Glycoconjugate Journal (1995) **12**: 780–786

Functional purification and characterization of a GDP-fucose: β-*N*-acetylglucosamine (Fuc to Asn linked GlcNAc) α1,3-fucosyltransferase from mung beans

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Received 3 April 1995, revised 22 April 1995

An α1,3-fucosyltransferase was purified 3000-fold from mung bean seedlings by chromatography on DE 52 cellulose and Affigel Blue, by chromatofocusing, gelfiltration and affinity chromatography resulting in an apparently homogenous protein of about 65 kDa on SDS-PAGE. The enzyme transferred fucose from GDP-fucose to the Asn-linked *N*-acetylglucosaminyl residue of an N-glycan, forming an α1,3-linkage. The enzyme acted upon N-glycopeptides and related oligosaccharides with the glycan structure $GlcNAc_2Man_3GlcNAc_2$. Fucose in α1,6-linkage to the asparagine-linked GlcNAc had no effect on the activity. No transfer to N-glycans was observed when the terminal GlcNAc residues were either absent or substituted with galactose. *N*-acetyllactosamine, lacto-*N*-biose and *N*-acetylchito-oligosaccharides did not function as acceptors for the α1,3-fucosyltransferase.

The transferase exhibited maximal activity at pH 7.0 and a strict requirement for Mn^{2+} or Zn^{2+} ions. The enzyme's activity was moderately increased in the presence of Triton X-100. It was not affected by *N*-ethylmaleimide.

Keywords: plant glycobiology, fucosyltransferase, α1,3-linked fucose

Abbreviations: α1,3-Fuc-T, GDP-fucose:β-*N*-acetylglucosamine(Fuc to Asn-linked GlcNAc)α1,3-fucosyltransferase; α1,6-Fuc-T, GDP-fucose:β-*N*-acetylglucosamine(Fuc to Asn-linked GlcNAc)α1,6-fucosyltransferase; PA-, pyridylamino; GnGn, GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc; $GnGnF^3$, GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc; $GnGnF^6$, GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc; $GnGnF^3F^6$, GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-3)[Fucα1-6]GlcNAc; MM, Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc; MMF^3, Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc; MMF^3F^6, Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-3)[Fucα1-6]GlcNAc.

Introduction

Carbohydrates of defined structure are a prerequisite for the investigation of glycoprotein glycan biosynthesis, for immunological studies or for the development of new analytical and preparative tools such as e.g. affinity matrices. In principle, several options exist to provide the desired structures in reasonable amounts and purity. However, in the case of the rather complex N-glycans, organic synthesis is extremely intricate and requries a considerable number of consecutive steps. Preparation from natural sources may fail to provide homogenous material in sufficient yield. In recent years, the use of glycosyltransferases has developed as a promising technology. It combines the advantages of technical simplicity and unparalleled specificity with the potential of converting easily accessible acceptor substrates into the desired products. As an example, the α1,2-, α1,3- or α1,3/4-fucosyltransferases (Fuc-Ts) involved in the synthesis of epitopes related to human blood group antigens have been effectively utilized to modify oligosaccharides for

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futher investigations [e.g 1–3]. Some of these glycosyltransferases have been cloned allowing large-scale production of recombinant enzymes [4–6].

Another group of Fuc-Ts transfers fucose to the asparagine-linked GlcNAc of N-glycans. This inner GlcNAc is α1,6-fucosylated in higher animals. N-glycans with an α1,3-fucosylated inner GlcNAc are typically found in plant glycoproteins but also in N-glycoproteins from insects which additionally may contain α1,6/α1,3-difucosylated structures [7–9]. The Fucα1-3GlcNAc moiety constitutes an essential part of an immunoreactive determinant [10–12]. Its reactivity with IgE from bee venom-allergic patients [10] and its presumably widespread distribution in plant and insect glycoproteins indicate the considerable significance of this structural feature. For further studies, mono- and difucosylated N-glycans or glycopeptides of high purity will be needed. Since their isolation from natural material such as bee venom is not feasible, it appears attractive to utilize an α1,3-Fuc-T to modify more common biantennary oligosaccharides. Enzymes with this specificity from honeybee venom glands, insect cells, plant cotyledons and plant cells have been discovered and partially characterized [13–16], but no purification procedure has yet been published.

