is realized, then according to Marcus and Sutin[13] eq 2 takes the form of eq 7

$$k_{12} = (P_{12}P_{21}k_{11}k_{22}K_{12}f/P_{11}P_{22})^{1/2} \quad (7)$$

where P_{11} and P_{22} are the stability constants of the precursor complexes for the homonuclear reactions, and P_{12} and P_{21} are the stability constants of the precursor complex and successor complex for the heteronuclear electron-transfer reaction, respectively. Due to the excellent correlations obtained without such considerations for the kinetic behavior of the five reductants of Figure 1 and ferrocyanide, these six species must exhibit a relatively constant value for the $P_{12}P_{21}/P_{11}P_{22}$ term. If it can be assumed that the association equilibrium constants of the various reductants with CcP are proportional to $(P_{12}P_{21}/P_{11}P_{22})^{1/2}$, then the cyt c'' data can be correlated. The association equilibrium constant for the binding of ferrocyanide and ferrocytochrome c to CcP has only been determined at pH 6.3[3] and 7,[35] respectively. However, the association equilibrium constants correspond well to the reciprocal of the Michaelis constants determined in the steady-state oxidation of ferrocyanide[4] and ferrocytochrome c.[35,39] The Michaelis constants have been determined over a wider range of pH and are relatively independent of pH (within an order of magnitude). At pH 5.25 the equilibrium association constants for ferrocyanide and ferrocytochrome c binding to CcP are estimated to be about 2×10^2 and 2×10^5 M^{-1}, respectively. Using these values of the equilibrium association constants, the calculated value for the ferrocytochrome c oxidation rate constant is corrected upward to 2×10^8 M^{-1} s^{-1} in comparison to the experimentally determined rate of 5×10^8 M^{-1} s^{-1}. These results suggest that complex formation may be very important in determining the rate of biological electron-transfer reactions.

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References and Notes

(1) Abbreviations appearing in the text are cytochrome c peroxidase = CcP; primary and secondary oxidized forms of CcP = CcP-I and CcP-II, respectively; ferro- and ferricytochrome c = cyt c'' and cyt c''', respectively; 1,10-phenanthroline = phen; 2,2′-bipyridine = bipy; 4,4′-dimethyl = DM; and 3,4,7,8-tetramethyl = TM.
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Catalysis of Internucleotide Bond Formation by Divalent Metal Ions

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Abstract: Adenosine 5′-phosphorimidazolide condensed in aqueous solution in the presence of various divalent metal ions to form short oligoadenylic acids. The effect of divalent metal ions on the synthesis of oligoadenylic acids is in the following order: $Pb^{2+} > Co^{2+} > Zn^{2+} \gtrsim Mn^{2+} > Ni^{2+} > Cd^{2+}$ $(Fe^{2+}) > Ca^{2+} \gtrsim Mg^{2+} \approx$ none $\approx Cu^{2+} \gtrsim Hg^{2+}$. When Pb^{2+} was used, the total yield of oligoadenylic acids was as high as 57%. The internucleotide linkage in the resulting pApA and pApApA were mainly 2′–5′.

An efficient procedure for the synthesis of polynucleotides from activated nucleotides in aqueous solution is very important both in prebiotic chemistry and biochemistry. Nucleoside 5′-polyphosphates are used as activated monomers in biological systems. ATP or ADP forms high molecular weight polynucleoides in aqueous solution in the presence of some enzymes. Without such enzymes, ATP and ADP hydrolyze but no polynucleotide can be obtained.

Adenosine 5′-phosphorimidazolide (ImpA) is an activated derivative of adenylic acid (pA) and hydrolyzes to pA and imidazole in the absence of a template or other catalysts.[1] In a previous communication,[2] we reported that Zn^{2+} ion cata-

Table I. R_f Values of the Compounds

Compd	System I[a]	System II[b]
ImpA	0.98	0.56
ImpApA	0.75	
AppA	0.63	0.78
pA	0.57	1.0
pApA	0.50	1.07
pApApA	0.36	
pApApApA	0.24	
pApApApApA	0.16	
Ap	0.66	1.04
pAp	0.42	
A	1.0	0

[a] R_f values with respect to A. [b] R_f values with respect to pA.

lyzes the condensation of nucleoside 5′-phosphorimidazolides in aqueous solution to form oligonucleotides even in the absence of a template polyU. The role of the Zn^{2+} ion is presumably to neutralize the charge of phosphate group by coordination and to orient the substrate. The present paper deals with the effect of various other divalent metal ions on the synthesis of oligoadenylic acids from ImpA.

