Effect of Azoles as a Nucleotide Activating Group on Uranyl (VI)-Ion Catalyzed Synthesis of Oligoadenylate in Aqueous Solution

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(Received February 15, 1990)

Various adenosine-5′-phosphorazolides were prepared from adenosine-5′-phosphate and azoles such as 2-methylimidazole, 4-methylimidazole, 1,2,4-triazole, 3-nitro-1,2,4-triazole, benzimidazole and 2-methylbenzimidazole. The adenosine-5′-phosphorazolides obtained were then polymerized in neutral aqueous solution using uranyl(VI) ion as a catalyst. Oligoadenylates up to the hexadecamer with 2′-5′-internucleotide linkage were formed in high yields under the optimum condition. The azoles worked as adenylate activating group, and had a large effect on the rate of polymerization, the yield and the chain length of the resulting oligonucleotides. Imidazolides showed the moderate reactivity and gave oligoadenylates of the longest chain length. From the phosphortriazolides, only short-chained oligoadenylates were obtained, as they are susceptible to the hydrolysis. Polymerization and hydrolysis reaction of the benzimidazolides were retarded.

Previously we reported that some divalent metal ions catalyze the polymerization of nucleoside-5′-phosphorimidazolide(ImpN) in neutral aqueous solution forming oligonucleotides from dimer to hexamer. The resulting oligonucleotides contained mainly 2′-5′ internucleotide linkage.[1] We applied the method for lead ion-catalyzed polymerization of adenosine-5′-phosphorimidazolide to the synthesis of 2′-5′ oligoadenylate which is related to the interferon's antiviral action.[2] Since then, we further explored other efficient catalyst for the oligonucleotide synthesis and found that uranyl(VI) ion catalyzes the polymerization very efficiently, giving the oligonucelotides up to the hexadecamer with high 2′-5′ regioselectivity.[3] The regioselectivity and the chain length of the resulting oligonucleotides vary depending on reaction conditions. In the coordination sphere of the metal ion, the 2′- or 3′-hydroxyl group of ImpA attacks the phosphorimidazolide of the proximate ImpA, forming the internucleotide linkage. Without metal ions, the phosphorimidazolide spontaneously hydrolyzes in aqueous solution.

Chandrasegaran et al. reported that use of imidazole as a catalyst, in a conventional phosphotriester method for oligonucleotide synthesis, produces the phosphorimidazolide bond as an intermediate. They have also mentioned that 1,2,4-triazole has a catalytic activity similar to imidazole, while tetrazole and 3-nitro-1,2,4-triazole promoted the internucleotide bond formation more efficiently than imidazole.[4] Tetrazole and triazole have also been used as activating agents for the phosphoramidite DNA synthesis.[5-7] These facts indicate that azole compounds have a large effect on the formation and reactivity of phosphorazolide bond.

Polymerization of guanosine-5′-phosphorimidazolide proceeds in the presence of poly C template in aqueous solution. Zn^{2+} and Pb^{2+} ion alter the regioselectivity of the internucleotide linkage of the resulting oligoguanylate.[8] Inoue and Orgel reported that, in the poly C-directed oligoguanylate synthesis, replacement of the guanosine-5′-phosphorimidazolide with guanosine-5′-phosphor-(2-methylimidazolide) as a starting monomer remarkably enhances the 3′-5′ regioselectivity and improves the chain length. Guanosine-5′-phosphor-(4-methylimidazolide) or guanosine-5′-phosphor-(2-ethylimidazolide) has no such effect of enhancement.[9] Effect of the substituent group of imidazole on the polymerization reaction is of interest.

In this study, we have prepared various adenosine-5′-phosphorazolides and carried out their polymerization using uranyl (VI)-ion as a catalyst. The effect of the azoles as nucleotide activating groups has been studied regarding the rate of the polymerization, the chain length and the regioselectivity of the resulting oligoadenylate.

