# THE SYNTHESIS OF THYMIDINE DIPHOSPHATE HEXOSES BY STREPTOCOCCUS FAECALIS GROWN ON D-GALACTOSE *

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Some microorganisms and plants contain an enzyme complex which is responsible for the synthesis of thymidine diphosphate glucose (dTDP-glucose) from thymidine triphosphate (dTTP) and α-D-glucose-1-phosphate (G-1-P) and for the conversion of the dTDP-glucose to dTDP-rhamnose (Pazur and Shuey, 1961; Glaser and Kornfeld, 1961). The first enzyme of the complex is a typical pyrophosphorylase in that it effects a transfer of the thymidyl moiety of dTTP to G-1-P. The presence of a new pyrophosphorylase, namely a thymidine diphosphate galactose (dTDP-galactose) pyrophosphorylase in extracts from Streptococcus faecalis adapted to D-galactose, has now been observed in our laboratory. The enzyme was not present in cells grown on D-glucose; hence the new pyrophosphorylase is an inducible enzyme. This pyrophosphorylase catalyzed the synthesis of dTDP-galactose from dTTP and α-D-galactose-1-phosphate (Gal-1-P). The non-purified enzyme preparation containing the pyrophosphorylase also catalyzed the conversion of the dTDP-galactose to dTDP-glucose and ultimately to dTDP-rhamnose. The preparation evidently contained a second inducible enzyme, a dTDP-galactose-4-epimerase, which effected the epimerization of dTDP-galactose to dTDP-glucose and the enzyme system required for the conversion of the dTDP-glucose to dTDP-rhamnose.

The S. faecalis was grown on nutrient broth containing 0.1% of D-glucose or D-galactose as the carbohydrate source. Examination of the cells from the

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two media by staining techniques and by biochemical tests showed that the cells grown on the two carbohydrate sources were identical. Enzyme extracts were prepared from the cells by methods previously described (Pazur and Shuey, 1961). These enzyme preparations were tested under identical conditions on mixtures of (1) uridine triphosphate (UTP) and G-1-P, (2) UTP and Gal-1-P, (3) dTTP and G-1-P and (4) dTTP and Gal-1-P. In the tests, reaction mixtures consisting of 2 mg of the nucleotide triphosphate and 2 mg of the hexose-phosphate in 0.1 ml of enzyme extracts buffered to pH 7.2 were incubated at room temperature. After six hours the mixtures were examined for new reaction products by paper chromatography in a solvent system of 7 parts ethyl alcohol and 3 parts 1 M ammonium acetate of pH 7.5. The nucleotide compounds were detected on the paper by UV absorption. The essential findings and the Rf values of the synthesized nucleotides are recorded in Table 1.

Table 1

Synthesis of nucleotide-hexoses by enzyme extracts of S. faecalis

| Reaction Mixture | Enzyme Ext. from G-adapted cells | Enzyme Ext. from Gal-adapted cells | Rf values of nucleotide-hexoses |
|---|---|---|---|
| UTP + G-1-P | UDP-glucose | UDP-glucose | 0.27 |
| UTP + Gal-1-P | UDP-galactose | UDP-galactose | 0.26 |
| dTTP + G-1-P | dTDP-glucose | dTDP-glucose | 0.38 |
| dTTP + Gal-1-P | none | dTDP-galactose | 0.37 |

The nucleotide-hexose compounds listed in the table were the initial products of the reactions. Because of the presence of epimerases in the enzyme preparations employed, these initial products were converted to other nucleotide-hexoses. As will be indicated in a later section, the dTDP-galactose isolated from a reaction mixture of the above type also contained a considerable amount of dTDP-glucose as well as some dTDP-rhamnose.

