Lanthanide Ion-Mediated Hydrolysis of DNA on Phosphate Bilayer Membrane

Nobuo Kimizuka, Eiji Watanabe, and Toyoki Kunitake*
Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 812-8581

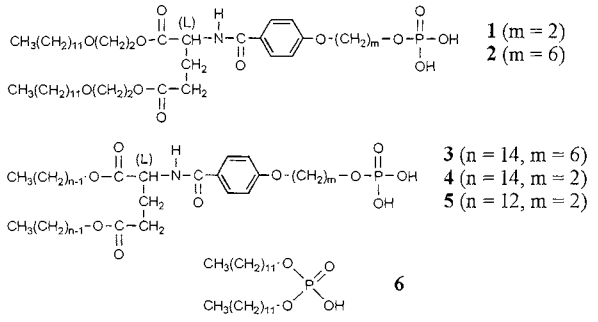
(Received August 31, 1998; CL-980669)

Binding of Ce^{3+} ion onto phosphate bilayer membranes and hydrolysis of plasmid DNA are examined. DNA cleavage is significantly promoted only when a particular phosphate bilayer is used in the liquid crystalline state.

Interaction with multivalent metal ions significantly alters physicochemical properties of bilayer membranes,[1,2] as well as their functions such as fusion,[3] phase separation[4] and ion transport.[5] We have reported that synthetic phosphate bilayers strongly bind divalent (Ca^{2+}, Mg^{2+}, Cu^{2+}) and trivalent metal (La^{3+}) ions.[1] These membrane-bound metal ions are accumulated on the two-dimensional bilayer surface, and are expected to show cooperative functions not attainable by monomeric, hydrated ions in solution. In contrast to the biological activity of metal ions displayed in biopolymer systems,[6,7] chemical functions of bilayer-bound metal ions are largely unexplored.

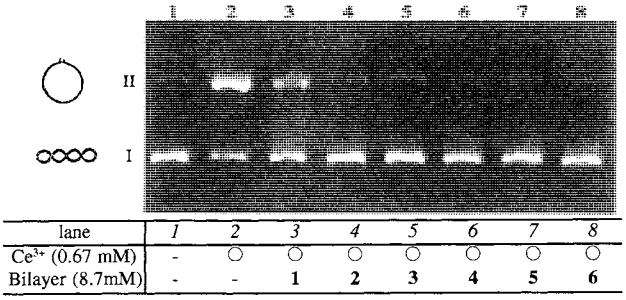
Recently, lanthanide ions such as Ce^{3+} are reported to hydrolyze phosphodiester linkages in nucleic acids.[8,9] In this study, we employed phosphate bilayer membranes as a two-dimensional matrix for Ce^{3+} ions, and investigated the effect of organization on hydrolytic scission of DNA.

DNA cleavage activity was investigated by the plasmid relaxation assay.[10] Plasmid DNA (pBR322, Nippon Gene) and cerium chloride (Wako Chemical) were used as received. Amphiphiles **1-6** provide typical phosphate bilayers and were prepared in these laboratories.[1,2,11] They were mixed with equimolar tris(hydroxymethyl)aminomethane (Tris), and the mixtures were dispersed in water by ultra-sonication (Branson Sonifier Model 250). Scission reactions of pBR322 DNA were performed in Hepes buffer (10 mM, pH 7). Aqueous $CeCl_3$ was first injected to a buffer solution of pBR322, and then aqueous phosphate bilayers were added. The resultant mixtures were incubated at given temperatures (pBR322, 45 μ M nucleotides, $CeCl_3$, 6.7 x 10^{-4} M, amphiphiles, 8.7 X 10^{-3} M). The reaction was quenched by the addition of EDTA. The bilayer component was removed from the reaction mixture by extraction with phenol, and gel electrophoresis was performed using 1% agarose gels under standard conditions. After electrophoresis, the gel was stained by ethidium bromide and



then visualized under UV light. Differencial scanning calorimetry (DSC) was performed for aqueous phosphate bilayers (20 mM) with a Seiko SSC/5200H calorimeter.

Figure 1 shows the result of cleavage of pBR322 by $CeCl_3$. After 12 h at 37 ℃, conversion of form I (supercoiled) to form II (nicked) DNA is apparent in the presence of 6.7 x 10^{-4} M



lane	1	2	3	4	5	6	7	8
Ce^{3+} (0.67 mM)	-	○	○	○	○	○	○	○
Bilayer (8.7mM)	-	-	**1**	**2**	**3**	**4**	**5**	**6**

Figure1. Relaxation of pBR322 DNA by Ce^{3+}- bilayer complexes; 45 μM nucleotides, 10 mM Hepes buffer (pH 7.0), 12 h incubation at 37 ℃.