Here we describe the purification and the properties of a Fuc-T from mung bean seedlings which acts upon N-linked oligosaccharides to produce N-glycans with an α1,3-fucosyl residue linked to the Asn-bound GlcNAc. The preparation of mono- as well as difucosylated oligosaccharides is demonstrated.

Materials and methods

Materials

Mung beans were purchased at a local food store. α-L-Fucosidase and N-glycosidase A were obtained from Boehringer Mannheim. GDP-L-[U-^{14}C]fucose (specific activity 270 mCi mmol^{-1}) was from Amersham. Jack bean β-*N*-acetylhexosaminidase, GDP-L-fucose, di-*N*-acetylchitobiose, tri-*N*-acetylchitotriose, lacto-*N*-biose and lacto-*N*-tetraose were purchased from Sigma.

The glycopeptides $Gal_2GlcNAc_2Man_3GlcNAc_2$, $GlcNAc_2Man_3GlcNAc_2$ (GnGn; see abbreviations) and $Man_3GlcNAc_2$ (MM) were prepared from bovine fibrin, whereas the corresponding α1,6-fucosylated N-glycopeptide ($GnGnF^6$) was isolated from human IgG as described [14]. Related oligosaccharides were obtained from these glycopeptides by digestion with N-glycosidase A. Reaction with 2-aminopyridine produced the pyridylaminated (PA-) oligosaccharides [8, 14]. Pyridylaminated reference oligosaccharides with α1,3- and α1,6-linked fucosyl residues, namely $GnGnF^3$-, $GnGnF^3F^6$-, MMF^3- and MMF^3F^6-PA were available from previous studies [8, 14].

Germination and extraction

Mung beans (100 g) were soaked in tap water overnight at 37 °C and then germinated in a ceramic sprouter in a dark and moist atmosphere at 37 °C for 3 days. The coats and ungerminated beans (less than 0.5%) were manually removed from the seedlings. After addition of 800 ml of buffer A (50 mM Tris/HCl pH 7.3, 250 mM sucrose, 0.5 mM 1,4-dithiothreitol) the seedlings were homogenized on ice with an IKA Ultra Turrax T25 at 15 000 rpm for 2 × 20 s. Triton X-100 was added to the resulting suspension to a final concentration of 1%. After stirring in the dark for 24 h at 4 °C, the suspension was filtered through coarse tissue. Insoluble material was removed by centrifugation at 27 500 × **g** for 30 min using a Sorvall GSA rotor. This and all subsequent steps were carried out at 4 °C. During the purification procedure, Fuc-T activity, protein concentration and several exoglycosidase activities of single and pooled fractions were analysed.

Ion exchange chromatography

The supernatant of the centrifugation was applied to a column containing 100 ml of DE 52 cellulose (Whatman) equilibrated in starting buffer (buffer A containing 1% Triton X-100) followed by one column volume of starting buffer. Fractions of 100 ml were collected and those containing Fuc-T activity were pooled and dialysed against buffer B (25 mM Tris/HCl, pH 7.5, 5% glycerol, 0.1% Triton X-100, 0.02% NaN_3). The binding fraction which did not contain transferase activity was eluted with 1.0 M NaCl.

Affigel Blue chromatography

The dialysate was applied onto a column of 100 ml Affigel Blue (100-200 mesh, Bio-Rad) equilibrated in buffer B. The column was eluted with 100 ml starting buffer followed by 150 ml buffer B containing 0.5 M NaCl and 200 ml buffer B containing 1.0 M NaCl. Fractions containing Fuc-T were pooled and dialysed against buffer C (25 mM ethanolamine/acetic acid, pH 9.4).

Chromatofocusing

A chromatofocusing step from pH 9.0–6.0 was performed using PBE 94 and polybuffer 96 (Pharmacia) according to the suppliers instructions. Briefly, the sample was applied to a column (1 × 20 cm) of PBE 94-gel equilibrated in buffer C and eluted with 200 ml of Polybuffer 96 (diluted 1:10 and adjusted to pH 6.0 with acetic acid). Fractions of 2.2 ml were collected and analysed for protein (280 nm), Fuc-T, α-mannosidase, β-galactosidase and β-*N*-acetylglucosaminidase. Fractions containing the transferase were pooled and ultrafiltrated to a final volume of about 2 ml. In this sample, protein was determined with the Coomassie plus protein assay reagent (Pierce) instead of the BCA method.

