A Novel Uridine Nucleotide Containing N-Acetylglucosamine and Galactose*

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While the yolk traverses the hen oviduct, albumen, shell membrane, and the calcified shell are successively secreted (9). The isthmus, a short section 2 to 8 cm in length, synthesizes and secretes the inner shell membrane, a lace-like envelope composed of fibers of an acid glycoprotein (see below).

A number of nucleotides have been shown to occur in the isthmus, including cytosine diphosphate choline, cytosine diphosphate ethanolamine, guanosine diphosphate mannosamine, uridine diphosphate (UDP) glucose, UDP-galactose, UDP-N-acetylglucosamine, UDP-N-acetylgalactosaminine, and UDP-N-acetylgalactosamine 4-sulfate (1). Some of these are identical with the glucosamine, UDP-N-acetylgalactosamine, and UDP-N-acetylglucosamine 4-sulfate. The product obtained from 50 g of isthmus from 10 laying hens essentially as described by Strominger (4, 5). In the present report the preparation from isthmus and characterization of a novel uridine nucleotide, containing both acetylgalactosamine and galactose, abbreviated here as UDP-GNAc-P-Gal, will be described.

EXPERIMENTAL PROCEDURE

Preparation of UDP-GNAc-P-Gal—This compound was prepared from 50 g of isthmus from 10 laying hens essentially as described by Strominger (5). UDP-GNAc-P-Gal (5 µmoles) was the fifth ultraviolet peak, located between UDP-glucose and UDP-N-acetylgalactosaminine 4-sulfate. The product obtained after adsorption and elution from charcoal was contaminated with a substance that had the properties of UDP-N-acetylgalactosaminine phosphate (see below). This contaminant, as will become evident, was probably produced by decomposition of UDP-GNAc-P-Gal during the manipulation. The two substances were separated by paper electrophoresis or by paper chromatography (cf. Table II), after which UDP-GNAc-P-Gal was eluted from the paper with water.

Other Nucleotides—UDP-glucose, UDP-N-acetylglucosamine, and GDP-mannose were prepared from bakers’ yeast by the methods of Leloir et al. (6-8) and were purified by paper chromatography in solvent A. UDP-GNAc-P-Gal was kindly contributed by Dr. C. Araki, Kyoto Textile College, Kyoto. Other carbohydrates were commercial preparations.

Yeast Cell Suspension—A cell suspension of n-galactose-adapted Saccharomyces fragilis, strain IFO, 0541, was prepared as follows. The cells were grown at 30° during 20 hours on 10 ml of agar medium, pH 5.6, containing a boiled extract from 2 g of potato, 0.3 g of bakers’ yeasts, and 0.15 g of n-galactose. They were harvested with 2 ml of chilled water and washed with another 2 ml. The cells were collected by centrifugation and suspended in 0.3 ml of water. The suspension thus prepared contained approximately 4 mg (dry weight) of cells per 100 µl and was immediately used for the experiment.

Enzymes—UDP-N-acetylglucosamine pyrophosphorylase was prepared from dry bakers’ yeast (12) as follows: 40 g of dried yeast were autoclaved with 120 ml of 0.1 M NaHCO3, for 17 hours at 30°. The mixture was centrifuged and the supernatant solution was saturated with ammonium sulfate. The precipitate was dissolved in 10 ml of water and dialyzed against running water for 3 hours. Snake venom pyrophosphatase and 5′-nucleotidase from Aeglebrondon blomhoffii were kindly contributed by Dr. S. Ivanaga, Kyoto University, Kyoto.

Paper Chromatography and Paper Electrophoresis—Descending paper chromatography was carried out on Toyo No. 50 (thick) filter paper in solvent A, consisting of 95% aqueous ethanol-1 M NaOH in absolute ethanol. The brown

* Supported by a grant from the Ministry of Education, Japan. Preliminary accounts have appeared (1, 2).
background was removed by washing the paper with 5% aqueous Na2S2O3 and then with a large amount of water. Sugar phosphates were detected as follows. The chromatogram was dipped into Burrows reagent (1 g of ammonium molybdate in 8 ml of water plus 3 ml of concentrated HCl and 3 ml of 70% HClO4, diluted to 100 ml with acetone) (14), then heated at 85°C for 3 minutes and illuminated with a mercury lamp. If the paper was exposed to ammonia vapors, the background and spots due to acid-stable phosphate esters, e.g. glucose-, galactose- and N-acetylglucosamine-6-P and 5'-UMP, were bleached, whereas spots due to acid-labile phosphate esters, e.g. glucose-, galactose- and N-acetylglucosamine-1-P, glucose-2-P, 5'-UDP, and UDP-sugar nucleotides, remained blue.

