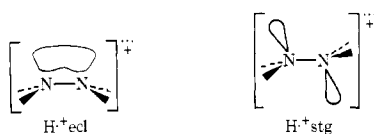


planar, and eclipsed at the alkyl groups (see  $H^+ecl$ ), although the staggered form ( $H^+stg$ ) is not very much higher in energy, because "double nitrogen inversion" in  $H^+ecl$ , which presumably goes through  $H^+stg$  form as an intermediate, has a very low activation energy.<sup>12</sup> Twisting about the "three electron  $\pi$  bond" to move  $\theta$  very far from 0 or 180° is costly in energy.



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Received March 3, 1975

## Oligonucleotide Synthesis Catalyzed by the $Zn^{2+}$ Ion

Sir:

Adenosine 5'-phosphorimidazolidine (ImpA) is an activated derivative of adenylic acid that polymerizes on a polyuridylylate template to form short oligoadenylic acids.<sup>1</sup> In the absence of a template or other catalyst, ImpA hydrolyzes to adenylic acid (pA) and imidazole. Here we report that the  $Zn^{2+}$  ion catalyzes the formation of oligonucleotides from nucleoside phosphorimidazolides in aqueous solution even in the absence of a template.

The imidazolides, ImpU and ImpA, were prepared by a modification of the procedures of Cramer et al.<sup>2</sup> Reaction mixtures (0.1 ml, pH 7.0), containing 0.025 M ImpA-8-<sup>14</sup>C (specific activity 0.12 mCi/mmol), 0.025 M  $ZnCl_2$ , and 0.2 M *N*-ethylmorpholine as a buffer, were prepared at 0° and kept at 0–50°. In all cases some precipitation occurred. Ali-

quots were withdrawn at various times and treated with 30  $\mu$ l of 0.25 M EDTA solution to break down  $Zn^{2+}$  complexes. The reaction mixtures were then subjected to paper chromatography in 1-propanol–15 M ammonia–water (55:10:35, v/v) and to electrophoresis in 0.03 M potassium phosphate buffer at pH 7.1. The yields of radioactive products were estimated by passing the chromatograms through a radiochromatogram scanner with integrator.

As the reaction progressed, ImpA disappeared from the reaction mixture, while adenylyl-adenosine 5'-phosphorimidazolidine (ImpApA)<sup>3</sup> and oligoadenylic acids with chain length up to four (pApA, pApApA, and pApApApA) were formed. In addition, the usual products of ImpA hydrolysis, pA, and a small amount of  $P_1P_2$ -diadenosine 5'-pyrophosphate (AppA),<sup>2</sup> appeared. ImpApA and oligoadenylic acids were identified by cochromatography with authentic samples. The identification of ImpApA was confirmed by its clean hydrolysis to pApA and imidazole under slightly acidic conditions. The oligoadenylic acids obtained in this reaction were completely degraded to pA by venom phosphodiesterase.<sup>4</sup>

The yields of the products are given in Table I. The maximum yield of oligoadenylic acids including ImpApA was 25.2%. ImpApA was formed after short reaction times and disappeared at later times as pApA and pApApA accumulated. The formation of the various products is explained in Scheme I. Pyrophosphate formation (eq 2) was always a minor reaction.

The ratio of the yield of oligoadenylic acids to that of pA expresses roughly the efficiency of phosphodiester bond formation. The maximum ratio was 0.45 at 0° in the presence of  $Zn^{2+}$  ion. In the control experiments in which  $ZnCl_2$  was omitted, the disappearance of ImpA was slow, and only small amounts of pApA and pApApA were formed. The  $Zn^{2+}$  ion increased the efficiency of phosphodiester-bond formation by a factor of as much as 10.

Table I. Yields of Products from Aqueous Solutions of ImpA in the Presence of  $ZnCl_2$