Experimental Section

Materials. Adenylic acid (Sigma Chemical Co.), [8-^{14}C]adenylic acid (Schwarz Mann), *N*-ethylmorpholine (Calbiochem), 1,1′-carbonylimidazole (Sigma Chemical Co.), venom phosphodiesterase (Worthington Biochemical), and RNase T_2 (Sankyo Co.) were obtained commercially. Metal chloride and metal nitrate were from Mallinckrodt. ImpA was prepared from pA and 1,1′-carbonylimidazole as a sodium salt by a modification of the procedure of Cramer et al.[3] Authentic samples of 3′–5′-linked oligoadenylic acids (pApA, pApApA, and pApApApA) were generous gifts of Dr. Jacques Ninio.

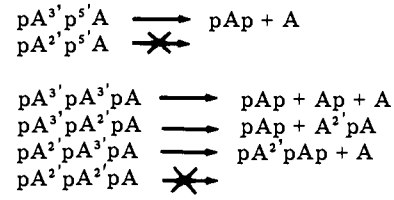
Paper Chromatography. Paper chromatography was performed on Whatman 3MM paper by the descending technique in solvent system I, 1-propanol–concentrated ammonia–water (55:10:35). Paper electrophoresis was carried out on Whatman 3MM paper in system II, 0.03 M potassium phosphate buffer at pH 7.1. R_f values of the compounds are listed in Table I. The chromatograms were passed through a Baird Atomic RSC 363 scanner with an integrator. The yields were determined as the percentage of the total radioactivity on the paper after correcting for background. When unlabeled starting materials were used, the yields were determined by elution of ultraviolet-absorbing spots from the chromatogram and measurement of these optical densities at 260 nm.

Condensation Reaction. A typical reaction mixture (0.1 ml) contained 0.05 M [8-^{14}C]-ImpA (specific activity, 0.12 mCi/mmol), 0.025 M metal chloride or metal nitrate, and 0.2 M *N*-ethylmorpholine buffer (pH 7.0). The solution was maintained at 0 °C or room temperature. Samples were withdrawn after 7 or 10 days and treated with 30 μl of 0.25 M EDTA solution to break down metal-nucleotide complexes. The reaction mixtures were then subjected to paper chromatography and paper electrophoresis.

Identification and Degradation of Products. The identification of oligoadenylic acids were carried out by cochromatography with authentic markers. pApA and pApApA were eluted from chromatograms with neutral water and subjected to enzyme digestion.

(a) Venom Phosphodiesterase Digestion. Eluted pApA and pApApA (2–5 optical density units at 260 nm) were digested at 37 °C for 2–4 h in 0.1 ml of reaction mixture containing venom phosphodiesterase (5 μl, 1 mg/ml), 0.1 M Tris acetate pH 8.8 (10 μl), and 0.1 M $MgCl_2$ (10 μl). The digests were evaporated to dryness, dissolved in 25 μl of water, and subjected to paper chromatography. Venom phosphodiesterase attacks both 3′–5′ and 2′–5′ phosphodiester linkages. pApA and pApApA obtained in the reaction degradated to pA completely. Control reactions without venom phosphodiesterase were also carried out under the same conditions and no degradation was detected.

