Formation of UDP-2-Acetamido-2-Deoxy-L-Galactose and UDP-2-Acetamido-2-Deoxy-L-Galacturonic Acid by *Pseudomonas aeruginosa*

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The O-specific polysaccharide from the lipopolysaccharide of *Pseudomonas aeruginosa* NCTC 8505 (IATS serotype O:3) consists of a tetrasaccharide repeating unit comprising L-rhamnose, N-acetyl-D-glucosamine (GlcNAc), bacillosamine, and N-acetyl-L-galactosaminuronic acid (L-GalNAcA) (Y. Tahara and S. G. Wilkinson, Eur. J. Biochem. 134:299–304, 1983). Incubation of GlcN or UDP-GlcNAc with cell extracts or EDTA-treated cells of *P. aeruginosa* NCTC 8505 yielded a mixture of UDP-ManNAc, UDP-GalNAc, UDP-GlcNAcA, UDP-ManNAcA, UDP-L-GalNAc, and UDP-L-GalNAcA. The last two compounds, here identified for the first time, may be intermediates in the synthesis of the L-GalNAcA moiety of the O-specific portion of the lipopolysaccharide of *P. aeruginosa*.

The O-polysaccharide chains of a number of gram-negative organisms contain unusual sugars such as dideoxyhexoses and hexosaminuronic acids. The biosynthesis of some of these carbohydrate residues (e.g., dideoxyhexoses) has been elucidated (14); however, nothing is known about the origin of certain others, in particular, L-hexosaminuronic acids. Commenting on this in a recent review, V. N. Shibaev observed that it seems difficult to suggest a reasonable pathway for the biosynthesis of L-galactosaminuronic acid (L-GalNA), which is found in some *Pseudomonas aeruginosa* lipopolysaccharides (14).

L-GalNA, found in the repeating unit of the backbone chain of the O polysaccharide of a number of different strains of *P. aeruginosa* (10, 11, 15), may be part of the antigenic determinants of the O polysaccharides from specific serogroups of *P. aeruginosa* (10). Since the most thoroughly characterized lipopolysaccharide which contains L-GalNA is that from *P. aeruginosa* NCTC 8505 (IATS serotype O:3), we chose this strain to study the origin of this compound. We have isolated two new nucleoside diphosphate sugars, UDP-L-GalNAc and UDP-L-GalNAcA, which are formed from GlcN or UDP-GlcNAc by EDTA-treated cells and cell extracts of *P. aeruginosa* NCTC 8505. It appears likely that these compounds are intermediates in the synthesis of the O-antigen portion of the lipopolysaccharide.

MATERIALS AND METHODS

Chemicals. [1-¹⁴C]GlcN was purchased from American Radiochemicals, St. Louis, Mo. It was converted to UDP-[¹⁴C]GlcNAc by the method of Lang and Kornfeld (12). [¹⁴C]GlcNAc was prepared by treating UDP-[¹⁴C]GlcNAc with a mixture of phosphodiesterase and alkaline phosphatase. UDP-Glc, UDP-GlcA, UDP-GalNAc, GlcA, GalN, GlcN, ManN, GalNAc, GlcNAc, and ManNAc were from Sigma Chemical Co., St. Louis, Mo. GlcA-1-P was prepared by treating UDP-GlcA with phosphodiesterase; the same procedure was used to prepare GlcNAc-1-P from UDP-

GlcNAc. All other chemicals were the purest commercially available. All enzymes were purchased from Sigma.

Preparation of bacteria. *P. aeruginosa* NCTC 8505 was grown at 34°C in nutrient broth to the mid-log phase and harvested by centrifugation at 5,000 × g for 30 min. After being washed by suspension in ice-cold 0.85% NaCl and centrifugation, some batches of cells were suspended in 0.01 M disodium EDTA (0.01 of the original culture volume) and stored frozen at –20°C (EDTA cells). EDTA treatment and freezing make cells permeable to nucleotides and nucleotide sugars without disturbing the ordered state of biosynthetic enzymes involved in monomer activation, nucleotide-sugar interconversions, and polysaccharide synthesis (7). Other batches were suspended in ice-cold 50 mM Tris, pH 7.5 (0.01 of the original culture volume), and disrupted by two passages through a French pressure cell at 0 to 4°C and 20,000 lb/in². The supernatant fluid obtained by centrifugation at 10,000 × g for 30 min (cell extract) was retained and stored at –20°C, and the pellet was discarded.

