Characterization of a core α1→3-fucosyltransferase from the snail *Lymnaea stagnalis* that is involved in the synthesis of complex-type *N*-glycans

Angelique van Tetering, Wietske E.C.M. Schiphorst, Dirk H. van den Eijnden, Irma van Die*

Department of Medical Chemistry, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands

Received 28 September 1999; received in revised form 22 October 1999

Abstract We have identified a core α1→3-fucosyltransferase activity in the albumin and prostate glands of the snail *Lymnaea stagnalis*. Incubation of albumin gland extracts with GDP-[^{14}C]Fuc and asialo/agalacto-glycopeptides from human fibrinogen resulted in a labeled product in 50% yield. Analysis of the product by 400 MHz ^{1}H-NMR spectroscopy showed the presence of a Fuc residue α1→3-linked to the Asn-linked GlcNAc. Therefore, the enzyme can be identified as a GDP-Fuc:GlcNAc (Asn-linked) α1→3-fucosyltransferase. The enzyme acts efficiently on asialo/agalacto-glycopeptides from both human fibrinogen and core α1→6-fucosylated human IgG, whereas bisected asialo/agalacto-glycopeptide could not serve as an acceptor. We propose that the enzyme functions in the synthesis of core α1→3-fucosylated complex-type glycans in *L. stagnalis*. Core α1→3-fucosylation of the asparagine-linked GlcNAc of plant- and insect-derived glycoproteins is often associated with the allergenicity of such glycoproteins. Since allergic reactions have been reported after consumption of snails, the demonstration of core α1→3-fucosylation in *L. stagnalis* may be clinically relevant.

Key words: Fucose; Complex-type *N*-glycan; Allergy; Glycosyltransferase

1. Introduction

Many different fucosyltransferases (FTs) can be involved in the biosynthesis of fucosylated glycans. One group of FTs catalyze the transfer of a fucose from GDP-fucose to the non-reducing terminus of a glycan, i.e. in α1→2 linkage to the terminal Gal, or in α1→3 or α1→4 linkage to the subterminal GlcNAc of *N*-acetyllactosamine units, thus forming blood group-related carbohydrate structures. Another group of FTs act on the asparagine-linked GlcNAc of *N*-glycans, in an α1→3 or α1→6 linkage.

The knowledge about specific invertebrate FTs, which are often involved in the biosynthesis of potentially immunogenic glycans, is limited in contrast to the knowledge about mammalian FTs. *N*-Glycans carrying a R-GlcNAcβ1→4-(Fucα1→3)GlcNAcβ1-Asn (core α1→3-Fuc) structure are typically found on plant, insect and helminth, but not mammalian glycoproteins [1–4]. Several studies show that the conserved *N*-linked core α1→3-Fuc antigen is an IgE binding epitope that may be responsible for some of the allergenic properties of glycoproteins from plants and insects [5–7]. Carbohydrate-based cross-reactivity has been observed for a long time in immunoassays of glycoproteins of plants, insects and mollusks [8,9]. The highly antigenic core α1→3-Fuc- and/or Xylβ1→2Manβ1→4GlcNAcβ1-R (β1→2-Xyl)-containing *N*-glycans have been shown to contribute to such cross-reactions [10]. Whereas the core α1→3-Fuc epitope appears a conserved epitope between plants and insects, the cross-reactivity observed between plants and mollusks was suggested to be due to the presence of *N*-linked β1→2-Xyl that was found to occur in both these organisms [8,11]. We report here the identification of a GDP-fucose:GlcNAc (Asn-linked GlcNAc) α1→3-fucosyltransferase (Fuc-T C3) in both the albumin and prostate glands of the snail *Lymnaea stagnalis*. Acceptor specificity studies and analysis of products obtained with this enzyme indicate its potential function in the biosynthesis of core α1→3-fucosylated *N*-glycans.