Analytical Procedures—Micro techniques for determinations of periodate consumption and formaldehyde liberation were described in a previous paper (15). The procedures for measurements of amino sugar and acetylamino sugar (16), galactose (17), phosphate (18), and reducing power (19) were modified to a micro scale so that the amounts of sample were in the range of 0.01 to 0.1 µmole. Infrared spectra were obtained with KBr pellets in a Perkin-Elmer spectrophotometer, model 21, with a NaCl prism. The amount of sample was 0.1 µmole, and 90% ethanol was used to dissolve it.

RESULTS

General Properties—The nucleotide had a typical uridine spectrum at both pH 2.0 and pH 12.0 and after bromine treatment. The ratio of uridine to acid-labile phosphate to total phosphate was 1:1.17:5.03 (Table I). Its electrophoretic mobility at pH 5.0 was slightly lower than that of 5'-UDP (Table II). On the electrophoretic strip and on chromatograms on silicic A and B, no other spots were detected either by ultraviolet photography or by staining for phosphate esters. After mild acid hydrolysis, the reducing equivalent was 1.53 relative to glucose (Table I). The following tests both on the intact nucleotide and on 0.1 n acid hydrolysates (80°, 60 minutes) were all negative: the thio-barbituric acid reaction for sialic acids (21), the carbazole reac-

### Table I

<table>
<thead>
<tr>
<th>Analysis of UDP-GNAc-P-Gal</th>
<th>µmoles/µmole uridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phosphate†</td>
<td>3.03</td>
</tr>
<tr>
<td>Acid-labile phosphate‡</td>
<td>1.17</td>
</tr>
<tr>
<td>Glucosamine§</td>
<td>0.91</td>
</tr>
<tr>
<td>Galactose*</td>
<td>1.06</td>
</tr>
<tr>
<td>Reducing value after hydrolysis§</td>
<td>1.53</td>
</tr>
</tbody>
</table>

a Estimated from absorbancy at 263 mμ by using the value, ε = 9800.
b Estimated by the method of Fiske and SubbaRow (18).
c Phosphate was liberated by 15 minutes of hydrolysis in 1 N H2SO4 and estimated by the method of Fiske and SubbaRow (18).
d The sugar was liberated by 16 hours of hydrolysis in 0.1 N HCl and estimated by a modified Elson and Morgan reaction (15).
e The sugar was liberated by 10 minutes of hydrolysis in 0.02 N HCl at 100° and estimated, after purification by paper electrophoresis, by the carbazole reaction (17).
f The value relative to a molar equivalent of glucose was estimated by the method of Park and Johnson (19). For this assay, the compound was previously hydrolyzed with 0.02 N HCl at 100° for 10 minutes. With this method, the reducing values of authentic d-galactose and synthetic N-acetylglucosamine-6-P are 1.00 and 0.42 relative to glucose, respectively.

### Table II

**Paper electrophoresis and paper chromatography of UDP-GNAc-P-Gal and its fragments obtained by chemical and enzymatic degradations**