Paper and thin-layer chromatography and electrophoresis. Paper chromatography and electrophoresis were done on EDTA-washed no. 1 or 3MM filter paper (Whatman, Inc., Clifton, N.J.). Thin-layer chromatography was done on cellulose (0.16-mm-thick) chromatogram sheets (Eastman Kodak Co., Rochester, N.Y.) with a 254-nm fluorescence indicator or on polyethylenimine (PEI) cellulose (0.1-mm-thick) MN 300 sheets with a fluorescence indicator (Machery-Nagel, Düren, Federal Republic of Germany). Chromatograms were developed in the following solvent systems: A, 1 M ammonium acetate (pH 7.5)–95% ethanol (2:3, vol/vol); B, n-butanol–pyridine–water (6:4:3, vol/vol/vol); C, ethyl acetate–pyridine–acetic acid–water (5:5:1:3, vol/vol); D, 0.4 M guanidinium hydrochloride; and E, a solution of 5.6 M ammonium sulfate, 35 mM ammonium bisulfate, and 1 mM EDTA. Buffers used for electrophoresis were F, 0.2 M ammonium formate (pH 3.6); G, 0.2 M ammonium acetate (pH 6.0); H, 50 mM sodium tetraborate (pH 10.3); and J, a solution made by adding 6 g of Na₂B₄O₇ · 10H₂O, 3 g of boric acid, and 25 ml of ethylene glycol to 70 ml of H₂O. Nucleotides were located on paper and thin layers by viewing at 254 nm. Glycosamines were located by spraying

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FIG. 1. Initial separation of reaction mixture components. See text for details.

with 0.2% ninhydrin in ethanol and heating at 100°C for 2 to 5 min. *N*-acetylated glycosamines were located by spraying with 0.1 M sodium hydroxide in ethanol-*n*-propanol (3:2, vol/vol), heating at 100°C for 5 min, and viewing the resultant fluorescent spot(s) at 360 nm. In addition, the ammoniacal silver nitrate spray method of Trevelyan et al. (16) was used to detect carbohydrates. Organic phosphates were visualized by the method of Hanes and Isherwood (8). Radioactive compounds were detected by autoradiography on Kodak XAR-5 no-screen X-ray film; they were eluted from paper and cellulose thin layers with water and from PEI thin layers with 0.5 M ammonium carbonate (pH 8.5). For scintillation counting, samples were dissolved in Scintiverse II (Fisher Scientific Co., Pittsburgh, Pa.) and radioactivity was determined with a Delta 300 scintillation spectrometer (Searle Analytic, Chicago, Ill.).

Chemical and enzymatic treatment of reaction products. GalNAc and UDP-GalNAc were oxidized to their 6-aldehyde derivatives by treatment with galactose oxidase (EC 1.1.3.9), which is specific for the *D*-galactose configuration (1). Typical reaction mixtures contained [¹⁴C]GalNAc or UDP-[¹⁴C]GalNAc, 200 µg of GalNAc or UDP-GalNAc, 20 IU of galactose oxidase, 100 IU of peroxidase (EC 1.11.1.7), and 20 µl of *o*-toluidine (0.5% in methanol) in a total volume of 100 µl of 15 mM potassium phosphate buffer, pH 7.0. After 20 h at 25°C, more galactose oxidase and peroxidase were added and incubation was continued until all the GalNAc or UDP-GalNAc was completely oxidized. UDP-*N*-acetyl hexosaminuronic acids were converted to the corresponding UDP-*N*-acetyl-glycosamines by esterification with diazomethane in ether followed by reduction with sodium borohydride as described by Biely and Jeanloz (3).

Preparation and initial separation of reaction products. Typical reaction mixtures contained 3×10^6 cpm of UDP-[¹⁴C]GlcNAc (55 Ci/mmol; 3 µmol), 5 µl of 0.2 M NAD, 1 µl

of 1 M MgCl₂, 50 µl of 0.1 M Tris hydrochloride (pH 8.2), and 150 µl of EDTA cells or cell extract (350 µg of protein) (5). Incubation was at 34°C for 4 to 5 h. When [¹⁴C]GlcN (3×10^6 cpm) was used as the substrate, 5 µl of 0.1 M UTP and 10 µl of 0.1 M ATP were added to the reaction mixtures.

