Extended x-ray absorption fine structure study on the cerium(IV)-induced DNA hydrolysis: Implication to the roles of 4f orbitals in the catalysis

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At various Ce(IV)/DPP (DPP: diphenyl phosphate) molar ratios, the structure of Ce clusters in aqueous solutions was studied by extended x-ray absorption fine structure measurements. Although the Ce–Ce bond was strong in the absence of DPP, the corresponding signal was significantly reduced when the Ce(IV)/DPP molar ratio was 1. The result indicates the existence of a strong interaction between Ce(IV) ions and DPP, and suggests the formation of a Ce–DPP structure in solution. With an increase in the molar ratio, the signal of Ce–Ce bonds increased again, and exceeded that observed in the absence of DPP, suggesting the formation of a Ce–Ce–DPP structure. Ce(IV) ions in the complexes were found to have ~ 0.67 $4f$ electrons. On the other hand, no measurable charge transfer was observed in the case of Ce(III) ions. These results demonstrate the role of the $4f$ orbital of Ce(IV) ions for its enormous activity for DNA hydrolysis. © *1999 American Institute of Physics.* [S0003-6951(99)01603-4]

Interest in the nonenzymatic hydrolysis of DNA has been growing rapidly, mainly because it could provide a novel tool for future biotechnology and molecular biology.[1] However, DNA is strongly resistant to hydrolysis (the intrinsic half life of its phosphodiester linkage at pH 7 and 25 °C is estimated to be 200 million years). It was only a few years ago that the hydrolytic activity of the Ce(IV) ion for DNA was demonstrated, when DNA hydrolysis was performed under physiological conditions.[2] The activity of Ce(IV) is significantly greater than those of other lanthanide(III) and non-lanthanide ions. However, the following points have remain to be clarified: (1) Why is the Ce(IV) ion far superior to other metal ions? (2) What is the structure of the complex formed between the Ce(IV) ion and DNA? (3) What kind of charge-transfer interaction takes place in the complex? and (4) Why does Ce(IV) have to exist in excess compared to the phosphodiester linkage for efficient catalysis? Information related to these points is crucial to the understanding of the catalytic mechanism and to design catalysts with improved activity.

In this study, the structure of Ce(IV) ions in complexes with diphenyl phosphate (DPP) is analyzed by extended x-ray absorption fine structure (EXAFS) measurements. The results of the electronic structure obtained from the study of x-ray absorption near edge structure (XANES) and x-ray photoemission spectroscopy (XPS) will be discussed together.

DPP was used as a model of DNA. Figure 1(a) shows the chemical structure of DNA and that of DPP. The two molecules have similar phosphodiester linkages. Therefore, a similar complex is expected to be formed by both compounds with Ce(IV) ions [Fig. 1(b)].[3] EXAFS and XANES experiments were performed using beam line 12*C* of the Photon Factory in Tsukuba.[4] The fluorescence yield from Ce atoms was measured using a 19-element solid-state detector. The samples were prepared by mixing DPP with $Ce(NH_4)_2(NO_3)_6$ in water at the required molar ratios, and subjected to analysis after being frozen in liquid nitrogen.

First, we discuss the results of EXAFS measurements. Figure 2 shows a series of Fourier-transformed Ce
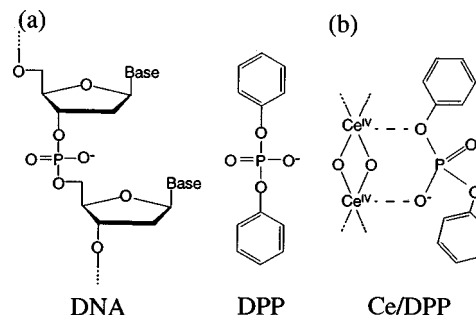

FIG. 1. (a) Chemical structures of the phosphodiester linkage in DNA and DPP. (b) Schematic model for the Ce/DPP complex.

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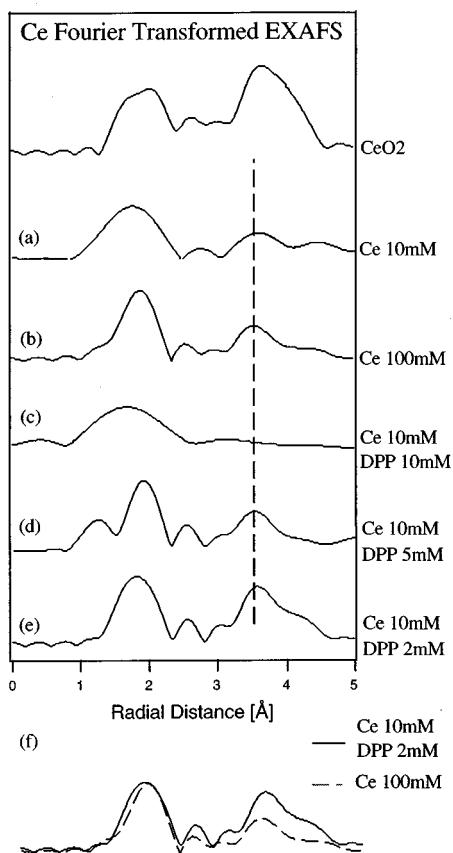[b)]Electronic mail: mkomi@chembio.t.u-tokyo.ac.jp

FIG. 2. Series of Fourier transformed Ce L_3-EXAFS spectra at different molar ratios [(a)–(e)], and (f) comparison of the spectra between (b) and (e).

