

Uranyl Ion as a Highly Effective Catalyst for Internucleotide Bond Formation

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(Received January 12, 1989)

Uranyl ion catalyzes the polymerization of adenosine-5'-phosphorimidazolidine very efficiently in aqueous solution. Up to 95% of the input adenosine-5'-phosphorimidazolidine is converted to oligoadenylates from dimer to hexadecamer when 1 mM of UO_2^{2+} is used as a catalyst. The oligomeric products are predominantly 2'-5' linked. The chain length and the regioselectivity of the resulting oligoadenylates varies greatly with the concentration of the UO_2^{2+} ion catalyst and the pH of the reaction medium. A high total yield of oligouridylylates up to dodecamer is also obtained by UO_2^{2+} ion catalyst with high 2'-5' regioselectivity. The oligonucleotide formation is mediated by UO_2^{2+} -nucleotide complex.

An efficient procedure for the nonenzymatic synthesis of oligonucleotides from an activated nucleotide in aqueous solution has been interesting subjects of prebiotic chemistry^{1,2)} and biomimetic chemistry of nucleic acid biosynthesis,³⁾ and may provide a new synthetic methodology for the oligonucleotides. Nucleoside-5'-phosphorimidazolidine is an activated nucleotide and hydrolyzes to nucleoside-5'-phosphate in the absence of a template or a catalyst. Previously we reported that metal ions such as Pb^{2+} and Zn^{2+} catalyze the oligomerization of nucleoside-5'-phosphorimidazolidine to yield short oligonucleotides even in the absence of a template.³⁾ Pb^{2+} ion has been the most active catalyst. Most of the resulting oligomers are 2'-5' internucleotide linked. The Pb^{2+} -ion catalyzed oligomerization was applied to the convenient synthesis of 2'-5' linked oligonucleotides.⁵⁾ The mechanistic role of the metal ion catalyst on the oligonucleotide formation is unclear. However, we tentatively believe that the metal ion could organize the nucleoside-5'-phosphorimidazolidine by coordination so as to promote internucleotide bond formation. The metal ion may also enhance the nucleophilicity of a OH group of the nucleotide. We have explored other efficient metal ion catalysts which can possibly play such roles, and found that uranyl ion is a highly effective catalyst for the oligonucleotide formation. Here we wish to report the uranyl ion-catalyzed oligonucleotide formation under various reaction conditions.

Experimental

Materials. Adenosine-5'-monophosphate and uridine-5'-monophosphate were purchased from Seikagaku Kogyo. *N*-Ethylmorpholine, from Tokyo Kasei was distilled before use. Imidazole, from Tokyo Kasei, was recrystallized from benzene. Uranyl nitrate was from Yoneyama Chemicals. Reagent grade triethylamine, triphenylphosphine, di-2-pyridyl disulfide, and lead nitrate were obtained commercially. Nuclease P1 (N.P1) was from Yamasa and venom phosphodiesterase (VPDase) from Worthington. Adenosine-5'-phosphorimidazolidine was prepared from adenosine-5'-monophosphate and imidazole using triphenylphosphine and di-2-pyridyl disulfide as a condensing agent as described previously.^{5a,6)} Uridine-5'-phosphorimidazolidine (ImpU)

was prepared from uridine-5'-monophosphate and imidazole by a similar procedure.^{5b)}

Standard Procedure for the Polymerization of ImpA. Reactions were carried out in an Eppendorf tube. The reaction mixture was prepared on an ice bath by the addition of compounds in the following order; buffer solution, ImpA solution and finally uranyl nitrate solution. Samples of the mixture were agitated vigorously and kept at 20 °C for various periods of times. A typical reaction mixture (200 μl) contained ImpA (50 mM) and a catalytic amount of uranyl nitrate (10 μM –12.5 mM) in *N*-ethylmorpholine- HNO_3 buffer (0.2 M, pH 7.3) or imidazole- HNO_3 buffer (0.2 M[†], pH 6.0–8.0). When the reaction was carried out at pH 5.5 and 8.9, the pH of the reaction mixture was adjusted to the appropriate value with 0.1 M HCl or 0.1 M NaOH. The reactions were stopped by adding of 20 μl of 0.25 M EDTA solution. Samples were analyzed by high performance liquid chromatography (HPLC).

