Lanthanide Ion as a Catalyst for Internucleotide Bond Formation

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The lanthanide ion-catalyzed oligomerization of adenosine 5′-(1-imidazolylphosphate) was performed to yield short-chained oligoriboadenylates. The effect of lanthanide ions on the synthesis of oligoriboadenylates increased nearly in the order of the atomic number: None $< Nd^{3+} \lesssim La^{3+} \lesssim Ce^{3+} \lesssim Pr^{3+} \lesssim Sm^{3+} \lesssim Eu^{3+} \lesssim Gd^{3+} < Tb^{3+} < Dy^{3+} \lesssim Ho^{3+} < Er^{3+} < Yb^{3+} < Lu^{3+}$. The total yield of the oligoriboadenylates was 49% when Lu^{3+} was used as a catalyst. The linkage in the resulting oligoribadenylate was mainly 2′–5′.

Nucleoside 5′-triphosphates are starting activated monomers for nucleic acid synthesis in biological systems. The enzyme, DNA, or RNA polymerase, which is Mg^{2+} and Zn^{2+} or Mn^{2+} ion dependent, catalyzes the nucleic acid synthesis.[1—6] Without such an enzyme, no nucleic acid can be formed from nucleoside 5′-triphosphates. The non-enzymatic process of olilgonucleotide synthesis from activated nucleotides in an aqueous medium is of particular interest from the perspectives of biomimetic chemistry and the prebiotic chemistry of nucleic acid, and may provide a new synthetic method for the oligonucleotides. Nucleoside 5′-(1-imidazolylphosphate) (ImpN) is an activated nucleotide which hydrolyzes to nucleoside 5′-phosphate and imidazole in the absence of a catalyst. We previously reported that Zn^{2+}, Pb^{2+}, or UO_2^{2+} catalyzes the oligomerization of nucleoside-5′-(1-imidazolylphosphate) to yield oligoribonucleotides.[7—10] The UO_2^{2+} ion is most active for oligoribonucleotide synthesis among the examined catalysts.[10] Oligomerization proceeds via an intermediate complex of ImpN with a divalent metal ion. From a study of the uranyl ion-catalyzed oligomerization of diastereomeric adenosine-5′-(1-imidazolylthiophosphate), we have suggested that the main function of the divalent metal ion for a oligoribonucleotide synthesis is to act as a template in orienting the reaction component to facilitate an attack of the OH group of ImpN in line with the leaving imidazolide of the adjacent ImpN.[11] The divalent metal ion has also been indicated to enhance the nucleophilicity of the OH group of ImpN by deprotonation.[8,10] We have further examined the catalytic effect of trivalent lanthanide ions, which can possibly play such roles for oligoribonucleotide synthesis. Lanthanide ions have large a coordination number,[12—14] and, hence, multi numbers of ImpN can coordinate to a lanthanide ion in the proximate position. The present paper describes the effect of various lanthanide ions on the synthesis of oligoriboadenylate from adenosine 5′-(1-imidazolylphosphate) (ImpA).

