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SYNTHESIS OF METHYL 6-(AMMONIUM 2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSYL PHOSPHATE)-α-D-MANNOPYRANOSIDE AND USE OF THIS COMPOUND FOR THE DETERMINATION OF *N*-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE*

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ABSTRACT

Methyl 6-(ammonium 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate)-α-D-mannopyranoside was synthesized and identified by ^{1}H-n.m.r. and ^{13}C-n.m.r. data, acid hydrolysis, and elemental analysis. It was utilized for the determination of UDP-*N*-acetylglucosamine-1-phosphotransferase in an assay procedure that employed methyl α-D-mannopyranoside as an acceptor. The assay product was identified and characterized by thin-layer chromatography with the title reference compound. The present technique does not require [^{32}P]UDP-*N*-acetylglucosamine, but effectively uses commercially available UDP-[^{14}C]GlcNAc.

INTRODUCTION

It has been well documented that D-mannosyl phosphate residues of lysosomal enzymes serve as an essential component of the recognition marker necessary for the binding of lysosomal enzymes to D-mannose 6-phosphate receptors and the translocation of these enzymes to lysosomes[1,2]. This phosphomannopyranosyl recognition marker is generated by the combined action of the two enzymes UDP-GlcNAc:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (GlcNAc-*P*-transferase) and *N*-acetyl-α-D-glucosamine phosphodiesterase. The former enzyme transfers a 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate residue to a D-mannose residue, and the latter enzyme removes the bound 2-acetamido-2-deoxy-α-D-glucopyranosyl group to generate a phosphoric monoester[3,4].

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However, detailed kinetic, substrate, and product analyses concerning these two enzymes have been restricted because of the limited amounts of phosphorylated, high-mannose type oligosaccharides that are available[4]. Hence, we have initiated a program to chemically synthesize various D-mannosyloligosaccharides sterically related to the high-mannose type units of lysosomal enzymes and couple these oligosaccharides to 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate (**1**). Such compounds will not only serve as substrates for α-GlcNAc-phosphodiesterase but will also be used as reference compounds for identification of the product formed when appropriate oligosaccharides are used as acceptors for GlcNAc-*P*-transferase.

As the first step towards this approach, we describe herein the chemical synthesis of methyl 6-(2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate)-α-D-mannopyranoside (**5**), as its ammonium salt (**6**), which is the expected product to be formed by the action of GlcNAc-*P*-transferase when methyl α-D-mannopyranoside is used as an acceptor. This synthetic compound has been successfully used for the determination of GlcNAc-*P*-transferase by a thin-layer chromatography technique.

RESULTS AND DISCUSSION

Synthesis. — The starting material for the synthesis of methyl 6-(ammonium 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate)-α-D-mannopyranoside (**6**) was 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate[5,6] (**2**), free of the β-D anomer (as shown by n.m.r. data), which was prepared *via* the oxazoline method. Compound **2** showed a clear coloring reaction when the t.l.c. plate was sprayed with the molybdate reagent[7], which generally gives a blue color for phosphates. However, compounds **4** and **6** showed a dull-blue color, and often the color could hardly be seen. The pyridinium salt of **2** was treated with methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**3**) in anhydrous pyridine in the presence of dicyclohexylcarbodiimide (DCC), as the condensing agent, to give the 6-substituted phosphoric diester derivative **4** in 28% yield. Other coupling reagents, such as triisopropylbenzenesulfonyl chloride (TPS) or mesitylenesulfonyl chloride, did not give better yields. Other methods using bis(1,2-dimethylethylene) diphosphate[8] and *N*-phosphoryl-*N'*-methylimidazolium salt[9] were not used because compound **2** was readily available in high yield through the oxazoline intermediate. The protecting groups of **4** were removed by two methods. In a first attempt, **4** was deacetylated with methanolic ammonia, followed by removal of the isopropylidene group with Amberlite IR-120 (H^+) in water[10]. In the second approach, treatment of **4** with trifluoroacetic acid in chloroform gave an acetylated intermediate that, upon further treatment with methanolic ammonia, provided the desired compound **6** in a better yield (53%).

