Purification, properties and partial amino acid sequence of the blood-group-A-gene-associated α -3-N-acetylgalactosaminyltransferase from human gut mucosal tissue

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An α -3-N-acetylgalactosaminyltransferase that transfers N-acetylgalactosamine from UDP-N-acetylgalactosamine to H-active structures to form A determinants was purified to homogeneity from human gut mucosal tissue of blood-group-A subjects. The mucosa was homogenized, then treated with Triton X-100, and the solubilized enzyme was purified by affinity chromatography on UDP-hexanolamine-agarose and octyl-Sepharose CL-4B. Enzyme activity was recovered in 44 % yield with a specific activity of approx. 7 μ mol/min per mg. The only effective acceptor substrates for the transferase were those containing a subterminal β -galactosyl residue substituted at the O-2 position with L-fucose. The purified enzyme had a weak capacity to transfer D-galactose from UDP-D-galactose to similar acceptors to make blood-group-B determinants. H.p.l.c. and SDS/PAGE analysis indicated an M_r of 40000 for the purified enzyme. For the first time a partial amino acid sequence Xaa-Ser-Leu-Pro-Arg-Met-Val-Tyr-Pro-Gln-Ile-Ser?-Val-Leu was obtained for the N-terminal region of the soluble α -3-N-acetylgalactosaminyltransferase.

INTRODUCTION

The demonstration in the 1950s that human blood-group-A- and -B-specificity is associated with carbohydrate structures made it evident that, despite the clear-cut Mendelian inheritance of the A and B characters, they are not the primary translated products of the genes at the ABO blood-group locus. Structural studies indicating the involvement of N-acetylgalactosamine in blood-group-A-specificity, together with the change in serological properties that occurred when this sugar was removed, led to the proposal in 1959 that the A gene encoded an enzyme that catalysed the addition of N-acetylgalactosamine to a precursor H structure (Watkins & Morgan, 1959). The validity of this proposal was supported by the characterization in human tissues of an α -3-N-acetylgalactosaminyltransferase (A transferase) with the distribution and acceptor-substrate-specificity predicted for the A-gene product (Kobata *et al.*, 1968; Hearn *et al.*, 1968). Since that time a number of investigators have studied the properties of this enzyme and the variant kinetic forms that occur in A subgroups and rare A phenotypes (reviewed in Cartron, 1976; Watkins, 1980). However, until now the low abundance of the A transferase in human tissues has made it difficult to isolate sufficient quantities of the purified protein for compositional and structural studies to be carried out.

Most attempts to purify the human A transferase have been made with plasma as the enzyme source and have utilized the observation by Whitehead *et al.* (1974) that the transferase binds specifically to the beaded agarose matrix Sepharose 4B (Pharmacia). The precise mechanism of this binding is not understood, but Nagai *et al.* (1978) achieved a 100000-fold purification of the A transferase in human plasma by a procedure in which the major step involved adsorption on, and elution from, this affinity absorbent. The enzyme was purified to apparent homogeneity and was reported to have an M_r of 100000. More recently two preliminary accounts have appeared concerning the purification of the A transferase from human lung tissue. One method utilized a Sepharose 4B affinity step, followed by f.p.l.c. ion-exchange chromatography (Clausen *et al.*, 1989) and the second

a procedure involving adsorption first on UDP-hexanolamine-agarose and then on octyl-Sepharose CL-4B (Navaratnam & Watkins, 1989a). Both laboratories reported an M_r of 40000 for the purified lung enzyme.

We now describe the purification, properties and partial amino acid sequence of the α -3-N-acetylgalactosaminyltransferase from human blood-group-A gut mucosal tissue.