2. Materials and methods

2.1. Materials

Human IgG was obtained from the CLB (Amsterdam, The Netherlands). Compound **4** (see Table 1) was derived by β-galactosidase treatment of the corresponding digalactosylated oligosaccharide which was donated by Dr. G. Strecker (Université de Lille, Villeneuve d'Ascq, France). Compound **5** was isolated from the urine of swainsonine-intoxicated sheep (supplied by Dr. C.D. Warren, Massachusetts General Hospital, Boston, MA, USA [12]) by filtration on Bio Gel P-4 and HPLC on Lichrosorb-NH2 using methodologies described previously [13]. GDP-[^{14}C]Fuc (New England Nuclear, Boston, MA, USA) was diluted with unlabeled GDP-Fuc to give the desired specific activity.

2.2. Preparation of glycopeptide acceptor substrates

Asialo/agalacto-glycopeptides from human fibrinogen (as/ag-GP-F2, compound **1**) were prepared from asialo-fibrinogen by pronase digestion as described previously [14] followed by enzymatic degalactosylation. Non-sialylated core α1→6-fucosylated glycopeptides from human IgG (ag-GP-IgGF$_6$) were prepared essentially as described in [15]. The glycopeptides were degalactosylated by treatment with jack bean β-galactosidase (1 unit/10 μmol glycopeptide). Glycopeptides containing a bisecting GlcNAc (compound **3**) were separated from glycopeptides without a bisecting GlcNAc (compound **2**) as described in [16].

2.3. Preparation of the enzyme and glycosyltransferase assays

Extracts of the albumin and prostate glands (10% w/v) of *L. stagnalis* were prepared by homogenization of the glands with a Polytron in 10 mM sodium acetate (pH 7), 10 mM $MnCl_2$, 0.5% Triton X-100, and incubation at 4°C for 2 h, under stirring. After centrifugation for 60 min at 20 000×*g* the supernatant (extract) was collected. Standard fucosyltransferase assays were performed in a 25 μl reaction mixture containing 2.5 μmol sodium cacodylate (pH 7.0), 0.25 μmol $MnCl_2$,

*Corresponding author. Fax: (31) (20) 4448144.
E-mail: im.van_die.medchem@med.vu.nl

PII: S0014-5793(99)01489-1

0.1 μmol ATP, 2.5 nmol GDP-[^{14}C]Fuc (2 Ci/mol), 0.1% (v/v) Triton X-100 and 5–10 μl of extract. The acceptor substrate concentration in Table 1 (compounds **1–5**) was 1 mM. Control assays lacking the acceptor substrate were carried out to correct for incorporation into endogenous acceptors. After incubation for 60–120 min at 37°C the reaction was stopped, and the product was separated from unincorporated label by chromatography on a 1 ml column of Dowex 1-X8 (Cl^- form) according to [14].

2.4. Product characterization

As/ag-GP-F2 (400 nmol) was incubated with 400 nmol GDP-[^{14}C]Fuc (1 Ci/mol), and 200 μl albumin extract under otherwise standard incubation conditions and incubated for 4 h at 37°C. The product was separated from unincorporated label by ion exchange on a 5 ml column Dowex 1-X8 (Cl^- form), isolated on a column (1.6×200 cm) of Bio Gel P4 (200–400 mesh) run in 50 mM ammonium acetate pH 5.2, and desalted on a column (0.7×45 cm) of Bio Gel P2 (200–400 mesh) run in water. The sample was treated with D_2O (99.75 atom%, Merck) three times with intermediate lyophilization. Finally, the product was redissolved in 400 μl of D_2O (99.5 atom%, Aldrich). 400 MHz ^{1}H-NMR spectroscopy was performed as described previously [17].

3. Results

3.1. Fucosyltransferase activity and kinetic parameters

In the albumin and prostate glands of *L. stagnalis* a FucT activity was detected, using GDP-fucose as sugar donor, as/ag-fibrinogen glycopeptide as acceptor substrate and assay conditions essentially as described by [18] for FucT assays of insect cells. The fucosyltransferase activity was found both in the albumin gland and in the prostate gland of the snail. To further characterize this enzyme activity, FucT assays were performed using a number of different glycopeptides and oligosaccharides as acceptor substrates (Table 1). The results show that as/ag-glycopeptides from human fibrinogen (as/ag-GP-F2, compound **1**) and core α1→6-fucosylated IgG (ag-GP-IgGF$_6$, compound **2**) can both serve as acceptors for the fucosyltransferase. Bisected ag-GP-IgGF$_6$ (compound **3**), however, appeared a very poor acceptor, and compounds lacking the peptide-linked GlcNAc or outer arm GlcNAc (compounds **4** and **5**) do not serve as acceptors.