High-Performance Liquid Chromatography on RPC-5. HPLC was taken with a Hitachi 638 apparatus with a stainless steel column (4 mm $\times$ 25 cm) packed with RPC-5 material. The RPC-5 was prepared from Adogen 464 and granular polychlorotrifluoroethylene (2–10 μm) by a modification of the method described by Pearson et al.⁷⁾ The elution was carried out with a linear gradient of NaClO_4 solution (0–0.05 M) buffered with 2.5 mM Tris-acetate (pH 7.5) and 0.1 mM EDTA in 60 min at a flow rate of 1.0 ml min⁻¹. The eluate was monitored by UV absorption at 260 nm. Linkage isomers of oligoadenylates and oligouridylylates with 2'-5' and 3'-5' internucleotide bonds were separated very well by HPLC on the RPC-5 column. Yields were calculated from peak integrals of the oligonucleotides on the HPLC chromatogram, after allowing for the hyperchromicity of each oligonucleotide. The hyperchromicity of the oligonucleotides up to the octamer was estimated by the ratio of UV absorption at 260 nm after and before alkaline hydrolysis as described previously.⁵⁾ We made no estimate of the hyperchromicity of the long oligonucleotides with chain length more than eight, using instead the value of the octamer for the calculation of the yield.

Characterization of Oligonucleotides. Identification of the resulting oligonucleotides was carried out by comparing the HPLC chromatogram with that of the authentic sample and by sequential enzyme and alkaline digestions. Authentic oligonucleotides up to the pentamer were

[†] 1 M=1 mol dm⁻³.

obtained as described previously.^{5a)} When no authentic sample was available, oligonucleotides were identified by degradation with enzymes, alkaline or acidic medium.

The enzyme nuclease P1(N. P1) degrades 3'-5' internucleotide linkage and is inactive to the 2'-5' linkage. Thus digestion of the oligonucleotides with N. P1 distinguishes between 2'-5' and 3'-5' linked products. After treatment of a total sample with Chelex-100 to remove uranyl ion, digestion of the resulting oligonucleotides with N. P1 gave a series of fully 2'-5' linked oligonucleotides. Digestion with N. P1 was performed at 37 °C for 2.5 h in a mixture (50 μ l) containing the oligonucleotide (0.1–1.0 ODU), 0.03 M Veronal-acetate buffer (pH 5.75) and an enzyme solution (5 μ g in 50 μ l).

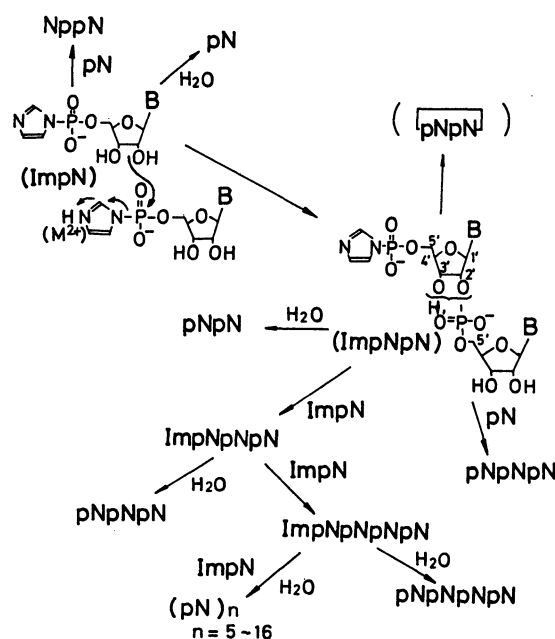
Venom phosphodiesterase (VPDase) degrades both 3'-5' and 2'-5' internucleotide linkage to give nucleoside-5'-monophosphate. It also cleaves a pyrophosphate bond. Degradation with VPDase was carried out at 37 °C for 2.5 h in a mixture (50 μ l) containing the substrate (0.1–0.3 ODU), 0.01 M Tris-acetate (pH 8.8) 0.01 M MgCl₂ and enzyme solution (0.02 unit).

Alkaline hydrolysis of the oligonucleotides was carried out at room temperature for 1 d in a 0.5 M NaOH solution. The hydrolysis of a (pA)_n oligomer containing both 2'-5' and 3'-5' linkage yielded on equivalent each of pAp and A and n-2 equivalent of Ap. The ratio of the resulting mononucleotides was determined by HPLC, which permits one to estimate the chain length. The HPLC of the mononucleotides was performed on a modified RPC-5 column with a linear gradient elution of NaClO₄ solution (0–0.01 M) buffered with 2.5 mM Tris-acetate (pH 7.5) and 0.1 mM EDTA in 15 min at flow rate of 1.0 ml min⁻¹.