MATERIALS AND METHODS

Materials

UDP-hexanolamine-agarose, UDP, UMP, cyclic UMP, uridine, CDP, GDP, fluorescein, fluorescein mercuric acetate and N-ethylmaleimide were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.). UDP-N-acetyl-D-[1-¹⁴C]galactosamine (2.3 GBq/mmol), UDP-D-[¹⁴C]galactose (11.4 GBq/mmol) and M_r markers for PAGE [non-radioactive Rainbow markers: lysozyme (M_r 14300), trypsin inhibitor (M_r 25000), carbonic anhydrase (M_r 30000), ovalbumin (M_r 46000), BSA (M_r 69000), phosphorylase *b* (M_r 92500) and myosin (M_r 200000)] were obtained from Amersham International (Amersham, Bucks., U.K.). Octyl-Sepharose CL-4B and the size-exclusion column TSK 3000 SW were purchased from Pharmacia (Milton Keynes, Bucks., U.K.). A silver staining reagent for polyacrylamide gels (2D silver staining kit) was from Koch-Light Laboratories (Haverhill, Suffolk, U.K.) and other chemicals used were of the best available grade from BDH Chemicals (Poole, Dorset, U.K.).

2'-Fucosylgalactose (Fuc α 1-2Gal) was a gift from (the late) Professor R. Kuhn (Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany). 2'-Fucosyl-lactose (Fuc α 1-2Gal β 1-4Glc), 3-fucosyl-lactose [Gal β 1-4(Fuc α 1-3)Glc], lacto-N-fucopentaose I (Fuc α 1-2Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc), lacto-N-fucopentaose II [Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc], lacto-difucohexaose I [Fuc α 1-2Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc], lacto-difucohexaose II [Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc], lacto-N-tetraose (Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc) and lacto-N-neotetraose (Gal β 1-

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4GlcNAc β 1-3Gal β 1-4Glc) were isolated from human milk as described by Donald & Feeney (1988) and were a gift from Dr. A. S. R. Donald (M.R.C. Clinical Research Centre, Harrow, Middx., U.K.). All the milk oligosaccharides were checked for purity by Dr. Donald by examination of their 500 MHz ^1H -n.m.r. spectra.

Purification of α -3-*N*-acetylgalactosaminyltransferase

All the purification procedures up to step 3 were carried out at 4 °C.

Step 1. Homogenization and Triton X-100 extraction. Mucosa was scraped from human blood-group-A stomach and duodenal tissues removed within 48 h of death and stored at -40 °C. About 40 g of mucosa was thawed and homogenized in 20 mM-Tris/HCl buffer, pH 7.2 (114 ml), containing 1 mM-EDTA, 1 mM-2-mercaptoethanol, 2 mM-MnCl₂, 0.1 M-NaCl, 50 μM -phenylmethanesulphonyl fluoride and 1% (v/v) Triton X-100 in an IKA Ultra-Turrax T25 homogenizer for 1 min in bursts of 10–20 s. The suspended material was further homogenized in a glass Dounce homogenizer (eight to ten strokes) and the homogenate was then filtered through glass-wool and centrifuged at 12000 *g* for 30 min in a Sorvall RC 2B centrifuge (first centrifugation). The supernatant was removed and further centrifuged at 48000 *g* for 60 min in a Sorvall RC 2B centrifuge (second centrifugation). The resultant pellets were re-extracted with the same buffer (57 ml), the extracts were centrifuged at 48000 *g* for 60 min as before (third centrifugation) and the second and third supernatants were pooled (100 ml).

Step 2. Chromatography of Triton-solubilized *N*-acetylgalactosaminyltransferase on UDP-hexanolamine-agarose. The Triton-solubilized enzyme was loaded on to a column of UDP-hexanolamine-agarose (2.5 cm $\times$ 10 cm) equilibrated with 20 mM-Tris/HCl buffer, pH 7.2, containing 1 mM-EDTA, 1 mM-2-mercaptoethanol, 2 mM-MnCl₂, 0.5 M-NaCl and 0.02% Triton X-100. The column was washed with the same buffer until the absorbance (at 280 nm) of the eluate was equal to that of the wash buffer. The bound enzyme was eluted with a UDP gradient (0–5 mM) in 60 ml of the wash buffer in which the MnCl₂ concentration had been increased to 10 mM. Fractions with enzyme activity were pooled, concentrated in an Amicon concentrator (10000-*M_r* cut-off) and dialysed for 16 h against four changes of 50 mM-sodium cacodylate/HCl buffer, pH 6.5, containing 1 mM-2-mercaptoethanol, 10 mM-MnCl₂ and 0.02% Triton X-100. Thorough dialysis to remove UDP is essential before proceeding to the next purification step.

