ISOLATION AND IDENTIFICATION OF AN UNUSUAL URIDINE NUCLEOTIDE SUGAR CONJUGATE [1]

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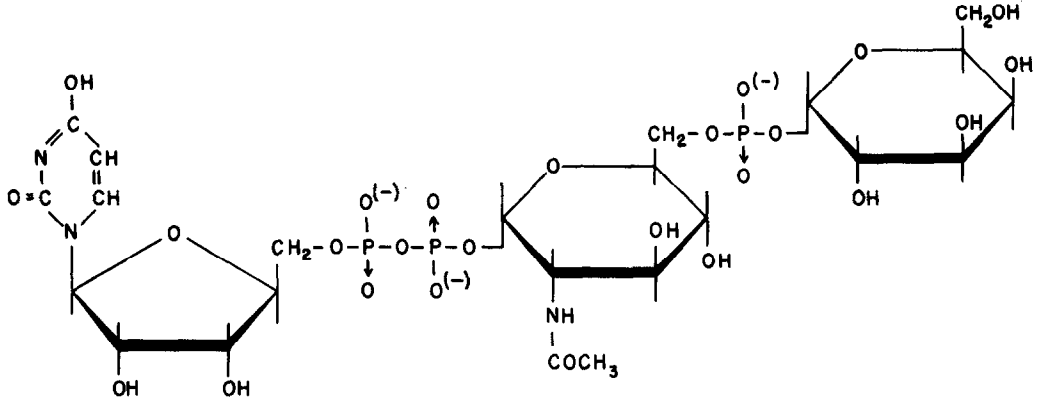
Strominger (1) reported the isolation of a sugar nucleotide from hen oviduct which was tentatively identified as UDP-N-acetylglucosamine-6-phosphate. The present paper is concerned with the isolation of a nucleotide from the same source which has been identified as UDP-N-acetylglucosamine-6-phospho-1-galactose (Fig. 1).



UDP-N-ACETYLGLUCOSAMINE-6-PHOSPHO-1-GALACTOSE

Hen oviducts, obtained from freshly killed laying hens, were extracted in boiling 80% ethanol for 30 minutes, filtered, concentrated to 1% of the

[1] During the course of the present investigation a similar observation has been reported by S. Sukuzi, Biochim. Biophys. Acta, 50, 395 (1961).

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original volume and extracted several times with ether. The residual aqueous extract was placed on a column of Dowex-1-Cl and eluted with a linear gradient employing 0.01 N HCl in the mixing flask and 0.01 N HCl plus 0.50 M NaCl in the reservoir. The unknown nucleotide was recovered together with UDP-glucuronic acid. Subsequent purification steps, including paper chromatography, high-voltage electrophoresis and fractional ethanol precipitation, resulted in the isolation of a single nucleotide sugar conjugate free from contamination with ultraviolet-absorbing or carbohydrate-containing impurities.

The intact sugar nucleotide exhibited the characteristic spectra of UDP at acid and alkaline pH ranges. Based upon an ϵ_M of 10×10^3 (at 262 mμ and pH 2.0, a ratio of UV absorption:total phosphorus:N-acetylglucosamine:galactose of 1:3:1:1 was obtained (Table I). When heated in 0.01 N HCl for 15 minutes at 100°, the compound was decomposed with the liberation of 1 mole each of UDP, N-acetylglucosamine-6-phosphate and free galactose (Equation a, Table I). These components were readily resolved and recovered by elution following high-voltage paper electrophoresis at pH 4.0. Subjection of the above isolated hexosamine phosphate to 3 N HCl at 110° for 18 hours in a sealed tube, followed by degradation of the resulting amino sugar with ninhydrin (2), lead to the formation of arabinose as the sole product.

A sequential enzymatic and chemical degradation of the original complex, accompanied by isolation and identification of the unique reaction products at each step, was carried out in the following manner.

Incubation of Compound I (UDP-N-acetylglucosamine-6-phospho-1-galactose) with semen pyrophosphatase [3/] resulted in the liberation of 1 mole of inorganic phosphate and the recovery of uridine and a new diphosphorylated disaccharide, Compound II (phospho-1-N-acetylglucosamine-6-phospho-1-galactose) (Equation b, Table I). The latter compound, after isolation, was treated with a partially purified phosphatase from E. coli [3/]. A second mole of inorganic phosphate was

[3/] The semen pyrophosphatase- and E. coli phosphatase preparations were generously provided by Drs. R. J. Hilmoe and D. R. Harkness.

Table I

Summary of the Degradation Procedures Employed and the Analytical Data Obtained for Each of the Isolated Products

(a) Compound I $\xrightarrow[15'\ 100^{\circ}]{\text{pH } 2.0}$ UDP + GNAc-6-P + galactose

(b) Compound I $\xrightarrow[\text{pyrophosphatase}]{\text{semen}}$ Uridine + P_i + P-1-GNAc-6-P-1-Gal (II)

(c) Compound II $\xrightarrow[\text{phosphatase}]{\text{E. coli}}$ P_i + GNAc-6-P-1-Gal (III)

(d) Compound III $\xrightarrow[1 \text{ hour } 100]{1 \text{ N KOH}}$ Gal-1-P $\xrightarrow[\text{phosphatase}]{\text{potato}}$ P_i + galactose

The figures recorded represent the calculated molar ratios for each of the constituents of the isolated compounds.

Compound	N-Acetyl-glucosamine (5)	Galactose (3)	Δ-7 P (6)	Total P (7)	Electrophoretic* migration
					cm
*I	1.0	1.1	1.06	3.06	+26.5
II	0.93	1.0	1.03	1.91	+23.0
III	0.91	1.0	0.0	1.09	+11.5
Galactose	0.0	1.0	0.0	0.0	-2.0
Gal-1-P	0.0	1.0	1.02	1.04	+17.0
GNAc-6-P	1.0	0.0	0.0	1.04	+16.0

* Electrophoresis carried out on Whatman No. 1 paper employing 0.05 M citrate buffer, pH 4.0, at 80 volts per cm for 30 minutes. The origin was 10 cm from the cathodic end of the paper. Migration (+) towards the anode; (-) towards the cathode.

liberated accompanied by the appearance of a new monophosphorylated disaccharide, Compound III (N-acetylglucosamine-6-phospho-1-galactose) (Equation c, Table I). Subjection of III to alkaline hydrolysis with 1 N KOH at 100° for 1 hour resulted in destruction of the amino sugar and permitted the recovery of galactose-1-phosphate. An aliquot of this material incubated with potato phosphatase, yielded a third mole of inorganic phosphate together with free galactose (Equation d, Table I). The latter hexose was identified by co-chromatography with authentic galactose in butanol:pyridine:water (3:2:1.5) and by the formation of a characteristic secondary absorption peak at 600 mμ in the cysteine-H_2SO_4 reaction (3). A second aliquot, treated with 1 N HCl for 7 minutes at 100°,

resulted in quantitative dephosphorylation; no evidence for a cyclic phosphate ester was obtained. A third aliquot was assayed enzymatically [4/] with Gal-1-P uridyl transferase in the presence of UDPG, phosphoglucomutase, zwischenferment and TPN (4). The rapid and stoichiometric formation of TPNH in this system, which showed an absolute dependence upon α-D-galactose-1-phosphate, confirmed the identification of the hexose phosphate arising from the alkaline degradation.

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[4/] The enzymatic assay was carried out by Dr. A. Grollman of this laboratory.