Isolation and Properties of α-D-Mannose:β-1,2-*N*-Acetylglucosaminyltransferase from Trachea Mucosa†

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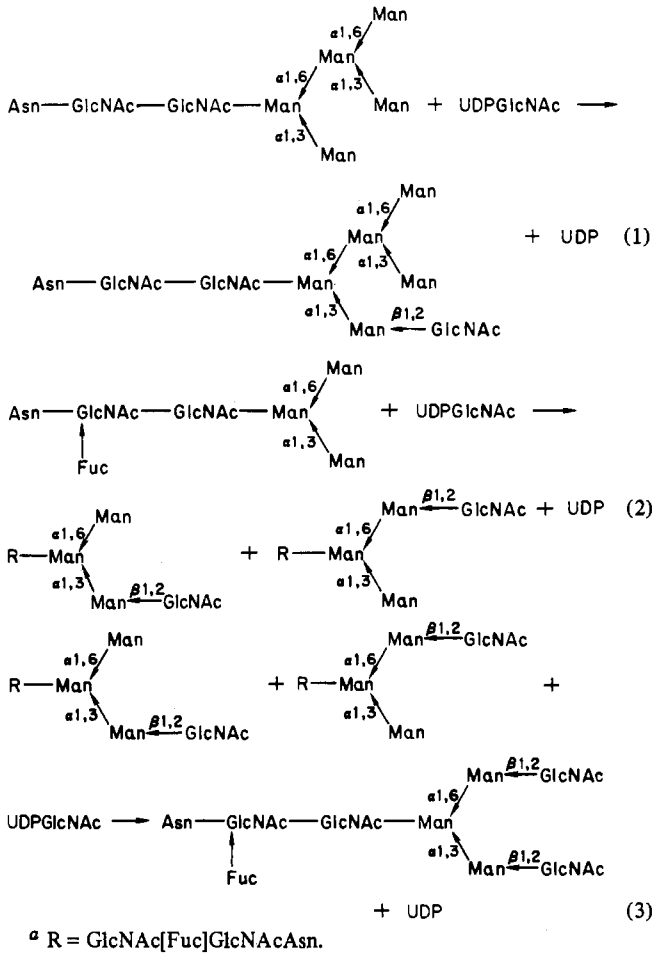
ABSTRACT: A β-1,2-*N*-acetylglucosaminyltransferase which transfers *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to the branched terminal mannosyl residues found in the complex type oligosaccharide units of N-linked glycoproteins was isolated from swine trachea mucosa. The enzyme was purified from microsomes after solubilization with 1% Triton X-100 and 1% Nonidet P-40, and it was also isolated from a soluble extract of this tissue. The enzyme was purified by chromatography on DEAE-cellulose, cellulose phosphate, and Sephacryl S300 columns and by affinity chromatography on a Sepharose 4B column containing covalently bound ovomucoid. The purified enzyme forms β-1,2 bonds between *N*-acetylglucosamine and terminal-branched mannosyl residues of glycoproteins and glycopeptides. The enzyme has an absolute specificity for terminal branched mannosyl residues, and no activity is observed with mannose, *p*-nitrophenyl α-D-mannoside, *p*-nitrophenyl β-*O*-mannoside, and glycopeptides which contain unbranched oligosaccharide chains terminating in free mannose. Evidence obtained by kinetic and structural analysis shows that the purified enzyme catalyzes the reactions shown in Scheme I.

The synthesis of secreted *N*-asparagine-linked glycoproteins occurs by a mechanism which involves the removal of external glucose and mannose units from highly mannosylated intermediates following the transfer of these oligosaccharide chains from dolichol phosphate intermediates to newly synthesized polypeptide chains (Leloir et al., 1973; Hsu et al., 1974; Waechter & Lennarz, 1976; Robbins et al., 1977). The sugars present in the outer chains of these secreted glycoproteins are then added sequentially by specific glycosyltransferases beginning with the addition of GlcNAc units to the terminal-branched mannosyl residues exposed during "processing" of the oligosaccharide chains by specific exoglycosidases (Spiro et al., 1979; Tabas & Kornfeld, 1978). The terminal stages in these "processing" reactions are catalyzed by specific α-mannosidases, and the completion of the outer chains of complex-type *N*-asparagine-linked glycoproteins is initiated by *N*-acetylglucosaminyltransferases. Mechanisms for the transfer of GlcNAc to the outer branched mannosyl residues of *N*-asparagine-linked oligosaccharide chains by sequential reactions catalyzed by two different GlcNAc-transferases have been proposed by Narasimhan et al. (1977). These and other studies with Chinese hamster ovary cells (Stanley et al., 1975; Li & Kornfeld, 1978) suggest that several GlcNAc-transferases with specificities for the α-1,3- and α-1,6-linked mannosyl residues may be present in this tissue. The present communication describes the isolation of a specific β-1,2-GlcNAc-transferase from trachea mucosa. The influence of the structure of glycopeptides prepared from porcine IgG and ovalbumin on the activity and specificity of the purified enzyme was examined. The results suggest that the purified β-1,2-*N*-acetylglucosaminyltransferase is highly specific for the branched α-1,3- and α-1,6-mannosyl residues attached to the β-linked mannose unit of *N*-asparaginyl-linked glycopeptides.

Scheme I[a]



[a] R = GlcNAc[Fuc]GlcNAcAsn.

Preliminary studies on the kinetic properties of this transferase have been presented (Reddy et al., 1979).

Experimental Procedures

Assay of N-Acetylglucosaminyltransferase Activity. The activity of the enzyme was determined by measuring the rate of transfer of GlcNAc from UDPGlcN[1-^{14}C]Ac to glycoprotein or glycopeptide acceptors. Reaction mixtures used in

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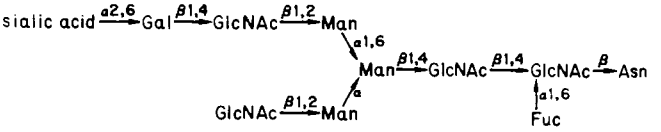
standard assays were incubated at 37 °C for 30 min and contained 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.5, 10 mM $MnCl_2$, 0.1 mM UDPGlcN[1-^{14}C]Ac (22 cpm/pmol), 2 mg of ovomucoid, and enzyme in a total volume of 0.3 mL. Triton X-100, 0.1%, was included in assays of membrane-bound enzymes, and 1 μmol of NAD^+ was added when crude enzyme preparations were assayed in order to provide an alternate substrate for pyrophosphorylases and thereby reduce the hydrolysis of UDPGlcNAc. The reaction was terminated by the addition of 3.5 mL of 2.8 N perchloric acid containing 2% phosphotungstic acid, and the precipitated glycoprotein was collected by centrifugation, washed twice, dissolved in scintillation solvent, and counted as described in our previous studies (Mendicino & Rao, 1975; Rao et al., 1976). The transfer of GlcNAc to glycopeptides and other low molecular weight acceptors was measured under the same conditions in a total volume of 0.1 mL as described previously (Rao & Mendicino, 1978). One unit of activity is defined as the amount of enzyme required to transfer 1 nmol of GlcNAc/min.

The initial rates obtained in these assays were directly proportional to the time of incubation and enzyme concentration. In the presence of 0.2 mM glycopeptide V and 0.1 mM UDPGlcNAc, 1, 2, and 3 μg of enzyme catalyzed the transfer of 0.9, 1.8, and 2.6 nmol of GlcNAc in 15 min, respectively. After 30 min of incubation, 1, 2, and 3 μg of enzyme transferred 1.7, 3.5, and 5.1 nmol of GlcNAc to the glycopeptide acceptor. In kinetic studies, the initial rates of purified GlcNAc-transferase were determined under the standard assay conditions by using five concentrations of UDPGlcNAc ranging from 5 to 25 μM and four concentrations of each glycopeptide ranging from 0.5 to 25 mM. The apparent K_m and other initial rate parameters for UDPGlcNAc and the glycopeptides were calculated from primary and secondary double-reciprocal plots of the data, and these are listed in the tables.