Gelfiltration

For size fractionation and in order to remove the polybuffer completely, the sample was subjected to gelfiltration on a Sephacryl S200 column (1.5 × 120 cm) using buffer D (buffer B without glycerol). Fractions of 2.4 ml were collected. Those containing Fuc-T activity were pooled and brought to a final concentration of 30 μM GMP. The S200 column was calibrated with bovine serum albumin (66 kDa), ovalbumin (45 kDa), trypsin inhibitor (20.1 kDa), and lysozyme (14.3 kDa).

Affinity chromatography on a GnGn-column

GnGn-glycopeptide was coupled to CNBr-Sepharose (Pharmacia) as recommended by the supplier. The column was equilibrated with 10 volumes of buffer D containing 30 μM GMP. Sample loading and elution was carried out as described by Voynow *et al.* [17]. Fuc-T containing fractions were pooled, ultrafiltrated to a final volume of 3 ml and desalted on the S200 column under the conditions described above. The pooled fractions were ultrafiltrated to 3 ml and bovine serum albumin and glycerol were added to final concentrations of 0.1 mg ml^{-1} and 10% (v/v), respectively.

Assay procedures

For the determination of Fuc-T activity, the standard incubation mixture contained, in a total volume of 20 μl, 0.5 mM acceptor (GnGn- or GnGnF^6-peptide), 0.25 mM GDP-[^{14}C]-fucose (5000 cpm nmol^{-1}), 0.1 M 2-(*N*-morpholino) ethanesulfonic acid/HCl at pH 7.0, 10 mM $MnCl_2$, 0.1% (w/v) Triton X-100, 0.2 M GlcNAc, 5 mM AMP and 5 μl of the appropriate sample. Incubations were carried out for 4 h at 37 °C. Termination of the reaction, processing over Dowex AG 1 × 8 (Cl^--form) and liquid scintillation counting was done as previously described [13]. Product formation was calculated after subtraction of control values performed without acceptor. One unit of activity is defined as the amount of enzyme catalysing the transfer of 1.0 μmol of fucose per min. Kinetic data were acquired using GnGn- or GnGnF^6-peptide at nine different concentrations ranging from 0.05 to 1.5 mM, with GDP-[^{14}C]-fucose kept constant at a concentration of 0.25 mM. K_m-values were obtained from Lineweaver-Burk plots. Sensitivity towards the inhibitor *N*-ethylmaleimide (10 mM) was determined by preincubation of the enzyme with *N*-ethylmaleimide for 1 h at 0 °C. All these assays were carried out in duplicate with appropriate controls.

The exoglycosidases α-mannosidase, β-galactosidase, β-*N*-acetylglucosaminidase and α-fucosidase were assayed using the appropriate 4-nitrophenyl glycosides at a concentration of 5 mM in 0.1 M Na-citrate buffer at pH 4.6. One unit of enzyme activity released 1.0 μmol of p-nitrophenol per min at 37 °C.

Protein was quantitated by the Micro-BCA protein assay (Pierce) or with the Coomassie plus protein assay reagent (Pierce). Bovine serum albumin was used as the standard. During the final steps of the preparation, protein content was estimated by amino acid analysis after derivatization with o-phthalaldehyde [18].

Product identification

Fucosyltransfers on a semi-preparative scale were carried out using the purified enzyme and 0.4 μmol of either GnGn- or GnGnF^6-peptide as acceptors. Incubation mixtures contained, in a total volume of 0.6 ml, 0.1 M 2-(*N*-morpholino) ethanesulfonic acid/HCl at pH 7.0, 10 mM $MnCl_2$, 0.1% (w/v) Triton X-100, 0.1 M GlcNAc, 0.5 mM GDP-[^{14}C]-fucose (860 cpm nmol^{-1}), and 8 μU Fuc-T. After incubation for 24 h at 37 °C, excess GDP-fucose was removed by passage over Dowex AG 1 × 8 as described for the transferase assay [13]. The glycopeptides were then lyophilized, digested with N-glycosidase A, pyridylaminated and the resulting PA-oligosaccharides were analysed by HPLC on a reverse phase and on a MicroPak AX-5 column [8, 14]. Degradations of PA-oligosaccharides (200 pmol) by jack bean β-*N*-acetylhexosaminidase and bovine kidney α-L-fucosidase were done as described [14]. A very high amount of fucosidase (8 mU) was used in order to substantially hydrolyse α1,3-linked fucosyl linkages [8]. Retention times are expressed as Glc-units with reference to pyridylaminated isomalto-oligosaccharides.

