Template-directed Synthesis of Oligoadenylates Catalyzed by Pb^{2+} Ions

H.L. Sleeper, R. Lohrmann and L.E. Orgel

The Salk Institute for Biological Studies, San Diego, California 92112, USA

Summary. The Pb^{2+} ion is an effective catalyst for the template-directed condensation of ImpA on poly(U). This reaction generates up to 35% of oligomers 5 or more units long. Furthermore, the product is predominantly 3'-5'-linked (75%) unlike that from the uncatalyzed reaction which is more than 90% 2'-5'-linked. The significance of metal-ion catalysis for prebiotic polynucleotide formation is discussed.

Key words: Adenosine 5'-phosphorimidazolide - Oligoadenylates - Poly(U)-directed condensation - Pb^{2+} ion catalysis - Isomer ratios - Prebiotic

Introduction.

In previous papers we have described a variety of experiments in which a poly(U) template facilitates the condensation reactions of ImpA (Orgel and Lohrmann, 1974). These reactions yield substantial amounts of (pA)$_2$ and (pA)$_3$, but only small quantities of higher oligomers. It is usually possible to detect some (pA)$_5$ and, occasionally, a trace of (pA)$_6$ and higher. In another set of experiments Sawai showed that a number of divalent metal ions facilitate the self-condensation of ImpA to give oligonucleotides in

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in the absence of templates (Sawai and Orgel, 1975; Sawai, 1976). The most effective metal ion was Pb^{2+}. Subsequently, we extended these observations and showed that the Pb^{2+} ion and Pb^{2+} minerals are catalysts for a variety of condensation reactions involving activated nucleotides (Sleeper and Orgel, 1979).

In the present paper we describe experiments in which Zn^{2+} and Pb^{2+} are added to our standard ImpA-poly (U) reaction mixtures. We show that Pb^{2+} is an effective catalyst — Zn^{2+} has no catalytic effect.

Experimental

Materials

Reagent grade chemicals were used throughout. Imidazole, purchased from Matheson, Coleman and Bell, was recrystallized from benzene before use. 1-Methylimidazole, obtained from Aldrich, was purified by redistillation. A and pA were purchased from Terra Marine Bioresearch. Ap and ATP were obtained from Sigma Chemical Company. The corresponding [^{14}C]-labelled compounds, $\overset{*}{A}$, p$\overset{*}{A}$, $\overset{*}{A}$p and $\overset{*}{A}$TP were purchased from Schwarz-Mann. $\overset{*}{A} > p$ was prepared by a published procedure (Smith et al., 1958). ImpA and Imp$\overset{*}{A}$ were prepared as described earlier (Lohrmann and Orgel, 1978). The oligomers $A(pA)_n$ and $(Ap)_n$, which were used as chromatographic markers, were prepared as described previously (Lohrmann and Orgel, 1979). Poly (U) was prepared by the procedure of Steiner and Beers (1958). Bacterial alkaline phosphatase (BAPF grade) was purchased from Worthington, and ribonuclease T_2 from Sigma.

Chromatography and Electrophoresis

Paper chromatography was carried out on Whatman 3MM paper by the descending technique. The following solvent systems were used: n-propanol, concentrated ammonia and water (55:10:35), System I; Saturated $(NH_4)_2SO_4$, 0.1M sodium acetate, pH 6.5, and isopropanol (79:19:2), System II. Paper electrophoresis was performed on Whatman 3MM paper at 3000 volts (55 volts/cm) using varsol as coolant. The buffer was 0.03M potassium phosphate, (pH 7.1), System III.

Aliquots of reaction mixtures were treated with sufficient Na_4EDTA at pH 8.0 to complex the divalent cations before chromatography or electrophoresis. The relevant chromatographic and electrophoretic mobilities of various compounds are listed in Table 1.

The nature of the reaction products was confirmed by comparison of their chromatographic and/or electrophoretic characteristics with those of authentic markers whenever possible. Quantitative estimates of the yields were obtained by running the chromatograms or electrophoretograms through a Baird Atomic RSC 363 scanner with integrator. Where necessary, results from several systems were collated to estimate the yields of individual compounds. The yields are expressed as the percentage of the total radioactivity on the paper, after allowing for the background. In cases where the amount of radioactivity on the chromatograms was too low to permit reliable estimates using the strip scanner, we cut out the radioactive zones and counted their radioactivity more accurately in a Beckman liquid scintillation counter.

