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Enzymatic Fucosylations with Purine-Diphosphate-Fucoses (PDP-Fucoses)

Gabi Baisch, Reinhold Öhrlein*, Andreas Katopodis

Central Research Laboratories CIBA AG, Schwarzwaldallee 211, CH-Basle (Switzerland)

ABSTRACT

Two cloned fucosyltransferases, Fuc-t III and Fuc-t VI, are probed on a preparative scale with non-natural donor-substrates, in which the guanosine of the natural donor guanosine-diphosphate-fucose is replaced by other purines. Surprisingly, the novel purine-diphosphate-fucoses (PDP-Fuc) are recognized by both enzymes as donor-substrates.

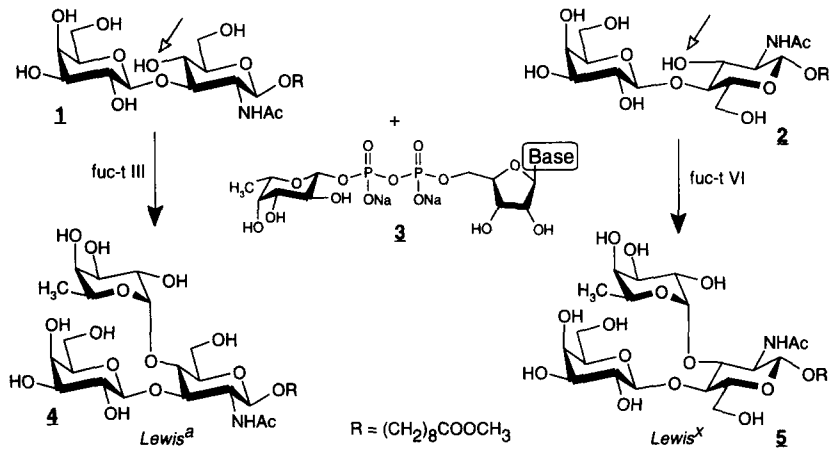
Oligosaccharides play a key role in cell-adhesion and developmental processes[1]. Altered or truncated oligosaccharides may lead to severe pathological disorders, or may be used to treat unwanted carbohydrate-based diseases[2,3,4]. The protocols for the chemical synthesis of oligosaccharides are diverse[5] but the synthesis of individual oligosaccharides remains tedious and unpredictable[6]. A combined chemo-enzymatic approach offers a less cumbersome access to this class of compounds[7].

A number of mammalian fucosyl-transferases (Fuc-t), either isolated from natural sources or cloned, have been explored for use in oligosaccharide synthesis[8,9]. The enzymes use β-guanosine-diphosphate-fucose (GDP-Fuc) to transfer a fucose-unit regio- and stereospecifically to a specific hydroxyl-group of an acceptor-saccharide *in vivo* and *in vitro* (e.g. see scheme).

A drawback of this elegant method, however, is the availability of GDP-Fuc. To circumvent this problem GDP-Fuc may be generated *in situ*[10] but this necessitates additional enzymes and thus limits the synthetic versatility. GDP-Fuc and some analogs have been prepared chemically[11] and a general protocol for the synthesis of the parent compound and a number of derivatives has been elaborated recently[12]. The synthetic applicability of Fuc-t's as efficient biocatalysts has been proven[9,11]. Even though these transferases were not well characterized at that time (e.g. mixtures of Fuc-t's from human milk) a number of natural and non-natural oligosaccharides could be

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

acceptors[8,9,13,14] and only a limited number of studies showed that the milk enzymes accepted GDP-Fuc-analogs, which had the parent fucose replaced by deoxyfucoses or 6-substituted fucoses[15,16].



Scheme: Enzymatic synthesis of *Lewis*^{a} and *Lewis*^{x}; base, see table.

Up to now five α1,3-Fuc-t's have been found and cloned[3] and are well characterized. Currently we are investigating the cloned ***Lewis-type enzyme*** (Fuc-t III)[17] (EMBL accession no. X53578) and the ***plasma enzyme*** (Fuc-t VI)[18] (EMBL accession no. L01698). Firstly, we are interested in the synthetic scope of these enzymes with respect to the acceptor and donor parts. Fuc-t VI, for example, tolerates a broad array of non-natural acceptors[14]. Secondly, in order to devise selective fucosyltransferase inhibitors[2,3,4], we synthesized a number of GDP-Fuc-analogs[12] (see table), which have the guanosine replaced by other bases. Thus we hope to get some insight into the binding sites of the enzymes.

Both Fuc-t III and Fuc-t VI are incubated[19] with the disaccharides **1**[20] and **2**[21] in the presence of the indicated donor-substrates (see scheme and table).

Surprisingly, both enzymes tolerate the exchange of the guanosine by other purines and handle those donors like GDP-Fuc to form the expected trisaccharides[22]. Neither the amino-group nor the carbonyl-group of guanosine seem to be essential for substrate recognition. The amino-group can be removed (IDP) without change in either reactivity or selectivity. The amino-group can even be replaced by a carbonyl-group (XDP) of reversed polarity without alterations in the reaction mode. Substitution of the carbonyl-group by an amino-group and omission of the amino-group in the original guanosine leads to the significantly altered ADP. Unexpectedly, also this substrate serves as

an excellent donor for both Fuc-t III and Fuc-t VI. In both cases the regio- and stereospecific formation of the single compounds **4** and **5** is observed. AMP is about ten times cheaper than GMP thereby reducing the cost ADP-fucose preparation.

However, GTP-Fuc is not accepted as a substrate by either transferase. In comparison to GDP this donor carries an additional charge and the distance between the fucose moiety and the purine-base is extended.

In conclusion our studies show for the first time the high promiscuity of two Fuc-t's, III and VI, *in vitro* towards the purine-base part of the donor-substrate. This demonstrates, in addition to previous reports[14,15,16], that Fuc-t's are versatile biocatalysts. Further evaluations are in progress and will be reported in due course.

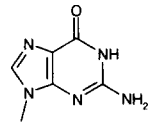
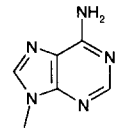
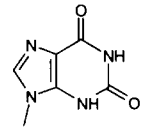
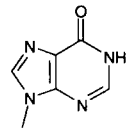
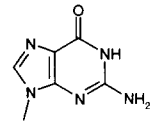
comp.	abbr.	base	Fuc-t III % , **4**	Fuc-t VI % , **5**
3a	GDP		96	83
3b	ADP		76	60
3c	XDP		73	62
3d	IDP		68	72
3e	GTP		0	0

Table: Investigated PD(T)P-fucoses.

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22) Representative incubation procedure: 9.1 mg (16.4 μmol) of the disaccharide **1**, 16.5 g (26.7 μ mol) ADP-Fuc and 2.1 mg bovine serum albumine (Boehringer) are added to a mixture of 450 μl of a 250 mM sodium-cacodylate-puffer (pH = 6.5), 150 μl of a 250 M $MnCl_2$-solution and 660 μl bidistilled water. The solution is briefly sonicated, then treated with 0.75 U (75 μl) of Fuc-t III and 30 U (2 μl) of calf intestine alkaline phosphatase (Boehringer No. 108146, 7500U/498μl) and incubated at 37°C overnight. The turbid mixture is then centrifuged and the supernatant passed over a short C-18 reversed-phase column, lyophilized and subsequently purified on silicagel (eluent: methylenchloride-methanol-water). A final lyophilization from dioxane gives 8.7 mg (76%) of pure trisaccharide **4** as a white powder, whose 1H and 13C NMR data are in agreement with reported ones[20], respectively[23] for **5**.
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