Phosphorylation with Inorganic Phosphates at Moderate Temperatures

Abstract. *Uridine phosphates may be obtained by heating uridine with inorganic phosphates for 9 months at temperatures as low as 65°C. Under similar conditions, uridine-5′-phosphate, in addition to forming uridine diphosphates, undergoes some dephosphorylation to give uridine.*

A number of publications (*1–4*) have dealt with the formation of nucleotides from nucleosides and inorganic phosphate by dry heating, but with one exception (*1*): they refer to experiments carried out at 160°C. Here we report quantitative results which show that these and related reactions proceed effectively, although more slowly at temperatures as low as 65°C.

Uridine was heated with inorganic phosphates in sealed ampules for 9 months at 65°C. Uridine monophosphates, together with small amounts of uridine diphosphates, were obtained with $Ca(H_2PO_4)_2$ (Table 1). No phosphorylated products (< 1 percent) were detected with $(NH_4)H_2PO_4$, $(NH_4)_2HPO_4$, NaH_2PO_4, Na_2HPO_4, or $CaHPO_4$. In similar experiments in unsealed ampules at 85°C for 6 months, a good yield of uridine monophosphate was obtained with $Ca(H_2PO_4)_2$, and a lower yield was obtained with $(NH_4)_2HPO_4$. However, NaH_2PO_4, Na_2HPO_4, KH_2PO_4, $Ca(NH_4)PO_4$, and $Mg(NH_4)PO_4$ gave negative results.

In an independent series of experiments, uridine-5′-phosphate was heated with inorganic phosphates in unsealed ampules for 6 months at 65°C. Under these conditions, dephosphorylation occurred; the yield of uridine was in the range of 10 to 20 percent, with Na_2HPO_4, KH_2PO_4, $CaHPO_4$, $(NH_4)_2HPO_4$, and $Mg(NH_4)PO_4$, and as high as 50 percent with NaH_2PO_4. Simultaneously, some diphosphates, presumably 2′,5′- and 3′,5′-, were formed. The best yield was obtained with $Ca(H_2PO_4)_2$, and smaller yields were obtained with $(NH_4)_2HPO_4$ and NaH_2PO_4 (Table 2); results with $Mg(NH_4)PO_4$, Na_2HPO_4, and KH_2PO_4 were negative.

In that $Ca(H_2PO_4)_2$ is precipitated only from acid solutions, it seems unlikely that it was ever a common mineral. However, if the oceans ever contained substantial amounts of ammonia and little calcium or magnesium, it is possible that some $(NH_4)_2HPO_4$ was formed by evaporation of shallow pools. When heated, this phosphate could have converted nucleosides to nucleotides. In this respect, $(NH_4)_2HPO_4$ is unique, for, although it is formed from neutral or slightly alkaline solutions, it loses ammonia on heating and then provides an acid environment.

If the hydrolysis of uridine-5′-phosphate to uridine that we observed is a general reaction of phosphate esters, it is unlikely that polymeric materials could ever be obtained under our reaction conditions. Perhaps polymers could be formed at higher temperatures or lower humidities.

Reaction mixtures were prepared by dissolving ^{14}C-labeled uridine or uridine-5′-phosphate in water, dissolving or suspending the inorganic phosphate, and lyophilizing the resulting solution or suspension.

The products were analyzed by paper chromatography in a mixture of isopropyl alcohol, concentrated ammonia, and water (70 : 10 : 20) and in a mixture of 95 percent ethyl alcohol and 1*M* ammonium acetate (7 : 3), *p*H 7.5. To confirm the formation of uridine diphosphates and diuridine phosphate, the products were eluted from the paper and subjected to paper electrophoresis (0.03*M* potassium phosphate buffer, *p*H 7.1, 4000 volts). Uridine monophosphates were eluted and chromatographed again in the isopropyl alcohol, concentrated ammonia, 0.1*M* boric acid system (70 : 10 : 20) to separate uridine-5′-phosphate from uridine-2′(3′)-phosphate.

The products on the chromatograms were located and estimated roughly by viewing under an ultraviolet light and using a radiochromatogram scanner with integrator. For quantitative analyses, the chromatograms were cut into segments, and the radioactivity was determined in a scintillation counter. The yields were calculated as the percentage of total ^{14}C on the paper.

Table 1. Products of reaction mixtures of uridine and inorganic phosphate. pU, uridine-5′-phosphate; Up, uridine-2′(3′)-phosphate; pUp, uridine diphosphate; and UpU, diuridine phosphate.

Temperature (°C)	Inorganic phosphate	Ratio of uridine to phosphate	Uracil	pU	Up	pUp	UpU
65	$Ca(H_2PO_4)_2$	1:1		2.3	0.5		
65	$Ca(H_2PO_4)_2$	1:5		11.3	3.3		1.4
85	$Ca(H_2PO_4)_2$	1:20	7.45	28.3	7.6	14.1	
85	$(NH_4)_2HPO_4$	1:20		2.9*			

* Up and pU were not separated.

Table 2. Products of reaction mixtures of uridine-5′-phosphate (pU) and inorganic phosphate (P_i) at 65°C. pUp, uridine diphosphate.

P_i	pU/P_i	Uridine	pUp
$Ca(H_2PO_4)_2$	1:20	14.7	12.1
$(NH_4)_2HPO_4$	1:20	13.5	4.1
NaH_2PO_4	1:20	45.5	3.4

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Motility of the Turtle Embryo, Chelydra serpentina (Linné)

Abstract. *Periodic motility of turtle embryos was observed during their incubation periods (60 ± 5 days). Cyclic activity was first observed between days 10 to 14; it increased to a peak level of 50 percent of the standard observation period on day 30 ± 5, then declined to low levels until hatching activities were initiated. During the first third of the incubation period, motility of the turtles closely resembled that previously described for chick embryos at similar stages of development.*

Motility of embryonic turtles removed from the nests of *Terrapene carolina* and *Caretta caretta* has been described by Tuge (*1*) and by Smith and Daniel (*2*). They reported spontaneous, nonreflexogenic activity of young embryos, but they were principally concerned with observations of reflex behavior following mechanical stimulation. My investigation of embryonic motility of the snapping turtle combined the methods for harvesting turtle eggs *ex utero* (*3*) with those developed for observing spontaneous periodic motility of the