All reaction mixtures were processed in an essentially identical fashion. A typical procedure for a reaction mixture containing UDP-[¹⁴C]GlcNAc and cell extract was as follows. After incubation, the reaction mixture was held at 100°C for 5 min and clarified by centrifugation. The supernatant fluid was dried by lyophilization and taken up in 1 ml of 1 M ammonium bicarbonate, pH 7.5. In order to convert any glycosyl phosphates present to free glycoses, alkaline phosphatase (EC 3.1.3.1) (15 U), which hydrolyzes phosphomonoesters but not phosphodiester, was added. After incubation at 37°C for 1 h, the solution was held at 100°C for 5 min and centrifuged, the supernatant fluid was subjected to electrophoresis in buffer F on Whatman 3MM paper at 15 V/cm for 4 h, and radioactive compounds were detected by autoradiography.

RESULTS

When EDTA cells or cell extracts were incubated as described with UDP-[¹⁴C]GlcNAc, the product contained two major radioactive bands which migrated toward the cathode in electrophoretograms. The rapidly migrating band, band II (3% of the input radioactivity), had a mobility similar to that of UDP-GlcA; the slowly migrating band, band I (19% of the input radioactivity), had a mobility equal to that of UDP-GlcNAc (Fig. 1). These two bands were examined as follows.

Band I was eluted and subjected to chromatography on PEI with buffer J. Two major radioactive bands were ob-

TABLE 1. Characterization of components I_f and I_s

Component	Treatment	Separation technique ^a (solvents or buffers)	Product(s) ^b
I_f	Phosphodiesterase	Electrophoresis (F), TLC (A)	<i>N</i> -Acetyl-[¹⁴ C]hexosamine-1-P
	Phosphodiesterase and alkaline phosphatase	TLC (B)	<i>N</i> -Acetyl-[¹⁴ C]hexosamine
		PEI ^c (D and E)	Uridine
	2 M HCl, 100°C, 40 min	TLC ^d (B)	[¹⁴ C]GlcNAc and [¹⁴ C]ManNAc
I_s		TLC ^e (C)	[¹⁴ C]GlcN and [¹⁴ C]ManN
	Phosphodiesterase	Electrophoresis (F), TLC (A)	<i>N</i> -Acetyl-[¹⁴ C]hexosamine-1-P
	Phosphodiesterase and alkaline phosphatase	TLC (B)	[¹⁴ C]GalNAc
		PEI ^c (D and E)	Uridine
	2 M HCl, 100°C, 40 min	TLC (C)	[¹⁴ C]GalN
	Galactose oxidase-peroxidase	PEI (J)	UDP-6-aldehydo-[¹⁴ C]GalNAc (35%) and UDP-L-[¹⁴ C]GalNAc (65%)

^a TLC, Cellulose thin-layer chromatography; PEI, PEI thin-layer chromatography.^b Identified by cochromatography with authentic compounds.^c Two-dimensional chromatography (4).^d Development repeated three times on sodium tetraborate-washed plates.^e Development repeated three times.

tained: a rapidly migrating band, band I_f , of mobility equal to that of UDP-GlcNAc, and a less rapidly migrating band, band I_s , of mobility equal to that of UDP-GalNAc (Fig. 1). (An additional radioactive band of minor intensity which migrated ahead of I_f , noted on some PEI thin-layer chromatograms, was not investigated further.) The chromatographic mobility of I_f was not changed by treatment with alkaline phosphatase at pH 7.5, demonstrating the absence of phosphomonoesters. Hydrolysis of I_f with phosphodiesterase (EC 3.1.4.1), which hydrolyzes phosphodiester but not phosphomonoesters, yielded a radioactive compound with electrophoretic mobility at pH 3.6 and the cellulose thin-layer chromatographic mobility (solvent A) of GlcNAc-1-P (Table 1); these results are consistent with the presence of phosphodiester (e.g., nucleoside diphosphate glycoses) in I_f . Hydrolysis for 2 h at 37°C with a mixture of alkaline phosphatase and phosphodiesterase (15 U of each in 1 M ammonium bicarbonate) converted I_f to (i) a radioactive component with the cellulose thin-layer-chromatographic mobility (solvent B) of GlcNAc and (ii) a single nonradioactive, UV quenching compound with a mobility like that of uridine in two-dimensional chromatography on a PEI thin layer in solvents D and E (4). Chromatography of the hydrolysate of I_f on cellulose thin-layer plates prewashed with 15 mM sodium tetraborate and developed three times in solvent B (a procedure that separates GlcNAc and ManNAc but not ManNAc and GalNAc) showed the presence of two radioactive components with chromatographic mobilities like those of GlcNAc and ManNAc. That these compounds were in fact GlcNAc and ManNAc was confirmed as follows. I_f was hydrolyzed in 2 M HCl at 100°C for 40 min, a procedure which converts the *N*-acetyl glycosaminyl moieties of UDP-*N*-acetyl glycosamines to free glycosamines. The products were separated by cellulose thin-layer chromatography in solvent C (development repeated three times). This chromatographic procedure effects unequivocal separation of GalN, GlcN, and ManN. Only [¹⁴C]GlcN and [¹⁴C]ManN were present in the hydrolysate. Thus, although I_f could not be separated into individual components by chromatography or electrophoresis, it contained a mixture of UDP-[¹⁴C]GlcNAc and UDP-[¹⁴C]ManNAc. These results are summarized in Table 1.

