methylamine (Mops-tris). After centrifugation for 10 minutes at 600*g*, the pellet was washed three times with fresh sucrose-EDTA buffer and finally homogenized vigorously with a Polytron Pt-20 homogenizer. In some cases the homogenate was then incubated with deoxyribonuclease I (10 μg/ml) (K. D. Philipson and D. M. Bers, personal communication) for 30 minutes at 22°C. After centrifugation at 12,000*g* for 10 minutes, the supernatant was layered over 0.6*M* sucrose, 0.5 m*M* EDTA, 10 m*M* Mops-tris (*p*H 7.0) and centrifuged at 113,000*g* for 90 minutes in a Beckman SW 27 rotor. The vesicles were collected at the 0.6*M*/0.3*M* sucrose interface, washed, and resuspended in 160 m*M* NaCl, 20 m*M* Mops-tris (*p*H 7.4). The vesicles were enriched more than 30-fold over the crude homogenate in $(Na^+ + K^+)$-activated adenosinetriphosphatase activity (40 to 60 μmole of adenosine triphosphate hydrolyzed per milligram of protein per hour), a well-characterized sarcolemmal marker. The Na^+-Ca^{2+} exchange activity has been shown to be a property of the sarcolemmal membranes within the vesicle preparation (*13*, *14*).

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Efficient Metal-Ion Catalyzed Template-Directed Oligonucleotide Synthesis

Abstract. *The Pb^{2+} and Zn^{2+} ions are efficient catalysts for the polycytidylic acid–directed polymerization of an activated guanylic acid derivative, guanosine 5′-phosphorimidazolide. The products include oligomers of 30 to 40 units in length. The nucleotide residues are predominantly 2′–5′ linked when Pb^{2+} is the catalyst, and predominantly 3′–5′ linked in the presence of Zn^{2+}. The significance of these results in the context of the prebiotic evolution of RNA polymerase is discussed.*

The DNA and RNA polymerases and DNA ligase differ from most other enzymes in that their specificity is dependent on the interaction of their substrates with a complementary template, as well as on the more normal interaction of substrate with enzyme. Naylor and Gilham were the first to use an analogous template principle to bring about a chemical condensation between oligonucleotides (*1*). They showed that poly(dA) (*2*) facilitates the condensation of two hexathymidylic acid molecules to form dodecathymidylic acid in aqueous solution. This reaction is in many ways analogous to that performed by a DNA ligase.

Organized helical structures are formed between polyuridylic acid and monomeric adenosine derivatives or between polycytidylic acid and monomeric guanosine derivatives (*3*). These structures, although often triple-helical, have much in common with double-stranded DNA and RNA helices. This suggested that it should be possible to carry out template-directed nonenzymatic reactions in which activated monomeric purine derivatives condense together to give oligonucleotides. Polymerase reactions should be susceptible to modeling more easily than most enzymatic oligomerizations, since part of the normal function of the enzyme is taken over by the template.

We have described the template-directed condensation reactions of adenosine derivatives (*4*, *5*) and shown that poly(U) directs the synthesis of oligoadenylic acids—at least up to the octamer—from a suitable adenosine derivative, the 5′-phosphorimidazolide, ImpA. The product formed in this nonenzymatic reaction is predominantly 2′-5′ linked (*5*). We have also shown that the Pb^{2+} ion catalyzes the formation of the longer oligomers, and increases the proportion of the natural 3′-5′ linkage in the products (*6*).

Until recently, we were unable to study the corresponding reactions of the 5′-phosphorimidazolide of guanosine (ImpG) in comparable detail, owing to the difficulty of separating oligo(G)'s in paper chromatographic systems. Our modification of an RPC-5 column (*7*) has permitted a rapid resolution of oligomers at least up to the 40-nucleotide polymer and has allowed separation of linkage isomers. This has enabled us to extend our work considerably. We now report a remarkable catalytic effect of Zn^{2+} and Pb^{2+} on the efficiency and stereoselectivity of the template-directed oligomerization of ImpG.

Polycytidylic acid was prepared by a modification of the method of Steiner and Beers (*8*); unlabeled and ^{14}C-labeled ImpG were prepared by modifications of the procedures of Lohrmann and Orgel (*9*). $(Gp)_n$ markers were prepared by partial alkaline hydrolysis of poly(G). All other reagents were analytical grade.