Preparation of Glycopeptide Acceptors for GlcNAc-transferase from Porcine IgC. The porcine immunoglobulin (Pentex) used in these studies was purified by chromatography on Sephadex G-200 and DEAE-cellulose columns, and the sample was digested with Pronase twice, as described in our previous studies (Rao & Mendicino, 1978; Rao et al., 1976). A crude glycopeptide fraction, molecular weight 2200, was isolated by chromatography on a Sephadex G-50 column (Spragg & Clamp, 1969). The desalted glycopeptide fraction was applied to a DEAE-cellulose column (5 × 75 cm), the column was washed with 1 L of 1 mM potassium phosphate, pH 6.7, and a monosialylated glycopeptide peak, 100 μmol, was eluted with 800 mL of 2 mM potassium phosphate, pH 6.7. The fractions in this peak were combined, concentrated, and desalted on a Bio-Gel P-10 column (2.5 × 30 cm). Paper electrophoresis at pH 6.5 showed the presence of a single major acidic component which stained with ninhydrin and periodate–alkaline silver nitrate sprays. The major glycopeptide in this preparation had structure I.

Another glycopeptide, 20–35% of the preparation, terminating in galactose in the incomplete chain was converted to glycopeptide I by treatment with highly purified β-galactosidase. A series of smaller glycopeptides with the structures shown below were prepared from glycopeptide I by sequential hydrolysis with specific exoglycosidases, and the products, in each case, were purified to homogeneity by repeated gel filtration on Bio-Gel P-6 (400 mesh) columns (2.2 × 200 cm) with 0.1 M pyridinium acetate, pH 5.5. The structures of the purified glycopeptides were examined by permethylation and



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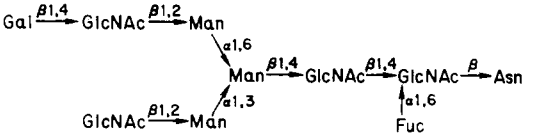

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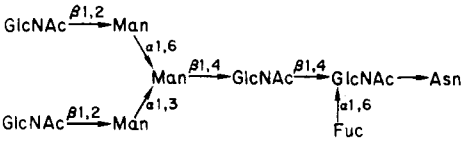

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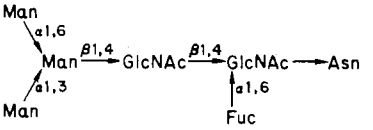

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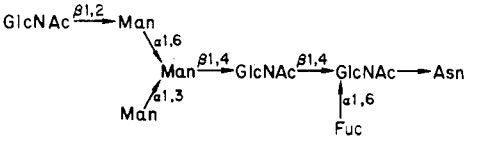

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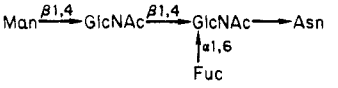

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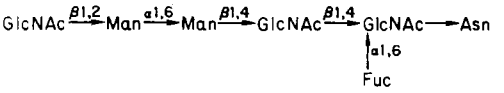

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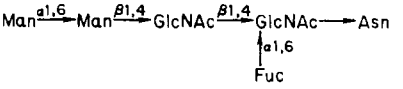

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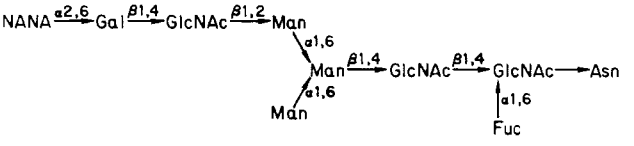


IX

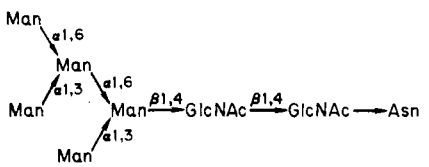
gas chromatography (Rao et al., 1976; Rao & Mendicino, 1978), and the results are summarized in Table I. Gas chromatographic analysis of the alditol acetate derivatives of the partially methylated sugars derived from glycopeptides VII and VIII showed that the penultimate and terminal mannoses were linked α-1,6 to the β-linked mannosyl residue. As seen in Table I, peaks were observed at retention times expected for 2,3,4-trimethylmannitol in the profile of this glycopeptide. Even when large samples of glycopeptides VIII and VII were analyzed, no peak corresponding to 2,4,6-trimethylmannitol was observed. The branch point in the oligosaccharide chain of porcine IgG contains two mannosyl residues which are attached through α-1,3 and α-1,6 linkages to a third mannosyl unit in the inner chain. One of these chains terminates in sialic acid, and the other less complete chain terminates in galactose or GlcNAc (Rao et al., 1976). The structure obtained for glycopeptide VIII and the methods used to prepare this glycopeptide establish that the more complete chain in these

Table I: Analysis and Kinetic Constants of Glycopeptides Prepared from Porcine IgG and Ovalbumin

component or methylated derivative	porcine IgG immunoglobulin glycopeptides									ovalbumin glycopeptides		
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
sialic acid	1.1	0	0						0.9			
galactose	1.0	0.9	0						0.9			
mannose	3.0	3.0	3.0	3.0	3.0	1.0	2.0	2.0	3.0	4.9	5.0	2.9
GlcNAc	3.9	3.7	3.8	1.9	2.8	1.9	2.9	2.0	2.8	2.0	2.9	3.0
fucose	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.8			
2,3,4-triCH$_3$-Fuc	0.8	0.8	0.7	0.8	0.7	0.8	0.8	0.7	0.8	0		
2,3,4,6-tetraCH$_3$-Gal	0.1	0.9	0						0.1	0		
2,3,4-triCH$_3$-Gal	0.9	0	0						0.8	0		
3,4,6-triCH$_3$-Man	2.0	2.0	1.9	0	0.9	0	0.9		1.0	0		
2,3,4-triCH$_3$-Man	0	0	0	0	0	0	0.8	0.7	0	0		
2,3,4,6-tetraCH$_3$-Man			0	1.9	1.0	0.9	0	0.9	0.9	2.8		
2,4-diCH$_3$-Man	0.8	0.9	1.0	0.9	1.0	0	0	0	0.8	2.1		
3,4,6-triCH$_3$-GlcNAc	0.9	1.1	1.9	0.1	0.9	0.1	1.0	0	0.1	0		
3,6-diCH$_3$-GlcNAc	2.0	1.9	0.8	0.9	0.8	1.0	0.9	0.8	1.9	1.8		
3-CH$_3$-GlcNAc	0.7	0.6	0.7	0.7	0.8	0.7	0.6	0.7	0.7	0		
activity [pmol min^{-1} (0.5 μmol of substrate)$^{-1}$]	0	0	0	30	39	0	0	0	2.1	25	0	34
apparent K_m (mM)				2.0	1.3				23	0.5		2.3

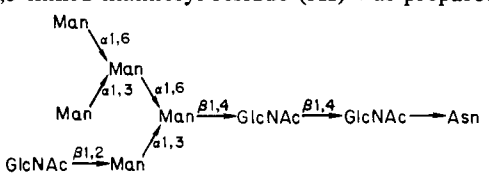
glycopeptides is attached to the α-1,6-linked mannosyl residue.

Preparation of Glycopeptide Acceptor for GlcNAc-transferase from Ovalbumin. Glycopeptides were prepared from crystalline ovalbumin (Sigma, Grade V) by Pronase digestion, and they were partially purified by chromatography on a Dowex 50W-X2 column as described by Huang et al. (1970). The crude glycopeptide mixture obtained from 30 g of ovalbumin was separated into five fractions by chromatography on a Dowex 50-X2 column (5 × 80 cm). The last glycopeptide fraction which emerged from this column contained (Man)$_5$(GlcNAc)$_2$Asn (Huang et al., 1970; Tai et al., 1975). This fraction was concentrated, and the major glycopeptide was purified to homogeneity by chromatography on Bio-Gel P-6 columns (2.2 × 200 cm) with 0.1 M pyridinium acetate, pH 5.5. The yield of (Man)$_5$(GlcNAc)$_2$Asn was 6.5 μmol. Carbohydrate analysis shown in Table I indicated that this glycopeptide (X) contained 2.45 mol of mannose/mol of glucosamine. Digestion with exoglycosidases and analysis by permethylation and gas chromatography showed that this glycopeptide has structure X.



