The enzymic synthesis of thymidine-linked sugars. IV. Thymidine diphosphate amino-sugars

In previous communications[1,2] we have reported the synthesis of TDP-D-glucose and TDP-L-rhamnose with partially purified enzymes from *Pseudomonas aeruginosa* (ATCC 7700). We wish to report in this communication the enzymic synthesis of TDP-glucosamine, TDP-*N*-acetylglucosamine, and TDP-*N*-acetylgalactosamine from α-glucosamine-1-*P* by the following pathway:

$$\alpha\text{-Glucosamine-1-}P + \text{TTP} \rightarrow \text{TDP-glucosamine} + \text{PP}_i \quad (1)$$

$$\text{TDP-glucosamine} + \text{acetyl-CoA} \rightarrow \text{TDP-}N\text{-acetylglucosamine} + \text{CoA} \quad (2)$$

$$\text{TDP-}N\text{-acetylglucosamine} \leftrightarrows \text{TDP-}N\text{-acetylgalactosamine} \quad (3)$$

Sonic extracts of *Pseudomonas aeruginosa* were prepared as previously described, and treated with protamine sulfate, followed by $(NH_4)_2SO_4$ fractionation[1].

When the 50–70% saturation $(NH_4)_2SO_4$ fraction was incubated with TTP and α-D-glucosamine-1-*P*, a new nucleotide was formed which had the properties of TDP-glucosamine. A typical reaction mixture contained 150 μmoles of Tris–HCl, 30 μmoles of $MgCl_2$, 3 μmoles of EDTA, 21 μmoles of α-glucosamine-1-*P*, 20 μmoles of TTP and enzyme (35 mg of protein) in a final volume of 12 ml; pH 8.0. After incubation for 1 h at 37°, the reaction mixture was deproteinized and the nucleotides were chromatographed on a Dowex-1-X8 formate column. 8.5 μmoles of TDP-glucosamine were recovered from the column.

The isolated nucleotide gave a single spot when chromatographed in the neutral ethanol–ammonium acetate solvent[3] with $R_{TMP} = 1.1$. The ratio of thymidine:total phosphate:acid-labile phosphate:amino-sugar was 1:2.04:1.02:1.1. Amino-sugar was determined by a slight modification of the method of LEVY AND MCALLAN[4] after 30-min hydrolysis in 1 *N* HCl. The amino-sugar obtained after hydrolysis was chromatographed on Dowex-50 H^+ by the method of GARDELL[5] and found to be eluted exactly in the region of glucosamine.

When TDP-glucosamine was incubated with a dialyzed sonic extract of *Pseudomonas aeruginosa* in the presence of acetate, CoA and ATP, a new nucleotide was formed which contained *N*-acetylamino-sugar as determined by the method of REISSIG *et al.*[6]. Synthetic acetyl-CoA could substitute for the mixture of acetate, ATP and CoA.

The nucleotide was isolated by column chromatography on Dowex-1-X8 formate. On analysis it gave ratios of thymidine:acid-labile phosphate:acetylamino-sugar of 1:0.97:0.75. *N*-Acetylglucosamine was used as a standard for the acetylamino-sugar determination; it is known that in this test *N*-acetylgalactosamine gives on a molar basis only one third the color of *N*-acetylglucosamine. On paper chromatography in the neutral ethanol–ammonium acetate solvent the nucleotide gave a single spot with R_{TMP} of 1.24.

After acid hydrolysis (pH 2.0, 15 min) the acetylamino-sugar from the nucleotide was chromatographed on borate-treated Whatman No. 1 paper, using butanol–pyridine–water as the solvent[7]. It gave two spots with the mobilities of *N*-acetylglucosamine and *N*-acetylgalactosamine.

Abbreviations: TMP, TDP, TTP, 5′-monophosphate, 5′-pyrophosphate and 5′-triphosphate of deoxyribosylthymine, respectively.

A preparation of the acetylated nucleotide was carried out using TDP-[^{14}C]-glucosamine. Residual TDP-glucosamine and the mixture of TDP-*N*-acetylglucosamine and TDP-*N*-acetylgalactosamine were isolated by column chromatography. The sugar from the TDP-glucosamine was chromatographed by the method of GARDELL and found to contain no galactosamine. The sugars from the TDP-*N*-acetylamino-sugar peak, after deacetylation, were chromatographed in the same way. The radioactivity was located in 2 peaks corresponding to galactosamine and glucosamine; the ratio of glucosamine:galactosamine was 7:3.

The acetylating system in the crude sonic extract will acetylate glucosamine-1-*P* at a rate 60% of that of TDP glucosamine. Glucosamine, galactosamine and glucosamine-6-*P* are not acetylated. Thus, the enzyme differs in specificity from the glucosamine-6-phosphate-*N*-acetylase of baker's yeast[8]. We have been unable to detect the formation of TDP-acetylglucosamine from acetylglucosamine-1-*P* and TTP.

The problem of whether the reaction of TTP with glucosamine-1-*P* is catalyzed by the same enzyme as the reaction of TTP with glucose-1-*P* is also being studied. Fractionation of the enzyme with $(NH_4)_2SO_4$ followed by adsorption and elution from calcium phosphate gel yielded fractions in which the ratio of activities of TDP-glucose pyrophosphorylase to TDP-glucosamine pyrophosphorylase varied from 10:1 to 2:1 suggesting that two separate enzymes are involved.

Work is now in progress to purify and separate the enzymes involved in these reactions. The finding of amino-sugars linked to deoxyribosylthymine provides an alternate series of nucleotides which should be considered in addition to the well known uridine series as possible precursors of amino-sugar-containing polysaccharides.

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The effect of fluoroacetate on cyanocobalamin absorption in the rat

There seems little doubt that cyanocobalamin, when administered in physiological amounts, is absorbed by some process involving active transport. Evidence has already been advanced that an enzyme reaction is probably concerned[1]. With this in view, it seems reasonable to assume that energy is required for absorption of the