

Letter to the Editor

Further Studies of Urea-Catalyzed Phosphorylation Reactions

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Summary. We have analyzed the products formed when mixtures of a nucleoside and ammonium dihydrogen phosphate are heated with an excess of urea. If there is more phosphate than nucleoside in the mixture, compounds containing pyrophosphate bonds are obtained. If uridine, as nucleoside, is in excess over phosphate, di- and oligonucleotides are formed.

Key words: Phosphorylation — Nucleotides — Nucleoside Polyphosphates — Oligonucleotides — Urea — Prebiotic.

Nucleosides are converted to phosphorylated derivatives in yields in excess of 90% when they are heated in dry mixtures with urea, sodium hydrogen phosphate, ammonium chloride, and ammonium bicarbonate (Lohrmann and Orgel, 1971). We have used X-ray methods to analyze the crystalline phases present in mixtures of this kind, and have shown that the effective components are ammonium dihydrogen phosphate and urea (Österberg, unpublished results). The phosphorylated products obtained from uridine and 2',3'-dideoxythymidine after heating for periods of up to 11 days at 100° with mixtures of urea and ammonium dihydrogen phosphate are described in this letter.

Experimental and Results

Reaction mixtures were prepared by evaporating to dryness solutions (1) of urea, $\text{NH}_4\text{H}_2\text{PO}_4$, and ^{14}C -labelled uridine (0.07 mc/mmole) or (2) of urea, ^{32}P -labelled $\text{NH}_4\text{H}_2\text{PO}_4$ (0.1 mc/mmole) and nucleoside (U, or *ddT*)¹ in open glass tubes at 30 to

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¹ *Abbreviations:* U, uridine; U > *p*, uridine cyclic 2',3'-phosphate; *pU*, uridine 5'-phosphate; U*p*, uridine 2'(3')-phosphate; *pUp*, uridine 5',2'(3')-diphosphate; *pU > p*, 5'-phosphouridine cyclic 2',3'-phosphate; U*pUp*, P₁,P₂-diuridine 5'-pyrophosphate; U*pU*, uridylyl-[2'(3') → 5']-uridine; *pUpU*, 5'-phosphouridylyl-[2'(3') → 5']-uridine; U*pUp*, uridylyl-[2'(3') → 5']-uridine 2'(3')-phosphate; *ddT*, 2',3'-dideoxythymidine (2',3'-dideoxy-riboseylthymine); *pddT*, 2',3'-dideoxy-thymidine 5'-phosphate; *p_nddT* (*n* = 2, 3, ...), 2',3'-dideoxy-thymidine 5'-polyphosphates; *ddTppddT*, dinucleoside 5'-phosphate of 2',3'-dideoxy-thymidine; *ddTppddT*, P₁,P₂-dinucleoside 5'-pyrophosphate of 2',3'-dideoxy-thymidine.

40°, using an IR-lamp. The samples were dried by maintaining them at reduced pressure (20 mm Hg) over P_2O_5 overnight. Each dry sample contained a total of about 100 μ moles reagents. No condensed or phosphorylated products were detected in the dried samples before heating.

Reactions were carried out in a dry nitrogen atmosphere, using a specially built closed glass apparatus assembled with ground glass joints. The glass apparatus was kept in an oil bath thermostated to $\pm 0.5^\circ$. The reactions were terminated by cooling the samples with a suspension of dry ice in acetone. They were stored at -20° until analyzed.

The products were separated by chromatography and high voltage electrophoresis on paper (Lohrmann and Orgel, 1971; Österberg and Orgel, 1972). The identifications were confirmed by acid and alkaline hydrolyses and enzymatic degradation with alkaline phosphatase, venom phosphodiesterase, and pancreatic ribonuclease (Sulston *et al.*, 1968; Lohrmann and Orgel, 1971).

a) Phosphate Excess

We first investigated the phosphorylation of a model compound, 2',3'-dideoxy-thymidine, a nucleoside analogue with only one hydroxyl group (Pfitzner and Moffatt, 1964). In this case only nucleoside polyphosphates and symmetric derivatives of the type $ddT-(p)_n-ddT$ can form. Our results are shown in Table 1.

The products obtained from uridine were much more complicated since the 2', 3' and 5'-OH groups can all be phosphorylated. In Table 2, we show the products obtained after relatively brief heating periods. We carried out a detailed analysis of a mixture that had been heated for a longer time. These results are given in Table 3.

Table 1. Reaction products formed by heating a mixture of 2',3'-dideoxy-thymidine (2.7 mole-%), urea (89.2 mole-%), and $NH_4H_2^{32}PO_4$ (8.1 mole-%) at 100° in a dry nitrogen atmosphere

Yields of nucleoside derivatives are given as percentages of the total 2',3'-dideoxy-thymidine initially in the reaction mixture. Yield of inorganic polyphosphate is given as percentages of the total phosphate in the mixture.