$CeCl_3$ (lane 2), as compared to the case of pBR322 alone (lane 1). Relaxation of pBR322 DNA is similarly observed in the presence of bilayer **1** under the standard experimental conditions (lane 3). The phosphate head group in amphiphile **1** is not hydrolyzed, as confirmed by TLC-FID analysis of the reaction mixture. In contrast, conversion of form I to the nicked II form was totally suppressed in the presence of bilayers **2-6** (lanes 4-8). Apparently, these bilayers suppress the Ce(III) activity that are seen in lanes 2 and 3. Lanthanide ions are typical hard acids, and they would be preferentially bound to phosphate groups in the periphery of DNA, when they are first mixed with pBR322. However, cerium ions will be transferred from DNA to the phosphate bilayers, and the resulting Ce^{3+}-bilayer (**2-6**) complexes seemingly possess no hydrolytic ability.

Binding of cerium ions to bilayers is supported by DSC measurement. Endothermic peaks due to the gel-to-liquid crystal phase transition are shown in Table 1. When $CeCl_3$ is

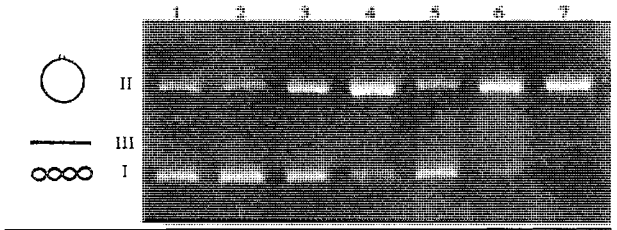
Table 1. Gel-to-liquid crystal phase transition data

Amphiphiles (Tris salt)	in pure water		amphiphile / Ce^{3+} = 3 : 1	
	Tc / ℃	ΔH / kJ·mol^{-1}	Tc / ℃	ΔH / kJ·mol^{-1}
1 (m = 2)	17.8	24.5	17.8	21.8
2 (m = 6)	52.3	45.0	not detected	
3 (n = 14, m = 6)	57.7	24.0	not detected	
4 (n = 14, m = 2)	49.0	36.5	50.3	4.8
5 (n = 12, m = 2)	36.6	40.1	not detected	
6	25.0	19.5	26.6	9.6

added to aqueous bilayers at the molar ratio of amphiphile : Ce^{3+} = 3 : 1, the peaks totally disappeared (for **2**, **3**, and **5**) or drastically suppressed (for **4** and **6**). Apparently, binding of cerium ions to these phosphate bilayers induces disorder in the bilayer structure[1] and may lead to concomitant changes in

aggregate morphology. On the other hand, the endothermic peak of bilayer **1** is essentially not affected in the presence of $CeCl_3$, showing that the bilayer structure of **1** is preserved in the presence of $CeCl_3$.

The unique cleavage activity of **1**-Ce^{3+} complex may be related to the physical state of the bilayer. The fraction of the nicked form Ⅱ relative to that of form I in the presence of bilayer **1** increases with elevating reaction temperature from 10 to 25 ℃ (Figure 2, lane 2 – 4), though form I is not fully converted to form Ⅱ. When a crystalline bilayer of **1** is present, (below Tc, lane 5, 10 ℃), both of form I and form Ⅱ are found,



lane	1	2	3	4	5	6	7
Ce^{3+} (0.67 mM)	-	○	○	○	○	○	○
1 (8.7 mM)	-	-	-	-	○	○	○
Temp. /°C	18	10	18	25	10	18	25

Figure 2. Temperature dependence of relaxation of pBR322 DNA; 45 μM nucleotides, 10 mM Hepes buffer (pH 7.0), 4 days incubation .

as is the case without bilayer (lane 2). On the other hand, at the phase transition temperature (18 ℃, lane 6), the fraction of form I is drastically reduced compared to that without bilayer (lane 3). The effect of bilayer **1** is even greater above the phase transition temperature. At 25 ℃, form I is completely converted to form Ⅱ, and a detectable amount of linear form Ⅲ is formed (lane 7). Extensive cleavage of form I and formation of linear form Ⅲ are not found under comparable conditions by the metal ion alone (lane 4). It is clear that the hydrolytic activity of $CeCl_3$ is enhanced when bound to the fluid bilayer of **1**. It is reported that divalent metal ions mediate

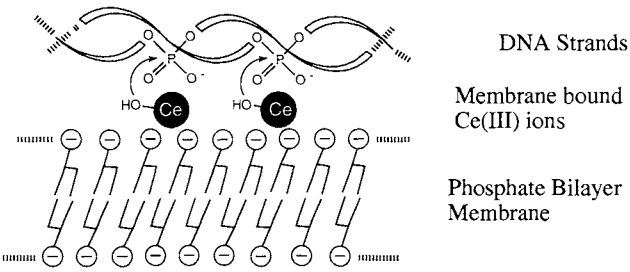


Figure 3. Schematic illustration of DNA/Ce^{3+}/**1** complex and concomitant hydrolysis of DNA .