In Table 2 the kinetic parameters of the fucosyltransferase from the albumin gland are shown. The K_m and V values of ag-GP-IgGF$_6$ (compound **2**) and GDP-fucose were estimated.

3.2. Product characterization

Incubation of albumin gland extract with GDP-[^{14}C]Fuc and glycopeptide ag-GP-F2 resulted in a radiolabeled product in 50% yield. The glycopeptide product formed was analyzed by 400 MHz ^{1}H-NMR spectroscopy (Fig. 1) and the chemical shift values and coupling constants of the structural reporter groups were compared with those for the glycopeptide substrate (Table 3). The introduction of a Fuc residue resulted in

Table 1
Acceptor specificity of *L. stagnalis* fucosyltransferase

	Acceptor substrate	Enzyme activity	
	(1 mM)	*Albumen gland* (μU/mg protein)	*Prostate gland* (μU/mg protein)
1	GlcNAcβ1-2Manα1\6 Manβ1-4GlcNAcβ1-4GlcNAc-GP (F2) GlcNAcβ1-2Manα1/3	110	240
2	GlcNAcβ1-2Manα1 Fucα1\6 \6 Manβ1-4GlcNAcβ1-4GlcNAc-GP (IgG) GlcNAcβ1-2Manα1/3	100	230
3	GlcNAcβ1-2Manα1 Fucα1\6 \6 GlcNAcβ1-4 Manβ1-4GlcNAcβ1-4GlcNAc-GP (IgG) GlcNAcβ1-2Manα1/3	<10	ND
4.	GlcNAcβ1-2Manα1\6 Manβ1-4GlcNAc GlcNAcβ1-2Manα1/3	-	ND*
5.	Manα1\6 Manβ1-4GlcNAcβ1-4GlcNAc Manα1/3	-	ND

*ND: not determined.