In agreement with previous observations[11], the ^{1}H-n.m.r. spectrum of **6** showed a double doublet ($J_{1',2'}$ 2.5, $J_{1',P}$ 8.0 Hz) at δ 5.31, attributable to the

anomeric proton of the 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate residue; the anomeric proton of the β-D-anomer was observed[11] as triplet ($J_{1',2'}$ = $J_{1',P}$ = 7.9 Hz) at δ 4.96. The doublet of doublets of the anomeric proton (α form) also indicated that the spatial arrangement of H-2–C-2–C-1–O–P was transanti-planar, which is in agreement with the favored conformation of the α-D isomer due to the exoanomeric efect[12,13].

In the ^{13}C-n.m.r. spectrum of **6** (Table I), the signal for C-6 occurred at a lower field (δ 64.31), as compared to that (δ 62.08) for methyl α-D-manno-pyranoside because of the substitution at C-6 with a phosphate group. Also the signals for C-5, -6, and -1′ showed the doublets, $^3J_{C,COP}$ 5.9, $^2J_{C,OP}$ 5.6, and $^2J_{C,OP}$ 5.7 Hz, respectively, typical for the coupling with the phosphorus atom.

Moreover, hydrolysis of **6** with acid gave (t.l.c.) 2-acetamido-2-deoxy-α-D-glucopyranose and methyl α-D-mannopyranoside 6-phosphate, as expected from a (1→6)-linked disaccharide.

Enzyme assay. — The availability of **6** may prove valuable to study the enzymes involved in lysosomal-enzyme targeting. It may be used as a model compound for the assay of enzyme removing 2-acetamido-2-deoxy-α-D-glucopyranosyl blocking groups from lysosomal enzymes, and it can also act as reference compound for identification of the product formed when methyl α-D-mannopyranoside is used

TABLE I

^{13}C-N M.R (25.2 MHz) CHEMICAL SHIFTS [a] (δ)

Atoms	*Methyl α-D-manno-pyranoside*[b]	*Methyl α-D-manno-pyranoside 6-phosphate*[c]	*Compound* **6**
C=O			169.65
C-1	101.89	101.77	100.94
C-2	71.68	71.31	70.39
C-3	71.03	70.71	69.97
C-4	67.88	67.09	66.13
C-5	73.63	72.00	72.46
$^3J_{C,COP}$ (Hz)		7.4	5.9
C-6	62.08	66.00	64.31
$^2J_{C,OP}$ (Hz)		5.0	5.6
C-1′			93.09
$^2J_{C,OP}$ (Hz)			5.7
C-2′			54.13
C-3′			70.39
C-4′			69.97
C-5′			72.91
C-6′			60.67
OCH_3	55.84	55.72	53.83
$COCH_3$			22.67

[a]Downfield from the signal of Me_4Si. Solvent D_2O, except $(CD_3)_2SO$ for **6**. The reference (Me_4Si) was internal for the solution in $(CD_3)_2SO$, and external for the solutions in D_2O. [b]From ref. 18. [c]Unpublished data from this laboratory.