Results and discussion

Initial investigations had shown that mung beans, which reportedly constitute a proper source for several N-glycan processing enzymes [19–23], also contain a Fuc-T acting upon Asn-linked oligosaccharides such as GnGn-peptide. A purification protocol was elaborated in order to isolate the enzyme in a form suitable for the examination of its specificity and properties but also for the purposes of preparative oligosaccharide modification.

Purification

Preceding experiments on the extraction of Fuc-T acting on the N-glycan substrate GnGn-peptide had revealed that excessive homogenization of the seedlings led to an increase of solubilized exoglycosidases but did not improve the yield of Fuc-T. Therefore, homogenization time was reduced to the minimum of about 20 s.

Following the removal of insoluble material by centrifugation, the extract was passed over DE-52 cellulose at pH 7.3. While most of the protein and the pigmented material bound to the ion exchanger, the Fuc-T was washed off with starting buffer. After this step, the transferase was free of α-fucosidase activity. The dialysed sample was now bound to Affigel Blue and eluted

with NaCl (Fig. 1). While this step did not result in a spectacular increase in specific activity (Table 1), it aided in concentrating the sample and rendered the Fuc-T free of pyrophosphatase, an especially awkward component of the initial extract. However, the sample still contained substantial amounts of other exoglycosidases such as α-mannosidase, β-galactosidase and β-*N*-acetylhexosaminidase, the latter being especially critical as it might inactivate the acceptor oligosaccharide (see Table 3). The Fuc-T was clearly separated from these three exoglycosidases by chromatofocusing (Fig. 2). From the pH measured at the elution position of Fuc-T, its isoelectric point was estimated to be around 8.5. α-Mannosidase, β-galactosidase and β-*N*-acetylhexosaminidase were eluted at around pH-values of 7.4, 6.9 and 6.5, respectively. Polybuffer adversely affected the measured activity of the Fuc-T; therefore, total activity was higher after removal of polybuffer by gelfiltration (Table 1).

After these four chromatographic steps, already providing a 1000-fold enrichment, the Fuc-T was sufficiently pure to be suitable for preparative applications. However, further purification could be achieved by affinity chromatography on immobilized GnGn-peptide, resulting in a 3100-fold purification of the enzyme. Under the conditions used, no exoglycosidase activity was found in the final enzyme preparation. In the presence of bovine serum albumin and glycerol, the enzyme was stable for at least 6 months at 4 °C without loss of activity.

Product characterization

The Fuc-T product was characterized by two-dimensional HPLC analysis of pyridylaminated oligosaccharides. This method has recently been proven to be particularly useful for the analysis of fucosylated N-glycans [8, 9, 14]. GnGn-peptide was incubated with the purified Fuc-T in the presence of GDP-[^{14}C]-fucose. The resulting mixture of residual acceptor substrate and product was digested with N-glycosidase A and pyridylaminated. The radioactive product eluted exactly at the position of reference oligosaccharide GnGnF^3-PA (5.9 Glc units) on the

