Hydrolysis of Nucleotides Using Actinoid Metal Ion

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Hydrolysis of phosphomonoester and phosphodiester bonds of dpC, dCp, and dCpC were markedly accelerated in the presence of Th^{4+} in acidic (pH 4 and 5) aqueous solution; this effect surpassed those of other metal ions that were hitherto known as metals having catalytic activity for organic phosphoester (Ce^{3+}, La^{3+}, Cu^{2+}).

For a few decades, the effect of polyvalent metal ions on the hydrolytic reactions of organic phosphoesters has been the subject of a number of research groups.[1] We have studied various kinds of polyvalent metal ions as a catalyst and pointed out that thorium ion significantly accelerated the hydrolysis of several phosphate monoesters.[2] Recent development of DNA chemistry reminded us the importance of the metal-assisted hydrolysis of deoxynucleotides and prompted us to use thorium (IV) ion for this reaction. Recently, Komiyama et al. succeeded in effective DNA hydrolysis using rare earth metals as catalysts.[3] Although they showed that the rare earth metals, in particular Ce(IV), significantly catalyzed the hydrolysis of DNA phosphodiester bond under neutral conditions, there is still a room for argument about the detailed mechanism such as the involvement of mixed valent cluster. Here we show the remarkable effect of thorium ion (Th^{4+}) on the phosphomonoester and phosphodiester hydrolysis of dpC, dCp, and dCpC. We can exclude the contribution of any mixed valent effect, because Th^{4+} is totally redox-inactive under ordinary aqueous reaction conditions. In addition, this is the first example of acceleration effect of actinoid metal for nucleotide hydrolysis as far as we know.

Figure 1 shows plasmid DNA (pBR322) cleavage in the presence of Th^{4+} in acidic medium. As the concentration of Th^{4+} increases, the relaxation of super-coiled form of pBR322 to open-circular form was accelerated markedly. No effect was observed whether this reaction was carried out in the presence or absence of molecular oxygen (data not shown).

In order to obtain more detailed information about the hydrolytic mechanism of nucleotides, dCp, dpC, and dCpC were used as substrates.[4] The reaction mixtures were kinetically analyzed using the reversed-phase HPLC equipped with multi-channel photo-diode array (MCPD) detector. Typical chromatogram obtained for dpC is indicated in Figure 2. It is known from a separate study that the substrate and the possible products, dCpC, dCp, dpC, cytidine, and cytosine, are clearly separated from each other. All the data followed pseudo first-order kinetics. Only Th^{4+} and Ce^{4+} ions promoted the hydrolytic reactions of nucleotides under these conditions (Table 1). The activity was very much prominent only for lanthanoid ions and Cu^{2+}, which are well known as effective catalysts of phosphoester hydrolysis so far.[1b-f]

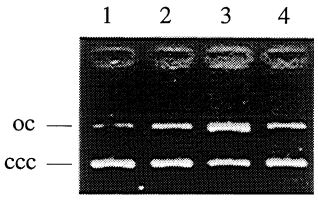

Figure 1. Relaxation of supercoiled pBR 322 DNA by $Th(NO_3)_4$ in pH 4.0 at 70 ℃ for 1 h. Sixteen μmol dm^{-3} (phosphate unit) of pBR322 DNA was incubated in 10 μdm^{-3} of acetate buffer containing various amount of Th^{4+} ion. lane 1, untreated pBR 322; lane 2 - 4, incubated DNA with 0.6, 6.0, and 0 μmol dm^{-3} of Th^{4+}, respectively. The mixture was analyzed by 1.0 % agarose gel electrophoresis.

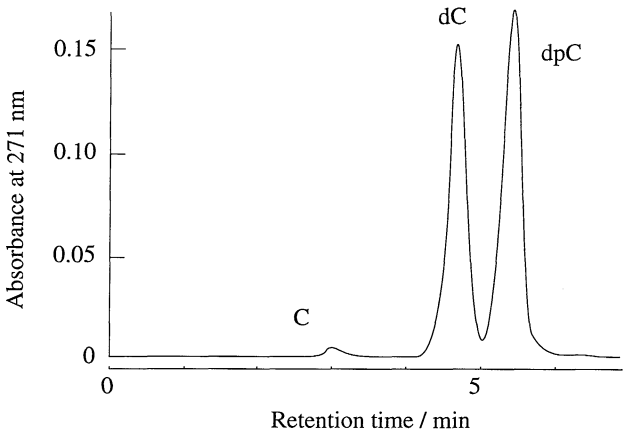

Figure 2. HPLC chromatogram[8] showing the cleavage of dpC (0.4 mmol dm^{-3}) by $Th(NO_3)_4$ (4.0 mmol dm^{-3}), pH 4.0 at 70 ℃ for 100 min.

