Template-Directed Synthesis with Adenosine-5′-phosphorimidazolide

Abstract. *Adenosine-5′-monophosphorimidazolide reacts efficiently with adenosine derivatives on a polyuridylic acid template, with the formation of internucleotide bonds.*

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride brings about formation of oligoadenylic acids from adenylic acid on a polyuridylic acid template (*1*, *2*), or of oligoguanylic acids from guanylic acid on a polycytidylic acid template (*3*). These reactions are base-specific (*2*, *3*). The efficiency of condensation is low; one internucleotide bond is formed for each 50 or so carbodiimide molecules hydrolyzed.

We have attempted these same reactions using "prebiotic" condensing agents (*4*), but without success. Cyanamide and cyanoguanidine, among the most plausible prebiotic condensing agents, cannot easily be tested in the laboratory since they react so slowly at the low temperatures required for formation of stable helices. Cyanogen, which is sufficiently reactive at 0°C, does not bring about template-directed synthesis.

An alternative approach is the use of preformed activated nucleotides. Adenosine triphosphate forms a stable helix with polyuridylic acid (*5*) but then undergoes hydrolysis without forming appreciable amounts of oligonucleotides. Adenosine-5′-phosphorimidazolide, however, reacts with remarkable efficiency on a polyuridylic acid template to give internucleotide bonds.

A solution (0.5 ml) containing adenosine-8-C^{14} (about 0.16 μc/μmole, 0.0125*M*), adenosine-5′-phosphorimidazolide (0.0125*M*), polyuridylic acid (0.05*M*), imidazole (0.2*M*), NaCl (0.2*M*), and $MgCl_2$ (0.075*M*) was prepared at 0°C, and portions were titrated to *p*H 6, 7, and 8 with 2*M* HCl. Samples were analyzed from time to time by methods described previously (*1*). Yields of ApA and ApApA (*6*) are given in Table 1 together with those from control experiments in which the polyuridylic acid was omitted. The ApA from an experiment carried out at *p*H 7 was then degraded enzymically (*1*) to determine the proportions of the different isomers. The major product was $A^{2'}$pA (95.9 percent); $A^{3'}$pA accounted for 1.8 percent and $A^{5'}$pA for 2.3 percent of total dinucleoside phosphate.

Solutions identical with those just described except that they contained 0.0125*M* adenylic acid-8-C^{14} (about 0.16 μc/μmole) in place of adenosine were analyzed by a method described previously (*1*); product yields appear in Table 2 along with those for controls in which the polyuridylic acid was omitted. The results (Tables 1 and 2) show that adenosine 5′-phosphorimidazolide reacts on a polyuridylic acid template to form phosphodiester bonds, with efficiency as high as 50 percent. In our control experiments only very small amounts of phosphodiesters were formed, although the pyrophosphate $A^{5'}$ppA was obtained in the self-condensation of pA.

Imidazoles are readily formed from simple precursors under potentially prebiotic conditions. Imidazole derivatives are obtained from sugars and ammonia (*7*); imidazoleglycerolphosphate, from ribose-5-phosphate and formamidine (*8*); histidine, from the hydrogen cyanide polymer (*9*); and imidazole, from cyanoacetylene and ammonia under ultraviolet radiation (*10*). Furthermore, activated phosphates react with imidazoles in aqueous solution to give *N*-phosphorimidazole derivatives; thus the prebiotic occurrence of such derivatives is not implausible.

Two phosphorylating enzymes, thiosuccinate kinase (*11*) and a hexose kinase (*12*), are known to involve *N*-phosphohistidine intermediates. Nothing is known about the mechanisms of action of the nucleotide polymerases, but they could be similar. We believe that our results, together with others showing that aminoacylimidazoles give peptides in aqueous solution (*13*), suggest that imidazoles may provide a link between prebiotic and biotic condensation reactions. However, there are several alternatives; for example, phosphoramidates, which we are investigating.

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Table 1. Percentage yields of ApA and ApApA formed on a polyuridylic acid template after 3 and 14 days.

*p*H	Polyuridylic acid	3 Days		14 Days	
		ApA	ApApA	ApA	ApApA
6	Without	0.7		1.2	
6	With	24.0	0.5	29.0	1.0
7	Without	0.9		1.7	
7	With	27.7	.7	41.7	2.05
8	Without	0.6		1.5	
8	With	26.3	.8	43.6	2.85

Table 2. Percentage yields of pApA, AppA, and trinucleotides formed on a polyuridylic acid template after 14 days.

*p*H	Polyuridylic acid	AppA	pApA	Trimer
6	Without	10.5	0.4	
6	With	11.2	4.9	0.2
7	Without	17.2	0.7	
7	With	8.4	19.8	2.45
8	Without	16.4	1.1	
8	With	4.25	27.6	3.8

References and Notes

1. J. Sulston, R. Lohrmann, L. E. Orgel, H. T. Miles, *Proc. Nat. Acad. Sci. U.S.* **59**, 726 (1968).
2. ———, *ibid.*, in press.
3. ———, unpublished.
4. R. Lohrmann and L. E. Orgel, *Science* **161**, 64 (1968).
5. H. T. Miles, F. B. Howard, J. Frazier, *Federation Proc.* **25**, 1853 (abstr.) (1966).
6. Abbreviations: A, adenosine; pA, adenosine-5′-monophosphate; ApA, adenylyl-adenosine (isomer not specified); ApApA, triadenosine diphosphate (isomer not specified); $A^{2'}$pA, adenylyl-(2′→5′)adenosine; $A^{3'}$pA, adenylyl-(3′→5′)adenosine; $A^{5'}$pA, adenylyl-(5′→5′)-adenosine; $A^{5'}$ppA, P^1,P^2-di(adenosine-5′)pyrophosphate.
7. K. Hofmann, *Imidazole and Its Derivatives* (Interscience, New York, 1953), part 1, chap. 2.
8. B. N. Ames, *J. Biol. Chem.* **228**, 131 (1957).
9. C. N. Matthews and R. E. Moser, *Nature* **215**, 1230 (1967).
10. J. P. Ferris, R. A. Sanchez, L. E. Orgel, *J. Mol. Biol.*, in press.
11. G. Kreil and P. D. Boyer, *Biochim. Biophys. Res. Commun.* **16**, 545 (1964).
12. W. Kundig, S. Ghosh, S. Roseman, *Proc. Nat. Acad. Sci. U.S.* **52**, 1067 (1964).
13. G. W. Anderson, *Ann. N.Y. Acad. Sci.* **88**, 676 (1960).
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