binding of oligonucleotides to anionic surface monolayers.[12]

The linear form Ⅲ observed in lane 7 is produced only by double strand scission of pBR322. Therefore, the two scission sites must be closely located. The high population of active metal ions at the two-dimensional bilayer surface is advantageous for this type of activity. The increased hydrolytic activity in the liquid crystalline state suggests that certain mobility of the cerium(Ⅲ) complex is required. Though the detailed mechanism of the bilayer-assisted DNA hydrolysis is not yet clear, it is possible that more than one cerium ions bound to the bilayer surface is involved in the active complex. Co-operative hydrolysis of phosphate ester bonds by two metal ions is known for a number of enzymes[6,7] and also reported for model systems.[13-15]

DSC data demonstrate that ordered bilayer assembly of **1** is retained in water upon binding with Ce^{3+} ion. In contrast, bilayer structures of **2** - **6** must be extensively deteriorated by Ce^{3+} binding. The hydrolytic activity is related to maintenance of the bilayer characteristics in the presence of Ce^{3+}. **1** possesses a rather low Tc (17.8 ℃) with a small ΔH value compared to those of amphiphiles **2** and **4**, in spite of their closely related chemical structures. This difference may be apparently ascribed to the short spacer methylene (m = 2) and the ether linkage present in alkyl chains. Shorter spacer methylenes result in less ordered bilayer organizations.[16] The ether linkage in alkyl chains impart aggregation morphology with surprising flexibility,[17] and ensures regular alkyl chain packing by decoupling it from the head group alignment.[18] These structural features of **1** are prerequisite for the retained bilayer organization upon complexation with Ce^{3+}.

Scission of DNA in the present system requires formation of a ternary complex mediated by cerium ion, as schematically shown in Figure 3. Maintenance of the bilayer organization in the active complex appears crucial. High density organization of Ce^{3+} ions at the bilayer surface is advantageous for enhanced activity and localized scission. To date, syntheses of metal-ion-containing DNA cleaving reagents have been a topic of active research.[14,15,19] The present bilayer-supported system provides a novel supramolecular approach to control the activity of lanthanide ions, and this may be applied to design a family of bilayer-based metal reagents.

References and Notes

1 N. Nakashima, R. Ando, H. Fukushima, and T. Kunitake, *Chem. Lett.*, **1985**, 1503.
2 T. Kunitake, J.-M. Kim, and Y. Ishikawa, *J. Chem. Soc., Perkin Trans.2*, **1991**, 885.
3 T. A. A. Fonteyn, D. Hoekstra, and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, **1990**, 112, 8870 and references therein.
4 M. Shimomura and T. Kunitake, *J. Am. Chem. Soc.*, **1982**, 104, 1757.
5 N. Kimizuka, T. Wakiyama, and T. Kunitake, *Chem. Lett.*, **1996**, 521.
6 E. E. Kim and H. S. Wycoff, *J. Mol. Biol.*, **1991**, 218, 449.
7 S. Doubli, S. Tabor, A. M. Long, C. C. Richardson, and T. Ellenberger, *Nature*, **1998**, 391, 251.
8 M. Komiyama, *J. Biochem.*, **1995**, 118, 665 and references therein.
9 Hydroxide bound to Ce(IV) ion formed under aerobic conditions is considered to be the active species : M. Komiyama, T. Shiiba, T. Kodama, N. Takeda, J. Sumaoka, and M. Yashiro, *J. Biochem.*, **1994**, 115, 809.
10 L. A. Basile, A. L. Raphael, and J. K. Barton, *J. Am. Chem. Soc.*, **1987**, 109, 7550.
11 T. Kunitake and Y. Okahata, *Bull. Chem. Soc. Jpn.*, **1978**, 51, 1877.
12 D. G. Rhodes and J. Liu, *Langmuir*, **1996**, 12, 1879.
13 A. Tsubouchi and T. C. Bruice, *J. Am. Chem. Soc.*, **1994**, 116, 11614.
14 K. G. Ragunathan and H. -J. Shneider, *Angew. Chem., Int. Ed. Engl.*, **1996**, 35, 1219.
15 M. Yashiro, A. Ishikubo, and M. Komiyama, *J. Biochem.*, **1996**, 120, 1067.
16 N. Nakashima, S. Asakuma, J. -M. Kim, and T. Kunitake, *Chem. Lett.*, **1984**, 1709.
17 N. Kimizuka, T. Takasaki, and T. Kunitake, *Chem. Lett.*, **1988**, 1911.
18 N. Kimizuka, T. Kawasaki, K. Hirata, and T. Kunitake, *J. Am. Chem. Soc.*, **1998**, 120, 4094 and references therein.
19 H. E. Moser and P. Dervan, *Science*, **1987**, 238, 645.