Experimental

Materials: Adenosine-5′-phosphate (pA) was from Seikagaku Kogyo. *N*-Ethylmorpholine (Tokyo Kasei) was distilled before use. Imidazole (Tokyo Kasei) was recrystallized from benzene. Uranyl(VI) nitrate was from Yoneyama Chemicals. Other reagents were obtained commercially and used without further purification. Adenosine-5′-phosphortriazolide (TrpA) was prepared from pA and 1,2,4-triazole by a similar procedure for the synthesis of adenosine-5′-phsophorimidazolide (ImpA).[10] In brief, pA was condensed with 1,2,4-triazole in dry DMF using triphenylphosphine and di-2-pyridyl disulfide as a condensing agent. The completion of phosphortriazolide bond formation and disappearance of pA was checked by cellulose F TLC (developing solvent, 2-propanol-concd aq-NH_3-H_2O=7:2:1); R_f, 0.73 (TrpA), 0.11 (pA). TrpA was isolated as a sodium salt by adding the above reaction mixture to the solution of dry ether and acetone containing sodium perchlorate. The resulting white precipitate was collected by filtration with a glass filter in a slow stream of dry nitrogen to exclude the moisture. The isolated yield was 61%. TrpA was kept in a desiccator stored in a freezer. Other adenosine-5′-phosphorazolides, adenosine-5′-phosphor-(2-methylimidazolide) (2-MeImpA), adenosine-5′-phosphor-(4-methylimidazolide) (4-MeImpA) adenosine-

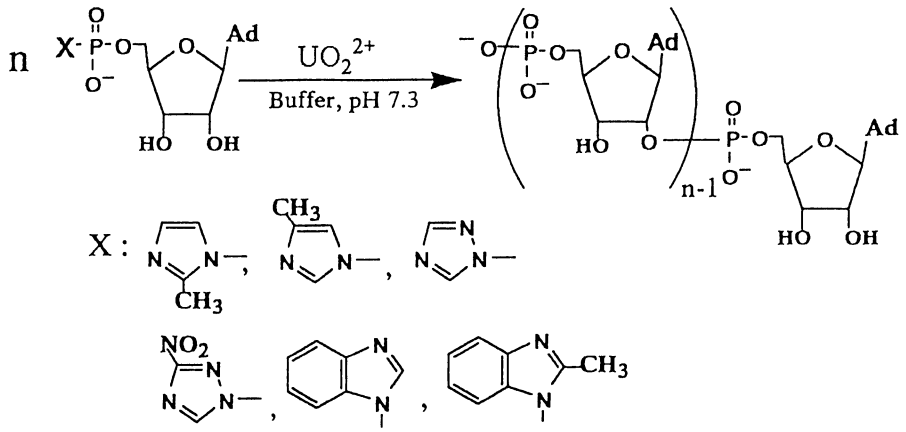
5′-phosphor-(3-nitro-1,2,4-triazolide) (3-NtTrpA), adenosine-5′-phosphorbenzimidazolide (BzImpA) and adenosine-5′-phosphor-(2-methylbenzimidazolide) (2-MeBzImpA), were prepared by the same method in 52—91% yield. The adenosine-5′-phsophorazolides, especially 3-NtTrpA, were susceptible to the hydrolysis by moisture. Their polymerization reaction was carried out immediately after the preparation. No hydrolysis of the phosphorazolide bond was checked by TLC and HPLC before the polymerization. An attempt to prepare adenosine-5′-phosphortetrazolide by a similar procedure was unsuccessful, because the phosphortetrazolide bond was too reactive and hydrolyzed to pA during the isolation as a sodium salt.

General Procedure of Polymerization of TrpA: The polymerization of TrpA by uranyl(VI) ion catalyst was conducted by a similar method to the one we used for that of ImpA previously.[3,10] A mixture (200 μl) containing TrpA (50 mM) and a catalytic amount of uranyl(VI) nitrate (0.01—10 mM) in *N*-ethylmorpholine buffer (0.2 M, pH 7.3) was prepared on an ice bath, agitated vigorously and kept at 20 °C. At the chosen time, the reaction mixture was quenched by addition of 0.25 M EDTA solution, and HPLC analysis was performed. Polymerization of other adenosine-5′-phosphorazolides was carried out by the same method.