Time	$pddT$	p_2ddT	p_3ddT	p_nddT $n > 3$	$ddTppddT$	$ddTpppddT$	$ddTppppddT$	P_N $N > 7$	Cycl P_3
1 h	6								
3 h	19								
6 h	29								
12 h	42	3							
24 h	53	13	4	2	5				
2 days	6	19	11	8	8	18	9	16	19
4 days	2	13	15	3	10	20	15	20	23

Table 2. Initial reaction products formed by heating ^{14}C -labelled uridine (2.7 mole-%), urea (89.2 mole-%), and $NH_4H_2^{32}PO_4$ (8.1 mole-%) at 100° in dry nitrogen atmosphere. Figures are expressed as percent of total radioactivity

Time	U	pU	Up	$U > p$	pUp	$pU > p$	Phosphorylation (%)
1 h	90	6	4				11
3 h	74	15	7	2	1	1	26
8 h	38	27	11	8	7	9	74
12 h	23	27	8	10	14	17	106
24 h	9	19	5	13	20	37	161

Table 3. Nucleoside phosphates and internucleotide bonds formed by heating ^{14}C -labelled uridine (2.7 mole-%), urea (89.2 mole-%), and $\text{NH}_4\text{H}_2\text{PO}_4$ (8.1 mole-%) for four days at 100° in a dry nitrogen atmosphere. Figures are expressed as percent of total radioactivity

$\cdot p$	$pU + Up$	$pU > p$	$p_n U > p$ $n \geq 2$	UpU	U^5ppU	U^5pppU^a	U^5ppUpU^a
	2	5	10	6	40	9	13

^a These materials were not studied in detail. They represent higher molecular weight products which must contain mixtures of isomers.

b) Nucleoside Excess

The products obtained from a mixture containing phosphate and an excess of uridine are given in Table 4. Enzymatic degradation showed that 50–60% of the internucleotide bonds are 3'-5' linked. The remainder was mainly 2'-5' linked, but a small proportion of 5'-5' linkages was also formed.

Table 4. Reaction products formed by heating a mixture of ^{14}C -labelled uridine (17.8 mole-%), urea (76.0 mole-%), and $\text{NH}_4\text{H}_2\text{PO}_4$ (6.2 mole-%) at 100° in dry nitrogen atmosphere. Yields show the percentage of total phosphate present in each product

ie	U ^a	pU	Up	$U > p$	pUp	$pU > p$	UpU	$pUpU^b$ ($UpUp$)	U^5ppU	$UpUpU$	Phosphate Incorporation (%)
1	85	22	15	3	2	1					46
1	77	36	21	5	5	3					74
1	67	44	25	13	15	6	1				103
1	66	43	17	20	10	11	2				102
1	66	43	14	23	7	13	4				103
lays	65	31	13	34	4	12	6		3		103
lays	61	19	7	39	1	12	10	5	4	2	99
lays	60	11	5	40		10	15	6	7	6	99
lays	59	5	3	45		3	19	8	11	7	102

^a The unreacted U is given as a percentage of total uridine present in the mixture.

^b $pUpU$ and $UpUp$ were not separated.

Discussion

After extended periods of heating with mixtures of urea and ammonium dihydrogen phosphate, nucleosides are converted to a variety of potentially important derivatives. When phosphate is in excess, large amounts of compounds containing pyrophosphate bonds are obtained. After four days of heating at 100° , 2',3'-dideoxy-thymidine is converted in 65% yield to molecules containing a pyrophosphate bond; the 5'-triphosphate, an analogue of deoxythymidine triphosphate, is obtained in 15% yield. A variety

of inorganic polyphosphates were formed in these experiments. The nucleoside catalyzes the formation of inorganic trimetaphosphate, a species which is not formed in appreciable amounts from simple mixtures of urea and ammonium dihydrogen phosphate (Österberg and Orgel, 1972).

When a mixture containing an excess of uridine over phosphate was used, a different spectrum of products was obtained. Di- and oligonucleotides accumulated gradually, and, after 11 days, made up about 33 % of the product. The majority of the internucleotide bonds were 3'-5' linked (50-60 %), as in naturally-occurring nucleic acids. However, large numbers of 2'-5' bonds and some 5'-5' bonds were also formed. These results are similar to those obtained by others in reactions carried out at much higher temperatures (Ponnamperuma and Mack, 1965; Moravek and Skoda, 1967). Experiments using $\text{NH}_4\text{H}_2^{32}\text{PO}_4$ showed that very little condensed inorganic phosphate is formed in these reactions.

The results show that phosphorylation with mixtures of urea and ammonium dihydrogen phosphate can be used to generate a variety of nucleotide derivatives of prebiotic interest, in addition to those reported previously (Lohrmann and Orgel, 1971). Preliminary experiments show that these same reactions occur more slowly at 85°, and are not inhibited by small amounts of water vapor.

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