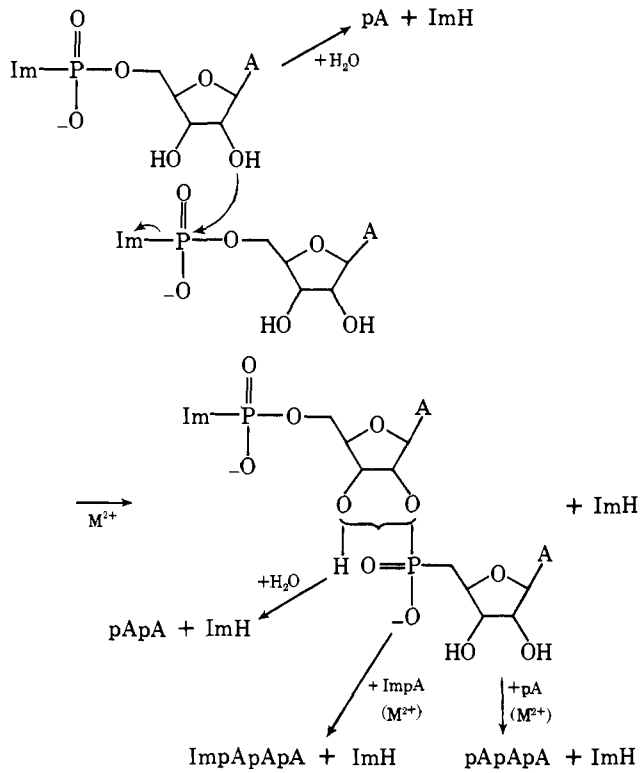
(b) RNase T_2 Digestion. pApA and pApApA, after elution from chromatograms, were digested with RNase T_2. Digestions were performed in a mixture (0.1 ml) containing the substrate (2–5 optical density units), RNase T_2 (10 μl, 500 units/ml), 0.2 M acetate buffer pH 4.75 (25 μl), and 0.02 M EDTA (15 μl). After incubation at 37 °C for 2–4 h, each aliquot was evaporated to dryness, dissolved in 25 μl of water, and chromatographed in system I. RNase T_2 degradates the 3′–5′ phosphodiester linkage but not the 2′–5′ linkage. Thus, pApA and pApApA obtained in the reactions were degradated by RNase T_2 in the following way.



The ratio of internucleotide linkages was calculated from the yields of digested products. No degradation was observed in the control reactions in which RNase T_2 was omitted.

Results and Discussion

The reaction mixtures containing Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+} ions were homogeneous throughout the reaction. Some precipitations occurred with Pb^{2+}, Hg^{2+}, Cu^{2+}, and Zn^{2+} ions. As the reaction progressed, the formation of oligoadenylic acids took place in addition to hydrolysis of ImpA to pA and imidazole.



Yields of oligoadenylic acids up to the pentamer are given in Table II together with the proportion of 2′–5′ phosphodiester linkage in pApA and pApApA. The total yield of oligoadenylic acid was as high as 57.8% in the experiment in which Pb^{2+} was used. The high activity of Pb^{2+} is interesting, since Pb^{2+} also promotes the depolymerization of ribonucleic acid more effectively than any other metal ions that have been studied.[4,5] Ni^{2+} and Co^{2+} decreased the rate of hydrolysis but enhanced phosphodiester bond formation. Cu^{2+} and Hg^{2+} accelerated the hydrolysis of ImpA but not the formation of phosphodiester bonds. The efficiency of phosphodiester bond formation, which is roughly expressed by the ratio of the total yield of oligoadenylic acids to that of pA, decreases in the order Pb^{2+} >

Table II. Yields of Products from ImpA in the Presence of Divalent Metal Ions

Temp, °C	Metal salt	Yield, %[c] pApA	pApApA	$(pA)_4$	$(pA)_5$	ImpApA	pA	AppA	ImpA	2′-5′ linkage, % pApA	pApApA
rt[a]	$CaCl_2$	5.6	0.4			0.7	85.6	2.7	5.0	89	
	$MgCl_2$	4.3	0.2			0.7	86.2	2.7	5.9	90	
	$MnCl_2$	15.4	3.2	0.5		1.2	73.9	1.6	4.1	95	93
	$FeCl_2$[d]	10.4	1.2			0.8	80.9	0.9	5.7	82	
	$CoCl_2$	19.9	5.7	0.5		2.0	60.1	1.8	9.9	92	87
	$NiCl_2$	5.7	0.5			7.2	55.5	0.9	30.2	82	
	$CuCl_2$	2.4	0.2			0.3	93.5	1.0	2.7		
	$ZnCl_2$	16.4	2.7			0.7	74.1	2.2	3.9	89	81
	$CdCl_2$	9.5	1.6	0.2		2.6	72.2	1.8	12.1	96	
	$HgCl_2$	2.8	0.3			0.3	92.2	2.1	2.3		
	$Pb(NO_3)_2$	30.7	16.3	6.4[e]	2.4[e]	2.0	39.5	2.8	4.0	94	93
	None	3.1	0.4			0.7	85.4	2.0	8.4		
0[b]	$MgCl_2$	1.3				0.7	74.5	2.3	21.2		
	$MnCl_2$	9.6	1.1	0.2		6.8	48.2	1.8	32.4	84	
	$CoCl_2$	3.0	Trace			7.7	33.8	1.2	54.2		
	$NiCl_2$	Trace				7.5	12.0	0.8	79.7		
	$ZnCl_2$	13.0	3.7	0.2		8.3	54.8	1.6	18.3	90	79
	$Pb(NO_3)_2$	26.0	9.8	5.2[e]	1.0[e]	7.1	39.4	4.0	6.7	94	91
	None	1.1	Trace			0.9	38.9	2.6	56.5		