Temp, °C	Time (days)	(ImpA): ( $ZnCl_2$ ) (molar ratio)	Yield (%)							(pA) <sub>n</sub> / pA	2'-5' linkage of pApA (%)
			ImpA	pA	AppA	ImpApA	pApA	pApApA	pApApApA		
0°	1	1:1	76.0	15.8	1.2	5.9	1.1			0.45	
	3		46.9	38.5	1.7	8.4	4.2	0.3		0.34	
	10		18.3	54.8	1.6	8.3	13.0	3.7	0.2	0.46	90
r.t.	1		15.8	64.9	1.8	4.4	10.9	2.3		0.27	
	7		2.8	72.3	1.8	0.9	16.7	4.3	1.2	0.32	87
37°	5		1.7	73.8	2.2	0.7	15.5	5.0	1.1	0.30	78
50°	1		3.1	75.6	1.1	0.3	14.6	4.6	0.7	0.27	76
0°	1	2:1	62.5	25.5	0.9	9.6	1.5			0.44	
	3		41.6	41.3	1.7	10.4	4.6	0.4		0.37	
	10		18.9	55.2	1.5	9.2	12.9	2.3	0.3	0.45	91
r.t.	1		20.7	59.6	1.4	6.0	10.1	2.2		0.31	
	7		3.9	74.1	2.2	0.7	16.4	2.7		0.27	89
50°	1		1.6	77.0	1.1	0.2	16.4	3.3	0.3	0.26	83
0°	10	1:0 <sup>a</sup>	56.5	38.9	2.6	0.9	1.1	Trace		0.05	
r.t.	7		9.3	86.2	1.6	0.5	2.1	0.3		0.03	
r.t.	7	1:1 <sup>b</sup>	8.3	84.9	3.0	0.8	2.6	0.4		0.04	

<sup>a</sup>Control reaction. <sup>b</sup> $MgCl_2$  was used. <sup>c</sup>r.t. = room temperature.

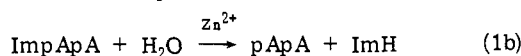
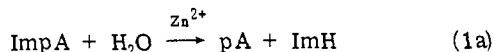
Table II. Yields of Products from Aqueous Solutions of ImpU in the Presence of  $MgCl_2$  or  $ZnCl_2$ <sup>a</sup>

(ImpU): ( $ZnCl_2$ ) (molar ratio)	Yield (%)							2'-5' linkage of pUpU (%)
	ImpU	pU	UppU	ImpUpU	pUpU	pUpUpU	(pU) <sub>n</sub> /pA	
1:1	5.8	81.4	3.6	0	8.8	0.4	0.11	86
2:1	3.2	83.3	1.9	0.2	10.1	1.3	0.14	86
1:1 <sup>b</sup>	11.7	84.2	2.2	Trace	1.9	0	0.02	Not studied
1:0	13.9	80.3	3.8	0	2.0	0	0.02	Not studied

<sup>a</sup>At room temperature for 7 days. <sup>b</sup> $MgCl_2$  was used.

## Scheme I

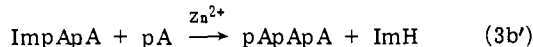
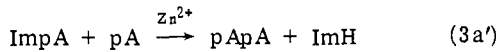
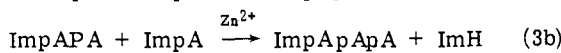
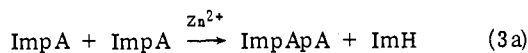
## 1. Hydrolysis



## 2. Pyrophosphate bond formation



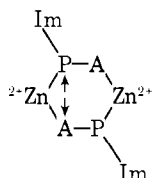
## 3. Phosphodiester bond formation



The pApA and pApApA formed in the reaction were analyzed by RNA'ase T<sub>2</sub> digestion,<sup>4</sup> and were found to contain 76–90% of unhydrolyzable 2'–5' phosphodiester bonds (Table I). The proportion of 2'–5' linkages and the efficiency of phosphodiester-bond formation were greater at lower reaction temperatures. Neither quantity was affected when ZnCl<sub>2</sub> was replaced by Zn(NO<sub>3</sub>)<sub>2</sub>.

When ImpU is used instead of ImpA in the Zn<sup>2+</sup>-catalyzed reactions discussed above, the qualitative features of the reaction are unchanged. However, the yields of corresponding oligonucleotides are smaller with ImpU than with ImpA (Table II).

The explanation of our results at a mechanistic level is unclear. On the one hand, it is possible that two imidazole molecules, bound in a single Zn<sup>2+</sup> complex, react together to form an internucleotide bond; a 2:2 complex can be formulated in which the 2'-OH of an ImpA approaches the 5'-phosphate of another.<sup>5</sup> On the other hand, the reaction



may occur between ImpA molecules attached to independent Zn<sup>2+</sup> ions in the precipitate. In either case ImpA coordinates to Zn<sup>2+</sup> ion and the internucleotide-bond formation takes place in the coordination sphere of Zn<sup>2+</sup> ion.