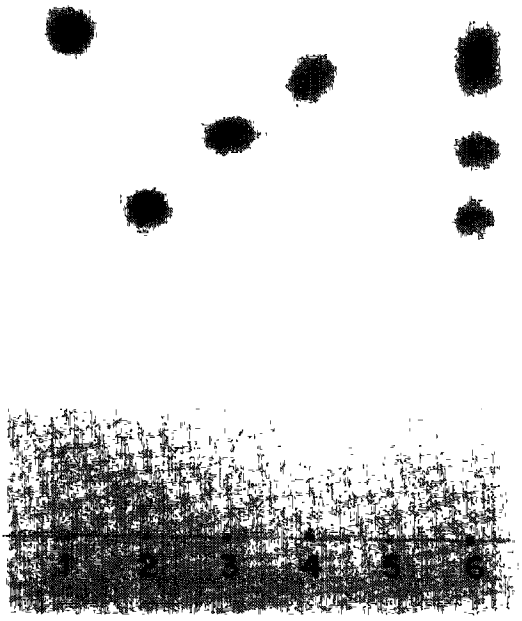


Fig. 1. T.l.c. of the enzymic reaction mixture components with synthetic and possible products of degradation. Lane 1, GlcNAc; 2, compound **1**; 3, compound **6**; 4, methyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside, and 5, UDP-GlcNAc. Lane 6 contains all five compounds. For the conditions, see the Experimental section.

as an acceptor for GlcNAc-*P*-transferase. Thus, we have developed a modified method for the determination of GlcNAc-*P*-transferase with the aid of **6**.

The enzyme GlcNAc-*1*-phosphotransferase is known to catalyze reaction *1*, in which R may be a mannosyl-containing oligosaccharide or a lysosomal enzyme. Interestingly, commercially available methyl α-D-mannopyranoside can also be used as an acceptor for the enzyme. Although the K_m for this acceptor is very high compared to that of the natural acceptor, it is being widely used to screen patients with I-cell diseases[14,15]. In the assay procedure reported earlier, the product of the enzyme was isolated by column chromatography on QAE-Sephadex (Q-25-120)[3,16].

$$\text{UDP-D-GlcNAc} + \alpha\text{-D-Man-}(1\rightarrow\text{OR}) \rightarrow \alpha\text{-D-GlcNAc-}(1\rightarrow P\rightarrow 6)\text{-}\alpha\text{-D-Man-}(1\rightarrow\text{OR}) \qquad (1)$$

In order to develop a convenient assay procedure for the enzyme, it was necessary to establish a chromatographic solvent system that would separate the acceptor methyl α-D-mannopyranoside, reference compound **6**, GlcNAc, and UDP-[^{14}C]GlcNAc, as GlcNAc could be expected as a by-product of phosphorylase degradation of UDP-GlcNAc. For t.l.c. on silica gel, the solvent system (v/v) 6:6:2:1 ethyl acetate–acetic acid–water–ammonium hydroxide was found to be suitable (Fig. 1). When radioactivity in the developed chromatogram of the

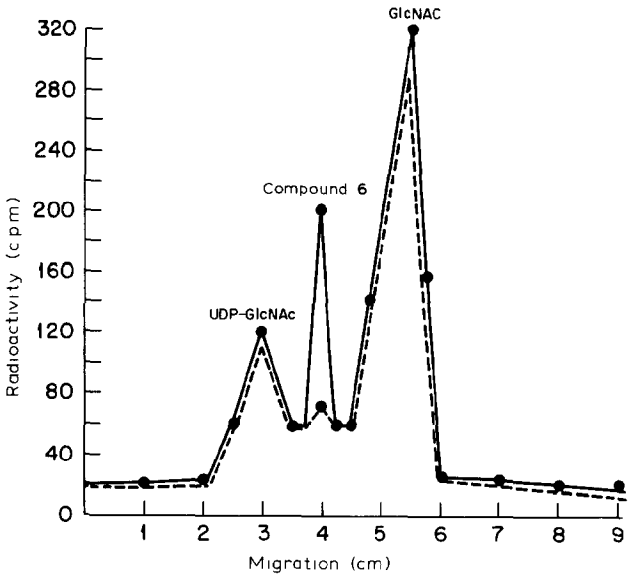


Fig. 2. Characterization, by t.l.c., of the reaction product of liver microsomes GlcNAc-*P*-transferase. After development, 0.5-cm portions of the chromatogram were evaluated for radioactivity content and the final figure was adjusted to show the actual migration of authentic compound **6**. (——) Test and (----) control.