Table 1.   Chromatographic and Electrophoretic Mobilities

Compound	System I[a]	System II[a]	System III[b]
A	1. 62	0. 45	0. 00
pA	1. 00	1. 00	1. 00
Ap	1. 12		1. 06
A >p	1. 57		0. 61
pAp	0. 70		1. 63
AppA	1. 14		0. 78
pA^2pA	0. 88	0. 64	1. 08
pA^3pA	0. 86	0. 34	1. 01
$(Ap)_2$	0. 85	0. 18	1. 05
$(Ap)_3$	0. 63	0. 07	1. 10
$(Ap)_4$	0. 44		
$(Ap)_5$	0. 26		
$(Ap)_6$	0. 16		
A^2pA	1. 28	0. 30	0. 37
A^3pA	1. 37	0. 11	0. 30
A^5pA	1. 20	0. 35	0. 37
$A(pA)_2$	0. 99	0. 04	0. 50
$A(pA)_3$	0. 75		0. 66
$A(pA)_4$	0. 50		0. 76
$A(pA)_5$	0. 34		0. 81
ImpA	1. 56		0. 55
$ImpA^2pA$	1. 27		
$ImpA^3pA$	1. 37		

[a] R_f values are given relative to pA.

[b] Electrophoretic mobilities are given taking $R_{adenosine} = 0$

   and $R_{adenylic acid} = 1$

Reactions

Self condensation of ImpÃ. Reactions (Tables 2-5; Fig. 1) were carried out in glass tubes with rubber stoppers. Reaction mixtures were made by addition of components in the following order: Mg^{++} salt, Na^+ salt, imidazole buffer, poly(U), [8-[14] C] ImpA, $Pb(NO_3)_2$, and HNO_3 to adjust the pH. All reaction mixtures were covered with 50 μl of toluene to prevent microbial contamination. For further details see Tables.

Table 2. Effect of Temperature on Yield of Oligoadenylates from the Selfcondensation of ImpA in the Presence of Poly(U) and Pb^{++}

Temp. (°C)	Time (days)	Salt	A	ImpA	ImpApA AppA	pA	pApA	pAp	$(pA)_3$	$(pA)_4$	$(pA)_5+$
0	3	$NaNO_3$		12.4	15.8	22.4	17.4		12.3	7.9	11.9
		$NaNO_2$		9.0	13.8	22.5	17.6		13.3	9.8	14.6
	5	$NaNO_3$	tr.	4.8	12.6	19.3	18.1	tr.	15.7	10.6	18.9
		$NaNO_2$	tr.	4.8	13.2	18.6	16.6	tr.	14.4	11.1	21.3
	10	$NaNO_3$	1.6	2.3	14.5	19.7	16.5	2.0	15.0	9.8	18.5
		$NaNO_2$	1.7	1.7	14.2	17.4	16.0	2.0	12.8	10.2	23.9
15	3	$NaNO_3$	tr.	6.5	11.1	24.9	17.2	tr.	14.9	9.3	16.0
		$NaNO_2$	tr.	5.7	8.6	20.3	15.6	tr.	14.8	10.9	24.1
	5	$NaNO_3$	1.8	2.4	12.2	21.8	17.9	2.1	13.7	9.4	18.8
		$NaNO_2$	1.6	2.1	10.9	19.1	13.8	2.0	14.4	9.7	26.4
	10	$NaNO_3$	4.5	2.7	13.9	26.5	14.9	4.2	11.0	7.0	15.1
		$NaNO_2$	3.5	1.8	12.1	22.8	14.4	3.7	13.3	9.5	19.0
30	3	$NaNO_3$	3.7	2.0	12.8	31.3	22.7	3.6	11.3	4.9	7.6
		$NaNO_2$	4.2	1.8	12.8	31.9	23.4	4.7	9.9	4.6	6.8
	5	$NaNO_3$	6.5	2.5	13.2	34.7	20.2	6.9	5.4	4.4	6.3
		$NaNO_2$	6.0	2.1	13.0	32.5	18.1	6.0	11.7	4.2	6.4
	10	$NaNO_3$	8.8	2.2	13.0	36.9	16.9	8.8	7.7	2.7	3.1
		$NaNO_2$	9.0	2.2	15.2	33.9	16.5	8.9	7.8	3.0	3.6

