# Monophosphate as Eminent Ligand to Bind Ce(IV)/EDTA Complex for Site-selective DNA Hydrolysis

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By the use of oligonucleotides bearing a terminal monophosphate, 10-base gap was formed in substrate DNA and the monophosphates were placed at the edge of gap. Upon the treatment of these systems with Ce(IV)/EDTA complex, the gap-site was selectively hydrolyzed. The monophosphates remarkably promoted both the selectivity and the efficiency of scission.

Site-selective scission of DNA is one of the most attractive themes of today, mainly because this technique opens the way to manipulation of huge DNA of higher animals and plants.[1–5] Of many catalysts hitherto proposed for DNA hydrolysis, Ce(IV) ion is characterized by its remarkable catalytic activity, and efficiently hydrolyzes DNA under physiological conditions.[6,7] Furthermore, Ce(IV)/EDTA complex is homogeneous and sufficiently active for the purpose (EDTA = ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate).[8] Recently, we found that gap-sites in DNA substrates are preferentially hydrolyzed by this homogeneous Ce(IV)/EDTA complex.[9] Although the Ce(IV) complex is not covalently bound anywhere, the gap-site is efficiently hydrolyzed since the phosphodiester linkages therein are more susceptible to the catalysis by the complex.[10] In the present paper, we show that this gap-selective DNA hydrolysis is enormously promoted by tethering monophosphate groups to the additive DNAs and placing these groups at the gap-site.

By using the additive DNAs in Figure 1, 10-base gap was formed in the middle of substrate $DNA^{(S)}$ (50-mer: $^{32}$P-labeled at the 5′-terminus). The hydrolysis of $DNA^{(S)}$ was achieved at pH 7.0 (Hepes buffer) and 37 °C. After the reactions, the mixtures were analyzed by denaturing polyacrylamide gel electrophoresis, and quantified with a Fuji Film FLA-3000G imaging analyzer.

In Lane 6 of Figure 2, $DNA^{(L)}$-P and $DNA^{(R)}$ were combined, and a monophosphate group was placed at the 5′-side edge of the 10-base gap in $DNA^{(S)}$. Under these conditions, the scission by the Ce(IV)/EDTA complex was strictly restricted to the gap-region (the three markers in lane M of the gel are for the 20-, 25-, and 30-mers which have the same sequences as the corresponding DNA fragments, and the gap is formed between M20 and M30). Similarly, gap-selective scission was successfully achieved by combining $DNA^{(L)}$ with P-$DNA^{(R)}$ and placing a monophosphate at another edge of the gap (Lane 7). In contrast, the scission was only marginal when two DNAs without the terminal monophosphate ($DNA^{(L)}$ and $DNA^{(R)}$) were combined (see Lane 5). Enormous promotion of gap-selective hydrolysis

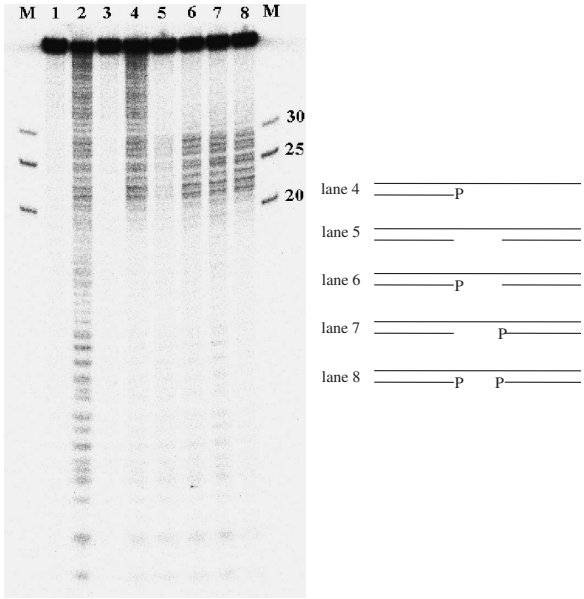


**Figure 2.** Gel electrophoresis patterns for the hydrolysis of $DNA^{(S)}$ at 10-base gap by combining Ce(IV)/EDTA complex with various additive DNAs. Lane 1, control; Lane 2, Ce(IV)/EDTA complex only; Lane 3, $DNA^{(L)}$-P/P-$DNA^{(R)}$ (without Ce(IV)/EDTA complex); Lane 4, $DNA^{(L)}$-P alone (with Ce(IV)/EDTA complex but without the second additive DNA); Lane 5, $DNA^{(L)}$/$DNA^{(R)}$; Lane 6, $DNA^{(L)}$-P/$DNA^{(R)}$; Lane 7, $DNA^{(L)}$/P-$DNA^{(R)}$; Lane 8, $DNA^{(L)}$-P/P-$DNA^{(R)}$. The 10-base gap is formed between the two markers M20 and M30. The structures in lanes 4–8 are schematically depicted in the right-hand side. Reaction conditions: $[DNA^{(S)}]_0 = 1.0\,\mu M$, [each of the additive DNAs]$_0 = 1.5\,\mu M$, $[NaCl]_0 = 100\,mM$, and [Ce(IV)/EDTA complex] = 0.5 mM at pH 7.0 (10 mM Hepes buffer) and 37 °C for 15.5 h (M = mol dm$^{-3}$).

