Isolation of thymidine diphosphosugar compounds from *Escherichia coli*

During the course of purification of a uridine nucleotide containing diaminopimelic acid from a mutant of *E. coli*[1] requiring this acid, three thymidine diphosphosugar compounds were separated from the uridine nucleotide. Their structures have been partially determined. The unusual nature of these substances prompts this preliminary communication.

On paper chromatography in neutral ethanol–ammonium acetate[2], TDP-X (mobility relative to UMP (R_{UMP}) = 1.94) and TDP-Y + TDP-Z (R_{UMP} = 1.67) were separated from the uridine nucleotide containing diaminopimelic acid. TDP-Y and TDP-Z were separated from each other in isobutyric acid–NH_3. 5–10 μmoles of each compound were obtained from the cells from 100 l culture. Each of the nucleotides had the following properties. U.v. absorption spectra in 0.01 *N* HCl and 0.05 *N* NaOH were similar to uridine nucleotides, but, after digestion with $HClO_4$, the base liberated was identified as thymine by paper chromatography in solvents 2, 3 and 5 of MARKHAM[3]. Identification of thymine was also obtained through the specific procedure of ROBERTS AND FRIEDKIN[4] in which acetol (derived from thymine after bromination) is determined as the fluorescent 3-hydroxyquinaldine. 0.75–1.0 mole acetol were obtained per mole of nucleotide. TDP (R_{UMP} = 0.75) was obtained by hydrolysis at 100° for 2 min in 0.01 *N* HCl and TMP (R_{UMP} = 1.52) was obtained by hydrolysis at 100° for 15 min in 1 *N* HCl. Analyses indicated 0.8–1.0 mole deoxyribose[5] and 1.85–2.15 moles phosphate/mole thymine. The compounds contained little or no ribose.

A reducing group, measured by ferricyanide reduction[6], was very rapidly liberated from each of the thymidine nucleotides in 0.01 *N* HCl at 100°, the half-time being about 30 sec. After hydrolysis, the reducing substances could be detected on paper chromatograms with the $AgNO_3$ spray, byt they were not detected with aniline hydrogen phthalate. In several solvents used for chromatography of sugars (*e.g.* butanol–pyridine–water (6 : 4 : 3)) they had high mobilities similar to 2-deoxyribose, 2-deoxyglucose, 6-deoxygalactose (fucose), 6-deoxymannose (rhamnose), 3,6-dideoxyglucose, and other dideoxysugars. The three sugars are similar to the 2-deoxysugars in the following properties*. They each gave the WEBB-LEVY reaction for a deoxynucleic acid[7], believed to be specific for 2-deoxysugars[8]. However, their extinction coefficients, like that of 2-deoxyglucose, were only about 10 % of that obtained with 2-deoxyribose. Following periodate oxidation, the WEBB-LEVY reaction was abolished, indicating that the compounds are not 3,6-dideoxyhexoses[8]. All three sugars also reacted with 3,5-diaminobenzoic acid to yield a fluorescent compound, a reaction believed to be specific for compounds with the structure R-CH_2-CHO[9]. Finally, X, but not Y or Z, reacted with diphenylamine[10] to give a pink-colored compound with a spectrum and extinction coefficient identical to that obtained from 2-deoxyglucose, but distinct from the blue-colored compound obtained from 2-deoxyribose. The following reactions, among others, were negative: anthrone for sugars, cysteine-

Abbreviations: TDP, thymidine diphosphate; TMP, thymidine monophosphate; UMP, uridine monophosphate; CDP, cytidine diphosphate.

* These reactions were not due to the 2-deoxyribose in the TMP moiety of the nucleotide. Under the conditions of the tests the N-glycoside linkage between thymine and 2-deoxyribose is stable, and TMP failed to give any of the indicated reactions.

sulfuric acid for 6-deoxyhexoses and heptoses, Morgan-Elson for acetylamino sugars, and cysteine-carbazole for ketohexoses (*cf.* ref.[10]). Failure to react with anthrone or with aniline hydrogen phthalate has also been observed with 2-deoxyglucose and 2-deoxyribose. All three sugars consumed 2.9–3.0 moles periodate while bound to TDP, and the consumption of Y and Z, but not X, increased by 0.6–0.7 mole when the sugar was liberated from the nucleotide by mild acid hydrolysis. After periodate oxidation, all three sugars failed to yield malonyl dialdehyde (measured by the thiobarbituric acid reaction[11]) while 2-deoxyglucose and 2-deoxyribose each produced 1 mole. Since the formation of malonyl dialdehyde requires free hydroxyl groups at both C-3 and C-4 of a 2-deoxysugar, other substituents must be present in the molecule, if these substances are 2-deoxysugars. At the present time it is clear that these sugars are unusual compounds and no structure has been written to accommodate all the experimental facts. Further investigation is in progress as are attempts to isolate larger quantities.

This paper is the first report of the isolation of thymidine diphosphosugar compounds, although the occurrence of unusual thymidine nucleotides in microorganisms has been recognized by OKAZAKI, OKAZAKI AND KURIKI[12]. They represent the second group of nucleotides containing a deoxynucleoside and hence they are related structurally to deoxynucleic acids. Previously, deoxy-CDP-choline[13] and deoxy-CDP-ethanolamine[14] have been isolated. Whether this structural relationship to deoxynucleic acid is also accompanied by some functional relationship is an exceedingly intriguing question. Deoxyhexoses have been encountered as components of specific antigens in *E. coli* and in various *Salmonellae*[15], and it might be suggested that the thymidine nucleotides isolated here act as carriers of the deoxysugars for synthesis of antigenic or other specific polysaccharides in *E. coli*, just as uridine nucleotides function as carriers of many types of sugars for a variety of transglycosylation reactions (*e.g.* ref.[16]).

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