Analysis of Products: Analysis of the resulting oligoadenylates were carried out by HPLC as described previously.[3] HPLC was taken with a Hitachi 638 equipment with an RPC-5 column (4 mm×25 cm). The elution was carried out with a linear gradient of $NaClO_4$ solution (0—0.15 M) buffered with 2.5 mM Tris-OAc (pH 7.5) and 0.1 mM EDTA in 60 min, and monitored by UV absorption at 260 nm. Yields were calculated from the peak integrals of the chromatograms after allowing for the hypochromicity of each oligoadenylate. The products were identified by comparing their retention times with those of authentic samples. Oligoadenylate-5′-phosphorazolides ($Az(pA)_n$, n=2,3,4) were identified by acidic hydrolysis to the corresponding oligoadenylates. The reaction mixture containing $Az(pA)_n$ was kept in 1 M acetate buffer (pH 4.5) for 1 d, and conversion to the corresponding oligoadenylates, $(pA)_n$, was checked by HPLC.

Results and Discussion

Condensation of pA with various azole compounds gave the corresponding adenosine-5′-phosphorazolides in substantial yields. The phosphorazolide



Scheme 1. Oligoadenylate formation from adenosine-5′-phosphorazolide.

Table 1. Oligoadenylates Formation from 2-MeImpA or 4-MeImpA Catalyzed by Uranyl Ion Complex[a]

$[UO_2^{2+}]$	Time	Yield/% (Percentage of fully 2′-5′ linked oligomer)[b]								
mM	d	MeImpA	pA	AppA	$(pA)_2$	$(pA)_3$	$(pA)_4$	$(pA)_5$	$(pA)_{6-8}$	$(pA)_{9-16}$
		2-MeImpA								
1	1	0.3	0.7	0.8	4.1	14.5	13.3	12.4	28.3	13.5
					(96)	(74)	(64)	(53)		
0.1	5	0.6	2.1	0.5	18.5	25.7	19.7	10.5	12.1	
					(97)	(71)	(49)	(35)		
0.01	10	4.8	26.2	2.3	46.1	14.6	3.5	0.3		
					(78)	(50)	(31)			
		4-MeImpA								
1	1	0.2	0.4	0.4	3.4	13.2	15.4	13.2	33.9	13.9
					(96)	(77)	(69)	(57)		
0.05	10	1.7	2.4	3.2	33.6	33.5	16.9	7.1	3.6	
					(94)	(71)	(42)	(28)		

a) Reactions were run at 20 °C in 0.2 M *N*-ethylmorpholine-HNO_3 buffer (pH 7.3). 50 mM of MeImpA was used in the reaction. b) Percentage of fully 2′-5′ linked oligomers is shown in the parenthesis below the yield data of each oligoadenylate.