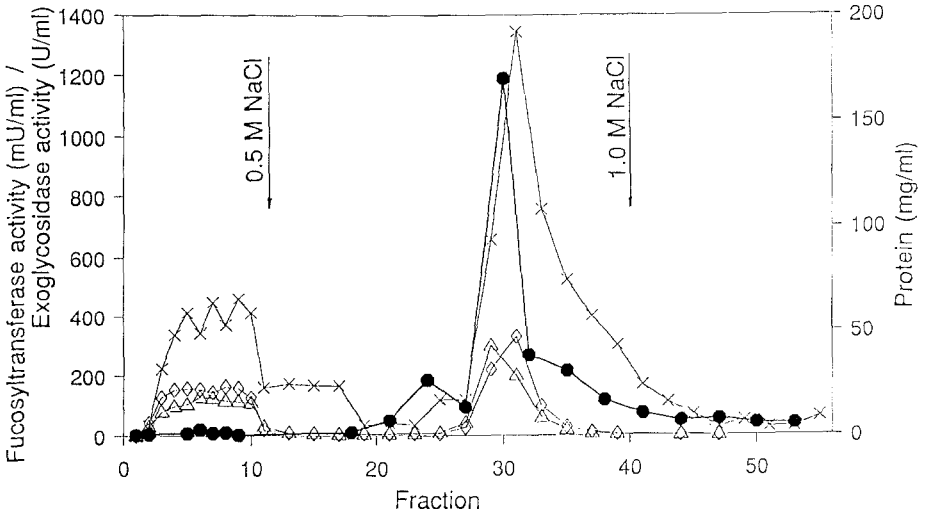

Figure 1. Purification of mung bean α1,3-fucosyltransferase activity by chromatography on Affigel Blue. The sample applied to the column had already been passed over DE-52 cellulose. During sample application fractions of 50 ml were collected (fractions 1–10). Thereafter, fraction size was changed to 5 ml. α1,3-fucosyltransferase (—●—), protein (—×—), α-mannosidase (—△—), β-*N*-acetylhexosaminidase (—◇—).

Table 1. Purification protocol for α1,3-fucosyltransferase from mung bean seedlings

Fraction	*Volume (ml)*	*Total protein (mg)*	*Total activity (mU)*	*Specific activity (mU mg^{-1})*	*Yield (%)*	*Purification (-fold)*
Crude extract	580	4640	105	0.023	100	1.0
DE-52 cellulose	430	860	38	0.044	36	1.9
Affigel Blue	55	193	16	0.083	15	3.6
Chromatofocusing	20	0.75	(2.2)[a]	(2.9)	(2.1)	(130)
Gelfiltration	33.5	0.134	3.0	22.5	2.9	980
GnGn-affinity	17	0.020	1.4	71.0	1.3	3090

[a]value too low, because Polybuffer reduces the apparent activity of fucosyltransferase.

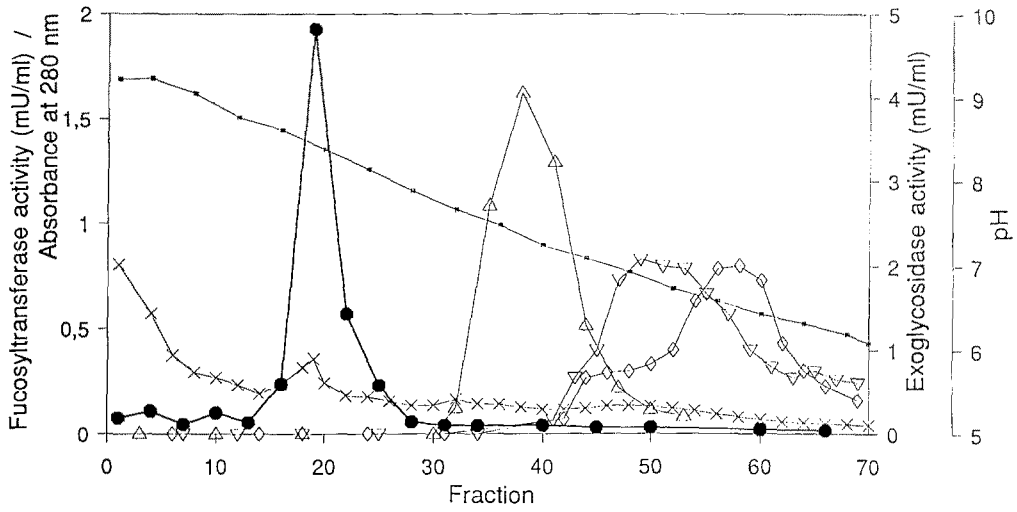

Figure 2. Purification of mung bean α1,3-fucosyltransferase activity by chromatofocusing. The sample applied to the column had already been passed over DE-52 cellusose and Affigel Blue. Proteins were eluted with a gradient from pH 9.0–6.0. Fractions of 2.2 ml were collected. α1,3-fucosyltransferase (–●–), absorbance at 280 nm (–×–), α-mannosidase (–△–), β-*N*-acetylhexosaminidase (–◇–), β-galactosidase (–▽–).