Oligonucleotide-5'-phosphorimidazolid (Im(pA)_n, Im(pU)_n; n=2, 3, 4) was identified by conversion of the phosphorimidazolid with 1 M acetate buffer (pH 4.5) to the corresponding oligonucleotide-5'-monophosphate by acidic hydrolysis for 1 d. The compounds ImpA2'p5'A and ImpA2'p5'A2'p5'A were further confirmed by comparing the HPLC chromatogram with those of the authentic samples which were prepared from pA2'p5'A and pA2'p5'A2'p5'A in a way similar to that of ImpA.^{5a)}

Results and Discussion

ImpA was gradually hydrolyzed to pA in neutral aqueous solution in the absence of a template or metal ion catalyst. Addition of a catalytic amount of uranyl nitrate enhanced the polymerization of ImpA greatly and gave a good yield of long, predominantly 2'-5' linked oligomers. A HPLC elution profile of the reaction mixture, in which 1 mM of uranyl ion ([ImpA]/[UO₂²⁺]=50) was used as a catalyst, is shown in Fig. 1. The formation of the simple hydrolyzed product, pA was less than 1%. Oligoadenylates from dimer to hexadecamer were obtained in more than 95% total yield. Digestion of the total reaction mixture with nuclease P1 resulted in the disappearance of the



Scheme 1. Reaction scheme of the oligonucleotide formation.

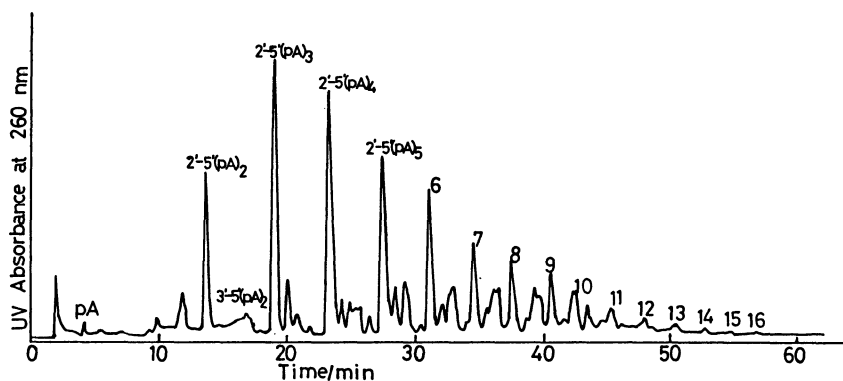


Fig. 1. HPLC elution profile of the products from the uranyl-ion catalyzed polymerization of ImpA. The reaction was run at 20 °C and pH 7.3 for 1 d. 50 mM of ImpA and 1 mM of uranyl nitrate were used for the reaction. The position of the fully 2'-5' linked oligoadenylates from dimer to hexadecamer are indicated.

subsidiary peaks, leaving only the series of main peaks, which indicates that the products are all 2'-5' linked oligoadenylylates. The subsidiary peaks to the right of the main peaks are oligomers of the same chain length containing one or more 3'-5' linkages. The regioselectivity of the 2'-5' internucleotide linkage decreased as the chain length of the oligomer increased. Thus the ratio of the fully 2'-5' linked oligoadenylylates was 98% in the case of dimer, and fell to 60% in the case of

octamer. The yields of all 2'-5' linked dimer, trimer, tetramer, pentamer, hexamer, heptamer and octamer were 5.6%, 13.9%, 11.9%, 10.4%, 6.9%, 4.1%, and 3.9%, respectively.

Figure 2 represents the time course of the polymerization of ImpA. Phosphorimidazolides of di- and triadenylate ($\text{Im}((\text{pA})_n, n=2, 3)$) formed initially in appreciable amounts, then gradually decreased. The chain length of the oligoadenylylates increased with reaction time. The polymerization of ImpA in the presence of 1 mM of $\text{UO}_2(\text{NO}_3)_2$ catalyst at room temperature was almost complete in 8 h. The time course of the reaction indicates that this oligoadenylylate formation proceeds stepwisely as shown in the Scheme 1.