[a] Reaction conditions: ImpA[*], 5 µmoles (0.12 mCi/mmole); poly(U), 10 µmoles; imidazole, 40 µmoles; $Mg(NO_3)_2$, 15 µmoles; $NaNO_3$ or $NaNO_2$, 40 µmoles; $Pb(NO_3)_2$, 2.5 µmoles; volume 100 µl, pH 8.0

Table 3. Effect of pH on Yield of Oligoadenylates from the Selfcondensation of ImpA Catalyzed by Pb^{2+} in the Presence and Absence of poly(U) [a]

pH	Polymer	A	ImpA	ImpApA	AppA	pA	pApA	pAp	$(pA)_3$	$(pA)_4$	$(pA)_5+$
5.0	poly(U)		tr.			84.5	11.4		3.1	1.0	
	poly(C)		0.9		1.4	84.4	14.2		1.3		
	none		tr.			88.9	10.3		0.8		
6.0	poly(U)		1.9		3.8	38.1	13.6		13.1	10.6	18.9
	poly(C)				2.6	71.9	20.2		4.9	0.4	
	none		1.0		4.1	60.8	24.9		6.6	2.7	
7.0	poly(U)	tr.	2.0		4.0	33.7	10.3	tr.	10.3	10.2	29.5
	poly(C)		2.3		6.8	50.0	25.7		9.3	4.9	1.9
	none		1.2		7.1	48.2	26.7		10.3	4.4	2.0
8.0	poly(U)	1.2	3.2		5.4	32.3	17.6	1.1	12.5	9.9	16.9
	poly(C)	0.3	4.3	6.2	6.0	36.4	25.2	tr.	11.8	6.7	3.0
	none	tr.	4.6		13.3	36.3	22.4	tr.	12.0	6.9	4.6

[a] Reaction conditions: [8-^{14}C] ImpA, 5 µmoles (0.12 mCi/mmole); poly(U) or poly(C), 10 µmoles; $Pb(NO_3)_2$, 2.5 µmoles; imidazole, 20 µmoles; $Mg(NO_3)_2$, 15 µmoles; $NaNO_2$, 40 µmoles, temperature 0°C, 6 days, final volume 100 µl

Table 4. Effect of Pb^{2+} Concentration on Yield of Oligoadenylates from the Selfcondensation of ImpA in the Presence of Poly(U) [a]

Time (hr.)	[Pb^{2+}] (M)	ImpA	ImpApA	AppA	pA	pApA	(pA)$_3$	(pA)$_4$	(pA)$_5$+
0.5	0.01	61.6	17.3		15.8	4.2	1.1	0.2	
	0.005	71.8	14.1		11.4	2.0	0.6		
	0.0025	78.3	10.8		9.2	1.7	0.2		
	0.001	81.9	9.8		7.4	0.9			
	0.0005	80.1	10.2		9.1	0.7			
	0.0	84.2	7.8		7.6	0.5			
	-poly(U) 0.01	79.7	11.0		8.5	0.8			
	-poly(U) 0.0	92.3			7.7				
1	0.01	44.9	20.4		20.6	9.4	2.7	1.6	0.4
	0.005	60.7	17.1		16.0	4.9	1.2	0.2	
	0.025	70.5	14.9		10.8	2.9	0.7	0.2	
	0.001	76.4	12.0		9.3	1.8	0.5		
	0.0005	78.0	11.0		8.8	1.3	0.9		
	0.0	82.2	8.0		8.2	1.3	0.2		
	-poly(U) 0.01	74.7	14.5		9.1	1.3	0.4		
	-poly(U) 0.0	92.6			7.4				
3	0.01	23.3	16.9		27.3	14.6	10.0	5.2	2.6
	0.005	38.5	20.2		24.5	9.5	5.1	1.3	0.7
	0.0025	52.6	17.8		18.6	7.1	2.8	0.8	0.2
	0.001	64.3	15.1		15.3	3.5	1.8		
	0.0005	67.4	14.9		13.5	4.2	1.0		
	0.0	74.3	13.1		10.5	1.9	0.3		
	-poly(U) 0.01	65.3	19.7		11.5	3.0	0.6		
	-poly(U) 0.0	90.5			9.5				
16	0.01	4.5	1.9	5.5	16.6	15.5	16.4	11.8	27.7
	0.005	8.5	5.1	4.7	22.5	16.2	15.5	12.0	15.4
	0.025	12.2	7.7	3.7	26.1	20.4	13.7	7.8	8.4
	0.001	19.8	10.1	4.5	31.5	16.7	9.1	5.5	2.9
	0.0005	22.3	10.8	3.2	32.1	16.9	9.1	3.5	2.0
	0.0	33.2	21.0		26.5	12.7	4.5	1.6	0.5
	-poly(U) 0.01	13.8	21.0	10.1	32.0	10.5	7.8	3.7	1.1
	-poly(U) 0.0	71.5			28.5	0.9			
96	0.01	3.1	1.2	8.1	13.5	11.6	13.1	14.3	35.1
	0.005	2.7	1.1	6.9	14.9	10.8	14.8	16.0	32.8
	0.0025	3.4	1.2	5.5	18.9	13.4	18.6	13.0	26.1
	0.001	2.8	1.7	4.2	23.7	15.6	20.6	12.2	19.0
	0.0005	2.7	1.7	4.7	26.0	17.5	19.6	11.8	15.9
	0.0	5.3	2.7	6.3	32.0	25.3	14.6	7.4	6.4
	-poly(U) 0.01	1.7	1.4	11.3	25.7	27.8	15.7	9.1	7.6
	-poly(U) 0.0	19.2			74.7	5.0	1.0		

