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Uridine diphosphate acetylglucosamine phosphate and uridine diphospate acetylgalactosamine sulfate

In the past few years a number of compounds containing uridine pyrophosphate linked to a sugar have been isolated from various sources. Three compounds of this type which contain a partially identified N-acetylamino sugar were isolated by PARK from penicillin-inhibited *Staphylococcus aureus*[1], and compounds of this type accumulate in penicillin-inhibited *Lactobacillus helveticus* 335 also[2]. In addition, uridine pyrophosphate N-acetylglucosamine has been isolated from yeast[3], and presumptively identified in liver[4,5]. The purpose of the present communication is to report the identification of two additional compounds, isolated from hen's oviduct, which contain uridine pyrophosphate and an N-acetylamino sugar.

Large amounts of N-acetylamino sugar esters were detected in several animal tissues using a modification of the MORGAN AND ELSON reaction[6]. A hot water extract prepared from one pound of oviduct from laying hens (which contained especially large amounts of these esters, about 0.5 μM/g) was brought to pH 9 and placed on a column of Dowex-1 Cl (2% cross-linked), 5 cm^2 × 15 cm. The column was eluted by gradient elution employing mixtures of NaCl and HCl*. Three peaks in the elution diagram (Fig. 1) which contained both 260 mμ absorption and N-acetylamino sugar were further purified by anion exchange chromatography under conditions different from the original elution and by paper chromatography. Analytical data for these three compounds (UDPA$_1$, UDPA$_2$, and UDPA$_4$) are presented in Table I. Hydrolysis of each of the compounds in 0.01 *N* and 1 *N* HCl yielded uridine diphosphate (UDP) and uridine-5′ phosphate (UMP) respectively, identified by paper chromatography and by chemical and enzymic analysis of the eluted spots**. The compounds therefore differ only in the nature of their N-acetylamino sugar fragments.

UDPA$_1$ has the same location on the chromatogram and the same R_F in ethanol-ammonium acetate as yeast UDP N-acetylglucosamine[3], and its sugar fragment has the same R_F in ethyl acetate-pyridine-

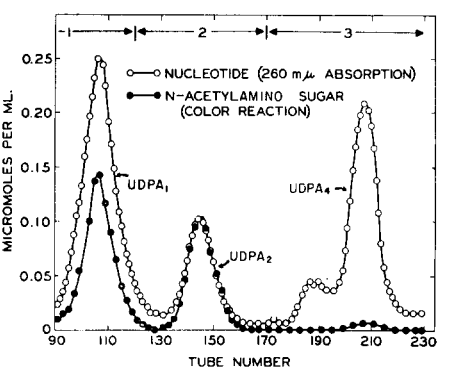

Fig. 1. Ion exchange chromatogram of hen's oviduct extract developed by gradient elution. In the portion of the chromatogram reproduced the gradients were: 1. 0.05 to 0.1 *M* NaCl in 0.01 *N* HCl, 2. 0.1 *M* to 0.25 *M* NaCl in 0.01 *N* HCl, 3. 0.01 *N* to 0.3 *N* HCl. The concentration of nucleotide was estimated approximately using the molar extinction coefficient for uridine, and N-acetylamino sugar was estimated using N-acetylglucosamine as the standard.

* The procedure used for the separation of tissue nucleotides, based on COHN's studies (*J. Cell. Comp. Physiol.*, 38 (Suppl. 1), 21 (1950)) of ion exchange chromatography of nucleotides, is very similar to the procedure employed by HURLBERT *et al.*[4]. The procedure differs in that 2% rather than 10% cross-linked resin has been used, and chloride rather than formate was the eluting anion.

** A third distinctly separate uridine nucleotide containing an N-acetylamino sugar (UDPA$_3$) was present between UDPA$_2$ and UDPA$_4$ on two occasions. Only small amounts of this compound were obtained, sufficient for identification of its hydrolysis products as UDP and UMP.

water[7] as N-acetylglucosamine, a procedure which does not separate N-acetylglucosamine and N-acetylgalactosamine. However, in the deacetylated compound galactosamine was detected as well as glucosamine (Table I). PONTIS VIDELA's observation[8] of the occurrence of UDP N-acetyl-galactosamine in some preparations of UDP N-acetylglucosamine (from liver in his case) is, therefore, confirmed.