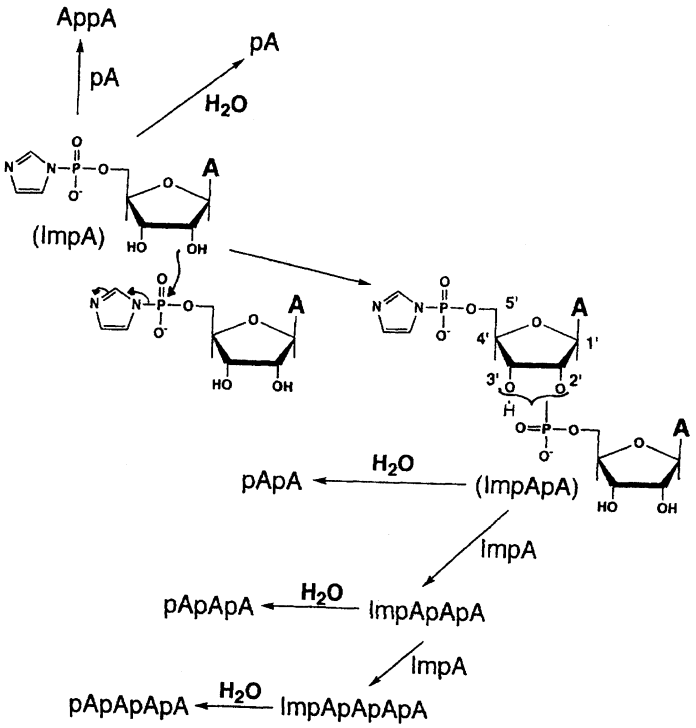
Experimental

Materials. Adenosine 5′-monophosphate (pA) was from Seikagaku Kogyo. *N*-Ethylmorpholine and imidazole were from Tokyo Kasei. $LaCl_3$, $CeCl_3$, $PrCl_3$, $NdCl_3$, $SmCl_3$, $EuCl_3$, $TdCl_3$, $DyCl_3$, $HoCl_3$, $ErCl_3$, $YbCl_3$, and $Lu(NO_3)_3$ were obtained from Wako Chemicals. Adenosine 5′-phosphorimidazolide was prepared from pA and imidazole using triphenylphosphine and di-2-pyridyl disulfide as a condensing agent, as previously described.[8,10]

Oligomerization of ImpA by Lanthanide Ion. 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 lanthanide salt solution. A typical reaction mixture (50 μl) containing ImpA (50 mM, 1 M = 1 mol dm^{-3}) and lanthanide salt (0.5—25 mM) in an *N*-ethylmorpholine buffer (0.2 M, pH 7.0) was kept at 24 °C for various periods of time. The reactions were stopped by adding 10 μl of a 0.25 M Versenol solution. Samples were then analyzed by high-performance liquid chromatography (HPLC) with a JASCO-800 apparatus on a RPC-5 column. The elution was carried out with a linear gradient of $NaClO_4$ solution (0—5 mM) buffered with 2.5 mM tris-acetate (pH 7.5) and 0.1 mM EDTA for 30 min at a flow rate of 1.0 ml min^{-1}. The eluate was monitored by UV absorption at 260 nm. Linkage isomers of oligoriboadenylates with 2′–5′ and 3′–5′ linkages were separated very well by HPLC on an RPC-5 column.[15] The yields were calculated from the peak integrals of the oligoriboadenylates on the HPLC chromatogram, after allowing for the hypochromicity of each oligoriboadenylate.[8,10] Identification of the resulting oligoriboadenylates was carried out by comparing the HPLC chromatograms with those of the authentic sample, and by enzyme digestion with nuclease p1 and venom phosphodi-esterase, as previously described.[8]

Results and Discussion

The 1-imidazolylphosphate bond of ImpA is labile. Without a catalyst, ImpA easily hydrolyzes in an aqueous solution to yield pA and imidazole (Scheme 1). The addition of lanthanide salt as a catalyst promoted the oligomerization of ImpA; oligoriboadenylates up to the tetramer were formed in addition to the hydrolyzed product, pA. The oligomerization of ImpA proceeds according to the follow-



Scheme 1.

ing scheme. Internucleotide bond formation involves a nucleophilic attack from the 2′- or 3′-OH of one ImpA to 1-imidazolylphosphate of another ImpA. 2′–5′ Linkage was preferentially formed in a reaction against the 3′–5′ linkage. The internucleotide bond formation competes with the hydrolysis of 1-imidazolylphosphate by an attack of nucleophilic water or hydroxide. The total yield of the oligoriboadenylates was as high as 49% in the lutetium ion-catalyzed oligomerization. Table 1 lists the yield data of oligoriboadenylates at several catalyst concentrations and at several reaction times, when lutetium ion was used as a catalyst. The lutetium ion showed catalytic activity at a concentration as low as 0.5 mM, a 100 : 1 molar ratio of ImpA to lutetium ion. The oligomerization reaction was almost complete within 2 d at 24 °C.

Table 1. Oligomerization of ImpA by Lutetium Ion

Time	$[Lu^{3+}]$	Yield/%						
d	mM	ImpA	pA	AppA	$(pA)_2$		$(pA)_3$[a]	$(pA)_4$[a]
					2′–5′	3′–5′		
2	25		56.0	9.9	21.4	8.0	5.0	0.1
2	10	2.0	44.9	6.9	31.7	3.6	6.7	0.2
2	2	6.5	50.2	8.0	24.6	1.8	3.3	0.1
2	1	8.7	58.3	2.8	16.0	2.3	0.2	
2	0	38.7	56.7	2.7	1.4	0.5		
1	10	7.6	43.3	5.7	26.7	1.7	5.2	0.1
5	10		44.4	6.8	32.2	4.7	10.6	0.9

a) Mixture of linkage isomers containing mainly 2′–5′ bond. Oligomerization of ImpA (50 mM) was done at 24 °C and pH 7.0 in the presence of lutetium nitrate.