Step 3. Chromatography on octyl-Sepharose CL-4B. The dialysed enzyme was loaded on to an octyl-Sepharose CL-4B column (1.5 cm $\times$ 2.5 cm) equilibrated with 50 mM-sodium cacodylate/HCl buffer, pH 6.5, containing 1 mM-2-mercaptoethanol, 10 mM-MnCl₂, 2 M-NaCl and 0.02% Triton X-100. The column was washed with 30 ml of this buffer and then with 50 ml of the same buffer except that the NaCl was omitted. The enzyme was eluted with 50 μM -UDP in the buffer used for the final wash, and 3 ml fractions were collected.

H.p.l.c. of the α -3-*N*-acetylgalactosaminyltransferase on a TSK 3000 SW size-exclusion column

The fractions with enzyme activity eluted from the octyl-Sepharose column were concentrated to 0.5 ml on a Centricon 10 (Amicon) microconcentrator. To this was added 2 ml of 50 mM-sodium cacodylate/HCl buffer, pH 6.5, containing 1 mM-2-mercaptoethanol, 10 mM-MnCl₂, 0.5 M-NaCl and 0.002% Triton X-100, and the solution was further concentrated to

0.5 ml. Two other batches of enzyme, similarly prepared, were added, and the pooled preparations were again concentrated to about 0.5 ml. The concentrated enzyme (500 μl) was loaded on to a TSK 3000 SW column (7.5 mm $\times$ 60 cm) equilibrated with the above buffer. Elution was performed with the same buffer, and fractions were collected at the rate of 0.4 ml/min for 70 min. The h.p.l.c. was carried out at room temperature, but all the buffers and the tubes in which the fractions were collected were kept in ice. Fractions with enzyme activity were stored at 4 °C.

Glycosyltransferase assays

For routine assays of the α -3-*N*-acetylgalactosaminyltransferase the reaction mixtures contained, in a total volume of 50 μl , 0.1 M-Mops/NaOH buffer, pH 7.4, 2 mM-2'-fucosyl-lactose, 20 mM-MnCl₂, 6.4 μM -UDP-*N*-acetyl[^{14}C]galactosamine (32000 c.p.m.) and up to 20 μl of the enzyme. When the enzyme activity was measured in crude homogenates 0.05 mM-ATP was included in the mixture. After incubation at 37 °C for 10–60 min the reactions were stopped with 50 μl of ice-cold EDTA and the tubes were kept in ice. The solutions were transferred quantitatively to Dowex 1 anionic resin (formate form) (0.8 ml) in glass columns (0.5 cm diam.), and the products were eluted with water (total 1.2 ml) into glass vials. After the addition of 10 ml of Liquiscint (National Diagnostics, Manville, NJ, U.S.A.) to the vials, radioactivity was counted in a Nuclear-Chicago mark II scintillation counter.

Protein determination

Protein concentrations were determined by the method of Peterson (1977), with BSA as the standard.

SDS/PAGE

PAGE was carried out in 12% polyacrylamide gels by the method of Laemmli (1970) in a Miniprotean II dual-slab-gel apparatus (Bio-Rad Laboratories, Watford, Herts., U.K.). Protein bands were identified with a silver staining reagent.