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Procedure for preparation</th>
<th>Location method on paper</th>
<th>Electrophoretic mobility</th>
<th>RUPM</th>
<th>Sglucosae</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-GNAc-P-Gal</td>
<td>Intact</td>
<td>I</td>
<td>23.5</td>
<td>0.81</td>
<td>0</td>
</tr>
<tr>
<td>UDP-GNAc-P</td>
<td>A, D</td>
<td>I, II</td>
<td>27.0</td>
<td>0.40</td>
<td>0</td>
</tr>
<tr>
<td>UDP-GNAc</td>
<td>D</td>
<td>I, II</td>
<td>21.0</td>
<td>1.55</td>
<td>0.10</td>
</tr>
<tr>
<td>UDP</td>
<td>E</td>
<td>I, III</td>
<td>25.5</td>
<td>0.54</td>
<td>0.17</td>
</tr>
<tr>
<td>UMP</td>
<td>D, E</td>
<td>I, III</td>
<td>16.0</td>
<td>1.00</td>
<td>0.64</td>
</tr>
<tr>
<td>Uridine</td>
<td>From UMP fragment by F</td>
<td>I</td>
<td>0.5</td>
<td>4.50</td>
<td></td>
</tr>
<tr>
<td>P-GNAc-P-Gal</td>
<td>From P-GNAc-P-Gal by A</td>
<td>II</td>
<td>22.0</td>
<td>0.75</td>
<td>0.03</td>
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<tr>
<td>P-GNAc-P</td>
<td>From P-GNAc-P by A</td>
<td>II</td>
<td>26.5</td>
<td>0.40</td>
<td>0.15</td>
</tr>
<tr>
<td>N-Acetylglucosamine-1-P</td>
<td>D</td>
<td>II</td>
<td>16.5</td>
<td>1.80</td>
<td>0.44</td>
</tr>
<tr>
<td>GNAc-P</td>
<td>B</td>
<td>III, IV, V</td>
<td>16.5</td>
<td>1.80</td>
<td>0.44</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>From UDP-GNAc by B</td>
<td>IV, V</td>
<td>&gt;4.3</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>Glucosamine</td>
<td>From GNAc-P by C</td>
<td>IV</td>
<td>To cathode &gt;4.3</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Gal-P</td>
<td>D</td>
<td>II</td>
<td>18.5</td>
<td>1.30</td>
<td>0.24</td>
</tr>
<tr>
<td>Inorganic phosphate</td>
<td>A</td>
<td>IV, V</td>
<td>0</td>
<td>&gt;4.3</td>
<td>0.85</td>
</tr>
<tr>
<td>Galactose</td>
<td>C, D</td>
<td>II</td>
<td>29.0</td>
<td>1.60</td>
<td></td>
</tr>
</tbody>
</table>

a A, Hydrolysis with Dowex 50-H+ at room temperature for 10 minutes; B, hydrolysis with 10% acetic acid or 0.01 N HCl at 100° for 5 minutes; C, hydrolysis with 6 N HCl at 100° for 12 hours; D, hydrolysis with concentrated ammonia at 100° for 2 to 6 hours; E, digestion with snake venom pyrophosphatase; and F, digestion with 5'-nucleotidase.
b I, Ultraviolet photography; II, staining for acid-labile phosphate; III, staining for acid-stable phosphate; IV, AgNO3 staining for reducing sugar; and V, aniline hydrogen phthalate staining for reducing sugar (20).
c Estimated in 0.05 M acetate buffer, pH 5.0, at 30 volts per cm for 90 minutes.
d Estimated on Toyo No. 50 (60 cm long) filter paper in solvent A at 10°.
e Estimated on Toyo No. 51A (60 cm long) filter paper in solvent B at 10°.
Identification of Sugar Components—Hydrolysis of UDP-
GNAc-P-Gal with 10% acetic acid at 100° for 5 minutes yielded
5'-UDP and two substances with reducing power. The 5'-UDP
was identified by paper chromatography and paper electrophores-
sis (Table II) and by chemical analysis of a chromatographically
isolated sample; the ratio of uridine to total phosphate to acid-
labile phosphate was 1:2.13:1.05. On paper electrophoresis at
pH 5.0, the two reducing substances were located at the origin
and at 16.5 cm toward the anode.

Further paper chromatography of the sample eluted from
the origin showed a single spot, the mobility of which was the same
as that of authentic p-galactose (Table III). The identity of the
sugar was also confirmed by electrophoresis on borate-buffered
paper (24) and by the carbazole reaction (17). The galactose
was also identical with p-galactose in its behavior toward p-gal-
actose-adapted yeast; i.e., both authentic p-galactose and the
galactose from the nucleotide were quantitatively decomposed by
washed cells of Saccharomyces fragilis, whereas no detectable re-
action was observed with l-galactose (Fig. 1).

The other reducing substance, located at 16.5 cm on the elec-
trophoretic strip, was eluted from the paper and further purified
by paper chromatography successively in solvents A and B. The
sample thus prepared, GNAc-P, was decomposed with 6 N HCI
at 100° for 16 hours to give inorganic phosphate and a reducing
compound with properties of hexosamine (Table II). Ninhydrin
degradation of the hexosamine (25) yielded a single pentose with
the mobility of p-arabinose. Differential colorimetric analysis of
the hexosamine (26, 27) showed that it gave the same color value
as authentic p-glucosamine. The value for p-mannosamine was
45% less. GNAc-P itself was found to give a positive reaction
for N-acetylhexosamine (16). The extinction coefficients at 585
mp were: for N-acetylgalactosamine, 20,000, and for GNAc-P,
22,000 (based on phosphate). It has been shown previously that
substitution with a methyl group or a sulfate at the 6-position of
N-acetylgalactosamine has no effect on the color reaction (14, 28).