reverse phase HPLC column (Fig. 3a) and exhibited an increase of 1.2 Glc units on the sizing column (MircoPak AX-5). Under the conditions employed, about 30% of the acceptor had been converted. To verify the identity of the product, the peak was collected, and aliquots were digested with either β-*N*-acetylhexosaminidase or a very high amount of α-fucosidase. The product of the α-fucosidase digestion was free of radioactivity and eluted at the position of GnGn-PA (11.5 Glc units) (Fig. 3b). The product of the β-*N*-acetylhexosaminidase degradation still contained the radioactive label and comigrated with reference oligosaccharide MMF^3-PA (5.3 Glc units) on reverse phase (Fig. 3c) as well as on MicroPak AX-5 (data not shown). Thus, the possibility of fucosylation of a non-reducing, terminal GlcNAc residue could clearly be excluded. We therefore conclude that fucose had been transferred in α1,3-linkage to the inner, Asn-linked GlcNAc – a structural feature typical for plant protein N-glycans.

The same procedure was carried out with $GnGnF^6$ as acceptor. On reverse phase, the resulting pyridylaminated product eluted at the position of reference oligosaccharide $GnGnF^3F^6$-PA which is, however, very close to that of GnGn-PA, because the opposite effects of the two fucosyl residues almost compensate each other (Fig. 3d) [8, 9, 14]. However, the radioactive label and the increase in size corresponding to 1.1 Glc units as determined on the sizing column confirmed the incorporation of an additional fucosyl residue. Digestion with fucosidase yielded two peaks on reverse phase, one eluting at the position of $GnGnF^3$-PA, the other of GnGn-PA (Fig. 3e). The first peak is regarded as an intermediate product because the hydrolysis of an α1,3-linked fucosyl residue

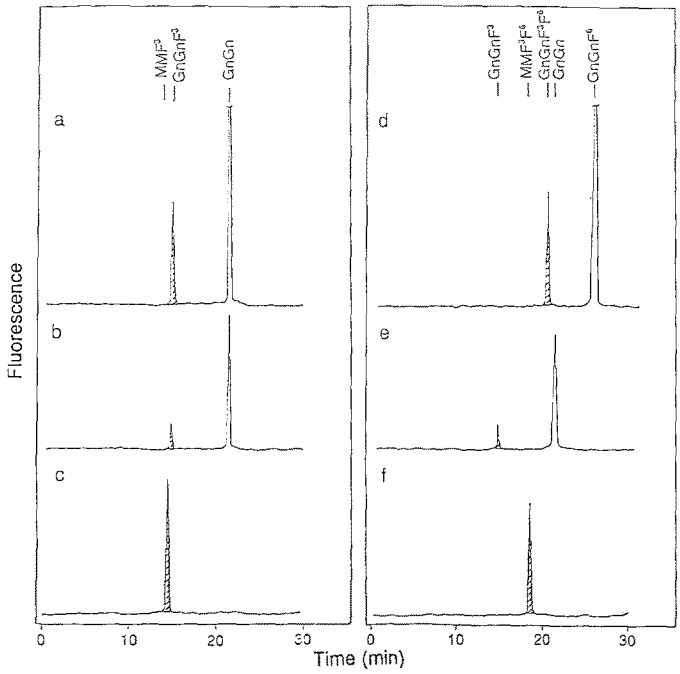

Figure 3. Analysis of transferase products by reverse phase chromatography. After incubation of glycopeptides with GDP-[^{14}C]-fucose and purified α1,3-fucosyltransferase, oligosaccharides were enzymatically released and pyridylaminated. Chromatograms on the left side were obtained with GnGn-peptide, those on the right side with $GnGnF^6$-peptide as acceptor. Shaded peaks contained radioactiviy. a and d: fucosyltransferase product and residual substrate. b and e: isolated fucosyltransferase product digested with α-fucosidase. c and f: isolated focosyltransferase product digested with β-*N*-acetylhexosaminidase. The arrows at the top of the figure indicate the elution positions of reference oligosaccharides.

proceeds extremely slow compared to the α1,6-linked residue [8]. Upon incubation with β-*N*-acetylhexosaminidase, the radioactively labelled product eluted at the position of MMF^3F^6 (8.6 Glc units) (Fig. 3f).