[a] Reaction conditions: [8-^{14}C] ImpA, 5 μmoles (0.12 mCi/mmole); poly(U), 10 μmoles; Im, 20 μmoles; Mg(NO$_3$)$_2$, 15 μmoles; NaNO$_2$, 40 μmoles. Volume 100 μl (pH 7.0). temperature 15°C

Table 5. Effect of Various Salts on the Self-condensation of ImpA on a poly(U)-template[a]
Catalyzed by Pb^{++}

Salt	A	ImpA	ImpApA	AppA	pA	$(pA)_2$	pAp	$(pA)_3$	$(pA)_4$	$(pA)_5$	$(pA)_6$+
NaNO₃ without Pb^{++}		27.9	15.8	tr.	24.2	18.8	tr.	8.5	3.6	1.2	
NaNO₃	1.8	4.3	6.1	tr.	21.5	14.1	4.9	14.1	12.9	10.4	9.8
NaF	1.8	9.7	5.7		25.4	12.7	6.6	13.6	10.1	10.5	4.0
NaCl	3.8	4.4	2.5	3.8	23.9	12.0	5.7	13.2	12.0	9.4	9.4
NaBr		2.8	3.2	tr.	10.1	12.9	tr.	17.5	15.2	24.0	13.4
NaI		6.2	5.3	1.8	25.6	19.4	tr.	17.6	11.5	5.7	7.1
Na-Acetate	2.1	3.2	1.1	3.7	23.3	11.1	3.7	16.9	13.8	10.6	10.6

[a] Reaction conditions: poly(U), 2.5 μmoles; ImpÅ, 1.25 μmoles (0.25 mCi/mmoles); Im-HNO₃
(pH 8.0),10 μmoles; in addition each reaction mixture contained 3μmoles of one of the salts
listed. Pb(NO₃)₂, 1 μmole, was added last to the precooled mixture with vigorous agitation.
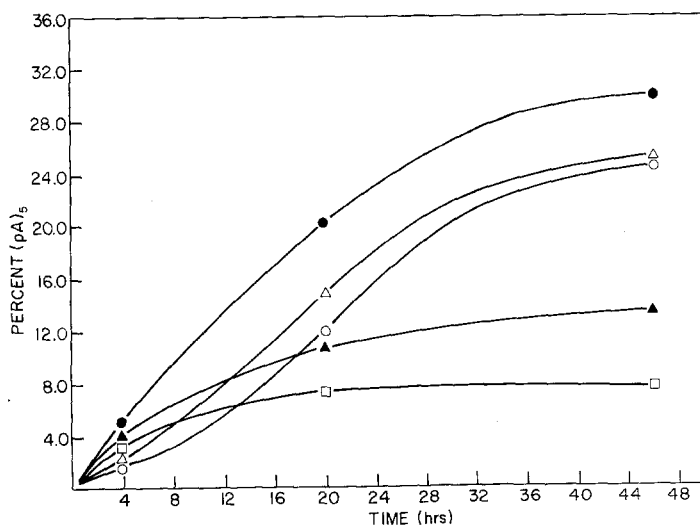Total volume 50 μl, 7 days at 0°C