Paper chromatography was carried out on Whatman 3MM paper, with a mixture

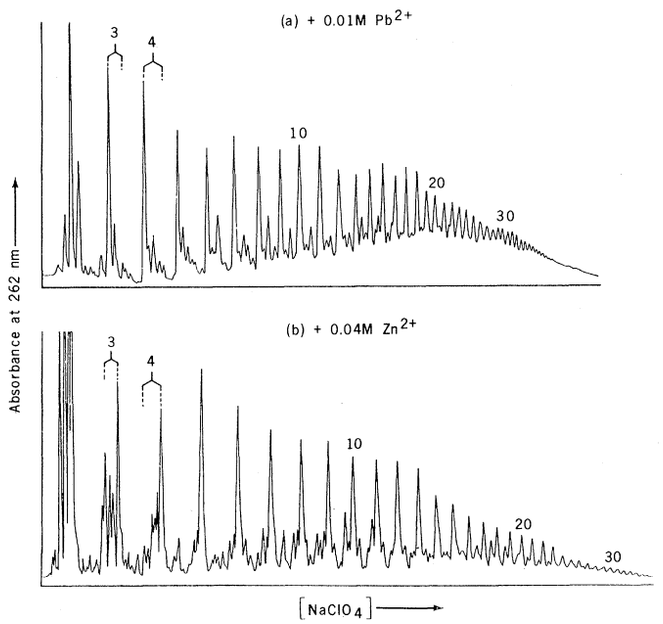

Fig. 1. Elution profiles of products from the template-directed self-condensation of ImpG in the presence of (a) 0.01*M* Pb^{2+} or (b) 0.04*M* Zn^{2+}. The positions of the major peaks of the 10-, 20-, and 30-nucleotide oligomers are indicated. The positions of all 2′-5′- and all 3′-5′-linked isomers of $(pG)_3$ and $(pG)_4$ are also indicated. The reaction conditions were as follows: 0.02*M* ImpG* (0.25 mCi/mmole), 0.04*M* poly(C), 0.4*M* $NaNO_3$, 0.5*M* $Mg(NO_3)_2$, 0.4*M* 2,6-lutidine buffer, *p*H 7.0. After the reaction mixture was held for 12 days at 0°C, excess EDTA was added, and the *p*H was adjusted to 7.9 with tris buffer. Pancreatic ribonuclease [0.25 mg per micromole of poly(C)] was added, and the solution was incubated at 37°C for 8 hours. Material on chromatograms (a) and (b) accounts for 86.2 and 74.6 percent of the products, respectively. The remaining product, pG, eluted with the void volume (not shown).

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of *n*-propanol, concentrated ammonia, and H_2O (55:10:35) as solvent. High-performance liquid chromatography (HPLC) (Waters) was carried out on an RPC-5 column. The column was eluted with a linear gradient (0 to 0.06*M*) of $NaClO_4$ at *p*H 12. Enzyme degradations with pancreatic ribonuclease and ribonuclease T1 were carried out by modifications of procedures described in (*10*).

All template-directed reactions were carried out at 0°C (*5*, *6*) (legend to Fig. 1). Pancreatic ribonuclease was used to degrade the poly(C) prior to analysis of oligo(G) products.

Analysis by paper chromatography of ^{14}C-labeled products separated pG, $(pG)_2$, and $(pG)_3$ from higher oligomers, which remained at the origin. The mean chain length of material sticking to the origin was determined by hydrolysis with 1*M* NaOH for 48 hours (at room temperature). This yielded G, Gp, and pGp in the ratio $1:(\bar{n}-2):1$, where $\bar{n}$ is the mean chain length.

The traces in Fig. 1, a and b, are representative of the HPLC elution profiles of the products obtained from the self-condensation of ImpG in a 2,6-lutidine buffer, in the presence of the Pb^{2+} ion (Fig. 1a) or the Zn^{2+} ion (Fig. 1b). Here we outline the procedures used for identifying the major products of these two reactions.

We first identified a variety of short oligomers in our products, including G^2pG, G^3pG, pG^2pG, pG^3pG, and a mixture of $(pG)_3$ isomers, by cochromatography with authentic markers. In the Zn^{2+}-catalyzed reaction, we then assumed that the regularly spaced major peaks were the oligomers $pG^3(pG)_n$. We confirmed this assumption by showing that for all values of n up to 35 the $(pG)_n$ oligomers had retention times close to those of the corresponding enzymatically prepared 3′-5′-linked $(Gp)_n$ oligomers. Furthermore, we showed that treatment of the reaction mixture with ribonuclease T1 destroyed the material responsible for the major peaks and yielded only monomer and small amounts of very short oligomers as hydrolysis products.