Table 1 shows the effect of the concentration of the uranyl ion catalyst on the oligoadenylylate formation. The catalyst concentration had large effect on the chain length and the internucleotide linkage of the resulting oligoadenylylate. High catalyst concentration ($\text{UO}_2(\text{NO}_3)_2=12.5$ mM; $[\text{ImpA}]/[\text{UO}_2^{2+}]=4$) gave preferentially short-chained oligomers from dimer to pentamer in addition to small amounts of long oligomers over heptamer. The yields of long-chained oligomers increased as the catalyst concentration decreased and reached a maximum at 1 mM of $\text{UO}_2(\text{NO}_3)_2$, and fell off substantially at lower concentrations. The highest yield of total oligoadenylylates was

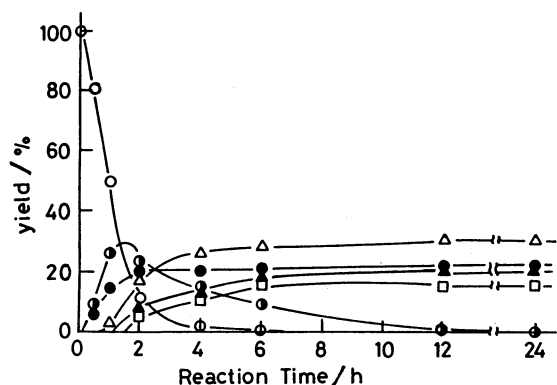


Fig. 2. Time course of the oligoadenylylates formation ImpA. Reaction conditions: ImpA, 50 mM; $\text{UO}_2(\text{NO}_3)_2$, 1 mM; 20 °C; pH 7.3 ImpA, (○); $\text{Im}(\text{pA})_2+\text{Im}(\text{pA})_3$, (●); $(\text{pA})_2+(\text{pA})_3$, (●); $(\text{pA})_4+$ $(\text{pA})_5$, (Δ); $(\text{pA})_6-(\text{pA})_8$, (▲); $(\text{pA})_9$, (□).

Table 1. Oligoadenylylate Formation Catalyzed by Uranyl Ion at Various Concentration

No.	UO ₂ (NO ₃) ₂	Time	Yield (%) (ratio of fully 2'-5' linked oligomer) ^{a)}											
	mM	day	ImpA	pA	AppA ^{b)}	Im(pA) _n ^{c)}	(pA) ₂	(pA) ₃	(pA) ₄	(pA) ₅	(pA) ₆	(pA) ₇	(pA) ₈	(pA) ₉₋₁₈
1	12.5	1	0.5	7.7	0.1	1.0	11.7 (98)	26.9 (74)	20.0 (70)	11.8 (64)	6.7 (54)	2.5	0.8	1.8 ^{d)}
2	5	1	0.9	4.2	0.1	1.1	8.7 (98)	19.5 (84)	18.0 (75)	13.6 (73)	10.7 (69)	5.1	3.0	8.9 ^{e)}
3	1	1	1.3	0.2	0.3	0.4	5.7 (98)	16.6 (83)	16.8 (71)	16.0 (65)	9.5 (73)	6.9	6.5	11.7 ^{f)}
4*	1	1	1.1	0.5	0.1	18.9	9.1 (98)	15.5 (79)	16.3 (68)	10.3 (59)	8.9 (60)	4.5	3.4	6.6 ^{g)}
5*	1	5	1.1	0.4	0.2	1.3	7.2 (97)	15.0 (82)	16.3 (72)	12.2 (62)	13.0 (57)	6.5	7.1	15.6 ^{h)}
6	0.25	1	1.4	0.5	0.4	4.3	19.8 (97)	29.0 (86)	20.8 (68)	10.7 (66)	5.1 (62)	2.9	0.8	1.7 ⁱ⁾
7	0.1	5	0.5	4.5	0.6	0.3	36.4 (95)	36.1 (82)	15.6 (57)	4.3 (58)	1.5			
8	0.05	5	1.3	14.3	2.0	1.9	50.8 (91)	21.0 (70)	6.1 (41)	1.1	0.3			
9	0.01	5	9.8	34.2	3.3	4.2	36.4 (70)	10.0 (35)	2.3 (25)	0.6				
10	0	14	9.2	81.8	8.0		0.9 (77)	0.1						
11	1 Pb(NO ₃) ₂ ^{j)}	5	26.1	37.9	2.7	4.1	22.3 (91)	4.3 (65)	1.0	0.2				