The PEI thin-layer-chromatographic mobility of I_s was not affected by treatment with alkaline phosphatase. In two-

dimensional chromatography on PEI (4), I_s yielded a single radioactive component which cochromatographed with authentic UDP-GalNAc. Phosphodiesterase treatment converted I_s to a radioactive compound with the paper-electrophoretic (buffer F) and cellulose thin-layer-chromatographic (solvent A) mobilities of GalNAc-1-P. Treatment with a mixture of alkaline phosphatase and phosphodiesterase yielded a single radioactive component with the paper- and cellulose thin-layer-chromatographic mobilities and electrophoretic mobility (buffer H) of GalNAc and a single nonradioactive component with a mobility like that of uridine as shown by two-dimensional PEI thin-layer chromatography with solvents D and E (4). Acid hydrolysis yielded a radioactive compound identical in its cellulose thin-layer-chromatographic properties to GalN (in solvent C). Thus, band I_s consisted of UDP-[¹⁴C]GalNAc (Table 1).

To determine the configuration of the glycosyl moiety, the UDP-[¹⁴C]GalNAc was diluted with authentic UDP-GalNAc and subjected to oxidation with galactose oxidase as described above. After exhaustive treatment with a mixture of galactose oxidase and peroxidase, all the added UDP-GalNAc was converted to UDP-6-aldehydo-GalNAc, as shown by the disappearance of a UV quenching band at the level of UDP-GalNAc and the appearance of a slower-running material (UDP-6-aldehydo-GalNAc) (PEI thin-layer chromatography with buffer J). The UDP-[¹⁴C]GalNAc, on the other hand, was only partially oxidized; 35% of it was converted to the 6-aldehydo derivative, with the remainder being unaffected by galactose oxidase. Exhaustive oxidation of the [¹⁴C]GalNAc produced by treatment of the UDP-[¹⁴C]GalNAc with phosphomonoesterase and phosphodiesterase yielded similar results, with 65% of the [¹⁴C]GalNAc being resistant to oxidation. These results show that band I_s contained both UDP-[¹⁴C]GalNAc and UDP-L-[¹⁴C]GalNAc (Table 1). The relative amounts of the components in band I were as follows: UDP-GlcNAc, 60%; UDP-ManNAc, 30%; UDP-GalNAc, 4%; and UDP-L-GalNAc, 6%.

Band I was eluted and resubmitted to electrophoresis at pH 6.0 (buffer G) on Whatman 3MM filter paper at 15 V/cm for 4 h. Two radioactive bands were obtained: a rapidly migrating band, band II_f , and a less rapidly migrating band, band II_s (Fig. 1). Upon prolonged paper electrophoresis (buffer G), band II_s sometimes seemed to contain two components, but no clear-cut separation could be consis-

TABLE 2. Characterization of components II_s and II_f

Component	Treatment	Separation technique ^a (solvents or buffers)	Product(s) ^b
II _s	Esterification and reduction	PEI (J)	II _{s-1} , UDP-[¹⁴ C]GlcNAc, II _{s-2} , UDP-[¹⁴ C]GalNAc
II _{s-1}	Phosphodiesterase and alkaline phosphatase 2 M HCl, 100°C, 40 min	TLC (B) PEI (D and E) TLC ^d (C)	[¹⁴ C]GlcNAc Uridine [¹⁴ C]GlcN
II _{s-2}	Phosphodiesterase and alkaline phosphatase 2 M HCl, 100°C, 40 min Galactose oxidase-peroxidase	TLC (B) PEI ^c (D and E) TLC ^d (C) PEI (J)	[¹⁴ C]GalNAc Uridine [¹⁴ C]GalN UDP-L-[¹⁴ C]GalNAc
II _f	Phosphodiesterase Phosphodiesterase and alkaline phosphatase Esterification and reduction	Electrophoresis (F) Electrophoresis (H) PEI (J)	[¹⁴ C]hexuronic acid-1-P [¹⁴ C]hexuronic acid II _{f-r} , UDP-[¹⁴ C]ManNAc
II _{f-r}	Phosphodiesterase and alkaline phosphatase 2 M HCl, 100°C, 40 min	TLC ^c (B) TLC ^d (C)	[¹⁴ C]ManNAc [¹⁴ C]ManN

^a TLC, cellulose thin-layer chromatography; PEI, PEI thin-layer chromatography.