The peak arising from cytosine, which is the typical product of (oxidative) radical degradation or hydrolysis of β-glycoside bond, was negligible compared with those of the unreacted substrates and deoxycytidine. The mechanism is, therefore, not associated with a redox reaction but should be due to a Th^{4+}-catalyzed hydrolytic reaction. Th^{4+} can interact with phosphoesters extending dual effects: (i) the electron withdrawing effect by the tetra-positive metal ion coordinated to the phosphate oxygen (large stability constants were reported for Th^{4+}-phosphate complexes[5]), and (ii) general-acid and -base catalysis by the water molecule coordinated to the metal ion (under this pH condition, one or two coordinated water molecules deprotonate; pK_{a1} and pK_{a2} is 3.89 and 4.20, respectively). In fact, the catalytic activity of Th^{4+} diminished when typical sequestering agents such as ethylenediamine-*N, N, N', N'*-tetraacetic acid (EDTA) and iminodiacetic acid (IDA) were present (Table 1). This is most certainly due to the so-called masking effect (anation) by these ligands on the coordinating site on Th^{4+}, so that both the binding affinity toward the substrate species and the Lewis acidity of the metal ion were

Table 1. Pseudo-first-order rate constants for the hydrolysis of nucleotides (0.4 mmol dm^{-3}) in the presence of polyvalent metal ions (4.0 mmol dm^{-3}) at pH 4.0

metal ion	$k \times 10^5$ / min^{-1}		
	dpC	dCp	dCpC
Th^{4+}	1000	280	27
+ IDA[a]	570	-[b]	-
+ EDTA[a]	480	-	-
Ce^{4+}	14570	-	98
Ce^{3+}	4.6	-	0[c]
La^{3+}	6.7	-	0[c]
Cu^{2+}	13	-	0[c]
control	7.2	7.0	0[c]

[a] Concentration of applied metal ligands, IDA and EDTA, were 4.0 mmol dm^{-3}. [b] Experiments under these condition were not carried out. [c] Hydrolytic products were scarcely observed except for negligible amount of cytosine.

considerably reduced.

The hydrolysis rate of dpC was faster than that of dCp. Although this preference is opposite to the result of Komiyama et al.,[3a] this is in line with the thermodynamics of alkoxy eliminations, i. e., the difference in acidity between primary- and secondary alcohol hydroxyl groups. However, other factors such as the stabilities of Th^{4+}-substrate complexes and/or the stereochemistry of transition state complexes have to be taken into account for the detailed understanding of this relatively large difference in reactivity. Actually, it is known that a Th^{4+} catalysis depends upon the structure of an alcoholic residue of the substrates.[1b, c] The hydrolytic behavior of dinucleotide is also in line with the above observation. Dinucleotide dCpC required much longer reaction time than mononucleotides, and the products detected were dC and a trace of dCp. Both dCp and dpC should have been formed in the first step of dCpC hydrolysis, but dpC obviously could not survive the reaction condition. The detection of dCp could mean that P-O bond cleavage in the phosphodiester also took place preferably at the primary alkyl side.

The activity of Th^{4+} was comparable to that of Ce^{4+} when the reactivity of dCpC was compared under the same condition (pH 4, 70 ℃). The reaction mixture using Th^{4+} was apparently transparent at pH 4; raising pH to 5 or 6 made it gradually opaque as the reaction proceeded with a slightly reduced reactivity as compared with that at pH 4. On the other hand, the reactivity of Ce^{4+} is rather high at neutral pH, where, of course, the reaction mixture contains a suspension of Ce^{4+} hydroxide gel. The metal ion olation depressed the hydrolysis of the nucleotides in the Th^{4+} catalyzed system, whereas, in the Ce^{4+} catalyzed system, the olation increased the catalytic activity. This suggests that the enormous acceleration effect of both metals is quite certainly based on their extreme charge density, the detail mechanisms and active species involved are different from each other.

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4 The reaction was carried out at 70 ℃ using sealed cell equipped with water jacket. In a typical run, aliquot (50 µl) of reaction mixture were removed and quenched with 50 µl of 0.2 mol dm^{-3} inorganic phosphate (pH 5.5).[1f] After removal of metal phosphate precipitate by centrifuge, 50 ml of supernatant was injected into HPLC (LiChrospher 100 RP-18(e), ϕ 4 x 250 mm) and eluted with 0.1 mol dm^{-3} phosphate buffer containing 5 mmol dm^{-3} tetra-*n*-butyl ammonium phosphate with a flow rate of 1.0 ml min^{-1}.

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