The catalytic effect of a series of lanthanide salts ($LaCl_3$, $CeCl_3$, $PrCl_3$, $NdCl_3$, $SmCl_3$, $EuCl_3$, $TdCl_3$, $DyCl_3$, $HoCl_3$, $ErCl_3$, $YbCl_3$, and $Lu(NO_3)_3$) was examined in order to find an efficient catalyst. The yields of the oligoribodenylates are given in Table 2 together with the proportion of 2′–5′ internucleotide linkage in the dimer (pApA). The catalytic efficiency, which is roughly expressed by the ratio of the total yield of the oligoriboadenylates to that of the hydrolyzed product (pA) increased nearly in the following order of atomic num-

Table 2. Oligomerization of ImpA by Lutetium Ion

Lanthanide	Yield/%					2′–5′ linkage
ion	pA	AppA	$(pa)_2$[a]	$(pa)_3$[a]	$(pa)_4$[a]	in pApA/%
La^{3+}	79.0	10.3	6.1	1.4		75
Ce^{3+}	76.0	10.7	7.8	0.8		82
Pr^{3+}	75.9	9.0	8.2	0.3		83
Nd^{3+}	79.4	13.1	5.9	0.7		90
Sm^{3+}	75.3	7.6	7.5	0.8		88
Eu^{3+}	78.9	5.6	9.6	0.7		79
Gd^{3+}	80.7	4.6	7.9	2.5		75
Tb^{3+}	84.6	6.0	12.7	1.2		68
Dy^{3+}	77.6	4.4	15.0	1.6	0.1	81
Ho^{3+}	77.7	4.6	15.5	1.5	0.1	83
Er^{3+}	71.4	4.0	19.3	2.7	0.3	85
Yb^{3+}	55.8	3.5	33.2	6.5	0.8	90
Lu^{3+}	44.4	5.8	36.9	10.6	0.9	87
None	84.7	5.6	1.2			91

a) Mixture of linkage isomers containing mainly 2′–5′ bond. Oligomerization of ImpA (50 mM) was done at 24 °C and pH 7.0 in the presence of lutetium nitrate.

ber: None $< Nd^{3+} \lesssim La^{3+} \lesssim Ce^{3+} \lesssim Pr^{3+} \lesssim Sm^{3+} \lesssim Eu^{3+} \lesssim Gd^{3+} < Tb^{3+} < Dy^{3+} \lesssim Ho^{3+} < Er^{3+} < Yb^{3+} < Lu^{3+}$. The efficiency with which different lanthanide ions promote the synthesis of the internucleotide bond is illustrated in Fig. 1. The efficiency of the lanthanide ions for the oligoriboadenylate synthesis was nearly the same from La^{3+} to Gd^{3+}, and greatly increased from Tb^{3+} to Lu^{3+}. The catalytic effect of lanthanide ions for oligomerization is interesting, since lanthanide ions also efficiently catalyze the hydrolysis of RNA,[16—22] which is a reverse reaction of internucleotide bond formation. It is noteworhty that the heavy lanthanide ions, which catalyze the internucleotide bond formation more efficiently than do any other lantahanide ions, also have a higher catalytic activity for the hydrolysis of oligoribonucleotide.[22] Similarly, the lead(II) ion catalyzes both the hydrolysis of RNA[23—25] and internucleotide bond formation.[7—9]

Although the mechanism of lanthanide ion-catalyzed oligomerization is not clear, we can propose that oligomerization take places via an intermediate ImpA–lanthanide ion complex. There have been very few studies of lanthanide complexes of nucleotides, and the characterization of an ImpA–lanthanide complex was unsuccessful. The lanthanide ions are strongly electropositive, and have comparatively large ionic radii.[12,13] Bonding between the lanthanide ion and the coordinating ligands depends primarily on the electronegativity of the bonded atom in the ligand.[12,13] Thus, ImpA could coordinate to a lanthanide ion with a phosphate group and an OH group.

The catalytic efficiency of the lanthanide ions on oligoribonucleotide synthesis is lower than that of the uranyl ion;[10] however, a comparison between the catalytic activities and the coordination properties of a series of lanthanide ions and the uranyl ion in an aqueous solution gives some insight into the roles of the metal ion for oligonucleotide synthesis.