Fig. 1. Yields of pentamer and higher oligoadenylates from ImpA catalyzed by Pb^{2+} at different concentrations in the presence of poly(U), at 0°C (pH 7.0). o—o—o, 0.005M Pb^{2+}, ●—●—●, 0.01M Pb^{2+}, △—△—△; 0.025M Pb^{2+}, ▲—▲—▲, 0.05M Pb^{2+}, □—□—□, 0.125M Pb^{2+}. Reaction mixtures contained [8-14C] ImpA, 5 μmole (0.12 mCi/mmole); poly(U), 10 μmoles; imidazole, 20 μmole; Mg(NO₃)₂, 15 μmole; NaNO₂, 40 μmoles, and Pb(NO₃)₂ as indicated

Reaction of Å with ImpA. $Pb(NO_3)_2$ was added with vigorous agitation to a cooled solution (0°) containing Mg++ salt, Na+ salts, Im-buffer, [8-14C]-adenosine, ImpA (Na-salt) and poly (U). (For details see Table 6.)

Table 6. Percentage Yields from Pb^{++} Catalyzed Reactions between [8-^{14}C]-adenosine and ImpA, with or without a poly(U)-template [a]

	$\overset{*}{A}$	$\overset{*}{A}pA$ [b]	$\overset{*}{A}(pA)_2$	$\overset{*}{A}(pA)_3$	$\overset{*}{A}(pA)_4$	$\overset{*}{A}(pA)_5$	$\overset{*}{A}(pA)_6+$
-poly(U)	75.0	22.6 (19.0)	2.4				
+poly(U)	27.8	27.8 (28.3)	17.3	11.6	8.1	4.6	2.9

[a] Reaction conditions: $\overset{*}{A}$, 0.125 µmole (4 mCi/mmole); ImpA, 1.12 µmole; poly (U), 2.5 µmole; Mg $(ClO_4)_2$, 15 µmole; $NaClCO_4$, 10 µmole; NaCl, 21 µmole; Im-$HClO_4$ (pH 8.0), 20 µmole, and $Pb(NO_3)_2$, 10 µmole, in 50 µl at 0°C for 3 days.

[b] The numbers given in parentheses indicate the percentage of the material that is [3'-5']-linked

In another series of experiments, 1M $Pb(NO_3)_2$ (10 µl) was added to 8 solutions (40 µl) each containing poly (U) (2.7 µmole), ImpA (1.14 µmole), $MgCl_2$ (10 µmole), NaCl (10 µmole) and Im-HCl (20 µmole, pH 8.5) at 0° with vigorous vibration. The mixtures were kept shaking at 0° for various times before 0.02M $\overset{*}{A}$ (2 µl, 10 mCi/mmole) was added, again with vigorous agitation. The mixtures were then kept at 0°, until all imidazolides had reacted. Aliquots were taken after a total of 5 days and chromatographed in System I. The results are given in Table 7.

Table 7. Reaction of $\overset{*}{A}$ with a Mixture of ImpA(pA)$_n$ preformed by Incubation of ImpA on a Poly(U) Template in Presence of Pb^{++} for Various Times. The Total Reaction Time was 5 Days.[a] The Figures Represent Percentage Yields Based on Total Radioactivity

Time of $\overset{*}{A}$ Addition (hr)	$\overset{*}{A}$	$\overset{*}{A}$pA	$\overset{*}{A}$(pA)$_2$	$\overset{*}{A}$(pA)$_3$	$\overset{*}{A}$(pA)$_4$	$\overset{*}{A}$(pA)$_{5+}$
0	20.7	24.0	26.0	13.2	8.7	7.5
1	20.9	24.8	26.0	12.0	7.8	8.5
2	19.7	25.8	27.5	12.5	6.9	7.7
4	19.5	23.7	28.8	11.9	7.6	8.5
8	24.1	22.4	23.7	12.5	7.5	10.0
14	30.5	17.3	20.6	14.0	7.0	10.7
24	35.2	13.3	23.4	12.5	7.0	9.4
48	43.7	8.5	18.3	12.7	7.0	9.9