^b Identified by cochromatography with authentic compounds.

^c Development repeated three times on sodium tetraborate-washed plates.

^d Development repeated three times.

^e Two-dimensional chromatography (4).

tently achieved. Therefore, all the material present in band II_s was esterified and reduced, and the reduced product was fractionated by PEI thin-layer chromatography in buffer J. Two radioactive components with the chromatographic mobilities of UDP-GlcNAc, II_{s-1} (70%), and of UDP-GalNAc, II_{s-2} (30%), were obtained. II_{s-1} was identical to UDP-GalNAc by the criteria used previously to identify UDP-[¹⁴C]GlcNAc (Table 2). II_{s-2} was electrophoretically and chromatographically identical to UDP-GalNAc and after enzymatic hydrolysis yielded a single radioactive component with a chromatographic mobility like that of GalNAc. Acid hydrolysis yielded a compound with a mobility like that of GalN. Upon exhaustive treatment with galactose oxidase-peroxidase, all the radioactive UDP-[¹⁴C]GalNAc resisted oxidation under conditions which afforded complete oxidation of UDP-GalNAc present in the same reaction mixture. These results show that II_{s-2} comprised UDP-L-GalNAc only and are consistent with the presence in II_s of UDP-[¹⁴C]GalNAcA in addition to UDP-[¹⁴C]GlcNAcA.

Upon treatment with phosphodiesterase, the more rapidly moving band, II_f, yielded a radioactive component with an electrophoretic mobility (buffer F) close to that of GlcA-1-P. Treatment of II_f with a mixture of phosphodiesterase and alkaline phosphatase yielded a radioactive component with electrophoretic mobility (buffer H) similar to that of GlcA. Esterification and reduction of II_f produced a radioactive compound (II_{f-r}) which had the PEI thin-layer chromatographic and paper electrophoretic properties of UDP-ManNAc. Hydrolysis of II_{f-r} with a mixture of phosphodiesterase and alkaline phosphatase yielded only [¹⁴C]ManNAc, which was characterized by the methods described above. These results show that II_f was UDP-[¹⁴C]ManNAcA. In summary, the relative amounts of UDP-*N*-acetyl hexosaminuronic acids present in the reaction products were as follows: UDP-ManNAcA, 70%; UDP-GlcNAcA, 20%; and UDP-L-GalNAcA, 10%.

When [¹⁴C]GlcN was used as the substrate with EDTA cells, 8% of the input radioactivity was recovered as UDP-*N*-acetyl hexosamines and UDP-*N*-acetyl hexosaminuronic acids; the latter represented 25% of the UDP sugars. The

relative amounts of UDP-*N*-acetyl hexosaminuronic acids were as follows: UDP-ManNAcA, 85%; UDP-GlcNAcA, 10%; and UDP-L-GalNAcA, 5%.

Qualitatively similar patterns of nucleotide sugar products were obtained in reaction mixtures in which EDTA cells were replaced by cell extract or in which UDP-[¹⁴C]GlcNAc was replaced by [¹⁴C]GlcN and UTP plus ATP. No products were formed, however, when UTP or ATP was omitted from reaction mixtures containing [¹⁴C]GlcN or when [¹⁴C]GlcN was replaced by [¹⁴C]GlcNAc. Omission of NAD from any reaction mixtures resulted in significant (up to 90%) reduction of the relative amounts of material in band II (UDP-*N*-acetyl hexosaminuronic acids).

DISCUSSION

At least four known UDP glycoses were among the reaction products when preparations of *P. aeruginosa* were incubated with UDP-GlcNAc plus NAD. These were UDP-ManNAc, UDP-GalNAc, UDP-GlcNAcA, and UDP-ManNAcA. When the substrate consisted of GlcN, ATP, UTP, and NAD, UDP-GlcNAc was also present as a reaction product. The nucleoside diphosphate *N*-acetyl hexosamines were identified by their electrophoretic and chromatographic properties and by the properties of the fragments released from them by enzyme- and acid-catalyzed hydrolysis. The UDP-*N*-acetyl hexosaminuronic acids were identified by similar methods as well as by reduction of their carboxyl groups, followed by characterization of the resulting UDP-*N*-acetyl hexosamines.