Phosphorylated sample, whereas 1 mole was formed from N-ac-
tetylgalactosamine. The interpretation of these data is essentially
the same as that used in a previous paper (15) for N-acetylgalac-
tosamine 6-sulfate. Synthetic and isolated N-acetylglucosamine
6-phosphates had identical infrared spectra. Bands at 820, 930,
1175, and 1375 cm⁻¹ were seen in both phosphorylated samples,
whereas they were not seen in N-acetylglucosamine.

Isolation and Identification of Galactose-free Fragment—On
treatment of UDP-GNAc-P-Gal in water with Dowex 50-H⁺ at
room temperature, galactose was liberated within 10 minutes,
and a new nucleotide with a faster mobility on paper electrophoresis at
pH 5.0 (Fig. 3) was obtained. The galactose-free nucleotide, UDP-GNAc-P, was eluted from the electrophoretic

<table>
<thead>
<tr>
<th>Substance</th>
<th>Rf</th>
<th>Electro-</th>
<th>Ex/Ea in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ph enol.</td>
<td>carbazole</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solvent B</td>
<td>reaction</td>
<td></td>
</tr>
<tr>
<td>Authentic p-galactose</td>
<td>0.85</td>
<td>1.14</td>
<td>0.85</td>
</tr>
<tr>
<td>Sugar from UDP-GNAc-P-Gal</td>
<td>0.85</td>
<td>1.13</td>
<td>0.85</td>
</tr>
</tbody>
</table>

* The ratio of optical density measured at 540 mp and 440 mp.

The ratio for p-glucose was 4.20, and that for p-mannose was 0.65.
Fig. 2. Rate of consumption of periodate during periodate oxidation of N-acetylglucosamine (O--O), synthetic N-acetylglucosamine-6-P (O--O), and GNAc-P from UDP-GNAc-P-Gal (X--X).

strip and purified by paper chromatography in solvent A (Table II). UDP-GNAc-P thus prepared was hydrolyzed with 10% acetic acid at 100° for 5 minutes to give 5'-UDP and N-acetylglucosamine-6-P, identifications of which have already been described. The electrophoretic mobility of UDP-GNAc-P at pH 3.4 was only 1 cm greater than that of UDP-GNAc-P-Gal, whereas at pH 5.0 it was much greater than that of the intact nucleotide. These electrophoretic mobilities suggest that UDP-GNAc-P has a dissociable secondary phosphate group not found in UDP-GNAc-P-Gal. Hence the galactose must be linked at the galactose residue to the rest of the molecule.

Isolation and Identification of UMP-free Fragment—Digestion of UDP-GNAc-P-Gal with snake venom pyrophosphatase yielded two products which were separated from the intact nucleotide and from each other by paper electrophoresis at pH 5.0 (Fig. 4). The slow spot, easily located by quenching of ultraviolet light, was identified as 5'-UMP by paper chromatography (Table II), by chemical analysis, and by treatment with 5'nucleotidase.

The fast spot, P-GNAc-P-Gal, was located by staining for acid-labile phosphate, but not by quenching of ultraviolet light. The electrophoretically isolated sample was further purified by paper chromatography in solvent A (Table II). Analysis of P-GNAc-P-Gal thus prepared showed that the ratio of acid-labile phosphate to N-acetylglucosamine-6-P to galactose was 1:0.88:0.91. From P-GNAc-P-Gal, galactose was liberated by treating with Dowex 50-H+ as easily as from UDP-GNAc-P-Gal, and a new sugar phosphate, P-GNAc-P, with an additional acidic group was obtained by paper electrophoresis at pH 5.0 (Table II).

Alkaline Hydrolysis—In contrast to the lability of the sample to acid, no detectable change was observed after heating UDP-GNAc-P-Gal for 10 minutes at 100° in concentrated ammonia (Fig. 5). Under these conditions, treatment of yeast UDP-glucose and UDP-N-acetylglucosamine led to a decomposition of the former substance but to no observable change in the latter. This behavior of UDP-glucose toward ammonia is typical of a pyrophosphoric ester which bears a cis-hydroxyl group on a carbon atom adjacent to that bearing the pyrophosphate residue (29). The cause of the greater stability of UDP-N-acetylglucosamine is that the hydroxyl group at position 2 is unavailable for cyclic phosphate formation (7). Therefore, the alkali stability of UDP-GNAc-P-Gal is in agreement with assignment of the pyrophosphate residue to the 1-position in the N-acetylglucosamine residue. In addition, these data also suggest that the phosphate at C2 in the 2-galactose residue is in a sterically unfavorable position for cyclic phosphate formation.