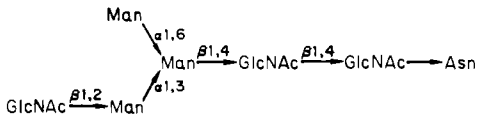
X

An intermediate with a single GlcNAc residue attached to the α-1,3-linked mannosyl residue (XI) was prepared from



XI

(Man)$_5$(GlcNAc)$_2$Asn by incubation with GlcNAc transferase. This product was treated with α-mannosidase at pH 6.5, and a glycopeptide (XII) was isolated by chromatography on



XII

Bio-Gel P-6 columns. The structure of this glycopeptide was similar to that of glycopeptide V except that it was devoid of fucose and the terminal GlcNAc unit was attached to the α-1,3-linked mannosyl residue.

Materials and Methods. Crystalline ovalbumin (grade V) and ovomucoid (type III-0) were obtained from Sigma. The activities and purity of the specific exoglycosidases used to sequentially hydrolyze the glycoproteins and glycopeptides in these studies have been reported previously (Rao et al., 1976; Rao & Mendicino, 1978). The anomeric purity and activity of these enzymes were examined with *p*-nitrophenylglycosyl derivatives of galactose, GlcNAc, and mannose. The neuraminidase from *Clostridum perfringens* (Sigma) contained protease activity; however, viral neuraminidase is free of protease activity and can be used with glycoproteins. Both preparations are free of other exoglycosidase activities. The β-galactosidase, β-*N*-acetylglucosaminidase, and α-mannosidase were completely free of contaminating exoglycosidase activities when measured at 10-fold greater enzyme concentrations under identical incubation conditions. As an added precaution, the *p*-nitrophenyl glycoside, 1 μmol, of the penultimate sugar of the glycopeptide being hydrolyzed was added to some reaction mixtures as an alternative substrate to ensure that the penultimate sugar was not released during long periods of incubation. In fact, no detectable hydrolysis of the alternative substrate was observed. The activity of some of the exoglycosidases decreased during incubation, and, in these cases, additional enzyme was added at various time intervals.

UDPGlcN[1-^{14}C]Ac (52 mCi/mmol), UDP[1-^{14}C]GlcNAc (50 mCi/mmol), and UDPGlcN[^{3}H]Ac (6.5 Ci/mmol) were prepared as described previously (Rao & Mendicino, 1978). Paper electrophoresis was carried out at pH 6.5 with pyridine–acetic acid–water (100:4:900) and at pH 8.5 with 0.1 M sodium tetraborate (Mendicino & Hanna, 1970). Glycopeptides were detected with ninhydrid and periodate–alkaline silver nitrate reagents. The anthrone and phenol–sulfuric acid methods were used to detect carbohydrate in intact glycoproteins and glycopeptides. The compositions of all of the glycopeptides used in the present studies were determined by specific colorimetric and enzymatic methods following acid hydrolysis (Rao et al., 1976). The positions of the glycosidic linkages were determined by permethylation and gas chromatographic analysis of methylated alditol acetate derivatives, as described in our previous studies (Rao et al., 1976; Garver et al., 1975).

Table II: Solubilization and Purification of β-1,2-GlcNAc-transferase from Swine Trachea Mucosa[a]

fraction	volume (mL)	total protein (mg)	total activity (nmol min^{-1})	specific activity (nmol min^{-1} mg^{-1})	yield (%)	purification (n-fold)
(1) crude extract	610	5490	278	0.056	100	
(2) microsomes	50	370	220	0.6	79	11
(3) chromatography on DEAE-cellulose	210	115	205	1.8	74	33
(4) chromatography on cellulose phosphate	10	12	185	15.4	67	275
(5) chromatography on Sephacryl S200	8	0.9	170	185	61	3300
(6) affinity chromatography	0.3	0.04	134	3370	50	60000

[a] The enzyme was assayed by the standard procedure, with native ovomucoid as the GlcNAc acceptor.

Results

Solubilization and Purification of GlcNAc-transferase from Swine Trachea Mucosa. Tracheas were obtained from a local supplier, and they were trimmed and placed on ice. Freezing tracheas for 3 months at −20 °C did not influence the recovery or specific activity of the purified enzyme. The trachea used for the isolation of this enzyme showed type A blood-group specificity in hemagglutination inhibition assays. The tracheas 1500 g, were sliced longitudinally, and the mucosal membrane was stripped off and rinsed with cold 0.05 M Tris-HCl, pH 7.5. All subsequent procedures were carried out at 4 °C. The membrane, 140 g, was cut into small pieces and homogenized in a Waring Blendor with 5 volumes of 0.05 M Tris-HCl, pH 7.5, and 0.25 M sucrose. The suspension was further dispersed in a Brinkman Polytron homogenizer, and it was then centrifuged at 27000*g* for 10 min. Microsomes were isolated from the supernatant solution by centrifugation at 100000*g* for 1.5 h. The pellets were suspended in 50 mL of 0.1 M Tris-HCl, pH 7.5, and 0.25 M sucrose and could be stored for at least 1 month at −20 °C without loss of activity. About 80% to 85% of the GlcNAc-transferase activity was consistently recovered in the microsomal fraction, and 15% to 20% of the activity remained in the 100000*g* supernatant fraction. The enzyme in the supernatant fraction was isolated by the same procedures used for the purification of the particulate enzyme described below. The initial specific activity of the enzyme in the soluble fraction was 10- to 20-fold lower than that in the microsome fraction. The soluble enzyme was concentrated by precipitation with ammonium sulfate at 0.7 saturation at 3 °C, and the precipitate was dissolved in 50 mL of 0.05 M Tris-HCl (pH 7.5)–0.25 M sucrose and dialyzed against 2 L of the same buffer.

The ability of different detergents, including TritonX-100, Nonidet P-40, sodium dodecyl sulfate, cholate, deoxycholate, Tween 80, and Tween 20, to solubilize GlcNAc-transferase from trachea microsomes was examined. The detergent was added to the microsomes at a final concentration of 1%, and the suspension was stirred at 3 °C for 15 min. Afterwards, the samples were centrifuged at 100000*g* for 90 min, and the supernatant was dialyzed against 0.05 M Tris-HCl(pH 7.5)–0.25 M sucrose and assayed for GlcNAc-transferase activity. Triton X-100 and Nonidet P-40 solubilized more than 90% of the GlcNAc-transferase activity whereas the other detergents were less effective.

The purification procedure for β-1,2-GlcNAc-transferase from swine trachea mucosa is summarized in Table II. Only data for the solubilization and purification of the enzyme present in microsomes are shown. The enzyme in the supernatant fraction was isolated by the same procedures with similar final yields and specific activity.

The isolated suspension of microsomes (fraction 2, Table II) or the concentrated soluble enzyme fraction in 50 mL of 0.05 M Tris-HCl (pH 7.5)–0.25 M sucrose–0.05 M NaCl was adjusted to 1% Triton X-100 and 1% Nonidet P-40, and it was stirred for 15 min. The solution was passed into a DEAE-cellulose column (5 × 5 cm) which was previously equilibrated against 0.02 M MES (pH 6.5)–0.25 M sucrose. The column was washed with 3 × 50 mL of 0.05 M Tris-HCl (pH 7.5)–0.25 M sucrose–0.05 M NaCl–0.5% Triton X-100. The filtrate and wash containing the activity were combined (fraction 3), 0.5 mg/mL albumin was added, and the solution at 3 °C was adjusted to 0.7 saturation with ammonium sulfate (47.2 g/100 mL). The suspension was centrifuged at 34000*g* for 10 min. The precipitate was dissolved in 20 mL of 0.05 M Tris-HCl (pH 7.5)–0.25 M sucrose–0.1% Triton X-100 and dialyzed twice against 1 L of this buffer.