Partial amino acid sequencing of the α -3-*N*-acetylgalactosaminyltransferase

H.p.l.c. fractions 43–47 were pooled and dialysed against 0.05% SDS in distilled and filtered deionized water (1 litre) over 3 days with several changes. The dialysed enzyme was freeze-dried and was subjected to *N*-terminal amino acid sequencing, as described by Findlay *et al.* (1989).

Briefly, the freeze-dried protein was dissolved in 0.035 ml of 0.1 M-NaHCO₃/0.1 M-Na₂CO₃/0.25% SDS, pH 10, and added to 10 mg of *p*-phenylene di-isothiocyanate-glass (17 nm pore size; 200–400 mesh). The glass was incubated for 60 min at 56 °C under N₂, then washed with water and methanol to remove non-covalently bound material. The glass-coupled peptide was then sequenced by automated solid-phase Edman degradation (Laursen, 1971) in the micro-sequence facility built by the Protein Sequence Unit, Department of Biochemistry, University of Leeds.

Anilinothiazolinone derivatives of amino acids were converted in aq. 30% (v/v) trifluoroacetic acid for 20 min at 70 °C (under N₂), and the corresponding phenylthiohydantoin derivatives of the amino acids were identified by reverse-phase (C₁₈) microbore h.p.l.c. (Brownlee Spheri-5 RP-18 column; 220 mm $\times$ 2.1 mm) on a Hewlett-Packard 1090 M h.p.l.c. system with a gradient of acetonitrile in 35 mM-acetic acid (adjusted to pH 4.9 with NaOH) at a flow rate of 0.21 ml/min. Data were collected at 269 nm and 313 nm by means of a diode-array detector. Serine and threonine residues were confirmed by the detection of their dehydro derivatives at 313 nm.

Table 1. Purification of α -3-*N*-acetylgalactosaminyltransferase from human gut mucosa

For experimental details see the Materials and methods section. One unit of activity is defined as the formation of 1 nmol of product/min.

Step	Volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Total purification (fold)	Yield (%)
1. Mucosal homogenate	100	737	40.4	0.055	1	100
2. UDP-hexanolamine-agarose	8	0.54	29.5	54.7	995	73
3. Octyl-Sepharose CL-4B	0.8	0.0026	17.9	6885	125 175	44

Effect of pH and Mn^{2+} concentration on the α -3-*N*-acetylgalactosaminyltransferase activity

To determine the optimum pH for the purified transferase the assays were carried out with 10 μ l of enzyme and the remainder of the reaction mixture described for the routine assays, except that the pH 7.4 buffer was replaced by a series of 0.1 M buffers over the pH range 5.3–8.9. Details are given in Fig. 4 legend.

To determine the optimum Mn^{2+} concentration the purified enzyme (10 μ l) was assayed in the presence of various concentrations of $MnCl_2$ (1–20 mM) in 0.1 M-Mops/NaOH buffer, pH 7.2, at 37 °C for 15 min.

Acceptor-substrate-specificity of the α -3-*N*-acetylgalactosaminyltransferase

The acceptor-substrate-specificity of the purified enzyme was tested with mono-, di- and oligo-saccharides essentially as described by Yates & Watkins (1982). The reaction mixtures contained, in a total volume of 100 μ l, 5 μ l of UDP-*N*-acetyl-[14 C]galactosamine (64000 c.p.m.; 0.64 nmol), 5 μ l of $MnCl_2$ (400 mM), 50 μ l of Mops/NaOH buffer, pH 7.4 (0.1 M), 5 μ l of acceptor substrate (50 mM), 15 μ l of water and 20 μ l of purified enzyme. The reaction mixtures were incubated at 37 °C for 20 min, and the products were separated by paper chromatography on Whatman DE-81 paper in propan-1-ol/pyridine/ethyl acetate/water (5:1:1:3, by vol.; solvent A). Radioactive peaks were detected with a Packard radiochromatogram scanner and their radioactivities counted in a Nuclear-Chicago mark II scintillation counter.

