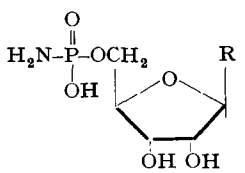
Short Communications

Synthesis of ribonucleoside-5'-polyphosphates

The preparation of ribonucleoside-5'-phosphoramidates (I) by a simple, one-step procedure[1] has provided the key intermediates for a new synthetic approach to unsymmetrical nucleoside-5'-pyrophosphates of biological interest such as ADP[2], UDP[3], FAD[4] and UDPG[4].



I

R = adenine, guanine, cytosine or uracil

All of the reactions which have been studied so far involve coupling a phosphoramidate with orthophosphoric acid or a phosphomonoester. This report describes the reaction of nucleoside-5'-phosphoramidates with pyrophosphoric acid to give nucleoside-5'-triphosphates.

Some selected data from studies on the reaction

$$AMP\text{–}NH_2 + PP \longrightarrow ATP + NH_3$$

are shown in Table I. The reactions were carried out in 12-ml centrifuge tubes under anhydrous conditions at 0° with rapid stirring. The products were precipitated with 1.4 ml petroleum ether (b.p. 30–60°), collected by centrifugation, washed twice with 1-ml portions of anhydrous ether and once with acetone. The residue was dissolved in 1.4 ml 1 *N* NH_4OH and fractionated by paper electrophoresis (Whatman 3 MM paper, saturated with 0.02 *M* citrate buffer, pH 4, 29 V/cm). The u.v.-absorbing spots were eluted from the paper and the absorbance was determined in a Beckman Model DU spectrophotometer. The presence of unreacted amide was detected by paper chromatography in isopropanol–ammonia–water (7 : 1 : 3)[5].

The results show that after 40 min considerable unchanged AMP–$NH_2$was still present (Expt. 1, monophosphate fraction). After 90 min (Expt. 2), the reaction was complete and increasing the reaction time had very little effect on the product distribution. Increasing the ratio of PP : AMP–NH_2 (Expt. 3) gave a slight improvement in the ADP–ATP yield, apparently by diminishing the formation of DAPP.

Abbreviations: AMP, adenosine-5'-monophosphate; AMP–NH_2, adenosine-5'-phosphoramidate; ADP, adenosine-5'-diphosphate; DAPP, P^1P^2-diadenosine-5'-pyrophosphate; ATP, adenosine-5'-triphosphate; CMP–NH_2, cytidine-5'-phosphoramidate; GMP–NH_2, guanosine-5'-phosphoramidate; UMP–NH_2, uridine-5'-phosphoramidate; UDPG, uridine diphosphate glucose; FAD, flavin-adenine dinucleotide; P_i, orthophosphoric acid; PP, pyrophosphoric acid.

TABLE I

REACTION OF NUCLEOSIDE-5-PHOSPHORAMIDATES WITH PYROPHOSPHORIC ACID

Expt.	Amide*	PP/amide**	Time	Solvent		Products			
				o-chloro-phenol***	n-butanol	Mono-phosphate	Di-phosphate	Tri-phosphate	Symm. pyro-phosphate
		mg/mg	min	ml	ml	%§	%	%	%
1	AMP–NH_2	2	40	0.7	0	66§§	16	9	9
2	AMP–NH_2	2	90	0.7	0	31	35	24	10
3	AMP–NH_2	5	90	0.7	0	32	40	28	0
4	AMP–NH_2	7	180	0.2	0.5	54	11	30	5
5	CMP–NH_2	2	180	0.2	0.5	37	18	24	21§§§
6	GMP–NH_2	3	210	0.2	0.5	46	10	32	12
7	UMP–NH_2	2	120	0.7	0	52	16	19	13

* GMP–NH_2 as its ammonium salt. All others as dicyclohexylguanidinium salts.

** Crystalline pyrophosphoric acid, City Chemical Corp., New York. This sample contained about 60% pyrophosphoric acid and 40% orthophosphoric acid identified by paper chromatography[11] and assayed by the method of FLYNN *et al.*[12].