enzyme-reaction mixture was located by scraping the silica gel every half centimeter, a distinct spot was observed that corresponded with the reference compound (Fig. 2). In addition to that spot, the chromatogram also showed radioactivity at two other locations that corresponded with UDP-GlcNAc and GlcNAc. However, no radioactivity was observed in the region corresponding to methyl-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside. Thus, it is unlikely for the enzyme β-GlcNAc-transferase, even if present, to transfer GlcNAc from UDP-GlcNAc to methyl α-D-mannopyranoside. This is not surprising as Vella *et al.*[17] demonstrated the requirement of an (1→3)-linked α-D-mannopyranosyl group for β-GlcNAc-transferase activity. As a result, UDP-[^{14}C]GlcNAc can be used for the study of GlcNAc-*P*-transferase with low-molecular-weight substrates and, thus, avoid [^{32}P]UDP-GlcNAc which has a limited shelf-life. In addition, the availability of an authentic product of enzyme action allows the specific quantitative determination of GlcNAc-*P*-transferase. Consequently, the results obtained by the t.l.c. technique are comparable to that obtained by the column chromatography procedure (Table II), even when UDP-[^{14}C]GlcNAc was used as a donor. The reaction rate of GlcNAc-*P*-transferase was found to be linear with respect to time from 0 to 60 min, and the rate of product formed was also proportional to the amount of liver-microsome protein content, from 10 to 350 μgm, when the enzyme was quantitatively determined by the t.l.c. technique.

The use of t.l.c. techniques for modified assay procedures of some glycosyltransferase has been reported previously from our laboratory[19,20].

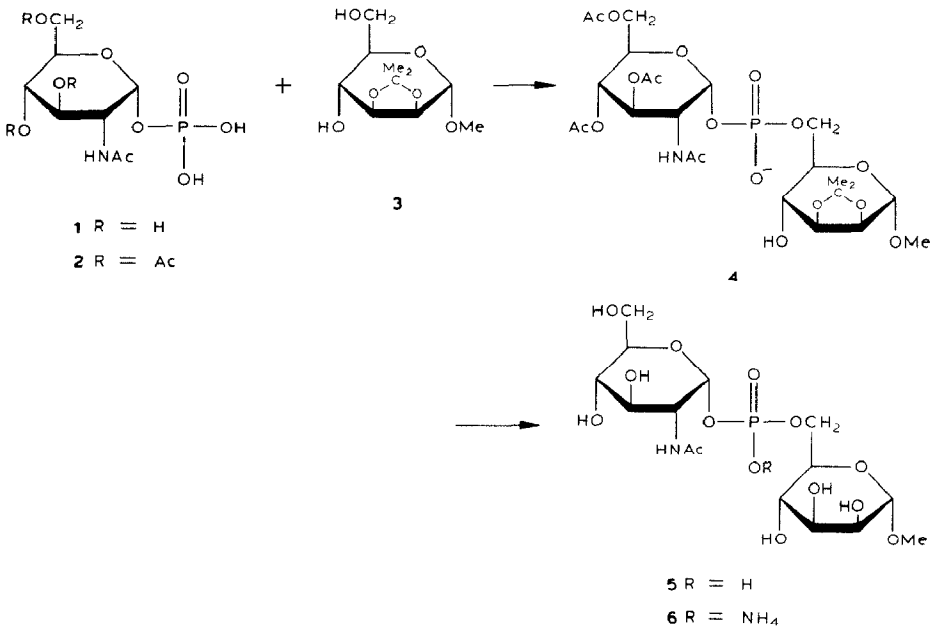
TABLE II

GlcNAc-*P*-TRANSFERASE ACTIVITY OF LIVER MICROSOMES, NORMAL AND ML II FIBROBLASTS

Enzyme source	*Product formed (pmol)/L/protein (mg)*[a]	
	Column chromatography	*T l c.*
Liver	162 ±56	288 ±33
Normal fibroblast (GM 1728)	165 ±34	325 ±61
ML II fibroblast	30 ±8	50 ±32