Fig. 3. Hydrolysis of UDP-GNAc-P-Gal with Dowex 50-H+. An electrophoretic strip, pH 5.0, is shown: 1, intact nucleotide (0.1 µmole); 2, after hydrolysis with Dowex 50-H+. To obtain the hydrolysate, 0.1 µmole of UDP-GNAc-P-Gal was dissolved in 0.1 ml of water and passed through a small column of Dowex 50-H+(approximately 1 ml) at room temperature. The effluents and washings were combined (approximately 1 ml) and concentrated under reduced pressure for paper electrophoresis. Compounds indicated by dark spots were seen under the ultraviolet lamp, and galactose was located by staining with AgNO3.
aqueous NaOH with a micropipette. An electrophoretic strip, pH 5.0, is shown:
1, intact nucleotide (0.15 pmole); 2, after digestion. To obtain the digest, 0.15 pmole of UDP-GNAc-P-Gal was incubated in 0.0025 M N-acetylglucosamine-1-P (Fig. 6).

The RR value of N-acetylglucosamine was 0.50; N-acetylmannosamine, 0.20; and the sugar from UDP-GNAc, 0.50. The identity of UDP-GNAc and yeast UDP-N-acetylglucosamine as standards. Paper spots were seen under the ultraviolet lamp, and the spot indicated by an open circle was seen only by staining for acid-labile phosphate.

However, by prolonged hydrolysis with ammonia, UDP-GNAc-P-Gal was decomposed to UDP-N-acetylglucosamine and a mixture of galactose-1-P and its isomer. More than 70% of UDP-GNAc-P-Gal was unaffected by treating with concentrated ammonia at 100° for 2 hours. However, two products of this hydrolysis, UDP-GNAc and GAL-P, which behaved as a nucleotide with a decreased negative charge and as a hexose monophosphate, respectively, could be detected on paper electrophoresis. At least three other minor products were also detected and will be considered later. UDP-GNAc so prepared had identical electrophoretic and chromatographic mobilities with yeast UDP-N-acetylglucosamine (7) (Table II) and contained, for each mole of uridine, 2.03 moles of total phosphate, 1.09 moles of labile phosphate, and 0.92 mole of N-acetylglucosamine after hydrolysis with 0.01 N HCl at 100° for 10 minutes.

The N-acetylglucosamine liberated by mild acid treatment was subjected to paper chromatography on borate-buffered paper in n-butanol-pyridine-water (6:4:3) (30) with authentic standards of galactose-1-P behaved on both chromatograms as a faster moving spot. 3

Although further work on the phosphate position in GAL-P was not attempted on account of the small amounts of material available, it should be noted that the electrophoretically isolated Gal-P gave two spots on paper chromatograms (Table II). A standard of galactose-1-P behaved on both chromatograms as the faster moving spot.

2 Since these results suggest that UDP-GNAc-P-Gal is hydrolyzed via a cyclic phosphate intermediate and previous studies have provided evidence favoring the view that the "leaving group," e.g. CMP residue of CDP-polyols, choline residue of glycerylphosphoryl choline, glycerol residue of phosphoinositides, and ribose residue of RNA, has no significant effect on the ease of cyclic phosphate formation, the high alkali stability of UDP-GNAc-P-Gal led to the speculation, as indicated in the text, that UDP-GNAc-P-Gal is hydrolyzed to some extent after heating for 2 hours. The degradation products identified were from TDP-L-rhamnose, TDP-a-D-glucose. The GDP-a-D-glucose was only hydrolyzed to some extent after heating for 2 hours. The degradation products identified were from TDP-L-rhamnose, TDP-a-D-glucose, GDP-a-D-glucose, GDP-mannose, GDP-inorganic phosphate, and mannose monophosphate. These experiments suggest that the GDP-a-mannose is in the a form (C₁ is cis to C₆), whereas the TDP-L-rhamnose is in the b form (C₁ is cis to C₆). The assignments are compatible with the facts that the GDP-D-mannose can be synthesized from GTP and GDP-a-D-glucose by the reverse reaction of pyrophosphorylase (32) and that TDP-L-rhamnose can be derived from TDP-a-D-glucose by multiple reactions, including epimerization at C₄ (33-35). Since a-D-glucose is in the cis form with respect to C₁ and C₆, the epimerization would afford the trans form, which is identical with the configuration for C₁ and C₆ of b-L-rhamnose.