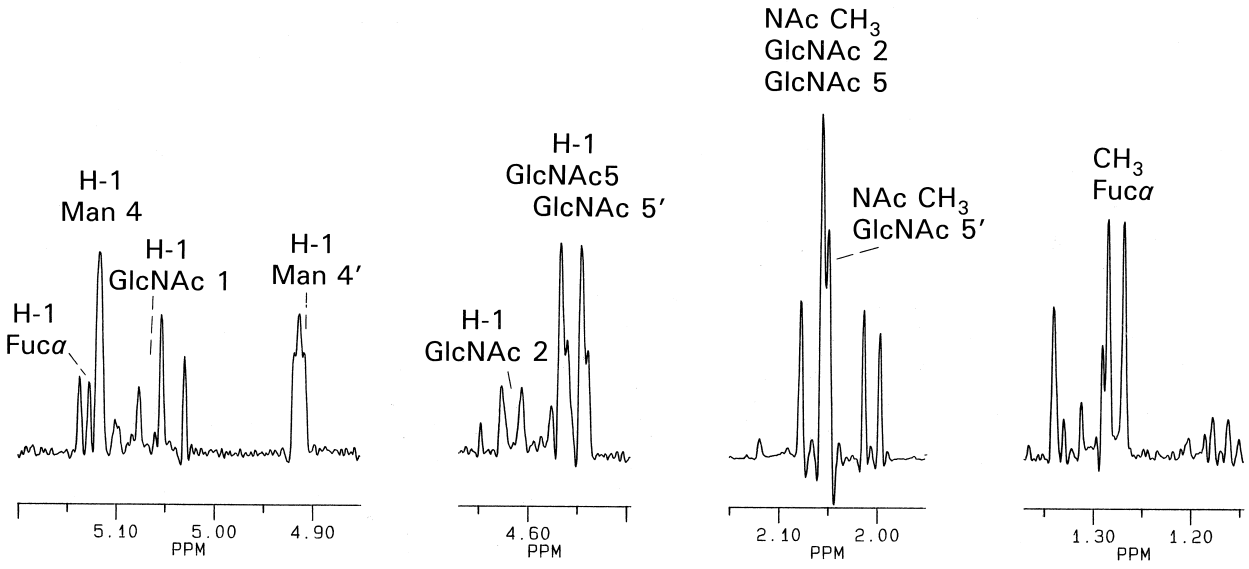

Fig. 1. Partial 400 MHz ^{1}H-NMR spectra showing the structural reporter group regions of agalacto diantennary glycopeptide GP-F2 fucosylated by Fuc-T C3 of *L. stagnalis* albumin gland. The assignments of resonances to specific residues in the product (+F) and unconverted glycopeptide substrate (−F) are indicated. The numbers represent the individual constituting monosaccharides as in [17,22].

the appearance in the spectrum of doublets for H-1 at $\delta = 5.132$ ppm (3.9 Hz) and CH_3 protons at $\delta = 1.276$ ppm (6.5 Hz), which are unique for a Fuc in α-anomeric configuration at C-3 of GlcNAc 1 of the core and quite different from the chemical shift values for a core α1→6-linked Fuc [18]. Furthermore, upfield shifts in the resonances of the H-1 of Man 4′ and GlcNAc 5′, the NAc-CH_3 of GlcNAc 1 and GlcNAc 2, and a downfield shift in the resonance of H-1 of GlcNAc 1 were observed, which are characteristic for the introduction of a core α1→3-Fuc [18]. Hence it can be concluded that the structure of the product is: GlcNAcβ1→2Manα1→3[GlcNAcβ1→2Manα1→6]Manβ1→4GlcNAcβ1→4[Fucα1→3]GlcNAcβ1-peptide.

4. Discussion

In this study we have identified and characterized a core α1→3-fucosyltransferase (Fuc-T C3), which catalyzes the transfer of a fucose residue from GDP-fucose to the Asn-linked GlcNAc of agalacto-glycopeptide acceptors. Characterization by ^{1}H-NMR of the product obtained after incubation in vitro of as/ag-glycopeptides of human fibrinogen with the *L. stagnalis* albumin gland extract showed that core α1→3-fucosylation was the only modification obtained. Since as/ag-glycopeptides of human fibrinogen also serve as acceptor for core α1→6-fucosyltransferase (Fuc-T C6) [19,20] these results suggest that the albumin gland of *L. stagnalis* does not contain detectable amounts of Fuc-T C6. Whereas the presence of core α1→3-fucosylated structures is a novel observation for mollusks, the lack of core α1→6-fucosylation is in agreement with the structural characterization of *N*-glycans from *L. stagnalis* hemocyanin, in which no core fucosylation has been found [11,21]. The presence of core α1→6-fucosylated structures has been demonstrated, however, in *Helix pomatia* [22] and difucosylation of the Asn-bound GlcNAc in *N*-glycans was shown in several invertebrates, e.g. from insects and helminths [2–4,23].

The *L. stagnalis* Fuc-T C3 resembles the Fuc-T C3 characterized from honeybee venom gland and mung bean [18,24]. All three Fuc-T C3 activities require the prior action of GlcNAc-T I and are active on both non-fucosylated and core α1→6-fucosylated glycopeptide acceptors. We show here that addition of a bisecting GlcNAc eliminates the potential of the *L. stagnalis* Fuc-T C3 to act subsequently. This feature has also been demonstrated for α-mannosidase II, GlcNAc-T II, GlcNAc-T IV, GlcNAc-T V and Fuc-T C6 [25].