Donor-substrate-specificity of the α -3-*N*-acetylgalactosaminyltransferase

In order to test the capacity of the purified enzyme to synthesize blood-group-B determinants, a reaction mixture containing 2'-fucosyl-lactose (2.5 mM), $MnCl_2$ (20 mM), Mops/NaOH buffer, pH 7.4 (0.2 M), UDP-[14 C]galactose (0.64 nmol/5 μ l) and 25 μ l of enzyme was incubated for 60 min at 37 °C. A parallel reaction mixture in which UDP-*N*-acetyl-[14 C]galactosamine (0.64 nmol/5 μ l) replaced UDP-[14 C]galactose was incubated with 5 μ l of the enzyme for 10 min at 37 °C in order to compare the activities with the two donor substrates. The reaction products were separated by chromatography on Whatman DE-81 paper in solvent A.

Enzyme inhibition experiments

Nucleotides and thiol-blocking reagents were tested for inhibition of α -3-*N*-acetylgalactosaminyltransferase activity. The purified enzyme (10 μ l) was mixed with UDP (50 μ M), with cyclic UMP (2 mM), with uridine (2 mM), with CDP (2 mM) and with GDP (2 mM) in 0.1 M-Mops/NaOH buffer, pH 7.4, and the enzyme activity in each case was measured by the routine assay procedure after incubation for 15 min at 37 °C.

The effects of fluorescein mercuric acetate and *N*-ethylmaleimide were studied by mixing at 0 °C, in a total volume of

50 μ l, purified enzyme (10 μ l), Mops/NaOH buffer, pH 7.4 (0.1 M), and either fluorescein mercuric acetate (1.4 nM or 2.8 nM) or *N*-ethylmaleimide (10 mM). Samples (10 μ l) were removed after 1 min and the enzyme activity was measured by the routine assay procedure after incubation at 37 °C for 20 min.

In order to test their protective capacity 2-mercaptoethanol (1 mM) or UDP (5 μ M) was added to enzyme reaction mixtures containing 1.4 nM-fluorescein mercuric acetate. Fluorescein (3 nM) was also included in a standard reaction mixture as a reagent control. The concentration of fluorescein mercuric acetate was measured by using a molar absorption coefficient at 499 nm of 7.8×10^4 M $^{-1}$ ·cm $^{-1}$ (Laidler *et al.*, 1982).

RESULTS

Purification of α -*N*-acetylgalactosaminyltransferase

A summary of the purification steps is given in Table 1. After chromatography on UDP-hexanolamine-agarose (results not shown) 73 % of the enzyme activity was recovered with about a 1000-fold purification. Most of the residual protein flowed straight through the octyl-Sepharose CL-4B column, but all the enzyme activity bound to the column and was eluted only on application of 50 μ M-UDP (Fig. 1). The yield in the final step was 44 % and a purification factor of 125000 was achieved.

H.p.l.c. of the α -3-*N*-acetylgalactosaminyltransferase on a TSK 3000 SW column

Elution profiles of the enzyme activity and the protein emerging from a TSK 3000 SW column are shown in Fig. 2. The arrows on this Figure show the elution positions of various M_r standards. The enzymically active peak corresponds to an M_r of approx. 40000, and SDS/PAGE analysis of the h.p.l.c. fractions with enzyme activity showed a major band with M_r 40000 (Fig. 3). Earlier fractions showed a band with M_r 65000, but this represented only a very minor component in the fractions that were concentrated for amino acid sequencing.

Fig. 1. Elution profile of α -3-*N*-acetylgalactosaminyltransferase activity from octyl-Sepharose CL-4B

Samples (10 μ l) of the fractions were incubated with 2'-fucosyl-lactose in the standard reaction mixture for 20 min at 37 °C.

