URIDINE DIPHOSPHATE DIHYDROXYACETONE

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All the types of Diplococcus pneumoniae so far investigated, when grown to the resting phase in the manner described (1), accumulate a uridine nucleotide which, by analytical and enzymatic criteria, is uridine diphosphate dihydroxyacetone.

The nucleotide was isolated from the cells of Diplococcus pneumoniae Type II, strain R19, by the methods of ion exchange and of paper chromatography described previously (2) and was located in the 0.01 N HCl-0.03 M NaCl eluate from a Dowex 1 Cl' column. The R_{UMP} value of the nucleotide by paper chromatography in the neutral ethanol-ammonium acetate solvent of Paladini and Leloir (3) was identical with that of UDP-acetylglucosamine (R_{UMP} = 1.62).

For analytical purposes, the nucleotide band was eluted from the paper chromatogram after washing the paper with 95% alcohol and the following analyses were carried out:

a.) Hydrolysis of the nucleotide with 0.01 N HCl for 20 min. at 100° to liberate the sugar component of the nucleotide, followed by the color reactions described by Dische (4) for sugar analysis, gave negative results for aldohexose, ketohexose, pentose, methyl pentose, 2-deoxypentose, heptose, heptulose, hexosamine and uronic acid. Tests for 3:6 dideoxy

sugars (5) and muramic acid (6) were also negative. The material liberated by the above hydrolysis was negative in the p-phenylenediamine reaction for aldehyde groups described by Feigl (7). Hydrolysis of the nucleotide with 1.0 N HCl for 30 min. at 100° resulted in a product giving a strongly positive reaction for aldehydes by this test. Commercial dihydroxyacetone reacted in an identical manner with both types of acid hydrolysis.

b.) Hydrolysis of the nucleotide with 0.01 N HCl for 20 min. at 100° with subsequent evaporation in vacuo and paper chromatography in ethanol-ammonium acetate (3), 2-butanol-acetic acid-acetone-water (8), n-butanol-ethanol-ammonia-water (9) and ionophoresis in borate buffer at pH 8.6 (10) liberated a reducing component which was located with the aniline hydrogen phthalate reagent. The R_F values of the reducing material in the above solvents corresponded with those of dihydroxyacetone which had been treated with 0.01 N HCl under identical conditions. The reducing material liberated from the uridine nucleotide by acid hydrolysis was also detectable on the chromatograms by spraying with 0.6% thiobarbituric acid, producing a yellow spot in visible light comparable to that obtained by dihydroxyacetone (11).

c.) Estimation of absorption at 262 mμ at pH 2, assuming a value for the molar extinction coefficient of 10,000, and estimation of total phosphorus gave the ratio: uridine/phosphorus = 1/2. The absorption spectrum was typical of a uridine compound with a well defined maximum at 262 mμ. Hydrolysis of the eluted material with 1.0 N HCl for 15 min. followed by evaporation in vacuo and subsequent chromatography in ethanol-ammonium acetate (3) and iso-propanol-HCl (12) showed UMP(5') to be the sole product.

d.) The nucleotide was rapidly hydrolyzed by snake venom organic pyrophosphatase and 5'-nucleotidase at pH 9.1 to uridine and a sugar phosphate. The latter was chromatographically identical with dihydroxyacetone phosphate in the solvents described above. The product of hydrolysis by snake venom pyrophosphatase readily oxidized DPNH in the presence of the α-glycerophosphate dehydrogenase preparation of Racker (13).

It is concluded from the above data that the compound isolated is uridine pyrophosphodihydroxyacetone.

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