Core α1→3-fucosylated *N*-glycans appear to occur widely on many (allergenic) plant proteins [10,26–29] and on glycoproteins from the parasitic helminths *Schistosoma mansoni*, *S. japonicum* and *Haemonchus contortus* [3,4]. It has been shown that IgE from sera of allergic individuals recognize core α1→3-fucosylated *N*-glycans [5–7]. Interestingly, several T cell clones have been identified from bee venom-sensitized subjects which proliferate in response to honeybee phospholipase A2 carrying core α1→3-fucosylated *N*-glycans, but not to its non-glycosylated variants, providing evidence for the involvement of a *N*-glycan in T cell recognition [30]. Recently, we have shown that parasite-specific IgE from sera of *H. contortus*-infected sheep bind to core α1→3-fucosylated *N*-glycans (I. Van Die, V. Gomord, F.N.J. Kooyman, T.K. Van den Berg, R.D. Cummings and L. Vervelde, submitted). Thus, data that suggest the significance of this glycan epitope

Table 2
Kinetic parameters of the *L. stagnalis* Fuc-T C3 from the albumin gland for acceptor and donor substrates

Acceptor/donor	K_m (mM)	V (μU/mg)	Kinetic efficiency (V/K_m)
Ag-GP-IgGF_6	0.49	180	368
GDP-fucose	0.065	100	1520

Table 3
400 MHz ^{1}H-NMR spectroscopic data of as/ag-GP-F2 and the fucosylated product formed from it by the action of Fuc-T C3 present in *L. stagnalis* albumin gland extract

Reporter group	Residue	Chemical shift and coupling constant			
		as/ag-GP-F2		core α1→3-fucosylated product	
		(ppm)	(Hz)	(ppm)	(Hz)
H-1	GlcNAc 1	5.044	9.7	5.065	8.8
	GlcNAc 2	4.615	7.9	4.616	8.6
	Man 4	5.115	1.4	5.118	1.0
	Man 4′	4.917	1.4	4.911	2.4
	GlcNAc 5	4.554	8.4	4.556	8.5
	GlcNAc 5′	4.554	8.4	4.549	8.2
	core Fuc	–		5.131	3.9
H-2	Man 3	4.247	2.5	4.245	2.4
	Man 4	4.187	3.4	4.188	3.4
	Man 4′	4.108	3.1	4.109	3.4
NAc-CH_3	GlcNAc 1	2.012		1.996	
	GlcNAc 2	2.076		2.055	
	GlcNAc 5	2.052		2.055	
	GlcNAc 5′	2.050		2.049	
CH_3	core Fuc	–		1.276	6.5

The numbering system of the monosaccharide residues is the same as in [17,22].

as a 'pan-allergen' are accumulating. Since allergic reactions have been reported after consumption of snails [31], the demonstration of core α1→3-fucosylation in *L. stagnalis* may be clinically relevant in countries where eating snails is common. The *L. stagnalis* core α1→3-fucosyltransferase will be a useful tool for the enzymatic synthesis of defined core α1→3-fucosylated glycans that will be applied to further elucidate the role of such glycans in immunogenicity in parasitic infections and in plant-, insect- or mollusk-induced allergies.

Acknowledgements: We gratefully acknowledge Carolien Koeleman for recording the NMR spectra, Drs. Thomas Norberg and Ola Blixt (University of Uppsala, Sweden) for the gift of GDP-Fuc, and Drs. G. Strecker (Université de Lille, Villeneuve d'Ascq, France) and C.D. Warren (Massachusetts General Hospital, Boston, MA, USA) for the gift of oligosaccharides.