$DNA^{(S)}$: 5'-CAATTAGAATCAGGAATGGC**TTATGCGCAC**GTGCAGACTGTCGACCTAAG-3'
$DNA^{(L)}$-P: 3'-GTTAATCTT AGTCC TTACCG-P-5'
P-$DNA^{(R)}$: 3'-P-CACGTCTGACAGCTGGATTC-5'
$DNA^{(L)}$: 3'-GTTAATCTT AGTCC TTACCG-5'
$DNA^{(R)}$: 3'-CACGTCTGACAGCTGGATTC-5'

**Figure 1.** Sequences of substrate DNA and additive DNAs. The nucleotides at the gap-site are bold-faced and underlined. $DNA^{(S)}$, substrate 50-mer DNA; $DNA^{(L)}$-P, the gap-forming 20-mer DNA additive which covers the left-hand side (5′-side) of $DNA^{(s)}$ and bears a monophosphate at its 5′-terminus; P-$DNA^{(R)}$, the gap-forming 20-mer DNA additive which covers the right-hand side (3′-side) of $DNA^{(S)}$ and bears a monophosphate at its 3′-terminus; $DNA^{(L)}$ and $DNA^{(R)}$, DNA additives which have the same sequence as $DNA^{(L)}$-P and P-$DNA^{(R)}$, respectively, but bear no monophosphate group at their termini.

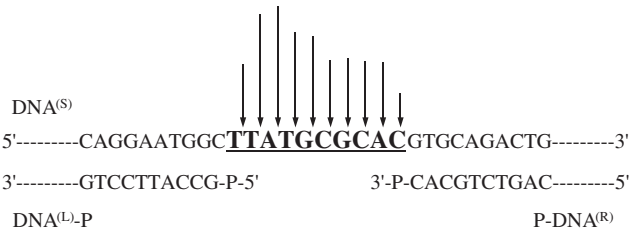


**Figure 3.** Quantitative analysis on site-selective scission of $DNA^{(S)}$ by Ce(IV)/EDTA complex in the presence of the $DNA^{(L)}$-P/P-$DNA^{(R)}$ combination (Lane 8 in Figure 2). The length of arrow corresponds to the efficiency of scission.

by the monophosphate groups has been conclusively substantiated.

The gap-selective DNA scission was still more efficient, when $DNA^{(L)}$-P and P-$DNA^{(R)}$ were combined and two monophosphate groups were placed at both edges of the gap (Lane 8). The results of quantitative analysis of this scission are presented in Figure 3. The scission is slightly more efficient near the 5′-edge of the gap.

According to kinetic analyses,[11] the phosphodiester linkages in gap-sites bind Ce(IV)/EDTA complex far more strongly than do those in the double-stranded portions, and thus gap-sites are preferentially hydrolyzed. In the present systems, this difference has been further magnified by the metal-binding activities of the monophosphate groups which are introduced to the gap-site. These arguments are supported by the fact that both of two additive DNAs are essential for the present gap-selective scission. When only either P-$DNA^{(L)}$ or $DNA^{(R)}$-P was used alone (without the coexistence of another oligonucleotide additive), the single-stranded portion was hydrolyzed almost randomly (see Lane 4 in Figure 2). Here, only the double-stranded portion in the substrate DNA was protected from the scission.[12] In the present gap-selective DNA hydrolysis, the $^{32}$P-labelled monophosphate ester at the 5′-end of $DNA^{(S)}$ was not hydrolyzed to a measurable extent. No $^{32}$P-labeled inorganic phosphates, which should be formed if this reaction were really taking place, were perceived in the bottom of electrophoresis gels (see Figure 2). The site-selectivity remained unchanged until the conversion of $DNA^{(S)}$ was around 50 mol % (the maximum value attained). Apparently, the phosphodiester linkages in $DNA^{(S)}$ are hydrolyzed preferentially to the monophosphate esters at the terminus of either $DNA^{(S)}$ substrate or additive DNAs.

In conclusion, DNA has been selectively and efficiently hydrolyzed by Ce(IV)/EDTA complex at the gap-site by attaching a monophosphate group to at least one of two oligonucleotide additives. Extensions of the present findings to developments of new tools for molecular biology, as well as detailed analysis on the roles of these monophosphates in these selective scissions, are currently under way in our laboratory.

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**References and Notes**

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