Reactions were run at 20 °C in 0.2 M *N*-ethylmorpholine- HNO_3 buffer (pH 7.3), except for Nos. 4 and 5 where reactions were run at 0 °C in the same buffer. 50 mM of ImpA was used in the reaction.

a) Ratio of the fully 2'-5' linked oligomers is shown in the parenthesis below the yield data of each oligoadenylylate. b) P^1, P^2 -diadenosine 5'-pyrophosphate. c) $\text{Im}(\text{pA})_2$, $\text{Im}(\text{pA})_3$, and $\text{Im}(\text{pA})_4$ are included. d) $(\text{pA})_9+(\text{pA})_{10}$. e) $(\text{pA})_9-(\text{pA})_{13}$. f) $(\text{pA})_9-(\text{pA})_{16}$. g) $(\text{pA})_9-(\text{pA})_{14}$. h) $(\text{pA})_9-(\text{pA})_{18}$. i) $(\text{pA})_9+(\text{pA})_{10}$. j) $\text{Pb}(\text{NO}_3)_2$ was used instead of $\text{UO}_2(\text{NO}_3)_2$.

obtained with 1—0.25 mM of $\text{UO}_2(\text{NO}_3)_2$. The proportion of the yield of the fully 2'-5' linked oligoadenylate to that of the total oligomers with the same chain length are shown in the parenthesis in Table 1. The 2'-5' regioselectivity of the oligoadenylates was almost the same when 12.5 mM—0.25 mM of uranyl ion was used as a catalyst. Hydrolysis of phosphorimidazolide competes with the internucleotide bond formation and decreases the chain length of the oligoadenylate at lower catalyst concentrations. Thus the yield of short-chained oligoadenylates reached a maximum at 0.25—0.05 mM of the uranyl ion catalyst. 0.05 mM of the uranyl ion gave the 2'-5' diadenylate in 46.2% yield. The 2'-5' linked trimer and tetramer were formed in significant yields at 0.1—0.25 mM of uranyl ion catalyst. The catalytic effect of $\text{UO}_2(\text{NO}_3)_2$ was still significant at concentrations as low as 0.01 mM, though a significant degree of hydrolysis of ImpA took place. Concentration of the catalyst below 0.1 mM decreased the 2'-5' regioselectivity. The lower the concentration, the lower the proportion of the 2'-5' linkage of the resulting oligoadenylates. Thus 0.01 mM of the uranyl ion gave 2'-5' and 3'-5' linked dimers in 25.4% and 11.0% yield, respectively.

We carried out the polymerization at 0°C in the presence of 1 mM of uranyl nitrate. The low temperature suppressed the hydrolysis of the phosphorimidazolide bond more strongly than the internucleotide bond formation. Therefore, completion of the reaction required long reaction time at 0°C. On the other hand the yield of long-chained oligoadenylates increased at 0°C, and oligoadenylates up to octadecamer were obtained at 0°C for 1 day.

Table 2 presents the effect of pH of the medium on the oligoadenylate formation. The reaction was car-

ried out in the presence of 1 mM $\text{UO}_2(\text{NO}_3)_2$ catalyst in imidazole buffer between pH 6.0 and 8.0. Replacement of *N*-ethylmorpholine buffer with imidazole buffer at pH 7.3 did not change the total yield of the oligoadenylate, but effected a small decrease in long-chained oligoadenylates. Imidazole coordinates to the UO_2^{2+} ion, which might suppress the UO_2^{2+} -ion catalyzed internucleotide bond formation to some extent. The reactions were also conducted at pH 5.5 and 8.9. At pH 5.5 and 6.0, only small amounts of dimer and trimer were formed with low 2'-5' regioselectivity. Instead, P^1, P^2 -diadenosine 5'-pyrophosphate formed in addition to the hydrolysis of ImpA to pA. The oligoadenylate formation was markedly enhanced at pH 6.5 and increased with the higher of pH of the medium. Optimum pH for the polymerization to obtain 2'-5' linked oligoadenylates was around pH 7.5. When the reaction was carried out at pH 8.9, both the rate of hydrolysis of phosphorimidazolide and the internucleotide bond formation slowed down, resulting in the low yield of oligoadenylates after 1 day.