[a]The activity was determined as described in the Experimental section both by column chromatography and t.l.c. The results are the mean±SE of seven sets of experiments in duplicate

When applied to the examination of GlcNAc-*P*-transferase activity in ML II fibroblasts, the present t.l.c. technique showed 25% of the normal fibroblast activity. This is comparable to the range of activity estimated by column chromatography (Table I). As reported by others[21,22] as well as observed by us, the fibroblasts from patients with I-cell disease (mucolipidosis II) or pseudo Hurler-polydystrophy (mucolipidosis III) are deficient in GlcNAc-1-phosphotransferase. The present method may likely be applied to the quantitative determination of the enzyme in clinical investigations of these diseases. It is also hoped that the availability of the well defined mannosyl phosphate derivative **6** will be useful for the study of the phosphorylated, mannose-specific receptor of the plasma membrane that is in-



volved in recognition and internalization of glycoproteins during metabolic turnover, in addition to its use in lysosomal-enzyme biosynthesis.

EXPERIMENTAL

Materials and methods. — Normal fibroblasts (GM 1728) and ML II fibroblasts were generously provided by Dr. Thomas Shows of the Dept. of Human Genetics at Roswell Park Memorial Institute. UDP-D-[^{14}C]GlcNAc was obtained from New England Nuclear, Boston, MA; methyl α-D-mannopyranoside from Sigma Chemical Co. (St. Louis, MO 63178), Permafluor III from Packard Instrument Co., Inc. (Downers Grove, IL 60515), and Bio-Solv from Beckman Instrument Inc. (Fullerton, CA 92634). All other materials were of the highest quality available commercially. Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at room temperature. Ascending t.l.c. was conducted on plates coated with a 0.25-mm layer of Silica gel 60 PF-254 (E. Merck, Darmstadt, Germany). The components were located by spraying the plate with 5% H_2SO_4 in ethanol, or the molybdate reagent[7] for phosphates, and heating. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN 37821). N.m.r. spectra were recorded with a Varian XL-100 instrument at 100 MHz with Me_4Si as the internal standard.

*Methyl 6-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-*D*-glucopyranosyl phosphate)-2,3-*O*-isopropylidene-α-*D*-mannopyranoside* (**4**). — 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate[5,6] (**2**, 0.95 g, 2.2 mmol) was dried by several co-distillations with anhydrous pyridine under reduced pressure <30°. To the resulting residue was added dry Amberlite IR-120 (pyridinium$^+$) ion-exchange resin (3 g) or Dowex 50 (pyridinium$^+$) ion-exchange resin[23] and methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[24,25] (**3**, 1.26 g, 5.4 mmol). The mixture was further rendered anhydrous by repeated evaporation with added dry pyridine (3 × 5 mL). A solution of the residue in anhydrous pyridine (30 mL) was stirred while dicyclohexylcarbodiimide (DCC, 2.23 g, 10.8 mmol) in dry pyridine (30 mL) was introduced dropwise over a 15-min period. The resultant mixture was stirred at room temperature protected from light for 3 days. A dropwise addition of water (60 mL) was made to a well cooled reaction mixture and stirring continued for 4 h. Resin and insoluble dicyclohexylurea were filtered off and the filtrate was evaporated to give a syrup. The remaining solvent was co-evaporated with toluene (3 × 5 mL) and the residue, dissolved in 40:1 (v/v) chloroform–methanol (20 mL) was applied to a dry silica gel column (3 × 80 cm), which was eluted with the same solvent (800 mL) to remove unreacted **3** and DCC by-products. Further elution with 4:1 (v/v) chloroform–methanol gave, on evaporation, **4** as a glossy white solid (0.36 g, 27.7%), $[\alpha]_D^{20}$ +43.8° (*c* 0.76, chloroform); t.l.c. (65:15:1 chloroform–methanol–water) R_F 0.15; ^{1}H-n.m.r. ($CDCl_3$): δ 5.20 (dd, 1 H, $^3J_{1',2'}$ 3, $^3J_{1',P}$ 8 Hz, H-1'), 4.86 (s, H-1), 4.22–3.43 (unresolved signals, 14 H), 3.37 (s, 3 H, OCH_3),