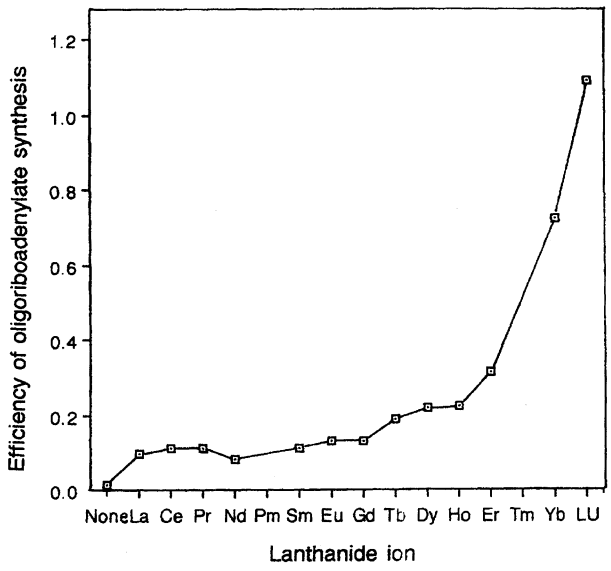


Fig. 1. Relative efficiency of lanthanide ions in catalyzing the oligoriboadenylate synthesis. Efficiency of the oligoriboadenylate synthesis is expressed by the ratio of yield of total oligoriboadenylates to that of pA.

The major role of the metal ion in oligomerization is likely to organize multi numbers of ImpA by coordination in such way as to promote internucleotide bond formation. The uranyl ion in aqueous solution tends to form an oxo or hydroxy bridged-polymeric complex,[26,27] to which ImpA can coordinate, thus successively enhancing long-chained oligoribonucleotide synthesis.[10,11] On the other hand, the lanthanide ions do not form such a polymeric structure, although the formation of dimeric or trimeric complexes has been reported for some lanthanide ions.[26] Because of their large size, lanthanide ions usually have a coordination number of 6 to 9,[12,13,26] and, hence, two or three molecules of ImpA can coordinate to a single lanthanide ion. This may facilitate either dimer or trimer formation. Thus, a template effect of a metal ion which orients the substrate ImpA, is likely to be essential for oligoribonucleotide synthesis.

Another role of the metal ion for internucleotide bond formation is an enhancement of the nucleophilicity of the reacting OH group of the substrate due to coordination. Lanthanide ions in aqueous solution form aquo-complexes, which tend to hydrolyze to give lanthanide–hydroxide complex.[12,14,26] The lanthanide ion, itself, or coordinated hydroxide, likely promotes deprotonation of the OH group of ImpA when coordinated to the lanthanide ion, and hence, enhances the nucleophilicity of the reacting OH group. The hydrolysis constant for an aquo-complex of lanthanide, pK_1, decreases from La^{3+} to Lu^{3+} approximately in the following order of atomic number: 9.33, 9.3, 8.82 or 8.5, 8.70 or 8.5, 8.61, 8.58, 8.62, 8.43, 8.37, 8.31, 8.26, 8.22, 8.19, and 8.17 or 6.6 for La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+}, and Lu^{3+}, respectively.[14] Thus, the Lu^{3+} ion, which has the best deprotonation ability of the OH group, is the most effective catalyst among the lanthanide ions. The hydrolysis constant for the uranyl-aquo complex is reported to be 4.1 to 4.3,[14] which indicates that the uranyl ion is more powerful for the deprotonation of the OH group enhancing the nucleophilicity. The lead(II) ion, which catalyzes the oligoribonucleotide synthesis,[7] also has a low hydrolysis constant of 7.2 for an aquo-complex.[14] On the other hand, the Mg(II) ion, which is inactive for oligoribonucleotide synthesis,[7] has a high hydrolysis constant of pK_1, 11.4.[14] These results imply that the catalytically active metal ion deprotonates the OH group under neutral reaction conditions and activates the reacting OH group.

The role of the metal ion for organizing of the substrate and activating the OH group is also proposed in the enzymatic RNA, or DNA synthesis.[5] RNA, or DNA polymerase, which catalyzes RNA or DNA synthesis, has been reported to have two divalent metal ions at the active site. One of the metal ions at the active site is hypothesized to promote the deprotonation of 3′ OH of the terminus of the growing strand, and to enhance the nucleophilic attack of the 3′ OH on the α-phosphate of the incoming nucleoside 5′-triphosphate.[5] The lanthanide ion in nonenzymatic oligoribonucleotide synthesis may mimic the role of the divalent metal ions in an enzymatic nucleic acid synthesis.

This is the first example of a catalytic effect of the lan-

thanide ions for oligonucleotide synthesis. The lanthanide ions are less effective than the UO_2^{2+} ion as a catalyst for long-chained oligoriboadenylates synthesis.[10] However, lutetium ion-catalyzed oligomerization may be applicable for the synthesis of short-chained 2′–5′ linked oligoriboadenylates, since this reaction does not require any protecting group, and is easy to perform.

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