In a similar way, the UO_2^{2+} ion catalyzes the oligomerization of ImpU, giving oligouridyates from dimer to dodecamer in high total yields, as shown in Table 3. The resulting oligouridyates contained mainly 2'-5' internucleotide linkage. Cyclic dimer and trimer were obtained in substantial amounts in addition to the linear oligomer.

The effective catalytic activity of the UO_2^{2+} ion for the internucleotide bond formation is surprising. Without a template or an enzyme, no long-chained oligonucleotide has been obtained in aqueous solution. The UO_2^{2+} ion catalyzes the oligonucleotide-bond formation far more effectively than the Pb^{2+} ion

Table 2. Effect of pH of Medium on Oligoadenylates Formation Catalyzed by Uranyl Ion

pH	Yield (%) (Percentage of fully 2'-5' linked oligomer) ^{a)}												
	ImpA	pA	AppA	Im(pA) ₂	(pA) ₂	Im(pA) ₃	(pA) ₃	(pA) ₄	(pA) ₅	(pA) ₆	(pA) ₇	(pA) ₈	(pA) ₉₋₁₄
5.5		63.6	24.3		8.8 (67)		3.2 (47)						
6.0		48.7	26.6		12.1 (66)		5.8 (48)						
6.5	0.1	0.8	0.1		13.3 (97)		24.5 (79)	21.4 (63)	13.2 (45)	7.9 (43)	4.9	23.2	2.3
7.0	0.4	1.2	0.5		10.2 (96)		17.8 (77)	19.2 (63)	14.0 (51)	9.4 (46)	7.0	4.8	5.4
7.5	0.1	0.2	0.5	0.3	5.8 (98)	0.2	16.5 (73)	17.3 (60)	13.3 (51)	9.7 (40)	6.9	6.3	8.5
8.0	0.5	0.3	0.3	3.1	5.7 (98)	3.5	16.0 (67)	18.5 (62)	13.6 (52)	9.2 (42)	6.3	5.7	6.8
8.9	15.9	0.1	tr	31.2	9.1 (98)	7.6	18.2 ^{b)} (41)	9.3 ^{c)} (52)	4.9 (43)	1.7	0.4	0.2	

Reactions were run at 20°C for 1 day in 0.2 M-imidazole- HNO_3 buffer. At pH 5.5 and 8.9, pH of the reaction mixture was adjusted with 1 M HCl or 1 M NaOH solution 50 mM of ImpA and 1 mM of uranyl nitrate were used in the reaction.

a) Ratio of the fully 2'-5' linked oligomer is shown in the parenthesis below the yield data of each oligoadenylate. b) Small amount of Im(pA)₄ is included. c) Small amount of Im(pA)₅ is included.

Table 3. Oligouridylates Formation Catalyzed by Uranyl Ion

UO ₂ (NO ₃) ₂ mM	Time day	Yield (%) (ratio of fully 2'-5' linked oligomer) ^{a)}										
		PU+ImpUUppU	tr	[pU ₂]	(pU) ₂	[pU ₃]	(pU) ₃	(pU) ₄	(pU) ₅	(pU) ₆	(pU) ₇	(pU) ₈₋₁₂ ^{b)}
1	1	5.1	tr	14.6	3.6 (97)	4.6	13.3 (65)	12.1 (62)	11.8 (36)	8.2	6.2	14.8
0.25	1	7.2	0.1	14.3	6.0 (98)	3.1	17.3 (69)	15.2 (59)	10.8 (29)	4.6	3.2	6.2
0.05	5	10.4	0.7	12.1	30.0 (94)	2.0	19.6 (64)	11.5 (36)	5.9 (27)	2.5		
0.01	5	44.1	1.6	4.9	29.7 (77)	1.0	9.1 (41)	3.1 (35)				

Reactions were run at 20 °C in 0.2 M imidazole-HNO₃ buffer (pH 7.5). ImpU (50 mM) was used in the reaction. a) Ratio of the fully 2'-5' linked oligomer is shown below the yield data of each oligouridylate in the parenthesis. b) Oligouridylates with chain length eight to twelve are included.