Fig. 2. Elution profile of α -3-*N*-acetylgalactosaminyltransferase activity from a TSK 3000 SW size-exclusion column

The arrows indicate the elution positions for the M_r markers: 1, alcohol dehydrogenase (M_r 150 000); 2, BSA (M_r 66 000); 3, ovalbumin (M_r 45 000); 4, carbonic anhydrase (M_r 29 000); 5, cytochrome *c* (M_r 14 500). —, A_{220} ; ●, α -*N*-acetylgalactosaminyltransferase activity.

Fig. 3. SDS/PAGE analysis of h.p.l.c. fractions with α -3-*N*-acetylgalactosaminyltransferase activity

The gel was stained with silver reagent. The fraction numbers correspond to those in Fig. 2.

Partial amino acid sequence of the α -3-*N*-acetylgalactosaminyltransferase

The purified enzyme (50 pmol) was subjected to automated solid-phase Edman degradation. A major sequence was obtained as follows: Xaa-Ser-Leu-Pro-Arg-Met-Val-Tyr-Pro-Gln-Ile-Ser-Val-Leu. The average repetitive yield was estimated to be 90% with 35 pmol of protein susceptible to sequence analysis.

Fig. 4. Effect of pH on the activity of the α -3-*N*-acetylgalactosaminyltransferase

The following 0.1 M buffers were used: sodium cacodylate/HCl, pH 5.35, 6.0 and 6.6; Mops/NaOH, pH 7.0 and 7.4; Tes/NaOH, pH 7.8 and 8.0; Tricine/HCl, pH 8.4; Bicine/NaOH, pH 8.9.

Table 2. Acceptor-substrate-specificity of the purified α -3-*N*-acetylgalactosaminyltransferase

Relative rates were determined at a fixed concentration of 2.5 mM acceptor substrate

Acceptor	Relative rate (%)
2'-Fucosyl-lactose	100
Galactose	0
<i>N</i> -Acetylgalactosamine	0
Lactose	0
3-Fucosyl-lactose	0
Fucosylgalactose	70
Lacto- <i>N</i> -fucopentaose I	83
Lacto- <i>N</i> -fucopentaose II	0
Lacto-difucohexaose I	0
Lacto- <i>N</i> -tetraose	0
Lacto- <i>N</i> -neotetraose	7
Lacto-difucotetraose	0

Effect of pH and Mn^{2+} concentration on the α -3-*N*-acetylgalactosaminyltransferase activity

The purified enzyme had a pH optimum between pH 7.0 and 7.4 (Fig. 4), and maximum enzyme activity was obtained with 10 mM- Mn^{2+} (results not shown).

Acceptor-substrate-specificity of the α -3-*N*-acetylgalactosaminyltransferase

Table 2 summarizes the acceptor-substrate-specificity of the purified enzyme. The results are similar to those obtained for the enzyme in extracts of submaxillary glands (Hearn *et al.*, 1968) and for the enzyme partly purified from human plasma (Yates & Watkins, 1982). The only effective substrates were those containing a subterminal β -galactosyl residue substituted at the O-2 position with L-fucose.

Capacity of the α -3-*N*-acetylgalactosaminyltransferase to make blood-group-B determinants

Substitution of UDP-[^{14}C]galactose for UDP-*N*-acetyl[^{14}C]galactosamine in the standard reaction mixture with 2'-fucosyl-lactose as the acceptor substrate failed to give a detectable product in 10 min. However, when the incubation time was extended to 60 min and the volume of enzyme was increased from 5 μ l to 25 μ l, a radioactive product was formed that was chromatographically indistinguishable from the blood-group-B-active tetrasaccharide Gal α 1-3(Fuc α 1-2)Gal β 1-4Glc. The

Table 3. Inhibitors of α -3-*N*-acetylgalactosaminyltransferase activity

Abbreviation: FMA, fluorescein mercuric acetate.