[a] Reaction conditions: poly(U), 2.7 μmole; ImpA, 1.14 μmole; MgCl$_2$, 10 μmole; NaCl, 10 μmole; Im-HCl, 20 μmole (pH 8.5); Pb(NO$_3$)$_2$, 10 μmole, in 50 μl kept at 0°C for indicated time, followed by addition of $\overset{*}{A}$, 0.04 μmole (10 mCi/mmole) in 2 ul

Preparation of Dimers and Trimers for Isomer Analysis. The reaction mixture contained 0.1M poly(U), 0.05M [8-^{14}C] ImpA (1.6 mCi/mmole), 0.01M Pb(NO$_3$)$_2$, 0.15M Mg(NO$_3$)$_2$, 0.4M NaNO$_3$, 0.2M imidazole and was maintained at 0°C pH 7.5 for 7 days. It was chromatographed on a A-25 QAE Sephadex (HCO$_3$ form) column using a linear triethylammonium bicarbonate gradient (0.05-1.0M, 2 x 1200 ml, pH 7.5). Fractions containing the dimer and trimer eluted at 0.28M for pA^{2}pA, 0.31M for pA^{3}pA and 0.39M for trimers. The fractions were pooled and evaporated in the flash evaporator. Repeated additions of water and flash evaporation ensured the complete removal of the carbonate buffer.

Degradation and Identification of Products

The chain lengths of oligomers (pA)$_n$ were determined by co-chromatography with authentic markers in System I. They were confirmed by eluting the materials, degrading them with BAP and re-running them in System I with authentic A(pA)$_n$ as markers. The isomeric composition of pApA and pApApA were determined by previously published methods (Lohrmann and Orgel, 1979).

Attempts to Extend Preformed Oligomers

ImpA was allowed to undergo self-condensation for 48 h at pH 8.0 and 0°C on a poly (U) template. The product was chromatographed in System I, and the material moving as (pA)$_5$ or slower, was eluted with water. The eluate was evaporated, and the product rechromatographed for 48 h in the same system. The well-separated spot of (pA)$_5$ was eluted and used in a chain-extension experiment.

Reaction mixtures (100 μl) containing $(p\overset{*}{A})_5$ (0.9 μmole, 0.044 mCi/mmole), ImpA (3.6 μmole), $Mg(NO_3)_2$ (13.5 μmole), $NaNO_2$ (36 μmole), $Pb(NO_3)_2$ (0.5 μmole) imidazole (20 μmole) with or without poly(U) (9.0 μmole) were covered with 50 μl of toluene and kept at pH 7.0 and $0^\circ C$ for 1.5 and 4 days. The products were analyzed by chromatography in System I.

In another template experiment we attempted to extend $(pA)_3$. This time we added reagents in a variety of different orders to make sure that no component was precipitated immediately from the reaction mixture and thus segregated from the other reactants. In no case did we observe extensive chain-elongation.

Results

Table 2 shows the effect of temperature on the rate of the $[Pb^{2+}]$-catalyzed self-condensation of ImpA on a poly (U) template. The maximum yield of oligomers is obtained after ten days at $0^\circ C$ or after five days at $15^\circ C$. We believe that the reduction in the yield of longer oligomers that is observed at $30^\circ C$ is due in part to hydrolysis of the products. The results indicate that the anion influences the yields; somewhat better yields are obtained with NO_2^- than with NO_3^-

The effect of pH on the template-directed formation of oligoadenylates from ImpA in the presence of Pb^{2+} is shown in Table 3. Under optimal conditions for synthesis, (pH 7.0), 29.5% of the starting material was found as pentamer and higher oligoadenylates after 6 days at $0^\circ C$. At lower pH's ImpA hydrolyzes rapidly to pA. At pH 8.0 the decreased yield is partly due to hydrolysis of oligomeric products. In the absence of a polymer, or in the presence of poly(C), very low yields of oligomeric product are obtained.

The effect of Pb^{2+} ion concentration on the yields of products obtained in the template-directed condensation of ImpA at pH 7 is illustrated in Fig. 1 and Table 4. The optimum yield is obtained with 0.01M Pb^{2+}. At this concentration 35.1% of the ImpA is converted to penta- and higher oligonucleotides. The effect of Pb^{2+} is clearly seen even when the Pb^{2+} concentration is reduced to .0005M.