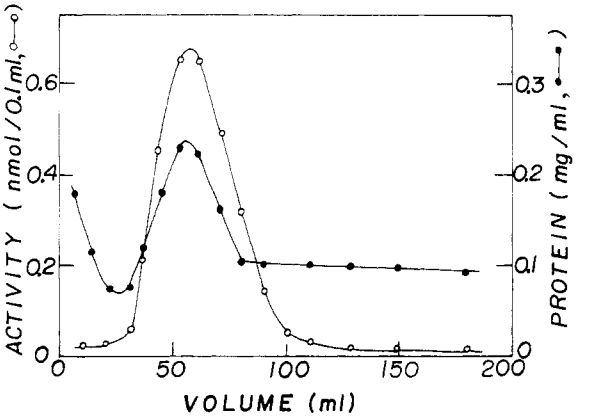


FIGURE 1: Elution profile of GlcNAc-transferase from a cellulose phosphate column. The enzyme was eluted from the column with buffer containing a linear gradient of NaCl from 0.2 to 2.0 M as described in the text at a flow rate of 35 mL/h. Fractions of 5 mL were collected, dialyzed against 0.05 M Tris-HCl (pH 7.5)–0.25 M sucrose, and assayed for transferase activity with ovomucoid as the GlcNAc acceptor.

The solution was then passed into a cellulose–phosphate column (2.5 × 12 cm) which was previously equilibrated against 0.05 M Tris-HCl, pH 7.5. The column was washed with 300 mL of 0.05 M Tris-HCl (pH 7.5)–0.25 M sucrose and eluted with the same buffer containing 0.1% Triton X-100 and 0.01% albumin, and a linear NaCl gradient formed with 200 mL of 0.2 M NaCl in the mixing chamber and 200 mL of 2.0 M NaCl in the reservoir. The elution profile of GlcNAc-transferase from this column is shown in Figure 1. The fractions containing transferase activity were dialyzed and concentrated to 10 mL by ultrafiltration with a PM-10 Amicon membrane (fraction 4).

The sample was applied to a Sephacryl S300 column (2.5 × 40 cm) which was previously equilibrated against 0.05 M Tris-HCl (pH 7.5)–0.25 M scurose–0.1% Triton X-100–0.1

Table III: Relative Activity of GlcNAc-transferase with Different Glycoprotein and Glycopeptide Substrates during Purification of the Enzyme[a]

fraction	ovalbumin (cpm/30 min)	ovomucoid (cpm/30 min)	ovomucoid/ ovalbumin ratio	glycopeptide IV (cpm/30 min)	glycopeptide V (cpm/30 min)	V/IV ratio
(2) microsomes	1050	6175	5.9	820	650	0.8
(3) chromatography on DEAE-cellulose	870	4680	5.4	560	610	1.1
(4) chromatography on cellulose phosphate	530	2980	5.6	480	430	0.9
(5) chromatography on Sephacryl S 200	590	3440	5.8	1010	1250	1.2
(6) affinity chromatography	610	3750	6.1	710	760	1.1

[a] Assays were carried out under standard conditions as described under Experimental Procedures with limiting concentrations of substrates. Reaction mixtures contained 4 mg of ovalbumin (molecular weight 45 000), 2 mg of ovomucoid (molecular weight 28 000), and 0.5 μmol of glycopeptide IV or glycopeptide V.

mg/mL albumin. The enzyme emerged from the column in a single peak between 45 and 58 mL, which was concentrated on a PM-10 Amicon membrane adjusted to 10 mM $MnCl_2$ and then adsorbed to an affinity column (2.2 × 5 cm) which contained ovomucoid covalently attached to Sepharose 4B by the procedure of Cuatrecasas (1970). The column was previously equilibrated against 0.05 M Tris-HCl (pH 7.5)–10 mM $MnCl_2$–0.1% albumin. The column was washed with 75 mL of this buffer, and the enzyme was then eluted with about 30 mL of 0.05 M Tris-HCl, pH 7.5, containing 25 mM EDTA. The final preparation was concentrated by ultrafiltration and dialyzed against 0.05 M Tris (pH 7.5)–0.25 M sucrose (fraction 6). The purified enzyme was stable for at least 1 month when stored at 3 °C in the presence of 0.1% Triton X-100 and 0.01% albumin. The addition of protein and detergent was essential for stability after solubilization of the enzyme. They did not influence the activity of the purified enzyme when added to the reaction mixtures. In the absence of these compounds, enzyme solutions became turbid with a concomitant decrease in transferase activity. The solubilized enzyme was unstable to freezing and thawing whereas the particulate could be frozen with little or no loss of activity. These observations suggest that albumin and detergent prevent the aggregation of the solubilized enzyme.

Relative Activity of GlcNAc-transferase with Different Glycoprotein and Glycopeptide Substrates during Purification. In order to determine whether GlcNAc-transferases with specificities for different glycoproteins and glycopeptides were present in trachea mucosa, the relative transferase activity at various stages of purification was measured with limiting amounts of different substrates. The results of these experiments are summarized in Table III. The ratio of the activity with native ovomucoid or ovalbumin only varied between 5.4 and 6.1 in the crude and purified enzyme preparations. Furthermore, the ratio of the activity with glycopeptide V, which contains only one terminal α-1,3-linked mannose, and glycopeptide IV, which contains both α-1,3- and α-1,6-linked terminal mannosyl residues, did not vary significantly during the course of purification of the enzyme. The ratio of the activity obtained with glycopeptide V to that observed with glycopeptide IV varied between 0.8 and 1.1. In these experiments, it was necessary to add 3 μmol of NAD^+ to incubation mixtures with crude enzyme preparations in order to provide an alternative substrate for hydrolyases and thereby decrease the rate of hydrolysis of UDPGlcNAc. Inclusion of NAD^+ did not affect the activity of the transferase. The activities listed in Table III are initial rates obtained with equal amounts of each of the substrates tested.

Since glycopeptides IV and V both contain a terminal α-1,3-linked mannosyl unit, there was a possibility that GlcNAc was only being transferred to this residue. The K_m of the enzyme for the α-1,6-mannosyl residue on glycopeptide IV could be much higher than that for the α-1,3-linked mannose, and, under conditions of limiting substrate, this transfer could escape detection. However, increasing the concentration of each glycopeptide 10-fold did not change the ratio of activities. Moreover, other experiments with these substrates, which will be discussed later, showed that the enzyme is able to transfer GlcNAc to both α-1,3- and α-1,6-linked mannosyl residues in glycopeptide IV. Taken collectively, these observations strongly suggest that the β-1,2-GlcNAc-transferase present in trachea mucosa is able to transfer GlcNAc to both terminal α-1,3- and α-1,6-mannosyl residues on branched glycopeptide chains.

Specificity of Purified β-1,2-GlcNAc-transferase. Glycoproteins containing large amounts of terminal highly branched mannosyl residues, such as ovomucoid and ovalbumin, were among the best macromolecular glycosyl acceptors for the purified enzyme. The specificity of the purified enzyme was examined with well-characterized glycopeptides prepared from porcine IgG. The transferase showed a very high degree of specificity for acceptors which contained terminal branched mannosyl residues. No activity was observed with glycosyl acceptors which contained only a single terminal mannosyl residue. Mannose, *p*-nitrophenyl β-D-mannoside, β-D-methyl mannoside, *p*-nitrophenyl α-D-mannoside, and glycopeptides Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc→Asn, Manα1→6Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc→Asn, and GlcNAcβ1→2Manα1→6Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc→Asn were inactive. A large number of glycopeptides containing other terminal sugar residues including GlcNAcβ→Asn, GlcNAcβ1→4GlcNAcβ→Asn, glycopeptide I, glycopeptide II, glycopeptide III, glycopeptide VII, Galβ1→3GalNAc, and GlcNAcβ1→4Galβ1→3GalNAc were also inactive. Glycopeptides with branched terminal mannosyl residues were excellent substrates for the purified enzyme, and the interaction of several of these glycopeptides with purified β-1,2-GlcNAc-transferase was examined in more detail. When glycopeptide IV, which was a good substrate for the enzyme, was subjected to limited hydrolysis with α-mannosidase to remove the α-1,6-linked mannosyl residue, the isolated linear glycopeptide containing a terminal α-1,3-linked mannose unit was inactive as a substrate for the transferase. Likewise, another glycopeptide prepared from IgG, Manα1→6Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc→Asn, which contains a similar oligosaccharide chain terminating in a α-1,6-linked mannosyl residue, and Manβ1→4GlcNAcβ1→-