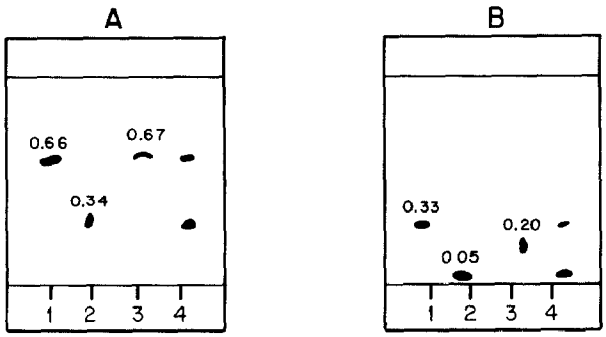


Fig 3. T.l.c. of the products of acid hydrolysis of compound **6**: (A) In 3·1:1 ethanol–conc ammonium hydroxide–water, (B) in 7:1:2 2-propanol–conc. ammonium hydroxide–water. Lane 1, GlcNAc; 2, methyl α-D-mannopyranoside 6-phosphate; 3, compound **6**, and 4, hydrolysis product

2.00 (m, 12 H, 3 OAc and NAc), 1.50 (s, 3 H, CH_3), and 1.30 (s, 3 H, CH_3).

Methyl 6-(ammonium 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate)-α-D-mannopyranoside (**6**). — A mixture of compound **4** (300 mg) in chloroform (4 mL), trifluoroacetic acid (0.2 mL), and water (0.05 mL) was stirred for 3.5 h at room temperature. The mixture was evaporated, final traces of trifluoroacetic acid and water were removed by repeated co-evaporation with toluene, and the residue was dried *in vacuo* to give methyl 6-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate)-α-D-mannopyranoside, t.l.c. (7:1:2 2-propanol–conc. ammonium hydroxide–water) R_F 0.68. Without further purification, this compound was treated with methanol (50 mL) saturated with dry ammonia at 0–5° (15M) in a sealed flask for 24 h at room temperature. The resultant mixture was evaporated to dryness, and the residue dissolved in the minimal amount of methanol (~10 mL) and applied onto a silica gel column (1 × 20 cm), which was eluted with 7:1:2 2-propanol–conc. ammonium hydroxide–water. After elution of fast moving components, the fractions showing on t.l.c. R_F 0.10 in 7:1:2 2-propanol–ammonia–water, 0.38 in 3:1:1 ethanol–conc. ammonium hydroxide–water, and 0.41 in 3:3:1:1 ethyl acetate–acetic acid–conc. ammonium hydroxide–water were pooled and evaporated to dryness. Crystallization from methanol gave **6** as white crystals (122 mg, 53.1%), m.p. 150–151°, $[\alpha]_D^{20}$ +75.6° (*c* 0.37, methanol); ^{1}H-n.m.r. (D_2O): δ 5.31 (dd, $^3J_{1',2'}$ 2.5, $^3J_{1',P}$ 8.0 Hz, H-1'), 4.97 (s, H-1), 4.20–3.38 (unresolved signals, 24 H), 3.27 (s, 3 H, OCH_3), and 1.91 (s, 3 H, NAc); ^{13}C-n.m.r.: see Table I.

Anal. Calc. for $C_{15}H_{31}N_2O_4P \cdot 1.5\ H_2O$: C, 34.55; H, 6.57; N, 5.37; P, 5.94. Found: C, 34.58; H, 6.57; N, 5.28; P, 6.12.