We studied the effect of a number of anions on the efficiency of condensation. The results are summarized in Table 5. Under the conditions of this set of experiments, Pb^{2+} in the presence of Br^- ion was the most effective catalyst.

While we concentrated on the reactions of $[8\text{-}^{14}C]$-labelled ImpA we carried out a few experiments in which non-radioactive ImpA condensed with $[8\text{-}^{14}C]$-adenosine. The results in Table 6 establish that there is a large template effect on the yields. The results given in Table 7 will be used in our discussion of the reaction mechanism.

In Table 8 we give the isomer ratios determined for pApA and pApApA. It is noteworthy that in the presence of Pb^{2+} the greater part of the product is 3'-5'-linked, while in the absence of this metal ion more than 90% is 2'-5'-linked. The numerical values for the proportions of isomers in the trimeric product are only approximate since the estimations depend on small differences between comparable quantities.

Table 8.   Ratios of Oligonucleotide Isomers Formed in the Template Reaction
           of ImpA in the Presence of Pb^{++} at $0^{\circ}C$, pH 7.5

|	
Dimer	
pA^2pA	22.5
pA^3pA	77.5
Trimer	
pA^2pA^2pA	9.1
pA^2pA^3pA	0.7
pA^3pA^2pA	51.0
pA^3pA^3pA	39.5

Discussion

General

Our results establish that the Pb^{2+} ion is a highly effective catalyst for the self-conden-
sation of ImpA or the condensation of ImpA with A on a poly(U) template. Under
optimal conditions more than 30% of the input ImpA is converted to pentamer and
higher oligomers in the presence of Pb^{2+} — in the absence of Pb^{2+} the yield is never
greater than 7%. Pb^{2+} did not catalyze the self-condensation of A > p or ATP on a
poly(U) template. Further experiments which we have not described in detail failed to
demonstrate any catalytic effect of the Zn^{2+} ion under similar conditions.

The Pb^{2+} ion is effective even at low concentrations. The data in Table 4, for ex-
ample, show that 0.0005M Pb^{2+} substantially increases the rate of formation of oligo-
mers from 0.05M ImpA. The decreased yield of oligomers obtained with high concen-
tration of Pb^{2+} (Fig. 1) is probably due to the destabilization of the 2 poly(U)-ImpA
triple-helix.

The data in Table 7 show that the terminal yield of higher oligomers of the type
$\overset{*}{A}(pA)_n$ is as high when the $\overset{*}{A}$ is added after 48 h as when $\overset{*}{A}$ is present throughout the
reaction. On the other hand, the yield of $\overset{*}{A}pA$ falls by a factor of three. This sug-
gests strongly that the imidazolides of oligomers are more stable than ImpA and that
the major mechanism for the formation of oligomers is

$$A + ImpA(pA)_{n-1} \rightarrow A(pA)_n$$
rather than
$$A(pA)_{n-1} + ImpA \rightarrow A(pA)_n$$
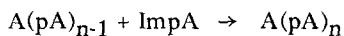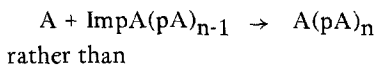
This conclusion is strengthened by our failure to extend $(pA)_3$ or $(pA)_5$ significantly
by further reaction with ImpA in the presence of poly(U) and Pb^{2+}.

The effect of Pb^{2+} on the isomer ratios among products is particularly interesting.
The self-condensation of ImpA on poly(U) in the absence of Pb^{2+} yields more than
90% of the 2'-5'-linked dimer. In the presence of Pb^{2+}, 77.5% of the

naturally-occurring 3'-5'-linked dimer is obtained. The precise ratio of isomers depends in a complicated way on the rates of formation and rates of removal of the two dimers, but it is clear, qualitatively, that the Pb^{2+} ion promotes the formation of 3'-5'-linkages more effectively than 2'-5'-linkages. It is interesting that the reaction of A with ImpA yields an excess of 2'-5'-linked ApA even in the presence of Pb^{2+}.