Inhibitor	Activity remaining (%)
None	100
UDP (50 μ M)	16
UMP (50 μ M)	17
Cyclic UMP (2 mM)	95
Uridine (2 mM)	66
CDP (2 mM)	62
GDP (2 mM)	84
Fluorescein (3 nM)	110
FMA (1.4 nM)	36
FMA (2.8 nM)	0
2-Mercaptoethanol (1 mM), then FMA (1.4 nM)	92
FMA (1.4 nM), then 2-mercaptoethanol (1 mM)	87
UDP (5 μ M), then FMA (1.4 nM)	46
<i>N</i> -Ethylmaleimide (10 mM)	87

amount of B tetrasaccharide synthesized, calculated as pmol of product/min per μ l of enzyme, was approx. 0.5 % of the amount of A tetrasaccharide made by the enzyme with UDP-*N*-acetylgalactosamine as the donor substrate.

Inhibitors of α -3-*N*-acetylgalactosaminyltransferase

The results obtained with various inhibitors are summarized in Table 3. UMP and UDP were strong inhibitors, whereas uridine, cyclic UMP, CDP and GDP gave little inhibition at the concentrations tested. Fluorescein mercuric acetate was a powerful inhibitor, but this inhibition could be prevented, or reversed, by the addition of 2-mercaptoethanol to the reaction mixture either before or after the addition of fluorescein mercuric acetate. Under the same conditions fluorescein had no inhibitory effect. Low concentrations of the inhibitor UDP did not protect the α -3-*N*-acetylgalactosaminyltransferase from inhibition by fluorescein mercuric acetate. *N*-Ethylmaleimide at 10 mM concentration had very little inhibitory action on the enzyme.

DISCUSSION

Human gastric mucosa is a potent source of ABH-blood-group-active glycoproteins (see Kabat, 1956), and the occurrence in microsomal preparations from gastric mucosa of blood-group-A individuals of an *N*-acetylgalactosaminyltransferase that is absent from similar preparations from blood-group-B and blood-group-O individuals was first described by Tuppy & Schenkel-Brunner (1969). The A transferase from human mucosa has now been solubilized and purified 125000-fold with a 44 % recovery.

The Triton X-100-solubilized enzyme was first concentrated and purified approx. 1000-fold by chromatography on UDP-hexanolamine-agarose, a reagent used for the affinity purification of a number of glycosyltransferases that utilize UDP-sugars as donor substrates (Beyer *et al.*, 1981). After this step attempts were made to exploit the unique property of the human α -3-*N*-acetylgalactosaminyltransferase to bind to Sepharose 4B (Whitehead *et al.*, 1974), but the recoveries were extremely low. Several other Sepharose 4B-based chromatographic media were therefore investigated (results not shown); octyl Sepharose CL-4B was found to be the most effective agent, and with this matrix excellent purification was achieved with good recovery (Table 1).

The two major subgroups of blood group A, namely A₁ and A₂, are associated with α -3-*N*-acetylgalactosaminyltransferases in plasma that differ in kinetic properties and pH optima

(Schachter *et al.*, 1973; Topping & Watkins, 1975; Cartron *et al.*, 1978). In A₁ individuals the plasma enzyme has a sharp optimum around pH 6, whereas in A₂ individuals it has a much broader optimum at pH 7–8. No blood samples were available from the cadavers from which the gut tissue was obtained, and hence the A subgroup could not be determined. The pH optimum of 7.0–7.4 found for the purified transferase (Fig. 4) resembles more closely the optimum found for the plasma A₂ enzyme, but several purified gut mucosal preparations from different blood-group-A donors had similar pH optima (results not shown); since only 20 % of blood-group-A individuals belong to the A₂ subgroup (Race & Sanger, 1975) it appears improbable that several samples from unrelated individuals would have originated from A₂ subjects.