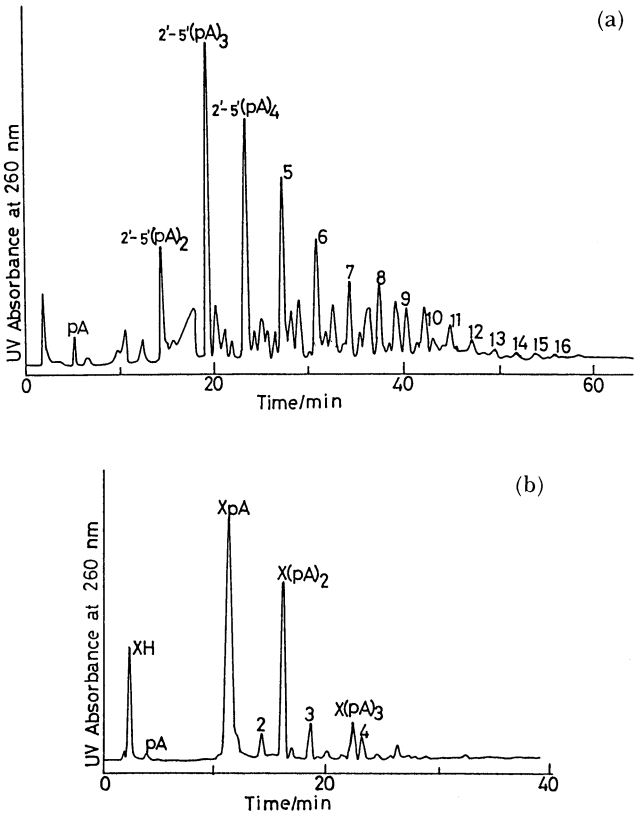


Fig. 1. HPLC profiles of the reaction products from adenosine-5′-phosphorazolide by uranyl(VI) ion catalyst.
a) The uranyl(VI) ion catalyzed oligoadenylates formation from 2-MeImpA at 20 °C and pH 7.3 for 1 d. 50 mM of 2-MeImpA and 1 mM of uranyl(VI) nitrate were used for the reaction. The number given to each peak denotes the chain length of fully 2′-5′ linked oligoadenylates. Small subsidiary peaks to the right of the main peaks are linkage isomers containing 2′-5 and 3′-5 bonds.
b) The uranyl(VI) ion catalyzed oligoadenylates formation from 2-MeBzImpA. The reaction conditions was the same as that in a). The peaks, XH, and X(pA)$_n$, denote the 2-methylbenzimidazole and 2-methylbenzimidazolide of mono and 2′-5′ oligoadenylates, respectively. The peak numbers, 2, 3, and 4, denote the fully 2′-5′ linked di-, tri- and tetraadenylates, respectively.

bond is susceptible to the moisture and hydrolyzes in neutral aqueous solution spontaneously forming pA. Adenosine-5′-phosphortetrazolide is too reactive toward hydrolysis to be isolated at room temperature. The reactivity of the phosphorazolide bond was in the following order; tetrazolide>3-nitrotriazolide>triazolide>imidazolides>benzimidazolides. The order is almost the same as that for the activating phosphoryl group in the internucleotide bond formation by phosphotriester method.[4]

Various adenosine-5′-phosphorazolides were polymerized in the presence of uranyl(VI) ion catalyst in neutral aqueous solution. Typical HPLC profiles of the reaction mixture, in which 2-MeImpA or 2-MeBzImpA was polymerized by uranyl(VI) ion catalyst, are shown in Fig. 1. Formation of pA by simple hydrolysis was very small in amount. The rate of the polymerization and the chain length of the oligoadenylates obtained varied greatly depending on the character of the adenosine-5′-phosphorazolide. Table 1 shows the yield data of the uranyl(VI) ion-catalyzed oligoadenylate formation from 2-MeImpA and 4-MeImpA. Oligoadenylates up to the hexadecamer were produced in the reaction. The 2′-5′ internucleotide linkage was preferentially formed. These results are somewhat similar to that of the uranyl(VI) ion-catalyzed oligoadenylate formation from ImpA.[3] However, replacement of nucleotide activating group, from imidazole to 2-methylimidazole or 4-methylimidazole, resulted in a small increase of the chain length and a small decrease of 2′-5′ regioselectivity of the oligoadenylates. The long chained oligoadenylates comprised mainly linkage isomers containing both 3′-5′ and 2′-5′ linkages. Inoue and Orgel reported the large effect of methyl substituent group of imidazole in the poly C-directed polymerization of guanosine-5′-phosphorimidazolide derivatives.[9] They found that 2-MeImpG increases the 3′-5′ regioselectivity and the chain length of the resulting oligoguanylate, while poly C-directed polymerization of 4-MeImpG, ImpG and 2-EtImpG gives short-chained oligoguanylate with low regioselectivity.