TABLE I

ANALYTICAL DATA

	μM μM uridine[a]				*Amino sugar, per cent*[f]	
	Labile[b] *phosphate*	*Total*[c] *phosphate*	*N-acetylamino*[d] *sugar*	*Sulfate*[e]	*Glucosamine*	*Galactosamine*
$UDPA_1$	1.04	1.86	0.78[g]	0	70–75	25–30
$UDPA_2$	0.94	2.88	1.04	0	100	0
$UDPA_4$	0.95	1.88	0.03[h]	0.96	0	100

[a] Estimated from 262 mμ absorption using $\epsilon = 9890$ (J. M. PLOESER AND H. S. LORING, *J. Biol. Chem.*, 178 (1949) 431). Complete spectra in acid and alkali corresponded to expected spectra for uridine.

[b] Phosphate liberated by 10 min hydrolysis in 1 *N* HCl estimated by method of C. H. FISKE AND Y. SUBBAROW (*J. Biol. Chem.*, 66 (1925) 575).

[c] Estimated by method of O. H. LOWRY *et al.* (*J. Biol. Chem.*, 207 (1954) 1).

[d] Estimated by an unpublished modification of the MORGAN AND ELSON reaction. For each fragment the color produced in the test has a spectrum identical to that produced by the standard, N-acetylglucosamine (*cf.* D. AMINOFF, W. T. MORGAN AND W. M. WATKINS, *Biochem. J.*, 51 (1952) 379).

[e] Sulfate was qualitatively identified as a material liberated by sealed tube hydrolysis in 4 *N* HCl and precipitable as a barium salt in 4 *N* HCl. I am indebted to Dr. ALBERT DORFMAN for the quantitative microsulfate analysis (procedure of L. ANDERSON, *Acta Chem. Scand.*, 7 (1953) 689).

[f] Attempts to separate the amino sugars by the procedure of CABIB, LELOIR AND CARDINI[3] were suggestive but difficult to reproduce. Separation and semiquantitative estimation was, therefore, carried out by the procedure of P. J. STOFFYN AND R. W. JEANLOZ (*Arch. Biochem. Biophys.*, 52 (1954) 373). I am very grateful to Dr. JEANLOZ for these determinations.

[g] The low value obtained for N-acetylamino sugar is due to the presence of UDP N-acetylgalactosamine, as in the test N-acetylgalactosamine has only about 30% of the color given by the standard, N-acetylglucosamine.

[h] The sugar fragment of this compound has a very low color yield in this test. This initially posed the question of whether this ester was actually linked to the UDP. However, it migrates with the uridine portion of the molecule on chromatography in several solvents, and has not been separated by any procedure other than hydrolysis. It has been concluded that the presence of sulfate is responsible for a very low color yield in the test.

$UDPA_2$ differs from $UDPA_1$ in that it contains one additional mole of stable phosphorus. The only amino sugar in this nucleotide is glucosamine. $UDPA_4$, which has a relatively high affinity for Dowex-1, contains one sulfate residue*. The only amino sugar in this compound is galactosamine. The UDP isolated from these compounds following hydrolysis in 0.01 *N* HCl did not contain additional phosphate or sulfate, and no phosphate or sulfate was liberated under these conditions. The additional phosphate and sulfate must, therefore, be attached to the N-acetylamino sugars of $UDPA_2$ and $UDPA_4$ respectively.

This evidence leads to the conclusion that these compounds are uridine diphosphate acetylglucosamine phosphate and uridine diphosphate acetylgalactosamine sulfate. The exact position of the additional substituents on the N-acetylamino sugars and whether or not they are monoester substituents remains to be determined**.

* Dr. H. M. KALCKAR first suggested to the author that the behaviour of this nucleotide on ion exchange chromatography might be explained by the presence of sulfate.