In common with many other glycosyltransferases (see Beyer *et al.*, 1981), the α -3-*N*-acetylgalactosaminyltransferase is inhibited by the product of enzymic transfer, UDP, and also by UMP (Table 3). Uridine itself is only slightly inhibitory, indicating that the UDP-*N*-acetylgalactosamine-combining site requires the phosphate group for effective binding. The purified transferase was also inhibited by very low concentrations of the thiol-blocking reagent fluorescein mercuric acetate (Table 3) under conditions in which fluorescein had no inhibitory effect. This inhibition was reversed by the presence of 2-mercaptoethanol, but not by UDP. These results support the inference that fluorescein mercuric acetate inhibits the enzyme by interaction with thiol groups, and that these thiol groups are not directly involved in the nucleotide-substrate-binding site but are important for preserving the conformation of the active site.

The blood-group B gene is believed to be an allelic form of the A gene at the ABO locus and to encode an α -3-D-galactosyltransferase responsible for the formation of B-active determinants in those who inherit this allele. The blood-group-A and -B characters are serologically and genetically so distinct that for some time it was considered that there could be no overlapping function between the enzymes encoded by the allelic genes. More recently, however, it was shown that serum from blood-group-B individuals contains an enzyme that, to a limited extent, utilizes UDP-*N*-acetylgalactosamine to make A structures (Greenwell *et al.*, 1986) and that serum from blood-group-A individuals has a very weak capacity to transfer D-galactose from UDP-D-galactose to make B structures (Yates & Watkins, 1982). The failure to detect any comparable activity in blood-group-O sera supported the inference that the enzymes involved were the glycosyltransferase products of the A and B genes, but the possibility could not be excluded that the weak activities detected resulted from the action of enzymes encoded at other loci. The demonstration that the highly purified α -3-*N*-acetylgalactosaminyltransferase from human gut mucosa has the capacity to transfer D-galactose to an H-active precursor confirms that the A-gene-encoded enzyme can under certain circumstances make blood-group-B determinants.

H.p.l.c. analysis of the purified enzyme on a size-exclusion column gave a native *M_r* for the α -3-*N*-acetylgalactosaminyltransferase of approx. 40000. A major band with a similar *M_r* was observed on SDS/PAGE analysis of the enzymically active h.p.l.c. fractions (Fig. 2). A minor band of *M_r* 65000 was also detected in some of the enzymically active h.p.l.c. fractions (Fig. 2), but it is not clear whether the enzyme exists in two forms or whether the higher-*M_r* species represents an impurity. The *N*-acetylgalactosaminyltransferase purified from human lung by two different procedures (Navaratnam & Watkins, 1989a; Clausen *et al.*, 1989) also had an *M_r* similar to that of the enzyme from gut mucosa. Moreover, in contrast with the recorded value of 100000 for the plasma enzyme (Nagai *et al.*, 1978), the A transferase purified 13×10^6 -fold from plasma (Navaratnam &

Watkins, 1989b) appeared on h.p.l.c. analysis on a gel-exclusion column to have an M_r of 40000 (N. Navaratnam, unpublished work).

Amino acid sequencing of the purified α -3-*N*-acetylgalactosaminyltransferase yielded a putative *N*-terminal sequence Xaa-Ser-Leu-Pro-Arg-Met-Val-Tyr-Pro-Gln-Ile-Ser?-Val-Leu. No significant homologies with known proteins were observed when the putative amino acid sequence was checked in the Leeds-Birkbeck Owl Sequence Database, Version 8.1. Repetition and extension of the sequence analysis should enable confirmation of the data and permit oligonucleotide probes to be synthesized for studies on the molecular genetics of the *ABO* genetic locus.

Since submission of this paper descriptions of the isolation to homogeneity of the α -3-*N*-acetylgalactosaminyltransferase from human lung tissue (Clausen *et al.*, 1990) and the cloning and characterization of DNA encoding this enzyme (Yamamoto *et al.*, 1990) have been published. The first nine amino acid residues in the partial sequence given in the present paper for the human gut α -3-*N*-acetylgalactosaminyltransferase are identical with amino acid residues 65–73 in the sequence deduced by Yamamoto *et al.* (1990) from the cDNA sequence corresponding to the lung *A* transferase.

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