Acid hydrolysis of **6**. — Compound **6** (2 mg) was dissolved in 10mM HCl (50 μL) and heated for 30 min at 100° to yield 2-acetamido-2-deoxy-α-D-glucopyranose and methyl α-D-mannopyranoside 6-phosphate, which were identified by t.l.c. comparison with authentic compounds (Fig. 3).

Enzyme source. — Normal fibroblasts (GM 1728) and ML II fibroblasts from patients with I-cell disease, maintained in Dulbecco's modified Eagle medium Alpha (Gibco Laboratories Grand Island, NY 14072) containing 7.5% bovine serum and 7.5% fetal bovine serum, were harvested by scraping with a rubber policeman in 20mM Tris-HCl, pH 7.45, containing 155mM NaCl. Rat liver microsomes were prepared according to Waheed *et al.*[26] from female Weistar rats (200–250 g). The microsomal fractions were washed with 20mM Tris · HCl, pH 7.45, containing 155mM NaCl.

Enzyme assay. — GlcNAc-*P*-transferase was assayed according to the conditions described by Waheed *et al.*[27] with minor modifications. Harvested fibroblasts or liver microsomes were homogenized in a minimal volume of 80mM Tris · HCl, pH 7.45, containing 1.2% (w/v) Triton X-100, 0.25mM dithiothreitol, 0.16mM leupeptin, and 3.2mM iodoacetamide by use of a Biosonic Sonicator for 90 s.

A standard incubation mixture contained UDP-[^{14}C]GlcNAc (25 pmol, 297 mCi/mol), UDP-GlcNAc (7.5 nmol), $MgCl_2$ (0.25 μmol), $MnCl_2$ (0.25 μmol), CDP-choline (0.5 μmol), ADP (0.1 μmol), methyl α-D-mannopyranoside (5.0 μmol) and the just mentioned homogenate (30 μL containing 100–300 μg of protein) was added to give a final volume of 50 μL. The control-assay tubes contained either water or methyl α-D-galactopyranoside instead of methyl α-D-mannopyranoside. The incubations at 37° for 30 min were terminated by adding either 95% ethanol (50 μL) (for t.l.c. application) or 40mM EDTA (for column application).

T.l.c. separation and estimation of the reaction product. — The assay mixtures were centrifuged at 6000 *g* for 15 min at 4° to remove the precipitated protein. The supernatant solution containing low-molecular-weight components and products of the reaction was used for estimation of the disaccharide phosphate formed. An aliquot (20 μL) was deposited on t.l.c. silica gel plates (50-mm thickness) along with the reference compound **6**. The chromatogram was developed with (v/v) 6:6:2:1 ethyl acetate–acetic acid–water–conc. ammonium hydroxide as the solvent system. The lane containing the reference compound alone was sprayed with 5% H_2SO_4 in 95% ethanol and heated at 110°. The spot corresponding to the reference compound in the lane containing the enzyme-reaction product was scraped and quantitatively determined by liquid-scintillation spectrophotometry in (v/v) 17:2:1 toluene–Permafluor III–Bio-Solv. (10 mL). Routinely, all enzyme assays were carried out in duplicate with four different control assays. These included a zero-time control in which assay components were assembled at 4° and immediately terminated, and assays without enzyme, substrate, or acceptor. The enzyme activity is expressed as pmol of product formed/h/mg of homogenate-protein.

Column-chromatography method. — Quantitative determination of the reaction product was carried out according to the established procedure[3]. EDTA-treated tubes were heated for 5 min in a boiling-water bath, diluted with 2mM Tris base (1 mL) and centrifugated at 6000 *g* for 15 min. The supernatant solution was applied to a column (0.5 cm × 1 cm) of QAE-Sephadex (Q-25-120), equilibrated

with 2mM Tris, and then washed with 2mM Tris (4 mL). Further elution with 30mM NaCl (6 mL) gave the disaccharide phosphate product. The eluant was lyophilized and the residue dissolved in water (1 mL). The radiolabelled product in the previously described "cocktail" was counted with a Beckman LS 9000 liquid-scintillation system.