*** Purified from practical grade (Matheson, Coleman and Bell, New York) by fractional distillation through a 4-foot packed column.

§ Results are expressed as percent of the total absorbance of all spots.

§§ Considerable AMP–NH_2 was present in this fraction.

§§§ This spot was not definitely identified as P^1P^2-dicytidine pyrophosphate.

Thus, under the most favorable conditions found so far, a total yield of ADP + ATP of 68 % has been obtained (Expt. 3).

Several solvents other than *o*-chlorophenol were also investigated. However, no single solvent was found which would dissolve the reactants and therefore the reaction was very slow. For example, when *n*-butanol was used, only traces of ADP and ATP could be detected after 3 days and large amounts of unreacted AMP–NH_2 were present. However, when a mixture of *n*-butanol and *o*-chlorophenol was employed, the reaction was complete in 180 min (Expt. 4). It is most interesting that this solvent mixture gave a product distribution completely different from *o*-chlorophenol alone. This change in ADP : ATP ratio from 1.4 (*o*-chlorophenol, Expt. 3) to 0.3 (*o*-chlorophenol + *n*-butanol, Expt. 4) was reproducible in 4 different experiments.

The reaction of other nucleoside-5'-phosphoramidates with pyrophosphoric acid has not been investigated as extensively as the AMP–NH_2 reaction. However, moderate yields of the corresponding di- and triphosphates have been obtained as shown in Table I (Expts. 5, 6 and 7).

No concerted effort has been made to isolate the products from these small-scale reactions. However, ion-exchange separation of the products expected from the AMP–NH_2 : PP reaction was investigated. The order of elution from a Dowex-1-chloride column (1 × 3 cm, 200–400 mesh, 8 % cross-linked) using linear gradient elution[6,7] (500 ml 0.003 *N* HCl in the mixer, 500 ml 0.003 *N* HCl + 0.1 *M* LiCl in the reservoir) was AMP, P_i, ADP, PP, DAPP and ATP. By this procedure, ADP and ATP can be separated easily from the other reaction products and similar separations should be possible with other nucleotides[8].

In attempting to formulate a mechanism to explain the product distribution, two points need to be emphasized. First of all, numerous experiments in this laboratory

have established that the amides are extremely sensitive to moisture in acidic solutions and in small-scale experiments such as those described here, it is virtually impossible to eliminate traces of water from the reaction mixtures. Thus, at least part of the AMP is probably produced by hydrolysis of AMP–NH_2. Further reaction of AMP with AMP–NH_2 could then account for the small amount of DAPP which is usually found. Secondly, samples of crystalline commercial pyrophosphoric acid employed in this work contained as much as 40 % orthophosphoric acid which could react directly with AMP-NH_2 to produce ADP. However, the change in ADP : ATP ratios and particularly the large amount of AMP formed when the solvent is changed is difficult to reconcile with this simple explanation. It is possible that exchange reactions of the type

$$2ADP \rightleftharpoons AMP + ATP \quad (1)$$

$$ATP + Pi \rightleftharpoons ADP + PP \quad (2)$$

also occur and influence the product distribution. If reaction (1) occurs, the solvent appears to play an important role since no ATP could be detected when AMP–NH_2 reacted with P_i in *o*-chlorophenol to give ADP[2]. Reaction (2) has been demonstrated by LOWENSTEIN in the presence of metal ions[9].

Recently, the biological counterpart of the work described here has been reported by ELLFOLK AND KATUNUMA[10]. These workers described crude enzyme preparations from several bacteria which catalyze the reaction

$$ATP + NH_3 \rightleftharpoons AMP\text{–}NH_2 + PP$$

Thus, the nucleoside-5'-phosphoramidates assume new importance as biological intermediates. It will be interesting to see what actual role these compounds play in metabolic reactions and if nucleoside phosphoramidates other than AMP–NH_2 have any biological activity.

This work was supported by a grant from the National Science Foundation (NSF-G-04964).

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Received August 24th, 1959

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