A number of DNA and RNA polymerases have been shown to contain tightly bound Zn<sup>2+</sup> ion<sup>6</sup> in the active site. It seems possible that the simple Zn<sup>2+</sup> ion-catalyzed polymerization of activated nucleotides is the forerunner of nucleic acid synthesis. In this connection, it is interesting that the Mg<sup>2+</sup> ion catalyzes the hydrolysis of ImpA and ImpU but does not enhance the formation of phosphodiester bonds. (Table I and Table II).

**Acknowledgment.** We wish to thank Dr. Jacques Ninio for his generous gift of authentic 3'–5' linked oligoadenylic acids (pApA, pApApA, pApApApA, and pApApApApA) and oligouridylic acids (pUpU and pUpUpU). This work was supported by NASA Grant NGR-05-067-001.

## References and Notes

- (1) B. J. Weimann, R. Lohrmann, L. E. Orgel, H. Schneider-Bernloehr, and J. E. Sulston, *Science*, **161**, 387 (1968).
- (2) F. Cramer, H. Schatter, and H. A. Ataab, *Chem. Ber.*, **94**, 1612 (1961).
- (3) Authentic ImpApA was prepared from pApA and 1,1'-carbonylimidazole (ref 2).
- (4) The enzymatic activity of venom phosphodiesterase (Worthington Bio-

chemical) and RNase T<sub>2</sub> (Sankyo Co.) were checked by the assay using 3'–5' linked ApA, pApA, pApApA, and pUpU.

- (5) This structure is one of the possible models for the 2:2 Zn<sup>2+</sup>-pA complex. J. M. Rifkind and G. L. Eichhorn, *J. Am. Chem. Soc.*, **94**, 6526 (1972).
- (6) A. S. Millivan, *Annual. Rev. Biochem.*, **43**, 850 (1974).

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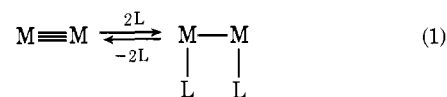
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Received March 24, 1975

### Reversible Addition of Carbon Monoxide to Tetracarbonylbis(η<sup>5</sup>-pentamethylcyclopentadienyl)-dimolybdenum(I)

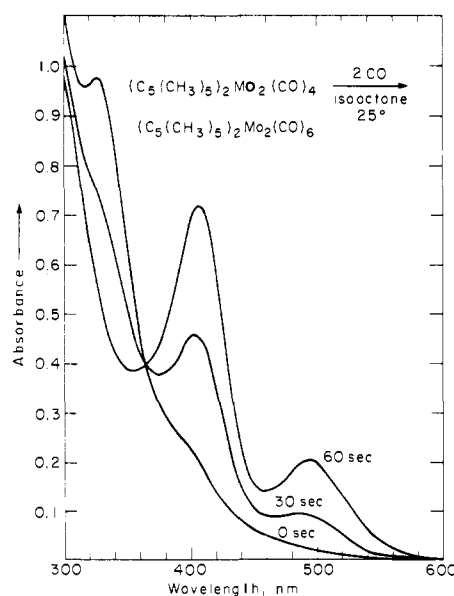
Sir:

We wish to report the first example of a reversible addition of simple, two-electron donor ligands to metal triple bond of a coordination compound. The general reaction is shown in eq 1 where L is a two-electron donor ligand and M



is a metal. This new reaction of metal-metal bonded complexes may have importance in catalysis and organometallic synthesis. Additionally, the work reported herein clarifies the uncertainties associated with previous studies<sup>1–3</sup> of tetracarbonylbis(η<sup>5</sup>-pentamethylcyclopentadienyl)dimolybdenum(I), I.

The complex I was prepared according to the literature procedure,<sup>1,2</sup> and as expected<sup>2</sup> the purest samples show only two CO stretching bands in the infrared. Purging iso-octane solutions of I with CO at 25° leads to the complete and rapid decline of the two CO stretching bands characteristic of I and the growth of two new CO stretching absorptions both higher in energy than those in I. Accompanying these spectral changes are uv-visible spectral changes like those depicted in Figure 1. The spectral changes indicate a half-



**Figure 1.** Uv-visible absorption spectral changes accompanying the conversion of I to II by purging an iso-octane solution of I with CO at 25°. The band at 409 nm in II is the  $\sigma_b \rightarrow \sigma^*$  transition analogous to that found at 388 nm in III.