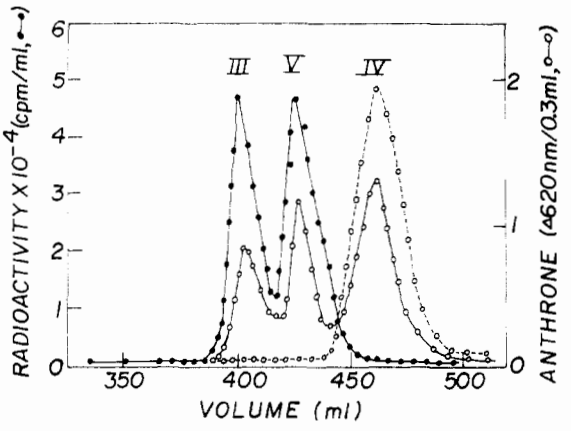


FIGURE 2: Elution profile of products formed by purified β1,2-GlcNAc-transferase from glycopeptide IV. After incubation for 2 h, the large-scale reaction mixture was adjusted to pH 3, and it was passed through a Dowex 1-Cl column (2 × 2.5 cm). The column was washed with 4 volumes of 0.001 N HCl. The filtrate and washes were combined, neutralized with NH_4OH, concentrated, and applied to a Bio-Gel P-6 column (200–400 mesh, 2.2 × 200 cm). The column was eluted with 0.1 M pyridinium acetate, pH 5.5. Radioactive profile (●—●); anthrone determinations for glycopeptide IV before incubation (O---O) and after incubation (O—O) with the transferase.

4(Fucα1→6)GlcNAc→Asn, which contains a β-1,4-linked terminal mannosyl residue, were also inactive. These results clearly demonstrated that the purified transferase was specific for terminal branched mannosyl residues on oligosaccharide chains.

Anomeric and Linkage Specificity of Purified Tracheal GlcNAc-transferase. In order to definitively characterize the nature of the reaction catalyzed by purified GlcNAc-transferase, it was necessary to elucidate the anomeric conformation and position of the linkage of the sugar transferred to the oligosaccharide substrate. Since the concentration of the product formed was very low in the usual reaction mixture, a large-scale incubation, 100 ×, was carried out under standard conditions with 5 μmol of glycopeptide IV, UDP[1-^{14}C]-GlcNAc (3.4×10^5 cpm/μmol), and 100 μg of the purified transferase for 3 h. The products were isolated by passing the incubation mixture at pH 3 through a Dowex 1-Cl column to remove radioactive reactants, and the filtrate and wash were combined, concentrated, and applied to a Bio-Gel P-6 column (2.2 × 200 cm). The individual peaks were collected, concentrated, and rechromatographed on a second Bio-Gel P-6 column (2.2 × 200 cm). As seen in Figure 2, the radioactive products formed in these reaction mixtures eluted in positions corresponding to glycopeptides III and V. The fractions in each peak were pooled and concentrated. About 2.9 μmol of reacted glycopeptide IV, 1.0 μmol of glycopeptide V, and 0.7 μmol of glycopeptide III were recovered. The specific activities of glycopeptide V and glycopeptide III were 3.1×10^5 and 6.7×10^5 cpm/μmol, respectively. These results suggest that the glycopeptide in peak V contains 1 mol of GlcNAc whereas the product in peak III contains 2 mol of GlcNAc/mol of glycopeptide. When aliquots of the radioactive glycopeptides were treated with β-*N*-acetylglucosaminidase, essentially all of the radioactivity was released. The radioactive component was identified as GlcNAc by paper chromatography in three different solvent systems (Rao et al., 1976). These products were also examined by permethylation analysis. In each case, a radioactive peak corresponding to 3,4,6-trimethyl-1,5-di-acetyl-*N*-methylacetylglucosaminitol was observed. A peak with the retention time of 3,4,6-trimethylmannitol was also found in the profile of the glycopeptides in peaks III and V. This peak was not present in the profile of the reisolated substrate, glycopeptide IV. The 3,4,6-trimethylmannitol peak disappeared when the isolated products, peaks III and V, were treated with β-*N*-acetylglucosaminidase. These results are consistent with the formation of a β1→2 bond between the transferred GlcNAc and terminal-branched mannosyl units in the glycopeptide.

Influence of the Concentration of Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc→Asn on the Activity of GlcNAc-transferase and Identification of the Products of the Reaction. The dependence of the activity of β-1,2-GlcNAc-transferase on the concentration of this glycopeptide, which contains two free terminal-branched mannosyl residues, was examined as a function of the concentration of UDPGlcNAc. Lineweaver–Burk plots of the data yielded a series of lines which intersected on the 1/[s] axis, and an apparent K_m of 2.0 mM (Table I) was calculated from primary and secondary reciprocal plots. The corresponding K_m for UDPGlcNAc under identical conditions was 18 μM.

The rate of formation of products of the reaction of GlcNAc-transferase with glycopeptide IV and UDP[1-^{14}C]-GlcNAc was also examined with large-scale reaction mixtures and gel filtration on Bio-Gel P-6 columns (2.2 × 200 cm). Two radioactive peaks corresponding to glycopeptides III and V were formed from this substrate, as seen in Figure 2. The amount of glycopeptide V formed was greater initially; however, as the steady-state level of glycopeptide V increased, the rate of formation of glycopeptide III also increased, and at longer times this glycopeptide was the major product of the reaction. The labeled glycopeptides in these peaks were permethylated and analyzed by gas chromatography. The glycopeptide in peak III had a profile which was identical with that of glycopeptide III (Table I). The intermediate product, peak V, contained a mixute of two glycopeptides with a single terminal GlcNAc attached to either the α-1,3- or α-1,6-linked mannosyl residue. Methylation analysis of the glycopeptides in peak V, Figure 2, yielded a profile which contained 2,3,4,6-tetra- 3,4,6-tri-, and 2,4-dimethylmannitol acetates, 3,4,6-tri-, 3,6-di-, and 3-methylglucosaminitol acetates, and 2,3,4-trimethylfucitol acetate in a ratio of 1.0:0.8:1.0:0.9:0.8:0.8:0.7. After the glycopeptides in peak V were treated with α-mannosidase, peaks corresponding to both 2,3,4- and 2,4,6-trimethylmannitol acetates were found. The glycopeptides in peak V were subjected to acetolysis, which usually removes α-1,6-linked glycosidic branches (Lee & Ballou, 1965), and the products were separated on a Bio-Gel P-6 column. About 50% of the radioactive GlcNAc was released as a disaccharide which contained equimolar amounts of GlcNAc and mannose. Mannose and two glycopeptides were also isolated. Methylation analysis showed that the larger radioactive glycopeptide contained 3,4,6-tri- and 2,4,6-trimethylmannitol acetates and 3,4,6-tri-, 3,6-di-, and 3-methylglucosaminitol acetates in a ratio of 1.0:0.8:1.0:0.9:0.6. The smaller unlabeled glycopeptide contained 2,3,4,6-tetra- and 2,4,6-trimethylmannitol acetates and 3,6-di- and 3-methylglucosaminitol acetates in a ratio of 0.9:1.0:0.8:0.7. The structures of these linear glycopeptides formed by acetolysis from products present in peak V suggest that this fraction contains a mixture of two glycopeptides, GlcNAcβ1→2Manα1→3(Manα1→6)Manβ1→4-R and GlcNAcβ1→2Manα1→6(Manα1→3)Manβ1→4-R. These results support the earlier observations which suggested that the purified enzyme was capable of transferring GlcNAc to either α-1,3- or α-1,6-linked terminal mannosyl residues in glycopeptide IV.