** A compound which contains two moles of phosphate per mole of N-acetylglucosamine has been derived from $UDPA_2$. Dr. J. L. REISSIG has kindly tested this fragment and found it to be a potent activator of *Neurospora* phosphoacetylglucosamine mutase. It does not activate phosphoglucomutase. These data strongly suggest that the derived compound is N-acetylglucosamine-1,6-diphosphate, and, therefore, that the additional mole of phosphorous in $UDPA_2$ is on the 6-position of N-acetylglucosamine.

A striking feature of the oviduct chromatogram is the predominance of uridine nucleotides. For example, adenosine triphosphate has not been found, and the amount present must be extremely small compared to any one of the uridine nucleotides. A major function of oviduct is the synthesis of glycoproteins containing N-acetylglucosamine and mannose[9]**. It also contains mucopolysaccharides (including both hyaluronic acid and chondroitin sulfate[10]). These facts suggest that these nucleotides may be the means of activation of glycosyl residues for incorporation into protein, and for synthesis of some mucopolysaccharides.

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* A mannose containing nucleotide, guanosine-5′ pyrophosphate mannose, has also been identified in oviduct (J. L. STROMINGER, *Federation Proc.*, 13 (1954) 307). This compound is identical to the compound isolated from yeast by CABIB AND LELOIR (*J. Biol. Chem.*, 206 (1954) 779).

Effect of substrate structure on the aldolase equilibrium*

The purpose of this communication is to call attention to a relationship between the structure of the aldehyde component and the equilibrium constant of aldolase reaction systems.

In the aldolase reaction

(1) D-3-phosphoglyceraldehyde + dihydroxyacetone phosphate $\rightleftharpoons$ D-fructose-1,6-diphosphate

various aldehydes may replace 3-phosphoglyceraldehyde (GAP) but dihydroxyacetonephosphate (DAP) appears to be specific[1,2,3,4,5]. When "foreign" aldehydes react with DAP, corresponding ketose-1-phosphates are formed (*i.e.* D-fructose-1-phosphate from D-glyceraldehyde[1,3] and D-xylulose-1-phosphate from glycolaldehyde[3]). MEYERHOF *et al.*[1] observed that the aldolase reaction was fully reversible with GAP or acetaldehyde. On the other hand, fructose-1-phosphate, readily formed from D-glyceraldehyde and DAP in muscle extracts, was not detectably split when added to such extracts, suggesting "irreversible" formation. More recent work[2,6] has shown that crystalline muscle aldolase does effect some cleavage of fructose-1-phosphate which is greatly enhanced by trapping or enzymic removal of reaction products.

We have examined the effect of "foreign" aldehydes on the equilibrium system resulting from the action of aldolase + isomerase on fructose-1,6-diphosphate. The course of the reactions toward new equilibria was followed by estimating DAP, other ketose-1-phosphates, and free aldehydes[1,7,8]. From the new equilibria reached the aldehydes tested fell into two categories: (A) aldehydes causing complete disappearance of DAP from medium (DL-glyceraldehyde, β-hydroxypropionaldehyde, and β-hydroxybutyraldehyde); and (B) aldehydes which caused establishment of a new equilibrium in which significantly large amounts of DAP remained (formaldehyde, acetaldehyde, propionaldehyde, β-methoxypropionaldehyde, DL-lactic aldehyde, and D-glyceraldehyde-3-phosphate).

If the "foreign" aldehydes condense with DAP in strict analogy with the "natural" aldolase reaction (no exceptions are known to date) then it is seen that the new ketose-1-phosphates formed from aldehydes of group (A) may exist in the stable pyranose configuration since a free hydroxyl group is present at carbon 6 (*i.e.*, D-fructose (L-sorbose) 1-phosphate from DL-glyceraldehyde; 5-desoxyfructose-1-phosphate from β-hydroxypropionaldehyde, *etc.*). However, the ketose-1-phosphates produced from aldehydes of group (B) have no hydroxyl at carbon 6 and must exist as the less stable furanoses, or as acyclic structures (*i.e.* fructose-1,6-diphosphate from D-glyceraldehyde-

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