Table 2. Oligoadenylates Formation from Adenosine-5′-phosphortriazolide or Adenosine-5′-phosphor-(3-nitrotriazolide) Catalyzed by Uranyl(VI) Ion Complex[a]

$[UO_2^{2+}]$	Time	Yield/%							2′-5′ linkage of $(pA)_2$ (%)
mM	d	TrpA	pA	AppA	$(pA)_2$	$(pA)_3$	$(pA)_4$	$(PA)_5$	
		TrpA							
1	1	0.2	1.6	3.3	19.6	35.5	14.7	9.7	94
0.1	5	5.2	10.0	7.0	36.1	19.4	4.3		88
0.01	10	22.8	43.2	9.4	19.1	2.0			58
0	1	67.6	17.0	14.6	0.5[b]				
		3-NtTrpA							
1	1	0.6	58.0	25.7	10.0	5.7			75
0.1	1	0.9	71.2	17.4	6.8	3.1			59
0	1	0.7	88.4	8.6	1.2				

a) Reactions were run at 20 °C in 0.2 M *N*-ethylmorpholine buffer (pH 7.3). 50 mM of TrpA or 3-NtTrpA was used in the reaction. b) $Tr(pA)_2$ is included.

Table 3. Oligoadenylate Formation from Adenosine-5′-phosphor-(2-methylbenzimidazolide) or Adenosine-5′-phosphorbenzimidazolide Catalyzed by Uranyl Ion Complex[a)]

No.	UO_2^{2+} mM	Time d	Yield/% (Percentage of fully 2′-5′ linked oligomer)[b)]								
			XpA	X(pA)$_n$	PA	(pA)$_2$	(pA)$_3$	(pA)$_4$	(pA)$_5$	(pA)$_{6-8}$	(pA)$_{9-14}$
			2-MeBzImpA								
1	1	1	30.8	36.9	0.8	2.9	9.8	7.9	4.0	3.1	
2	1	14	3.2	10.3	1.1	10.4 (91)	25.2 (60)	16.5	8.4	14.4	
3	0.1	14	20.6	35.3	2.1	14.5 (90)	13.3 (71)	4.5	1.2		
4	0.01	14	68.6	13.7	6.9	6.4	0.4				
5	0	14	86.2		9.6						
6*	1	1	1.7[c)]	6.5[d)]	0.8	5.6 (93)	18.2 (72)	17.9 (68)	11.7	22.4	6.9
7*	0.1	5	1.4[c)]	7.4[d)]	4.7	22.9 (95)	18.3 (75)	13.0	5.7	5.8	
			BzImpA								
8	0.1	14	17.7	14.7	8.1	32.0 (90)	18.4 (71)	6.5	1.7	0.3	

a) Reactions were run at 20 °C in 0.2 M *N*-ethylmorpholine buffer (pH 7.3) except for Nos. 6 and 7 where reactions were run at 20 °C in 0.2 M imidazole-HNO_3 buffer (pH 7.3). 50 mM of 2-MeBzImpA or BzImpA was used in the reaction. AppA was also formed in small amounts in the reactions. b) Percentage of fully 2′-5′ linked oligomers is shown in the parenthesis below the yield data of each oligoadenylate. c) ImpA is included. d) Im(pA)$_n$ is included.

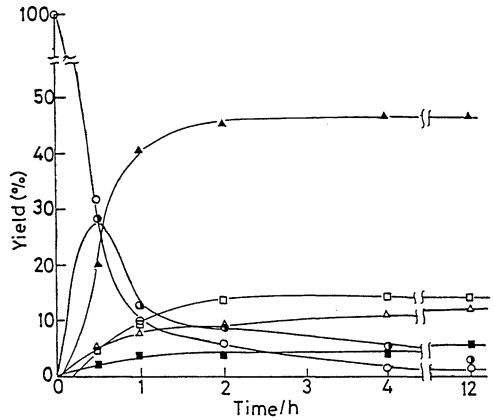


Fig. 2. Time course of polymerization of 2-MeBzImpA (50 mM) at 50 °C and pH 7.3 in the presence of uranyl(VI) nitrate (10 mM). (○), ImpA; (◑), Im(pA)n; (■), pA; (△), (pA)$_2$; (▲), (pA)$_{3,4}$; (□) (pA)$_{5-8}$.