[a] Reactions were run at room temperature (rt) for 7 days; 0.05 M ImpA and 0.025 M metal salt were used. [b] Reactions were run at 0 °C for 10 days; 0.025 M ImpA and 0.025 M metal salt were used. [c] Yields are expressed as percentages of the initial ImpA. [d] Fe^{2+} was oxidized to Fe^{3+} in the course of the reaction. [e] These materials were not analyzed in detail.

$Co^{2+} > Zn^{2+} > Mn^{2+} > Ni^{2+} > Cd^{2+} > (Fe^{2+}) > Ca^{2+} \gtrsim Mg^{2+} \approx$ none $\approx Cu^{2+} \gtrsim Hg^{2+}$.

The efficiency with which different metal ions promote the synthesis of phosphodiester bonds is illustrated in Figure 1. The pattern is completely different from the pattern of activity for normal Lewis acid catalysis; for such reactions Cu^{2+} has the highest activity.[6] The pattern of activity is similar to that obtained with some divalent-metal-substituted zinc enzymes.[6]

The stereochemistry of the complexes formed by the different metal ion with ImpA is probably the most important factor determining the course of the reaction. It may also be significant that the most efficient catalysts are ions that occupy an intermediate position in the HSAB classification;[7] perhaps they bind both to the base group and to the phosphate group. The major phosphodiester linkage is 2′-5′. The nature of the metal ion has little influence on the proportion of 2′-5′ linked product. The formation of a precipitate is not an essential factor of the reaction.

General Discussion. Nucleoside phosphorimidazolides are suggested as intermediates of enzymatic nucleotidyl transfer reactions.[8] A histidine residue localized in the active site of the enzyme is the primary acceptor of the nucleotide. Then the nucleotide residue bound to the enzyme transfers to the acceptor. The metal ion works as a catalyst in the transfer reaction. ImpA may serve as a model of the substrate-enzyme complex.

ImpA can be obtained from ATP and imidazole under plausibly prebiotic conditions.[9] Prebiotic syntheses of imidazole derivatives are well known.[9] Thus we can propose that polymerization of ImpA catalyzed by metal ions could occur in ponds or sea on the primitive earth. Among the metal ions which have high catalytic activity for the internucleotide bond formation, Zn^{2+} ion is most abundant in sea water.[10] Egami has suggested that the metal ion which is abundant in sea water was incorporated into protoenzymes, which have evolved to metalloenzymes.[10] A number of RNA and DNA polymerases have been known to contain Zn^{2+} ion in the catalytic active site.[11] The Zn^{2+} ion catalyzed condensation reaction might be regarded as a link between prebiotic and biotic synthesis of nucleic acids.

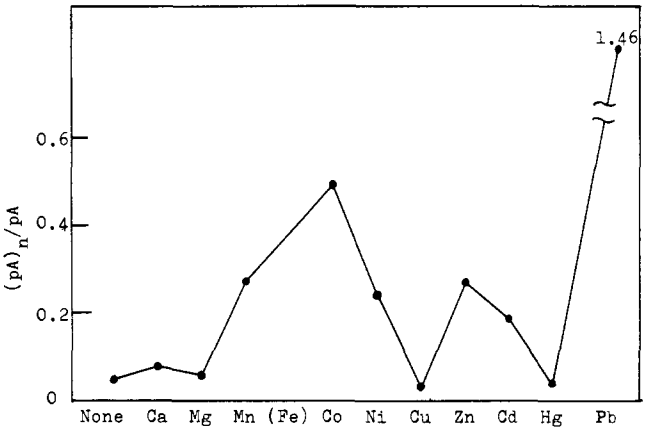


Figure 1. Relative effectiveness of metal ions in catalyzing the synthesis of oligoadenylic acids.

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