Obviously, the mung bean α1,3-Fuc-T was capable of transferring fucose to an already α1,6-fucosylated Asn-linked GlcNAc, thus generating the difucosylated core structure previously found in insect glycoproteins [8, 9].

Properties of the enzyme

Incorporation of fucose into GnGn-peptide was proportional to the time of incubation over a period of 4 h and increased with time for at least 24 h. The enzyme had a broad pH-optimum around pH 7.0 and did not require the addition of detergent although the activity was slightly enhanced with 0.1–0.5% Triton X-100 (an enzyme sample free of Triton X-100 was available from a separate experiment where GnGn-affinity was performed without detergent). The enzyme depended strictly on the presence of divalent cations. The best activators were Mn^{2+} or Zn^{2+} (Table 2). With Mn^{2+}, optimal activity was observed at concentrations of 10–15 mM (data not shown). *N*-ethylmaleimide caused no detectable inhibition.

The mass of the Fuc-T was inferred from gelfiltration to be 64 kDa. SDS-PAGE revealed the presence of one major polypeptide with an apparent mass of 65 kDa (data not shown).

The apparent K_m values for GnGn- and $GnGnF^6$-peptide were estimated to be 0.26 and 0.06 mM, respectively.

Table 2. Effect of divalent cations and EDTA on the activity of α1,3-fucosyltransferase from mung beans. The various additives were present at a concentration of 10 mM

Additive	*Relative activity (%)*
None	0
EDTA	0
$MnCl_2$	100
$CaCl_2$	31
$MgCl_2$	26
$CdCl_2$	0
$CoCl_2$	50
$CuCl_2$	38
$NiCl_2$	38
$ZnCl_2$	92

Acceptor specificity studies

Various glycopeptides and oligosaccharides were tested as acceptors for the pure α1,3-Fuc-T (Table 3). No

Table 3. Substrate specificity of purified α1,3-fucosyltransferase and cude extract. N-glycan structures are given in a short form where Gn denotes GlcNAc; M, Man; G, Gal; F, Fuc

Acceptor substrate		*Relative activity of*	
		Pure enzyme (%)	*Crude extract* (%)
Gn-M(α1-6)M-Gn-Gn	-peptide	100	100
Gn-M(α1-3)	-oligosaccharide	93	
	-pyridylaminated	<1	
Gn-M(α1-6)F(α1-6)M-Gn-Gn	-peptide	99	70
Gn-M(α1-3)	-oligosaccharide	95	
	-pyridylaminated	<1	
M(α1-6)M-Gn-Gn	-peptide	<0.1	
M(α1-3)			
G-Gn-M(α1-6)M-Gn-Gn	-peptide	<0.1	
G-Gn-M(α1-3)			
Galβ1-4Glc		<0.1	
Galβ1-4GlcNAc		<0.1	
Galβ1-3GlcNAc		<0.1	26
Galβ1-3GlcNAcβ1-3Galβ1-4Glc		<0.1	42
GlcNAcβ1-4GlcNAc		<0.1	
GlcNAcβ1-4GlcNAcβ1-4GlcNAc		<0.1	

transfer to *N*-acetyllactosamine or lacto-*N*-biose, the standard acceptor substrates for α1,3-Fuc-Ts from human blood or milk was observed with the purified mung bean enzyme. In the crude mung bean extract, however, at least one additional Fuc-T activity was detected (Table 3). According to its substrate specificity, this enzyme resembles the Le^a-specific Fuc-T described by Crawley *et al.* [24]. For the pure enzyme, only N-glycans with GlcNAc residues at the non-reducing terminus acted as acceptors, glycopeptides acting slightly better than reducing oligosaccharides (Table 3). In analogy to the recently described substrate specificity of kidney bean α1,3-Fuc-T [15], we assume that it is the GlcNAc residue at the α1,3-antenna which is required by the mung bean transferase. Glycopeptides without terminal GlcNAc or with Gal-substituted GlcNAc residues were inactive (Table 3). Reductively aminated (pyridylaminated) oligosaccharides likewise did not act as acceptors which is not surprising in view of the dramatic structural change exerted by this modification at the reducing terminus of the oligosaccharide.