The analysis of the oligomers obtained with Pb^{2+} was more difficult since no authentic 2′-5′-linked oligomers were available. Alkaline hydrolysis of the total material on the origins of paper chromatograms yielded only G, Gp, and pGp, establishing that we were dealing with a family of 2′-5′- and 3′-5′-linked oligonucleotides. We isolated, separately, material from the main peaks and from the clusters of subsidiary peaks that accompany the main peaks. The oligomers responsible for the main peaks were completely resistant to ribonuclease T1 and, therefore, entirely 2′-5′ linked. The material giving the minor peaks was degraded to shorter oligomers, showing that they contained 3′-5′ links.

We have also studied the uncatalyzed and the Pb^{2+}-catalyzed reactions in 1-methylimidazole buffer (*4*) and found that both reactions are substantially more efficient than in 2,6-lutidine buffer. The Pb^{2+}-catalyzed reaction yields up to 85 percent of predominantly 2′-5′-linked oligomers that are longer than the trimer and have a mean chain length as high as 17. The distribution of major and minor peaks resembles closely that obtained with lutidine buffer. The uncatalyzed reaction gives up to 50 percent of oligomers that have a mean chain length up to 5.5. The Zn^{2+}-catalyzed reaction cannot be studied in methylimidazole buffer, since the Zn^{2+} ion forms stable coordination complexes with the buffer.

In control reactions in which the template poly(C) was omitted, only small yields of dimer and trimer were obtained when the Zn^{2+} ion was present. Oligomers up to the octamer (eight nucleotides in length) were obtained with Pb^{2+}, with the higher oligomers in very low yield.

Several mechanisms have been proposed to account for the Zn^{2+} ion catalysis of enzymatic and nonenzymatic phosphorylation reactions (*11*). These include activation of the phosphate donor, activation of the OH group of the acceptor, and orientation of the donor and acceptor in a coordination complex in such a way as to promote reaction. Any or all of these explanations could account for our results; in addition, the metal ions might change the structure of the poly(C)-ImpG helix, by interacting at a point distant from the reactive phosphate and OH groups.

Whatever the mechanism, it is clear that metal ions have a profound effect on the efficiency and stereoselectivity of template-directed reactions. The formation of 3′-5′-linked oligomers in the presence of poly(C) and Zn^{2+} is particularly impressive, since the 2′-OH is six to nine times as reactive as the 3′-OH in the absence of a template (*9*).

It does not seem possible to predict which isomer will be favored by a given metal ion; the Pb^{2+} ion favors the 3′-5′ linkage in the poly(U)-ImpA system, but favors the 2′-5′ isomer in the poly(C)-ImpG system. All DNA and RNA polymerases are Zn^{2+} enzymes. It may be significant, therefore, that Zn^{2+} catalysis yields the 3′-5′-linked oligo(G)'s in the poly(C)-ImpG system.

Our results have implications for theories of the origins of life. It is a striking fact that catalysts as simple as the Pb^{2+} and Zn^{2+} ions, together with a poly(C) template, can bring about an efficient, linkage-specific oligomerization of the imidazolide of the complementary base, ImpG, to give oligomers almost in the "biological" molecular weight range. We do not believe that this reaction was necessarily important on the primitive earth, since other metal ions and other activated nucleotides will probably substitute more or less well for the compounds that we used. We are, however, encouraged to find that at least one template-directed reaction proceeds efficiently without the help of complicated catalysts. This makes it seem more plausible that polymerases could have evolved on the primitive earth. We have shown that under some conditions, the template discriminates efficiently (at least 200:1) against "wrong" bases (*12*).

Finally, we note that, if a comparably efficient template-directed synthesis of oligomers containing both G and C could be achieved, most of the major obstacles to the development of a nonenzymatic, self-replicating system would be overcome.

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2. Abbreviations: G, guanosine; Gp, guanosine 2′ (or 3′)-phosphate; pG, guanosine 5′-phosphate; ImpG, guanosine 5′-phosphorimidazolide; pGp, 5′-phosphoguanosine 2′(3′)-phosphate; the numbers given as superscripts between G and phosphate indicate the type of internucleotide linkage, for example, G^2pG, guanylyl-[2′→5′]-guanosine; $(pG)_n$ (n = 2, 3 . . .), oligomer of pG;$(Gp)_n$ (n = 2, 3 . . .), oligomer of Gp; poly(G), polyguanylic acid; poly(C), polycytidylic acid; oligo(G), oligomer of G; EDTA, ethylenediaminetetraacetic acid; U, uridine; C, cytidine; and dA, deoxyadenylate.
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