experiments suggest either that if there is a soluble RNA for each amino acid, it may exist in two forms, or that there may be more than one soluble RNA for each amino acid.

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Enzymic transfer of pyruvate to uridine diphosphoacetylglucosamine

Uridine nucleotides containing a substituted acetylglucosamine are present in normal *Staphylococcus aureus* and accumulate in the penicillin-inhibited organism[1]. This substituted amino sugar also occurs in the spores and cell walls of many bacteria. STRANGE[2] proposed that the substituent on the 3-position of the acetylamino sugar is lactic acid linked as an ether through its 2-position. This structure has been confirmed by synthesis[3]. A nucleotide form of the sugar is presumed to be a precursor of a part of the bacterial cell wall, and the mechanism of action of penicillin may be related to inhibition of a reaction in which a more complex derivative of this sugar is transferred from nucleotide to cell wall[4].

A direct enzymic transfer of enolpyruvate from 2-phosphoenolpyruvate (PEP) to the 3-position of acetylglucosamine, bound to the nucleotide uridine diphosphoacetylglucosamine (UDPAG), has now been observed in extracts of *S. aureus*, *Escherichia coli*, and *Aerobacter aerogenes* (Eqn. (1)).

$$\text{UDPAG} + \text{PEP} \longrightarrow \text{UDPAG-pyruvate} + \text{inorganic phosphate} \qquad (1)$$

This reaction is measured by means of a colorimetric method specific for 3-substituted acetylamino sugars, modified from a previously published method for acetylglucosamine estimation[5]. UDPAG-pyruvate, the product of this reaction, is presumably a precursor of UDPAG-lactic ether, one of the nucleotides which accumulates in penicillin-inhibited *S. aureus*.

The reaction requires both PEP and UDPAG. To provide evidence that PEP and UDPAG are the specific substrates, other compounds which might be formed from them by bacterial enzymes were tested in the reaction, and found to be inactive (Table I).

In a study of the stoichiometry of the reaction with a 7-fold purified enzyme preparation from *S. aureus*, the disappearance of 56 mμmoles of UDPAG and 54 mμmoles of PEP was accompanied by the appearance of 48 mμmoles of UDPAG pyruvate and 59 mμmoles of inorganic phosphate as required by eqn. 1. Attempts at demonstrating phosphorolysis or arsenolysis of UDPAG-pyruvate have been unsuccessful.

40 μmoles of the nucleotide product were isolated from an incubation mixture containing PEP, randomly labelled with ^{14}C, by anion-exchange chromatography followed by charcoal adsorption and elution. The compound contained both uridine diphosphate and an acetylamino sugar, and had the following additional properties:

1. It had a specific activity of 12,700 counts/min/μmole compared to a value of 11,800 counts/min/μmole for the PEP used in the incubation mixture.

2. On paper electrophoresis in 0.1 *M* acetate buffer, pH 4.6, the intact nucleotide had a mobility relative to UDPAG of 1.32 compared to a mobility of 1.22 for UDPAG-lactic ether.

3. In the specific color reaction the new acetylamino sugar had an extinction coefficient, $\varepsilon_{580\ m\mu} = 4500$, compared to values of 430 for acetylglucosamine, 4150 for 3-O-methyl-N-acetylglucosamine and 9,000 for the acetylamino sugar in UDPAG-lactic ether.

TABLE I

REQUIREMENTS FOR THE FORMATION OF A 3-SUBSTITUTED ACETYLGLUCOSAMINE

The incubation mixture contained 25 μl 0.1 M maleate buffer, pH 6.8, 25 μl 0.063 M UDPAG (0.15 μmole), 4 μl 0.07 M PEP (0.28 μmole), 10 μl 0.1 M KF, 25 μl enzyme prepared from *S. aureus* (9.7 mg protein/ml) and water to 90 μl. After 2-h incubation, the mixtures were assayed for 3-substituted acetylglucosamine.

Incubation mixture	*Absorbance 580 mμ*
Complete	0.410
Minus UDPAG	0.003
Minus PEP	0.040
α-D-acetylglucosamine-1-phosphate substituted for UDPAG*	0.010
2-phospho-DL-lactate substituted for PEP*	0.031

* Similarly, uridine diphosphoacetylgalactosamine[6] and acetylglucosamine did not substitute for UDPAG, and 2-phospho-D- or 2-phospho-L-glycerate, 3-phospho-D-glycerate, oxaloacetate, and pyruvate or lactate plus ATP did not substitute for PEP.

4. Both glucosamine and pyruvate could be recovered from the new nucleotide by acid hydrolysis. By contrast the ether linkage between glucosamine and lactic acid in UDPAG-lactic ether is not hydrolyzed under the conditions employed. Hydrolysis in 6 N HCl for 3 h in a sealed tube at 100° yielded an amino sugar identified as glucosamine by the following criteria: (a) It had an absorption maximum at 525 mμ in the Elson-Morgan reaction. The amino sugar from UDPAG-lactic ether yields a chromogen with an absorption maximum at 505 mμ under these conditions. (b) Paper chromatography in *n*-butanol–acetic acid–water (4:1:5) or 80% aqueous pyridine revealed a ninhydrin-positive compound with the mobility of glucosamine. (c) Following degradation with ninhydrin[7], only arabinose, the pentose corresponding to the last 5 carbon atoms of glucosamine, was obtained.

5. The ease of recovery of glucosamine from this new amino sugar suggested that the pyruvate present might have a lability similar to that found in PEP. Hydrolysis was carried out in 1 N HCl in a sealed tube at 105°, and pyruvate liberated was measured with lactic dehydrogenase and reduced diphosphopyridine nucleotide. Maximum recovery was at 30 min and amounted to 0.80 μmole pyruvate/μmole nucleotide.

6. The acetylamino sugar prepared by hydrolysis in 1 N HCl at 100° for 30 min was chromatographed in *n*-butanol–pyridine–water (6:4:3) on borate-treated paper. Its R_F was 0.26 compared to values of 0.26 for N-acetylglucosamine, 0.12 for N-acetylgalactosamine and 0.12 for N-acetylmannosamine[8].

These facts establish that the enzymically synthesized nucleotide is UDPAG-pyruvate. In all probability the pyruvate is present in an ether linkage from the 3-position of the nucleotide form of acetylglucosamine to the 2-position of enolpyruvate. The enzyme which catalyzes its synthesis has been named pyruvate-UDPAG transferase. Although this reaction is the fifth known enzymic reaction of PEP, it is the first involving pyruvate transfer.

Investigation of the possible reduction of UDPAG-pyruvate to UDPAG-lactic ether by a bacterial enzyme is in progress.

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