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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of this kind, and have shown that the effective components are ammonium dihydrogen phosphate and urea (Österberg, unpublished results).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, $NH_4H_2PO_4$, and ^{14}C-labelled uridine (0.07 mc/mmole) or (2) of urea, ^{32}P-labelled $NH_4H_2PO_4$ (0.1 mc/mmole) and nucleoside (U, or *dd*T)[1] in open glass tubes at 30 to

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1 *Abbreviations:* U, uridine; U>*p*, uridine cyclic 2′,3′-phosphate; *p*U, uridine 5′-phosphate; U*p*, uridine 2′(3′)-phosphate; *p*U*p*, uridine 5′,2′(3′)-diphosphate; *p*U>*p*, 5′-phosphouridine cyclic 2′,3′-phosphate; U*pp*U, P_1,P_2-diuridine 5′-pyrophosphate; U*p*U, uridylyl-[2′(3′)→5′]-uridine; *p*U*p*U, 5′-phosphouridylyl-[2′(3′)→5′]-uridine; U*p*U*p*, uridylyl-[2′(3′)→5′]-uridine 2′(3′)-phosphate; *dd*T, 2′,3′-dideoxythymidine (2′,3′-dideoxy-ribosylthymine); *pdd*T, 2′,3′-dideoxy-thymidine 5′-phosphate; p_n*dd*T ($n=2, 3, \ldots$), 2′,3′-dideoxy-thymidine 5′-polyphosphates; *dd*T*pdd*T, dinucleoside 5′-phosphate of 2′,3′-dideoxy-thymidine; *dd*T*ppdd*T, P_1,P_2-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 μmoles 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 ±0.5°. 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 *dd*T-$(p)_n$-*dd*T 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	*pdd*T	p_2*dd*T	p_3*dd*T	p_n*dd*T $n>3$	*dd*T*pdd*T	*dd*T*ppdd*T	*dd*T*pppdd*T	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 $NH_4H_2PO_4$ (8.1 mole-%) at 100° in dry nitrogen atmosphere. Figures are expressed as percent of total radioactivity

Time	U	*p*U	U*p*	U > *p*	*p*U*p*	*p*U > *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