In conclusion, an α1,3-Fuc-T acting upon the Asn-linked GlcNAc residue of N-glycans can be purified from mung bean seedlings. The enzyme acts upon acceptor glycopeptides or glycans with the structure GnGn or $GnGnF^6$ which are easily obtainable from bovine fibrin and human IgG (or equine fibrinogen), respectively. Incubation of products with β-*N*-acetylhexosaminidase yields MMF^3 and MMF^3F^6 oligosaccharides or glycopeptides which are likely to represent the immunologically significant carbohydrate structures of insect glycoproteins such as honeybee phospholipase A_2 [10].

Acknowledgements

This work was financed by the Fonds zur Förderung wissenschaftlicher Forschung Project Nr.: P10611-GEN. We thank Dr Iain B.H. Wilson for reading the manuscript.

References

1. DeVries T, van den Eijnden DH, Schultz J, O'Neill R (1993) *FEBS Lett* **330**: 243–48.
2. Natunen J, Niemelä R, Penttilä L, Seppo A, Ruohtula T, Renkonen O (1994) *Glycobiology* **4**: 577–83.
3. DeVries T, van den Eijnden DH (1994) *Biochemistry* **33**: 9937–44.
4. Schachter H (1994) In *Molecular Glycobiology* (Fukuda M, Hindsgaul O, eds) pp. 128–37. Oxford: IRL Press.
5. Natsuka S, Gersten KM, Zenita K, Kannagi R, Lowe JB (1994) *J Biol Chem* **269**: 16789–94.
6. Sasaki K, Kurata K, Funayama K, Nagata M, Watanabe E, Ohta S, Hanai N, Nishi T (1994) *J Biol Chem* **269**: 14730–37.
7. Staudacher E, Altmann F, März L, Hård K, Kamerling JP, Vliegenthart JFG (1992) *Glycoconjugate J* **9**: 82–85.
8. Kubelka V, Altmann F, Staudacher E, Tretter V, März L, Hård K, Kamerling JP, Vliegenthart JFG (1993) *Eur J Biochem* **213**: 1193–204.
9. Kubelka V, Altmann F, Kornfeld G, März L (1994) *Arch Biochem Biophys* **308**: 148–57.
10. Tretter V, Altmann F, Kubelka V, März L, Becker WM (1993) *Int Arch Allergy Immunol* **102**: 259–66.
11. Kurosaka A, Yano A, Itoh N, Kuroda Y, Nakagawa T, Kawasaki T (1991) *J Biol Chem* **266**: 4168–72.
12. Faye L, Gomord V, Fitchette-Lainé AC, Chrispeels MJ (1993) *Anal Biochem* **209**: 104–8.
13. Staudacher E, Altmann F, Glössl J, März L, Schachter H, Kamerling JP, Hård K, Vliegenthart JFG (1991) *Eur J Biochem* **199**: 745–51.
14. Staudacher E, Kubelka V, März L (1992) *Eur J Biochem* **207**: 987–93.
15. Johnson KD, Chrispeels MJ (1987) *Plant Physiol* **84**: 1301–8.
16. Tezuka K, Hayashi M, Ishihara H, Akazawa T, Takahashi N (1992) *Eur J Biochem* **203**: 401–13.
17. Voynow JA, Kaiser RS, Scanlin TF, Glick MC (1991) *J Biol Chem* **266**: 21572–77.
18. Altmann F (1992) *Anal Biochem* **204**: 215–19.
19. Szumilo T, Kaushal GP, Elbein AD (1986) *Arch Biochem Biophys* **247**: 261–71.
20. Szumilo T, Kaushal GP, Hori H, Elbein AD (1986) *Plant Physiol* **81**: 383–89.
21. Kaushal GP, Szumilo T, Pastuszak I, Elbein AD (1990) *Biochemistry* **29**: 2168–76.
22. Szumilo T, Kaushal GP, Elbein AD (1986) *Biochem Biophys Res Comm* **134**: 1395–403.
23. Szumilo T, Kaushal GP, Elbein AD (1987) *Biochemistry* **26**: 5498–505.
24. Crawley SC, Hindsgaul O, Ratcliffe RM, Lamontagne LR, Palcic M (1989) *Carbohydr Res* **193**: 249–56.