Influence of the Concentration of GlcNAcβ1→2Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc→

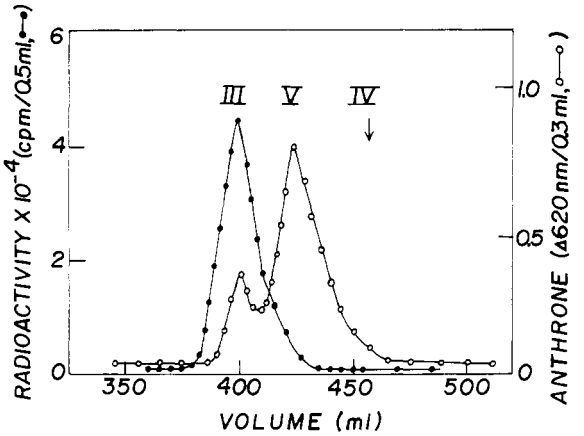


FIGURE 3: Elution profile of products formed by purified β1,2-GlcNAc-transferase from glycopeptide V. After incubation, the reaction mixture was adjusted to pH 3, and the glycopeptides were passed through a Dowex 1-Cl column, concentrated, and applied to a Bio-Gel P-6 column (2.2 × 200 cm). Radioactive profile (●); anthrone determinations (○). The position of glycopeptide IV is shown by the arrow.

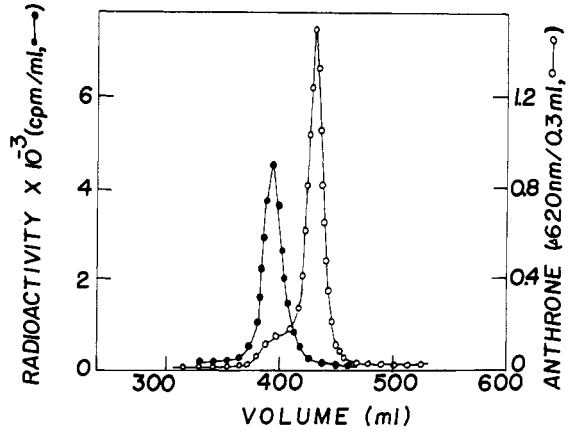


FIGURE 4: Separation of the product formed by incubation of purified GlcNAc-transferase with UDP[1-^{14}C]GlcNAc and GlcNAcβ1→2Manα1→3(Manα1→6)Manβ1→4GlcNAcβ1→4GlcNAc→Asn under standard assay conditions. The elution profiles of radioactivity (●) and anthrone (○) from a Bio-Gel P-6 column are shown. Fractions of 5 mL were collected at a flow rate of 30 mL/h.

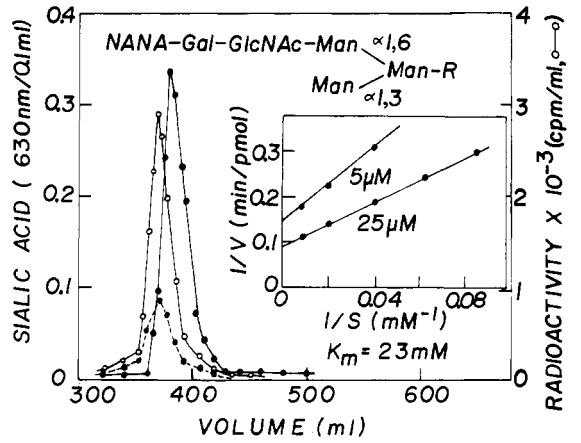


FIGURE 5: Elution profiles of NANAα2→6Galβ1→4GlcNAcβ1→2Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)-GlcNAc→Asn and the radioactive product formed by incubating this glycopeptide with purified GlcNAc-transferase and UDP[1-^{14}C]-GlcNAc. The radioactive product was previously passed through two Bio-Gel P-6 columns. Fractions of 5 mL were assayed by determination of sialic acid (●—●), unlabeled glycopeptide IX and radioactivity (○—○), and sialic acid (●---●), for the labeled product. (Insert) Influence of the concentration of this glycopeptide on the activity of β1,2-GlcNAc-transferase under the standard assay conditions with the amounts of glycopeptide and UDPGlcNAc shown in the figure.

Asn on the Activity of GlcNAc-transferase. The effect of increasing concentrations of glycopeptide V on the activity of purified β-1,2-GlcNAc-transferase as a function of the concentration of UDPGlcNAc was measured under identical conditions. Lineweaver–Burk plots yielded a series of lines which intersected on the 1/[S] axis. An apparent K_m of 1.3 mM (Table I) was obtained for this glycopeptide, and an apparent K_m of 21 μM for UDPGlcNAc was calculated from replots of the data. The K_m for glycopeptide V is only slightly lower than that obtained with glycopeptide IV, which contains two terminal mannosyl residues. The data summarized in Figure 3 further show that the enzyme transfers GlcNAc to the free terminal α-1,3-mannosyl residue in glycopeptide V to form glycopeptide III which contains two terminal GlcNAc residues. In this case, only one radioactive peak corresponding to glycopeptide III was found by chromatography on Bio-Gel P-6 columns (2.2 × 200 cm). No specificity or order of addition for either of the branched mannosyl residues was observed in these experiments.

The corresponding isomer with a single GlcNAc residue attached to the β-1,3-linked mannosyl residue was prepared from $(Man)_5(GlcNAc)_2Asn$ by incubation with GlcNAc-transferase and by limited hydrolysis with α-mannosidase at pH 6.5, as described under Experimental Procedures. Glycopeptide XII was also a good substrate for the enzyme and showed nearly the same activity as glycopeptide V when assayed at identical concentrations in the standard reaction mixture. As seen in Table I, the apparent K_m for this isomer was 2.3 mM, which compares favorably with that obtained for glycopeptide V. Only one radioactive product with an elution volume corresponding to that of authentic glycopeptide III (Figure 4) was formed from this glycopeptide. Taken collectively, these observations indicate that the purified enzyme is able to transfer GlcNAc to either of the terminal mannosyl residues at the branch point in these oligosaccharide chains in any order and at comparable rates. Furthermore, the enzyme can also transfer a second GlcNAc residue to the products of the first reaction which contain only one free mannosyl residue at similar rates of reaction.

The effect of increasing concentrations of a glycopeptide containing galactose and sialic acid attached to one of the GlcNAc residues (glycopeptide IX) on the activity of the GlcNAc-transferase was examined. This glycopeptide was prepared from glycopeptide I by treatment with purified β-*N*-acetylglucosaminidase, and it was purified by chromatography on a Bio-Gel P-6 column as shown in Figure 5. An apparent K_m of 23 mM was obtained with this glycopeptide (Table I). The presence of a galactose and sialic acid residue on one of the branched oligosaccharide chains increased the K_m of the enzyme by nearly 10-fold. The presence of sialic acid on one chain of a branched oligosaccharide has previously been shown to decrease the activity of a β-1,4-galactosyltransferase acting to complete an adjacent chain (Rao & Mendicino, 1978).