References

[1] Lerouge, P., Cabanesmacheteau, M., Rayon, C., Fischettelaine, A.C., Gomord, V. and Faye, L. (1998) Plant Mol. Biol. 38, 31–48.
[2] Staudacher, E., Altmann, F., März, L., Hård, K., Kamerling, J.P. and Vliegenthart, J.F.G. (1992) Glycoconjug. J. 9, 82–85.
[3] Haslam, S.M., Coles, G.C., Munn, E.A., Smith, T.S., Smith, H.F., Morris, H.R. and Dell, A. (1996) J. Biol. Chem. 271, 30561–30570.
[4] Khoo, K.-H., Chatterjee, D., Caulfield, J.P., Morris, H.R. and Dell, A. (1997) Glycobiology 7, 663–677.
[5] Tretter, V., Altmann, F., Kubelka, V., März, L. and Becker, W.M. (1993) Int. Arch. Allergy Immunol. 102, 259–266.
[6] Fotisch, K., Fah, J., Wuthrich, B., Altmann, F., Haustein, D. and Vieths, S. (1998) Allergy 53, 1043–1051.
[7] Garcia-Casado, G., Sanchezmonge, R., Chrispeels, M.J., Armentia, A., Salcedo, G. and Gomez, L. (1996) Glycobiology 6, 471–477.
[8] Faye, L. and Chrispeels, M.J. (1988) Glycoconjug. J. 5, 245–256.
[9] Aalberse, R.C. (1997) Allergy Immunol. 15, 375–387.
[10] Wilson, I.B.H., Harthill, J.E., Mullin, N.P., Ashford, D.A. and Altmann, F. (1998) Glycobiology 8, 651–661.
[11] Van Kuik, A., Sijbesma, R.P., Kamerling, J.P., Vliegenthart, J.F.G. and Wood, E.J. (1987) Eur. J. Biochem. 169, 399–411.
[12] Sadeh, S., Warren, C.D., Daniel, P.F., Bugge, B., James, L.F. and Jeanloz, R.W. (1983) FEBS Lett. 163, 104–109.
[13] Blanken, W.M., Hooghwinkel, G.J.M. and Van den Eijnden, D.H. (1982) Eur. J. Biochem. 127, 547–552.
[14] Nemansky, M. and Van den Eijnden, D.H. (1993) Glycoconjug. J. 10, 99–108.
[15] Narasimhan, S., Wilson, J.R., Martin, E. and Schachter, H. (1979) Can. J. Biochem. 57, 83–96.
[16] Narasimhan, S., Freed, J. and Schachter, H. (1986) Carbohydr. Res. 149, 65–83.
[17] Neeleman, A.P., Van der Knaap, W.P.W. and Van den Eijnden, D.H. (1994) Glycobiology 4, 641–651.
[18] Staudacher, E., Altmann, F., Glössl, J., Marz, L., Schachter, H., Kamerling, J.P., Hard, K. and Vliegenthart, J.F.G. (1991) Eur. J. Biochem. 199, 745–751.
[19] Kornfeld, R. and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664.
[20] Staudacher, E. and März, L. (1998) Glycoconjug. J. 15, 355–360.
[21] Van Kuik, J.A., Sijbesma, R.P., Kamerling, J.P., Vliegenthart, J.F.G. and Wood, E.J. (1986) Eur. J. Biochem. 160, 621–625.
[22] Lommerse, J.P., Thomas-Oates, J.E., Gielens, C., Preaux, G., Kamerling, J.P. and Vliegenthart, J.F. (1997) Eur. J. Biochem. 249, 195–222.
[23] Hassani, O., Loew, D., Van Dorsselaer, A., Papandreou, M.J., Sorokine, O., Rochat, H., Sampieri, F. and Mansuelle, P. (1999) FEBS Lett. 443, 175–180.
[24] Staudacher, E., Dalik, T., Wawra, P., Altmann, F. and Marz, L. (1995) Glycoconjug. J. 12, 780–786.
[25] Schachter, H. (1986) Biochem. Cell Biol. 64, 163–181.
[26] Wilson, I.B.H. and Altmann, F. (1998) Glycoconjug. J. 15, 1055–1070.
[27] Ohsuga, H., Su, S.N., Takahashi, N., Yang, S.Y., Nakagawa, H., Shimada, I., Arata, Y. and Lee, Y.C. (1996) J. Biol. Chem. 271, 26653–26658.
[28] Kubelka, V., Altmann, F., Staudacher, E., Tretter, V., März, L., Hård, K., Kamerling, J.P. and Vliegenthart, J.F.G. (1993) Eur. J. Biochem. 213, 1193–1204.
[29] Kubelka, V., Altmann, F. and März, L. (1995) Glycoconjug. J. 12, 77–83.
[30] Dudler, T., Altmann, F., Carballido, J.M. and Blaser, K. (1995) Eur. J. Immunol. 25, 538–542.
[31] De Maat-Bleeker, F., Akkerdaas, J.H., Van Ree, R. and Aalberse, R.C. (1995) Allergy 50, 438–440.