![Figure 4. Digestion of UDP-GNAc-P-Gal with snake venom pyrophosphatase. An electrophoretic strip, pH 5.0, is shown: 1, intact nucleotide (0.15 pmole); 2, after digestion. To obtain the digest, 0.15 pmole of UDP-GNAc-P-Gal was incubated in 0.0025 M MgCl₂ with 30 μg of the enzyme in a final volume of 12 ml for 2 hours at 37°. During incubation, the pH of the reaction was maintained between 8.0 and 9.0 by occasional addition of aqueous NaOH with a micropipette. Compounds indicated by open circles were seen only by staining for acid-labile phosphate.](http://www.jbc.org/)

![Figure 5. Hydrolysis of UDP GNAc P-Gal with concentrated ammonia. An electrophoretic strip, pH 5.0, is shown: 1, intact nucleotide (0.2 pmole); 2, after heating with 0.2 ml of concentrated ammonia in a sealed tube at 100° for 10 minutes; 3, for 2 hours; 4, for 6 hours. Spots indicated by closed and open circles are as described in Fig. 4.](http://www.jbc.org/)
Finally, UDP-GNAc-P-Gal was quantitatively destroyed by treating for 6 hours with concentrated ammonia. Electrophoresis of the reaction mixture indicated the appearance, in addition to UDP-N-acetylgalactosamine and galactose monophosphate, of products with mobilities of inorganic phosphate, UDP-GNAc-P, N-acetylgalactosamine-1-P, 5'-UMP, and uridine, respectively (Fig. 5). The identities of these products were further confirmed by paper chromatography (Table II). Analysis of the chromatographically isolated N-acetylgalactosamine-1-P indicated an acid-labile phosphate to N-acetylgalactosamine ratio of approximately 1.

To summarize these observations, the following structure (Fig. 7) is proposed for UDP GNAc-P-Gal. Apparently, the bond which is most easily cleaved by alkali is the one marked (d) in the formula, so that hydrolysis for 2 hours yields predominantly UDP-N-acetylgalactosamine and galactose monophosphates. However, the bonds marked (a), (b), and (e) are also susceptible to destruction by alkali, particularly on prolonged treatment. On the other hand, the bond marked (b) is the one cleaved by snake venom pyrophosphatase. The bonds marked (c) and (e) are both acid-labile, although the latter is predominantly cleaved by treatment with Dowex 50-H+.

**DISCUSSION**

The isthmus is concerned physiologically with the synthesis of the egg shell membrane. The structure of this membrane is of considerable interest, and some preliminary data have been obtained. After treatment of the membrane with a protease of *Streptomyces griseus* (37), a fibrous material (approximately half of the whole membrane) remained unaffected. Hydrolysis of the fiber with 1 N HCl at 100° yielded glucosamine, galactosamine, glucose, galactose, mannose, an oligosaccharide containing galactose and hexosamine, sulfate, phosphate, and amino acids. UDP-GNAc-P-Gal might be involved in the simultaneous or successive addition to some polymer of N-acetylgalactosamine, phosphate, and galactose. This polymer might be secreted during formation of the egg shell membrane.

UDP-galactose (38) is a widely distributed compound. Recently, GDP-galactose has been isolated from red algae (39). However, the data so far available have indicated that in the former compound the galactose is in the α-D form, whereas in the latter it is in the L form. Unlike these compounds, the galactose of UDP-GNAc-P-Gal is possibly in the β-D form. In addition, comparative experiments (cf. footnote 3) with GDP-N-mannose (yeast) and TDP-rhamnose (E. coli) suggest that the N-mannose is in the α form and that the L-rhamnose (6-deoxy-α-mannose) is in the β form. Therefore, more generally, the sugar residue attached to each different nucleotide aglycone might have a stereochemically unique structure. For example, the glucose of GDP-glucose (40) may not be identical with the glucose of UDP-glucose (29) with respect to α, β, o, L, pyranose, or furanose forms. Similarly, the same criteria may distinguish the mannose of TDP-mannose (41) from that of GDP-mannose (8) and the rhamnose of UDP-rhamnose (42) from that of TDP-rhamnose (7). It certainly will be desirable to clarify these stereochemical relationships.

**SUMMARY**

A novel uridine nucleotide has been isolated from the isthmus of hen oviduct. It has been shown, by a series of degradative procedures, to be a β-galactose 1-phosphate derivative of uridine diphosphoacetylglucosamine, in which galactose and N-acetylgalactosamine are linked by a 1,6-phosphodiester linkage.

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Sakaru Suzuki


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