Influence of the Concentration of Manα1→6(Manα1→3)Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc→Asn on the Activity of GlcNAc-transferase and Identification of the Products of the Reaction. The effect of increasing concentrations of $(Man)_5(GlcNAc)_2Asn$ on the activity of β-1,2-GlcNAc-transferase as a function of the concentration of UDPGlcNAc was examined. An apparent K_m of 0.5 mM for glycopeptide X (Table I) was calculated from primary and secondary plots of these data, and an apparent K_m of 27 μM

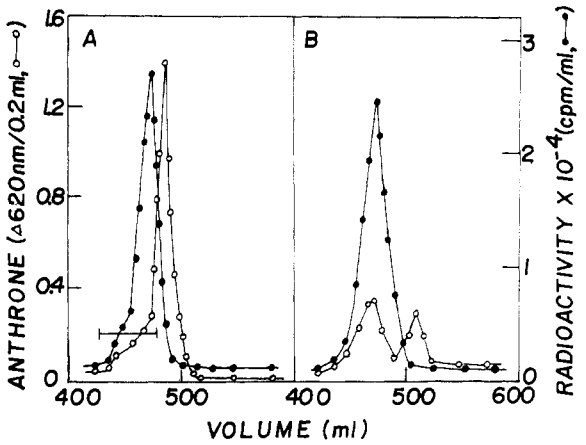
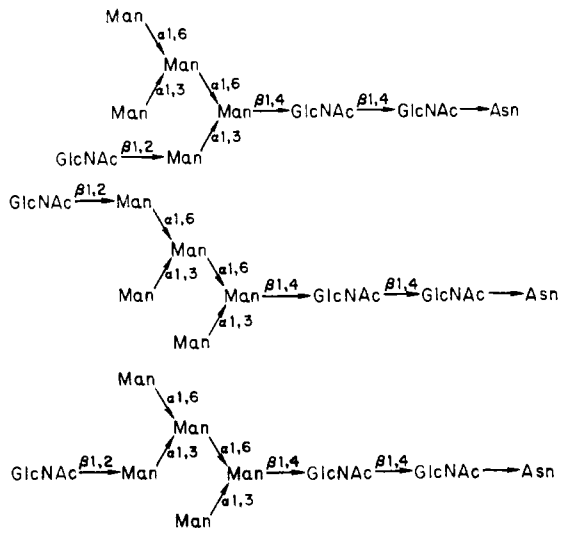


FIGURE 6: Isolation of the product formed by incubating purified GlcNAc-transferase with $(Man)_5(GlcNAc)_2Asn$ and UDP[1-^{14}C]-GlcNAc. After incubation, the reaction mixture was adjusted to pH 3, passed through a Dowex 1-Cl column, neutralized, concentrated, and applied to a Bio-Gel P-6 column. The fractions between the hatch marks (frame A) were collected, concentrated, and rechromatographed on a second Bio-Gel P-6 column (frame B).

for UDPGlcNAc was calculated from replots of the data. Surprisingly, the apparent K_m for this substrate, which contains three branched mannosyl residues attached to the α-1,6-linked mannosyl residue, was somewhat lower than those of glycopeptides IV and V.

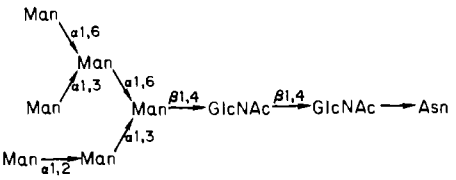
The radioactive product formed from $(Man)_5(GlcNAc)_2Asn$ in large-scale reaction mixtures was isolated by gel filtration on Bio-Gel P-6 columns, and the elution profile is shown in Figure 6. The radioactive peak was further purified by rechromatography on a Bio-Gel P-6 column. The specific activity of the isolated glycopeptide, 3.9×10^7 cpm/μmol, compared favorably with the specific activity of the UDP[1-^{14}C]GlcNAc, 4.0×10^7 cpm/μmol, used in this reaction mixture. The position of elution from the Bio-Gel P-6 column and the specific activity of the isolated product correspond to a product with an empirical structure of [^{14}C]GlcNAc-$(Man)_5(GlcNAc)_2Asn$. The product which contains only 1 mol of terminal GlcNAc/mol of glycopeptide could contain the following glycopeptides.



Trachea β-1,2-GlcNAc-transferase did not transfer a second GlcNAc residue to this glycopeptide substrate even though two terminal branched mannosyl residues were still available. This is an interesting finding, especially since the enzyme is able to convert glycopeptide V, which contains only one free terminal mannosyl residue, to glycopeptide III.

Acetolysis of the radioactive product yielded mannose, Manα1→3Man, and a linear radioactive glycopeptide with the structure [^{14}C]GlcNAcβ1→2Manα1→3Manβ1→-4GlcNAcβ1→4GlcNAc→Asn. This same linear glycopeptide was formed when the product was treated exhaustively with α-mannosidase. More than 80% of the radioactivity was recovered in the linear glycopeptide peak, and treatment with β-*N*-acetylglucosaminidase released essentially all of the radioactive GlcNAc. Incubation of this product with α-mannosidase released 1.9 equiv of mannose, and the resulting glycopeptide was identified as Manβ1→4GlcNAcβ1→-4GlcNAc→Asn. These results suggest that the principal product formed from $(Man)_5(GlcNAc)_2Asn$ by the purified transferase has the structure Manα1→6(Manα1→3)-Manα1→6([^{14}C]GlcNAcβ1→2Manα1→3)Manβ1→-4GlcNAcβ1→4GlcNAc→Asn.

Further evidence for the inability of purified β-1,2-GlcNAc-transferase to transfer GlcNAc to branched mannosyl residues attached to α-linked mannose groups was obtained by testing other glycopeptides prepared from ovalbumin. The enzyme did not transfer GlcNAc to a glycopeptide with the structure.



Apparently the enzyme only transfers GlcNAc to the α-mannosyl residues attached to the β-linked mannose residue in the chain. Thus, the product formed from $(Man)_5(GlcNAc)_2Asn$ after the transfer of one GlcNAc to the β1→-3-linked mannosyl residue did not satisfy the structural requirements of the enzyme for additional transfers.

Discussion

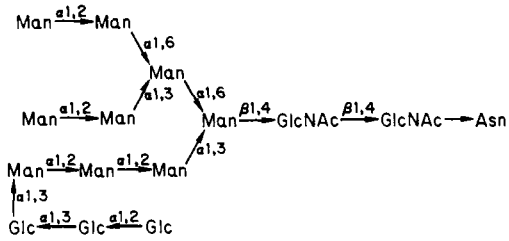
The β-1,2-GlcNAc-transferase isolated from swine trachea showed a high degree of specificity for its glycopeptide substrates. The purified enzyme had an absolute specificity for terminal-branched mannosyl residues of the "core portion" of complex-type glycoproteins. Glycopeptides containing only a single terminal mannosyl residue did not act as GlcNAc acceptors for the purified enzyme. However, this appeared to be the only strict requirement. The enzyme preparation transferred GlcNAc to both α-1,3- and α-1,6-linked terminal mannosyl units in glycopeptides in any order. It also added a second GlcNAc residue to the product of the first transfer reaction to form branched chains with two terminal GlcNAc residues. The specificity of the β-1,2-GlcNAc-transferase preparation was not influenced by the molecular weight of its substrates; however, the enzyme has a much lower K_m for macromolecular substrates. Apparent K_ms of 45 μM and 19 μM were obtained for ovalbumin and the solubilized heavy chain of IgG whereas glycopeptide IV, glycopeptide V, and $(Man)_5(GlcNAc)_5Asn$ had apparent K_ms of 2.0 mM, 1.3 mM, and 0.5 mM, respectively. Similar activities and apparent K_ms were observed with Manα1→6(Manα1→3)Manβ1→-4GlcNAcβ1→4(Fucα1→6)GlcNAc→Asn, GlcNAcβ1→-2Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→-6)GlcNAc-Asn, Manα1→6(GlcNAcβ1→2Manα1→3)-Manβ1→4GlcNAcβ1→4GlcNAc-Asn, and Manα1→6-(Manα1→3)Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→-4GlcNAc→Asn. An examination of the structures of the

glycopeptides prepared from ovalbumin and porcine IgG, which served as substrates for the transferase, revealed that they all contained at least one free mannosyl group attached to a β-linked mannose unit. The purified enzyme transferred GlcNAc only to the mannosyl residues attached to the β-linked mannose. These observations suggest that the enzyme may be specific for terminal branched mannosyl residues which are bound to this β-linked mannose unit or for the chitobiose group attached to asparagine. The presence or absence of fucose linked to GlcNAc of the chitobiose unit did not influence the activity or specificity of the transferase.