In order to obtain sufficient amounts of the new nucleotide produced from dTTP and Gal-1-P for characterization purposes, a reaction mixture of

30 mg of dTTP and 30 mg of Gal-1-P in 2 ml of enzyme extract from the D-galactose-adapted cells was prepared. The material in this mixture at Rf value 0.37 was isolated by paper chromatographic methods in overall yield of about 30% based on the initial dTTP employed. On acid hydrolysis, the nucleotide fraction was converted to thymidine monophosphate, inorganic phosphate and to the reducing hexoses, D-galactose, D-glucose and L-rhamnose. These reducing compounds were initially detected by their characteristic Rf values 0.59, 0.65, 0.88, respectively, after three ascents of the solvent system of n-butyl alcohol, pyridine and water (45:25:45 by volume). Additional evidence for these hexoses was obtained by use of galactose oxidase (Avigad, et al, 1962), glucose oxidase (White and Secor, 1957), and the Dische-Shettles reaction (Dische and Shettles, 1948). Adequate controls established that D-glucose and L-rhamnose did not yield a positive test with galactose oxidase while the validity of the latter methods have been well established. Apparently the nucleotide fraction isolated from the above digest consisted of a mixture of thymidine diphosphate hexoses of which the major components were thymidine diphosphate galactose, thymidine diphosphate glucose and thymidine diphosphate rhamnose and the minor components, nucleotide hexoses which are intermediates in the transformation of D-galactose to L-rhamnose.

It can be seen from the results in Table 1 that S. faecalis whether grown on D-glucose or D-galactose possessed the pyrophosphorylase responsible for the synthesis of dTDP-glucose from dTTP and G-1-P as well as the pyrophosphorylases responsible for the synthesis of the uridine nucleotide hexoses. However, only the D-galactose-adapted cells produced the enzyme which catalyzed the synthesis of dTDP-galactose from dTTP and Gal-1-P. That the former enzymes were responsible for the synthesis of dTDP-galactose is unlikely for several reasons. First, since the enzyme preparations from both types of cells contained the UDPG- and UDPGal-pyrophosphorylases, the dTDPG-pyrophosphorylase and the UDPGal-4 epimerase (determined in another series of experiments), a synthesis of thymidine diphosphate hexose from dTTP and Gal-1-P should have occurred with the enzyme extracts from both types of cells.

Second, when UDP-glucose and dTTP were incubated with the enzyme preparation from the D-galactose-adapted cells no synthesis of thymidine diphosphate hexose occurred. This result was interpreted to indicate that under the conditions of our experiments, an interconversion of the uridine and thymidine nucleotide hexoses did not occur. Finally, as already indicated, D-galactose was detectable chromatographically and by means of galactose oxidase in the hydrolysate of the thymidine diphosphate hexose fraction produced from dTTP and Gal-1-P. The chromatographic procedure employed for isolating the thymidine diphosphate hexose fraction (Rf value, 0.37) eliminated the possibility of contamination of the nucleotide fraction with unreacted Gal-1-P (Rf value 0.18). Repeated experiments in which enzyme extracts from cells grown on D-glucose were tested on dTTP and Gal-1-P showed that enzyme extracts from D-glucose-adapted cells did not produce the dTDP-hexose even on prolonged incubation of the digests. The dTDP-galactose pyrophosphorylase was always present in the enzyme extracts from the D-galactose-adapted cells, although in some experiments longer incubation periods were required to obtain an appreciable synthesis of dTDP-hexoses.

The energy needs of S. faecalis grown on D-galactose were undoubtedly satisfied by the reactions of glycolysis and the tricarboxylic-acid cycle. Since the organism adapted to D-galactose possessed the enzymes for the uridine pathway of galactose metabolism, this pathway was most probably employed for the conversion of D-galactose to D-glucose which, in turn, was metabolized via the normal cycles. It is therefore unlikely that the dTDP-galactose pyrophosphorylase is required for the energy yielding reactions. Rather, the suggestion is advanced that the new pyrophosphorylase and the dTDP-galactose 4-epimerase are required for the conversion of the intact D-galactose unit to the L-rhamnose unit via the thymidine diphosphate hexose pathway. Evidently, a direct connection between the uridine nucleotide hexoses and the thymidine nucleotide hexoses does not exist. The thymidine diphosphate hexoses are used for the synthesis of essential cellular

constituents such as deoxy-nucleic acids (Okazaki, 1959) cell wall heteropolysaccharides (Pazur and Shuey, 1960) or plant glycosides (Barber and Neufeld 1961). Studies to assess the role of thymidine diphosphate galactose in the growth and development of organisms adapted on D-galactose are being continued.

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