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FORMATION OF OLIGONUCLEOTIDES DURING HEATING OF A MIXTURE OF URIDINE 2′(3′)-PHOSPHATE AND URIDINE

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Considerable attention has been recently focussed on methods for specific synthesis of polyribonucleotides containing the 3′→5′ interribonucleotide bonds, especially of dinucleotides and biochemically important[1-4] trinucleotides. Generally applicable methods have been developed, making it possible to prepare all the dinucleoside monophosphates or trinucleoside diphosphates, derived from the four principal ribonucleotides[5].

These several-step synthetic processes require a temporary protecting of preselected positions of the reacting nucleotide or dinucleotide and nucleoside for achieving the specific 3′→5′ internucleotide bond. Their application to the preparation of labelled dinucleotides and trinucleotides of high specific activity for biochemical research involves great difficulties. The amounts of starting radioactive nucleosides or nucleotides that are usually available are mostly below 10 μmol.

For this reason, we studied in our earlier work the formation of dinucleoside monophosphates during the very simple thermal phosphorylation of unprotected nucleosides by inorganic phosphate. We succeeded then in isolating among other compounds uridylyl-(3′→5′)-uridine and 6-azauridylyl-(3′→5′)-6-azauridine.

In selecting the procedure to be applied to the preparation of oligonucleotides by the thermal phosphorylation method of nucleosides we proceeded

from the following facts:

1. The thermal phosphorylation of nucleosides by inorganic phosphate revealed a high thermal stability of nucleotides[6,7] as well as dinucleotides[6] under the conditions used (160°C).

2. Of the six theoretically possible types of internucleotide bonds, only the 3′→5′ and 2′→5′ bonds are formed during thermal phosphorylation by inorganic phosphate[6].

3. The pK values of nucleoside monophosphates are close to the pK values of inorganic phosphate[8] and it can be assumed that under the conditions of thermal phosphorylation a reaction between a nucleotide and a nucleoside can take place giving rise to internucleotide bonds.

On the basis of these premises we thought it feasible to investigate the possibilities of a simple preparation of oligonucleotides by a thermal reaction of unprotected nucleotides and nucleosides.

Heating a mixture of dry uridine 2′(3′)-phosphate and uridine-^{14}C(G) (molar ratio 5:1) to 160°C for 5 min in vacuo of an oil pump yielded a mixture of products, the resolution of which by repeated chromatography on Whatman No. 3 in isopropyl alcohol:ammonia:water (7:1:2) is shown in Fig. 1.

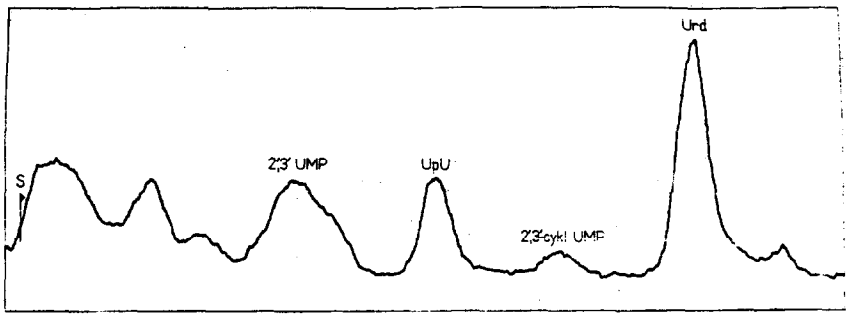

Fig. 1: Distribution of Radioactivity on a Chromatogram of a Reaction Mixture Obtained by Heating Uridylic Acid and Uridine-^{14}C(G)
S - start, UMP - uridine 2′(3′)-phosphate, UpU - uridylyluridine, 2′,3′-cycl.UMP - uridine-2′,3′ cyclic phosphate, Urd - uridine.

A comparison of the chromatographic and electrophoretic mobilities of the individual fractions with authentic samples permitted us to identify, in

addition to nonreacted uridine and uridine-2′(3′)-phosphate, also uridine--2′,3′ cyclic phosphate and uridylyluridine.

The fraction of lower chromatographic mobility than that of UMP (Fig. 1) was completely split by snake venom enzymes. Hence it contains only 2′→5′ and/or 3′→5′ internucleotide bonds, no terminal phosphates being present. An exact identification of compounds present in this fraction, one of them having the same chromatographic and electrophoretic mobility as authentic UpUpU, is under way.

Cleavage of uridylyluridine with snake venom enzymes and bovine pancreatic ribonuclease, and electrophoresis in borate buffer showed (in common with the thermal reaction of uridine with inorganic phosphate[6]) that we are dealing here with a mixture of uridylyl-(2′→5′)-uridine and uridylyl-(3′→5′)-uridine in a ratio close to 1:1. The uridine and uridylic acid formed by cleavage with bovine pancreatic ribonuclease were labelled, the molar specific radioactivity of uridine being almost twice higher. The yield of uridylyluridine (17% with respect to the original uridine) obtained by this procedure is more than twice higher than during thermal phosphorylation with inorganic phosphate[6]. A cleavage of selected fractions after chromatographic separation by snake venom phosphodiesterase or by 1N NaOH permits to obtain uridine 5′-phosphate-^{14}C or another fraction (a total of 40% of radioactivity used) of uridine 2′(3′)-phosphate-^{14}C.

The formation of radioactive uridine 2′(3′)-phosphate (Fig. 1) and the detection of radioactivity in both uridine moieties of the uridylyluridine molecule prepared as described above indicates that the overall reaction is not a simple esterification of the type of Up + U → UpU. We are apparently dealing here with a more complex set of reactions, involving probably a transient formation of 2′(3′) → 2′(3′) internucleotidic bonds or an intermolecular shift of the phosphate residue. For an explanation of the reaction mechanism further experimental data are required.

The thermal reaction of uridylic acid with uridine is specific in the sense that the primary phosphate of the nucleotide successfully attacks only the 5′-hydroxyl of the sugar residue of the molecule with which it reacts.

Preliminary evidence was obtained that the above described procedure can be applied to the preparation of homogeneous as well as mixed oligonucleotidic compounds, derived from other natural, as well as anomalous nucleosides and nucleotides of the pyrimidine type. Uridylyluridine was also obtained by heating a mixture of uridine-2′(3′)-phosphate and uridine in dimethylformamide. The main reaction product here is 2′,3′ cyclic phosphate. The application of these findings to a simple cyclization of nucleoside-2′(3′)-phosphates or to an increase of the yield of dinucleoside phosphates by suitably masking position 2′ of the nucleotide will be studied in the future.

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