Our results do not establish the mechanism of Pb^{2+}-catalysis. However, analogy with a variety of known metal-catalyzed phosphorylations of alcohols (Rifkind and Eichhorn, 1972; Mildvan and Grisham, 1974) suggests that the Pb^{2+} ion co-ordinates to the reactive OH group and facilitates its ionization. If this is correct the nucleophile is a Pb^{2+} complex:
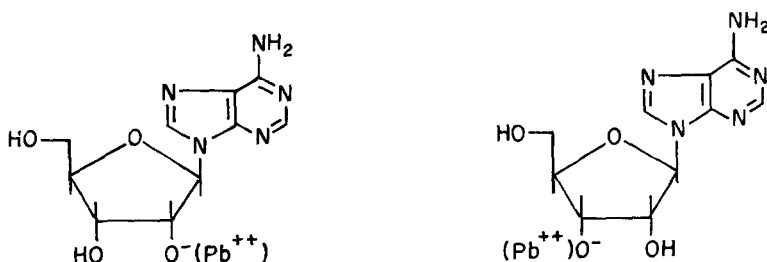
The different isomer ratios obtained with ImpA or A as acceptor would thus reflect different modes of co-ordination of Pb^{2+} to these molecules.

Prebiotic Significance

The results described in this paper establish that metal ions can act as catalysts in template-directed reactions and, perhaps more importantly, that they can direct the synthesis of 3'-5'-linked oligomers even when the spontaneous reactions gives more than 90% of the 2'-5'-linked isomers. Since all DNA and RNA polymerases that have been studied adequately have been shown to be metallo-enzymes (Zn^{2+} enzymes) and since metal ions must have been abundant on the primitive Earth, it seems probable that polynucleotide synthesis has been a metal-catalyzed reaction from the earliest times.

We emphasize that we do not believe that the precise reactions that we have de-scribed are likely to have been important on the primitive Earth. They involve triple-rather than double-helices, the effective metal ion is Pb^{2+} rather than Zn^{2+}, and the active nucleotide, ImpA, may not have been important for biochemical evolution. Furthermore, the chains grow from the 3' to the 5'-terminal rather than from the 5'-to the 3'-terminal as in the biological reactions. Nonetheless, our results are sufficiently encouraging that we plan to test a variety of Pb^{2+} and Zn^{2+} complexes as catalysts in more realistic model systems that involve double- rather than triple-helices.

The efficiency achieved in these reactions is encouragingly close to what would be needed for the synthesis of substantial amounts of predominantly 3'-5'-linked high molecular weight material. The self-condensation of ImpA in 0.01M solution gives only a few percent of the dimer pApA because hydrolysis of ImpA is 10-100 times more

effective than condensation under these conditions. The dimer is about 90% 2'-5'-linked. The presence of a poly(U) template greatly increases the rate of condensation, but gives a product that is almost entirely 2'-5'-linked (Orgel and Lohrmann, 1974). The presence of Pb^{2+} further increases the efficiency of the reaction and, more importantly, gives a product that contains more than 75% of natural 3'-5'-linkages. An analysis of our data shows that the Pb^{2+} ion increases the reactivity of the 3'-OH group by a factor of about 50. A further increase in the reactivity of this hydroxyl, say by a factor of 5, would lead to the synthesis of substantial amounts of relatively high molecular weight material having the natural linkage.

References

Lohrmann, R., Orgel, L.E. (1978). Tetrahedron **34**, 853–855
Lohrmann, R., Orgel, L.E. (1979). J. Mol. Evol. **3**, 237–257
Mildvan, A.S., Grisham, C.M. (1974). Structure and Bonding **20**, 1–21
Orgel, L.E., Lohrmann, R. (1974). Accounts Chem. Res. **7**, 368–377
Rifkind, J.M., Eichhorn, G.L. (1972). J. Am. Chem. Soc. **94**, 6526–6534
Sawai, H. (1976). J. Am. Chem. Soc. **98**, 7037–7039
Sawai, H., Orgel, L.E. (1975). J. Am. Chem. Soc. **97**, 3532–3533
Sleeper, H.L., Orgel, L.E. (1979). J. Mol. Evol. **12**, 357–364
Smith, M., Moffatt, J.G., Khorana, H.G. (1958). J. Am. Chem. Soc. **80**, 6204–6212
Steiner, R.F., Beers, R.F. Jr. (1958). J. Polym. Sci. **30**, 17–28