The reaction product initially identified as UDP-GalNAc actually consisted of a mixture of two compounds, the known compound UDP-GalNAc and UDP-L-GalNAc, which is described here for the first time. The latter differs from its diastereoisomer in its resistance to oxidation at C-6 by galactose oxidase, an enzyme specific for aldohexoses with the *D*-galactose configuration (1). Yet another new compound, UDP-L-GalNAcA, was also present in the reaction

FIG. 2. Two possible reaction sequences leading to the formation of UDP-L-GalNAcA. See text for details.

products. Interestingly, the corresponding D isomer (UDP-GalNAcA) was not detected.

UDP-[^{14}C]GalNAc isolated from reaction mixtures could conceivably have contained UDP sugars other than UDP-[^{14}C]GalNAc and UDP-L-[^{14}C]GalNAc. Such compounds would not be susceptible to oxidation by galactose oxidase. However, the possibility that the UDP-[^{14}C]GalNAc was contaminated with other UDP sugars seems remote, since only one radioactive compound was noted on two-dimensional PEI chromatography of UDP-[^{14}C]GalNAc in the system of Bochner and Ames (4), which gives clear separation of nucleoside diphosphate sugars (see also Table 1). UDP-[^{14}C]GalNAc also cochromatographed with authentic UDP-GalNAc in yet another solvent system (Table 2), and [^{14}C] GalNAc and [^{14}C]GalN obtained from UDP-[^{14}C]GalNAc were identical in electrophoretic and chromatographic behavior to the authentic compounds in systems (buffer H and solvent C) which unequivocally separate *N*-acetyl hexosamines and hexosamines.

L-GalNAcA and GlcNAc, together with L-rhamnose and bacillosamine, compose the O-antigen repeating unit of the lipopolysaccharide of *P. aeruginosa* NCTC 8505 (15). In accord with the general scheme for synthesis of bacterial lipopolysaccharides (14), it is likely that UDP-GlcNAc and UDP-L-GalNAcA serve as glycosyl donors in the synthesis of the O antigen and that TDP-L-Rha is the donor of the L-Rha moiety. The donor of bacillosamine, the fourth component of the O antigen, might be the putative UDP glucose observed in reaction mixtures in quantities too small to permit identification.

Although our results show that *P. aeruginosa* can convert GlcN or UDP-GlcNAc to the UDP derivatives of ManNAc, ManNAcA, GlcNAcA, and L-GalNAc, none of these residues are known to be present in the O chains or outer core (which contains Glc, GalN, and L-rhamnose [13]) of the lipopolysaccharide or in other polysaccharides of the organism. A somewhat similar situation exists in *Micrococcus luteus*, in which UDP-GlcNAc is converted via UDP-ManNAc to UDP-ManNAcA; the last compound serves as a donor in teichoic acid synthesis. In a separate reaction, *M. luteus* oxidizes UDP-GlcNAc to UDP-GlcNAcA; however, since *M. luteus* does not contain GlcNAcA residues, no function has yet been assigned to UDP-GlcNAcA (9).

On the basis of our results and in analogy with known enzymatic interconversions of nucleoside diphosphate glycoses, at least two reaction sequences leading to the formation of UDP-L-GalNAcA can be suggested (Fig. 2). Scheme a provides functions for UDP-ManNAc and UDP-L-GalNAc. Although the formation of the latter has not been previously demonstrated, the analogous conversion of GDP-Man to GDP-L-Gal catalyzed by an enzyme preparation from *Chlorella pyrenoidosa* is known (2). UDP-L-GalNAcA would then be formed from UDP-L-GalNAc by the usual type of four-electron transfer oxidation (6).

Scheme b is an alternative possibility in which the known compound UDP-ManNAcA would be converted by 3,5-epimerization to the final product. We consider this sequence less likely because it provides no function for UDP-L-GalNAc and because 3,5-epimerization at the nucleoside diphosphate hexuronosyl level is not a known reaction. Whether either of the proposed routes to UDP-L-GalNAcA actually exists in *P. aeruginosa* cannot be determined until sufficient quantities of intermediates are available and separation of the enzymes involved is possible so that each of the reactions leading from UDP-GlcNAc to UDP-L-GalNAcA can be demonstrated.

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