 4 - 0.5, vol - vol - vol). A final lyophilization of the tetrasaccharide containing fractions from dioxane yields 9.9 mg (83%) of tetrasaccharide **2c** as a white powder. The incubations with the parent GDP-fucose donor can be terminated after one day of incubating.
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