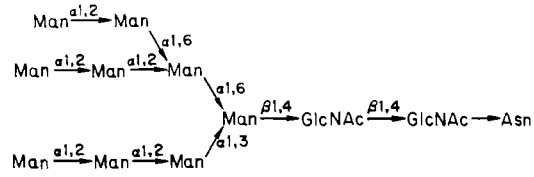
The results obtained in several studies suggest that two different GlcNAc-transferases with specificities for the α-1,3- and α-1,6-linked mannosyl residues in branched oligosaccharide chains are present in Chinese hamster ovary cells (Li & Kornfeld, 1978; Narasimhan et al., 1977; Stanley et al., 1975). The conclusions were based on evidence obtained by analyzing the radioactive products formed by mutants of this cell line and by differences in the activity of partially purified enzyme preparations toward mixtures of several different glycopeptide substrates. More recently, GlcNAc-transferase I has been completely separated from GlcNAc-transferase II (Harpaz & Schachter, 1980). This purified enzyme has a preference for the α-1,3-linked mannosyl residue, but at high concentrations of substrate it also transferred GlcNAc to α-1,6-linked mannose. The K_m for glycopeptide IV was 0.2 mM, and the K_m for Manα1→6(GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAc(Fucα1→6)GlcNAc→Asn was 10 mM. The values obtained in the present study for these glycopeptides were 2.0 mM and 2.3 mM, respectively. A GlcNAc-transferase has also been partially purified from rabbit liver (Oppenheimer, 1980). This enzyme has a K_m of 3.6 mM for Manα1→6(Manα1→3)Manβ1→4GlcNAc and a K_m of 0.38 mM for Manα1→6(Manα1→3)Manα1→6(Manα1→3)Manβ1→4GlcNAc. These values are similar to those observed with the enzyme from trachea. The liver enzyme showed no activity with Manα1→6(GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAc when assayed at a concentration of 2 mM. Because of their closely related functions, the two β-1,2-GlcNAc-transferases may exist as a tightly associated complex in some tissues. Thus the results reported in the present communication are not necessarily at variance with the studies of Schachter, Kornfeld, and their colleagues, who demonstrated the existence of two distinct GlcNAc-transferases, specific for the α-1,3- and α-1,6-linked mannose residues.

The findings described in the present study provide evidence for a mechanism which allows for the transfer of GlcNAc units to the α-1,3- and α-1,6-linked terminal mannosyl units in growing oligosaccharide chains, even while α-mannosidase is removing excess mannosyl groups from these chains. This simultaneous synthesis and "processing" of oligosaccharide chains are possible because the specificity and activity of these enzymes are significantly influenced by the structures of intermediate oligosaccharide chains. The purified β-1,2-GlcNAc-transferase preparation has an absolute specificity for terminal branched mannosyl residues on glycopeptide chains. The enzyme is able to transfer GlcNAc to a number of glycopeptide chains which are present in the later stages of processing of glycoproteins.

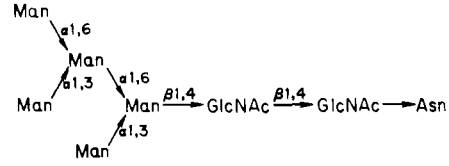
The most complete oligosaccharide chain transferred from a lipid intermediate to a nascent polypeptide chain may have the structure (Li et al., 1978; Spiro et al., 1979)



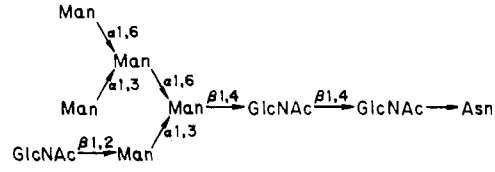
This oligosaccharide chain may be processed in at least two different ways. If α-glycosidases act first, then an intermediate devoid of glucose may be formed. This α-glycosidase may initiate the first step in the series of sequential hydrolyase reactions involved in this "processing" pathway (Spiro et al., 1979).



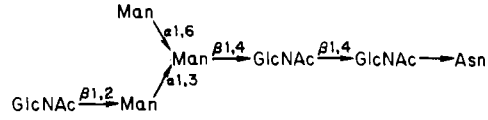
This intermediate can then be converted to the following glycopeptide by the action of a specific α-1,2-mannosidase which has been isolated from rat liver Golgi membranes (Tabas & Kornfeld, 1979).



This glycopeptide which is present in both ovalbumin and ovomucoid can act as a substrate for purified β-1,2-GlcNAc-transferase.



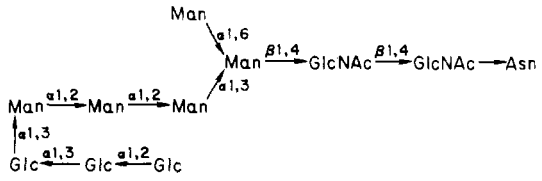
This intermediate can then be hydrolyzed by α-mannosidase to yield glycopeptide XII. The transfer of GlcNAc to the



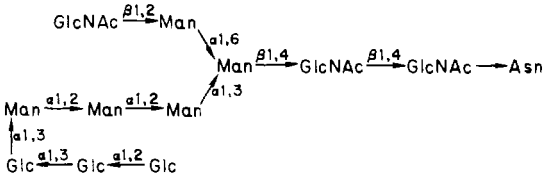
XII

α-1,3-linked mannosyl unit in this glycopeptide promotes the removal of the two mannosyl residues on the α-1,6-linked chain (Tabas & Kornfeld, 1978). An intermediate with this structure has been isolated from bovine rhodopsin (Fukuda et al., 1979). The results presented in this report show that tracheal β-1,2-GlcNAc-transferase can transfer GlcNAc to this intermediate to form glycopeptide III. Galactosyl- and sialyltransferases then complete the outer chains (Rao & Mendicino, 1978; Rao et al., 1976).

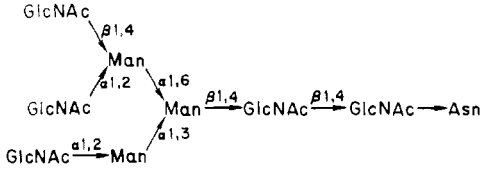
Alternatively, the "processing" may begin with the removal of four mannosyl residues from the transferred oligosaccharide to form an intermediate with a free α-1,6-linked mannosyl residue.



This compound has been isolated from mouse lymphoma cells which have a block in the synthesis of the oligosaccharide chain transferred from lipid intermediates (Kornfeld et al., 1979). On the basis of the properties of the β-1,2-GlcNAc-transferase described in the present studies, this intermediate might serve as a substrate for the enzyme to form the intermediate



Hydrolysis by α-glucosidase and α-1,2-mannosidase would convert this intermediate to glycopeptide V, and β-1,2-GlcNAc-transferase can convert this oligosaccharide chain to glycopeptide III. The addition of a second GlcNAc residue to the α-1,6-linked mannosyl unit by a specific β-1,4-GlcNAc-transferase could also give rise to a glycopeptide with the structure



The transfer of galactose and sialic acid to this intermediate could then yield complex oligosaccharides which contain three branched chains, such as those found in fetuin (Baenziger & Fiete, 1979) and other more highly branched glycoproteins.

In previous studies with β-1,4-galactosyltransferase, we showed that the rate of transfer of galactose to terminal GlcNAc residues in incomplete branched oligosaccharide chains was dependent on whether a galactosyl residue was already present on one of the chains and on the rate of transfer of sialic acid to this galactosyl residue (Rao & Mendicino, 1978). The presence of galactose on one chain greatly decreased, 10-fold, the rate of transfer of a second galactose unit to the remaining GlcNAc residue on an adjacent chain. This property could be responsible for the marked microheterogeneity often found in the galactosyl and sialic acid units of secreted glycoproteins. In marked contrast, the activity of β-1,2-GlcNAc-transferase is not significantly influenced by the presence of a GlcNAc residue on an adjacent chain, and, interestingly, relatively little microheterogeneity is observed in these residues of secreted glycoproteins. These observations are consistent with the properties of β-1,4-galactosyltransferase described in previous studies and with the specificity and properties of β-1,2-GlcNAc-transferase presented in this report. The evidence further suggests that the structures of the oligosaccharide intermediates formed during the synthesis and "processing" of incomplete glycoprotein chains may play an important role in regulating the activity and perhaps the specificity of the glycosyltransferases and exoglycosidases which participate in glycoprotein biosynthesis.

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