The orientation of 2-MeImpG on poly C template to promote the 3′-5′ internucleotide bond formation has been proposed for the efficient and regiospecific polymerization.[11)] Present research revealed that such a remarkable substituent effect cannot be observed in the uranyl(VI) ion-catalyzed polymerization of adenosine-5′-phosphorimidazolide derivatives.

Table 2 lists the oligoadenylate formation from TrpA and 3-NtTrpA. Uranyl(VI) ion-catalyzed polymerization of TrpA yielded short-chained oligoadenylates in substantial yields, but no long ones. Fully 2′-5′ linked dimer, trimer and tetramer were formed from TrpA in 18, 32 and 10% yields, when 1 mM of uranyl(VI) nitrate was used as a catalyst. No efficient polymerization took place from 3-NtTrpA. Hydrolysis of phosphor-3-nitro-triazolide bond and formation of pyrophosphate bond (AppA) were predominant in this case. The phosphor-3-nitrotriazolide bond hydrolyzed rapidly in aqueous solution giving pA which reacted with 3-NtTrpA to produce AppA. The hydrolysis reaction suppressed the chain elongation of the oligonucleotide.

Table 3 shows the oligoadenylate formation from 2-MeBzImpA and BzImpA by uranyl(VI) ion catalyst. 2′-5′-Linked internucleotide linkage was predominant in the oligoadenylates. The steric hindrance and resonance effect of 2-MeBzImpA and BzImpA stabilized the phosphorbenzimidazolide bond and suppressed both the polymerization and hydrolysis of 2-MeBzImpA and BzImpA. Long reaction time was required for the completion of the polymerization in *N*-ethylmorpholine buffer. Chain length did not exceed octamer. High reaction temperature accelerated the reaction rate of 2-MeBzImpA. Time course of the polymerization at 50 °C and at 10 mM catalyst concentration is shown in Fig .2. The polymerization was almost complete in 4 h at 50 °C giving oligoadenylates from dimer to octamer in more than 55% total yield. The polymerization reaction of 2-MeBzImpA was also accelerated when the medium was replaced from *N*-ethylmorpholine buffer (pH 7.3) to imidazole buffer (pH 7.3). 2-MeBzImpA was almost disappeared in the imidazole buffer at room temperature for 1 d and oligoadenylates up to tetradecamer were formed. The results suggest that exchange reaction of imidazole with 2-MeBzImpA took place rapidly in the imidazole buffer forming ImpA which

was polymerized to oligoadenylates by uranyl(VI) ion catalyst. The formation of ImpA was confirmed in the initial stage of the reaction.

Character of the azole in adenosine-5′-phosphorazolides decides the efficiency of their uranyl(VI)-ion catalyzed polymerization. However, it has little effect on the regioselectivity of the internucleotide linkage. The formation of 2′-5′- internucleotide linkage is always predominant, though the percentage of the 2′-5′ linkage in the dimer varies 58 to 98%. The high 2′-5′ regioselectivity is explained by the fact that the nucleophilicity of 2′-hydroxyl group is stronger than that of 3′-hydroxyl group in the ribonucleotide system.[10,12] The geometrical orientation of the adenosine-5′-phosphorazolides in the coordination sphere of uranyl(VI) ion may also be favorable for the formation of 2′-5′ internucleotide linkage. The uranyl(VI) ion-catalyzed polymerization method is very useful for the synthesis of 2′-5′ linked oligonucleotide. For the synthesis of 3′-5′ internucleotide linkage, however, different orientation of the starting nucleotide is necessary.

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