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FORMATION OF URIDYLYL(3′→5′)URIDINE, URIDYLYL(2′→5′) URIDINE, 6-AZAURIDYLYL(3′→5′)6-AZAURIDINE AND 6-AZA-URIDYLYL (2′→5′)6-AZAURIDINE BY THERMIC PHOSPHORYLATION OF THE CORRESPONDING NUCLEOSIDES WITH INORGANIC PHOSPHATE

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It was recently observed by Ponnamperuma and Mack (1) that the heating of a mixture of natural nucleosides with inorganic phosphate to 160°C results in a complex mixture of products. The two authors have so far identifie the presence of nucleoside monophosphates in the mixture and, on the basis of chromatographic and electrophoretic mobility, obtained preliminary evidence on the presence of other substances, among others dinucleoside monophosphates. The paper quoted (1) represents a continuation of Ponnamperuma′s studies of the formation of nucleic acid components under abiotic conditions.

It was considered to be of interest

(1) to analyze in detail the reaction products of thermic phosphorylation of nucleosides with the objective of fully identifying particularly the products of dinucleoside monophosphate mobility, including an analysis of the nature of their internucleotide bonds;

(2) to investigate the applicability of this very simple method to phosphorylation of anomalous nucleosides altered either in the nitrogen base or in the sugar component;

(3) to develop a simple separation procedure for isolating the individual products as an important prerequisite for employing thermic phosphorylation for preparing small amounts of highly radioactive nucleotidic compounds.

Thermic phosphorylation products of uridine-^{14}C (G) (equimolar amounts of the nucleoside and of NaH_2PO_4 had been heated for 3 hrs. to 160°C) were isolated in the pure form by paper chromatography (uridine-5′-phosphate 8%, uridine-2′,3′-cyclic phosphate 13%, uridine-2′(3′)-phosphate 2%, uridylyl uridine 7%). The separation scheme as shown in Fig. 1 may be applied to isolating products of an analogously conducted phosphorylation of 6-azauridine-4,5-^{14}C. Enzymatic degradation of the individual products using Russell′s viper venom enzymes and bovine pancreatic ribonuclease confirmed the identity of the monophosphates

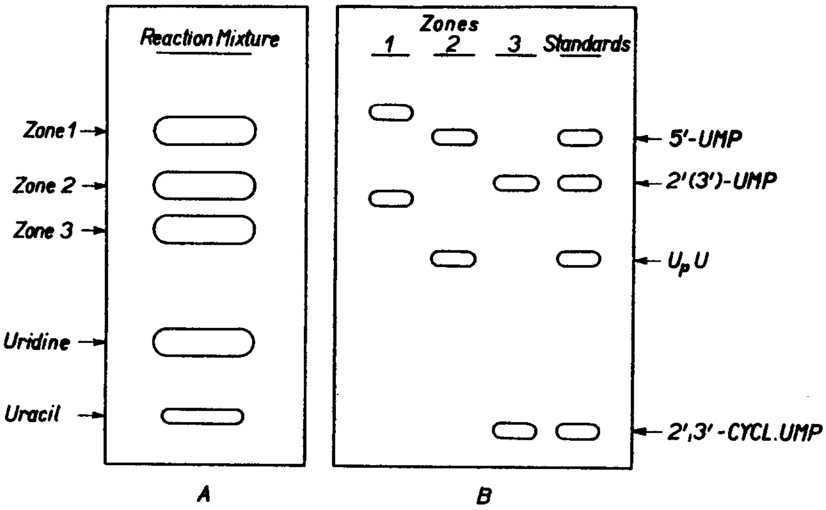

Fig. 1: Chromatographic Isolation of the Principal Products of Thermic Phosphorylation of Uridine-^{14}C (G) with NaH_2PO_4.
A Paper chromatography on Whatman no. 3MM in a mixture of isobutyric acid-ammonia-water (66 : 1.5 : 33).
B Chromatography of eluates from zones 1-3 of the preceding separation (A) on Whatman no. 3MM in a mixture of isopropanol-ammonia-water (7 : 1 : 2).

formed (ref.1 and Fig.1). Substances with chromatographic and electrophoretic mobilities identical with those of authentic samples of uridylyl(3′→ 5′)uridine and 6-aza-uridylyl(3′→ 5′)6-azauridine were completely cleaved by the Russell′s viper venom enzymes to the corresponding nucleosides. On the basis of this finding and in view of the fact that comparative electrophoresis of both analyzed fractions in borate buffer (pH 9) showed no substances of higher mobility than the authentic samples of dinucleoside monophosphates it was assumed that the substances can contain only 3′→5′ and/or 2′→5′ internucleotidic bonds. In agreement with this assumption it was found that pancreatic ribonuclease splits one-half of the fractions to an equimolar mixture of the nucleoside and of nucleoside-3′-phosphate whereas the other half of the fractions remain intact. It was deduced from these findings that thermic phosphorylation of uridine and 6-azauridine yields the corresponding dinucleoside monophosphates, indirect evidence on which had been obtained by Ponnamperuma and Mack. It was further shown that these dinucleoside monophosphates contain only 3′→5′ and 2′→5′ internucleotidic bonds at a ratio of approximately 1 : 1. The mixtures can be readily resolved by ion-exchange chromatography (2).

As was shown by orientation experiments with a number of anomalous nucleosides (3) the greatest part of these substances undergo thermal phosphorylation, sometimes in a relatively high yield (6-azauridine, 6-azacytidine, 5-hydroxyuridine, 5-bromuridine, tubercidin, uracil xyloside). On the other hand, e.g. orotidine and 6-mercaptopurine riboside were not phosphorylated under the conditions described.

In view of the simplicity of thermic phosphorylation of unprotected nucleosides and of the ready chromatographic isolation of the individual products, as well as nonreacted material the procedure appears to be of special advantage for preparing radioactive labelled nucleotides of high specific activity. The preparation of these substances by the conventional multi-stage procedures is very difficult because of the small quantities of substances usually involved in the work with highly radioactive materials. Since most of the products of thermic phosphorylation belong among substances in great demand the formation of a mixture of well separable labelled nucleotides presents no disadvantage.

The finding of the $3' \rightarrow 5'$ internucleotidic bond in the products of thermic phosphorylation of nucleosides is remarkable in connection with Ponnamperuma's hypothesis on the possible importance of thermic phosphorylation in the formation of nucleic acid components under primitive earth conditions.

The detailed account of this work will be published in the Collection of Czechoslovak Chemical Communications.

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