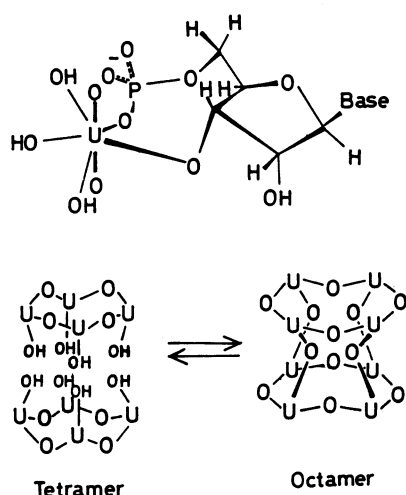


Fig. 3. Structure of the uranyl complex of 5'-nucleotide proposed by Kainosho et al.

which has been considered the most efficient catalysis.⁴⁾ High concentration of Pb²⁺ ion is required for its catalytic activity. 0.1 mM of Pb²⁺ ion has a negligible effect on the oligoadenylate formation. 12.5 mM of Pb²⁺ ion catalyst gives oligoadenylates up to hexamer.⁴⁾

Our results do not permit us to draw any conclusions about the reaction mechanism. However the oligoadenylate formation is probably mediated by a uranyl complex of ImpA. The structure of the UO₂²⁺-ImpA complex remains unclear, but probably is similar to that of the uranyl complex of pA. Two unique structures for the UO₂²⁺-pA complex have been proposed from NMR studies.^{8,9)} In an early study, Feldman et al. proposed a dimeric structure in which 2'- and 3'-hydroxyl groups of ribose ring coordinate to the uranyl ion with removal of protons.⁸⁾ The 3'-oxo group coordinates to both uranyl ions working as a bridging ligand. Later Kainosho et al. reported a revised structure from the study of 500 MHz NMR and C-13 NMR of the complex from [N¹⁵]pA.⁹⁾ They claimed that the uranyl complex of nucleoside-5'-monophosphate is composed of several oligomeric

species with hydroxo or oxo bridges. Each species has a common structural unit as shown in Fig. 3. The primary coordination sites are phosphate and 3'-hydroxyl group with a removal of a proton.

We tentatively postulate that the uranyl complex of ImpA could form a polymeric structure similar to that claimed for the UO₂²⁺-pA complex.⁹⁾ The activation of 2'-OH of the ribose moiety in the complex by hydrogen bond with 3'-oxo group may be responsible for the efficient 2'-5' internucleotide bond formation. The formation of polymeric UO₂²⁺ complex of ImpA could be favorable for the long oligoadenylates formation, since ImpA could line up on uranyl ions in the polymeric complex. The polymeric uranyl ion probably works as a kind of a template. The detailed discussion of the mechanistic roles of UO₂²⁺ ion for the oligonucleotide formation needs further study of the uranyl complex of ImpA and other nucleotides.

The uranyl ion-catalyzed oligoadenylate synthesis from ImpA has a potent synthetic utility. Since the isolation of 2'-5' linked triadenylate (2-5A) from interferon-treated cells and the discovery of its strong biological activity,¹⁰⁾ 2-5A and its analogues have been synthesized by a number of groups.¹¹⁾ Recently, Torrence et al. reported that 2'-5' linked hexadenylate inhibits DNA topoisomerase reaction.²⁾ They suggested that 2'-5' linked oligoadenylates of sufficient chain length may be involved in mediating the interferon-induced translational regulation by an inhibition of DNA topoisomerase. The oligoadenylate synthesis with UO₂²⁺ ion catalysts seems to provide the most efficient and convenient method for making the 2'-5' linked oligoadenylates with desired chain length. Any chain length of the oligomer from dimer to decamer can be obtained by changing the catalyst concentration or pH of the medium.

Finally we mention the significance of this reaction in the context of prebiotic chemistry. Egami has suggested that the metal ion which is abundant in seawater was incorporated into protoenzymes, which have evolved to contemporary metalloenzymes.¹³⁾ The abundant metal ions in seawater are generally

essential for life. The UO^{2+} ion is more abundant in seawater than the Co^{2+} ion which is essential for many living organisms.¹³⁾ No long-chained oligonucleotide has been obtained in the model reactions of prebiotic synthesis without a template. The UO^{2+} might have worked as a catalyst for oligonucleotide formation in the stage of chemical evolution, though its function disappeared during the later evolution.

This work was supported partially by a Grant-in-Aid from the Ministry of Education, Science and Culture.

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