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REFERENCES

1 W S SLY AND H. D. FISCHER, *J. Cell Biochem* , 18 (1982) 67–85.
2 M. REITMAN AND S KORNFELD, *J. Biol. Chem.*, 256 (1981) 4275–4281.
3 M. REITMAN AND S KORNFELD, *J. Biol Chem.*, 256 (1981) 11977–11980
4 A VARKI AND S. KORNFELD, *J Biol. Chem* , 255 (1980) 8398–8401.
5 T. YAMAZAKI, C. D. WARREN, A HERSCOVICS AND R W JEANLOZ, *Can. J Chem.*, 59 (1981) 2247–2252.
6 C. D WARREN, A HERSCOVICS AND R. W. JEANLOZ, *Carbohydr Res* , 61 (1978) 181–196.
7 J. C. DITTMER AND R. L LESTER, *J Lipid Res* , 5 (1984) 126–127
8 F RAMIREZ, S B. MANDAL, AND J F. HARECEK, *J. Am. Chem. Soc.*, 104 (1982) 5483–5486.
9 H TAKAKU, Y. SHIMADA, AND H. OKA, *Chem. Pharm. Bull* , 21 (1973) 1844–1845.
10 T A. W KOERNER, JR., R J. VOLL, AND S. YOUNATHAN, *Carbohydr. Res.*, 70 (1979) 165–168.
11 M. INAGE, H. CHAKI, S KUSUMOTO, AND T. SHIBA, *Tetrahedron Lett.*, 22 (1981) 2281–2284.
12 O. OGAWA AND A. SETA, *Carbohydr Res* , 110 (1982) c1–c4.
13 R U LEMIEUX AND S. KOTO, *Tetrahedron*, 30 (1974) 1933–1944
14 O T MUELLER, N. K HONEY, L E LITTLE, A. L. MILLER AND T B. SHOWS, *J Clin. Invest.*, 72 (1982) 1016–1023.
15 N. K. HONEY, O. T. MUELLER, L. E. LITTLE, A. L. MILLER AND T. B. SHOWS, *Proc. Natl. Acad. Sci. U S.A* , 79 (1982) 7420–7424.
16 Y BEN-YOSEPH, M. S. BAYLERIAN, AND H. L. NADLER, *Anal. Biochem.*, 142 (1984) 297–304
17 G J. VELLA, H. PAULSEN AND H. SCHACHTER, *Can. J. Biochem. Cell Biol.*, 62 (1984) 409–417.
18 S. S RANA, J J BARLOW AND K. L. MATTA, *Carbohydr. Res.*, 96 (1981) 79–85.
19 R. A. DICIOCCIO, J J BARLOW, AND K L MATTA, *Clin. Chim Acta*, 108 (1980) 41–48.
20 D A. SYKES, S. S RANA, J. J. BARLOW, AND K. L. MATTA, *Carbohydr Res* , 112 (1983) 221–232
21 A. HASLIK, A. WAHEED AND K VON FIGURA, *Biochem. Biophys Res Commun* , 98 (1981) 761–767
22 M REITMAN, A. VARKI AND S. KORNFELD, *J Clin Invest.*, 67 (1981) 1574–1579
23 R LOHRMANN, D SOLL, H HAYATSU, E OHTSUKA AND H G KHORANA, *J Am Chem Soc.*, 88 (1966) 819–829
24 M E. EVANS AND F W. PARISH, *Carbohydr Res* , 54 (1977) 105–114.
25 R G. AULT, W. N. HAWORTH, AND E. L. HIRST, *J. Chem Soc* , (1935) 517–518.
26 A WAHEED, A. HASLIK, AND K. VON FIGURA, *J. Biol. Chem.*, 256 (1981) 5717–5721.
27 A WAHEED, A HASLIK, M CANTZ AND K VON FIGURA, *Hoppe-Seyler's Z. Physiol Chem.*, 363 (1982) 169–178