FIG. 3. Ce L_3-XANES spectra obtained for (a) Ce/DPP (Ce 10 mM, DPP 5 mM), (b) Ce(III)/DPP (Ce 10 mM, DPP 5 mM), and (c) Ce(IV)+Ce(IV) samples.

L_3-EXAFS spectra of the $k^3 - \chi$ function at different molar ratios; (a) Ce 10 mM; (b) Ce 100 mM; (c) Ce 10 mM, DPP 10 mM; (d) Ce 10 mM, DPP 5 mM; and (e) Ce 10 mM, DPP 2 mM. Two large peaks at about 0.18 and 0.36 nm exist in the spectra. By comparison with the crystal structure of CeO$_2$ (also shown in Fig. 2), these peaks are attributed to Ce–O and Ce–Ce bonds, respectively. CeO$_2$ has a structure similar to CaF$_2$, and Ce–O and Ce–Ce bond length in the nearest neighbors obtained by the x-ray diffraction method (our EXAFS measurement) are 0.234 (0.235) and 0.383 (0.384) nm, respectively. The bond length obtained from the curve fitting of EXAFS χ function with the theoretical phase shifts and backscattering amplitude calculated with FEFF.[5]

As shown in Figs. 2(a) and 2(b), Ce–Ce bonds exist in the Ce 10 mM and Ce 100 mM samples without DPP. Therefore, Ce ions themselves form some cluster structure in solution. However, as is clearly shown in Fig. 2(c), the signal of Ce–Ce bonds is markedly reduced to almost zero, when the Ce/DPP ratio is 1:1 (Ce 10 mM, DPP 10 mM). The observed results indicate the existence of a strong interaction between Ce(IV) ions and DPP molecules in solution, and suggest the possibility of the formation of a Ce–DPP complex with a molar ratio of 1:1.

When the molar ratio of Ce(IV) ions to DPP was increased, the Ce–Ce bond appeared again and increased as shown in Fig. 2(d) (Ce 10 mM, DPP 5 mM) and (e) (Ce 10 mM, DPP 2 mM). This result indicates that this Ce–Ce bond is caused by the additional Ce ions. The observed Ce–Ce bonds may be ascribed to the formation of a Ce–Ce–DPP structure in solution, as shown in Fig. 1(b). However, since
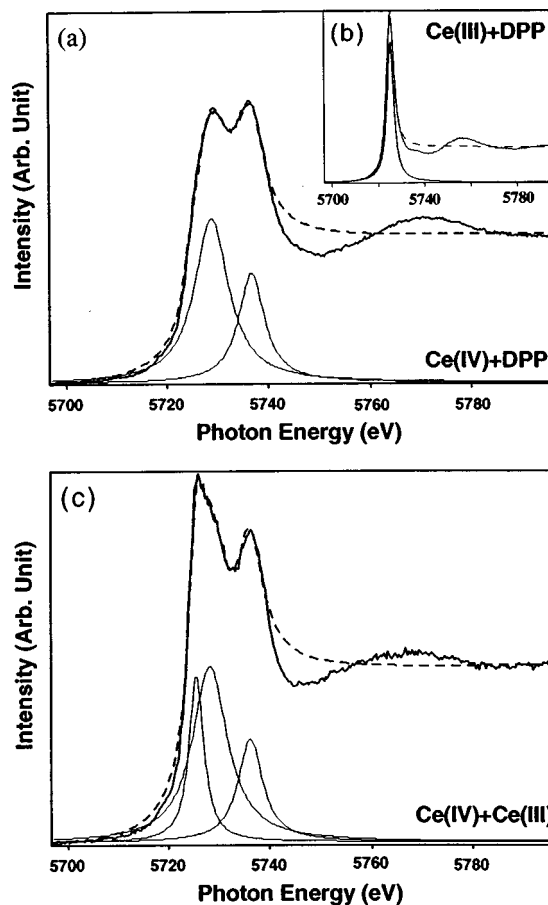
Ce–Ce bonds exist in Ce solution without DPP from the beginning as shown in Figs. 2(a) and 2(b), we have to clarify whether the observed Ce–Ce bonds that appear in Figs. 2(d) and 2(e) are related to the structure including DPP.

A comparison between Figs. 2(b) (Ce 100 mM) and 2(e) (Ce 10 mM, DPP 2 mM) is shown in Fig. 2(f). The Ce–Ce bond exists in both cases; however, the signal increases almost twofold, when DPP molecules exist in solution. From the results obtained, as shown in Fig. 2(f) and the finding that the Ce–Ce bond almost disappears when the molar ratio of Ce/DPP is 1, we concluded that the Ce–Ce bonding in Figs. 2(d) and 2(e) is related to the formation of a structure including DPP molecules. It remains to be clarified whether the effective Ce–Ce bond in the complex corresponds to the formation of the (Ce)n-DPP structure or the simple Ce–Ce–DPP structure. Further experiments are necessary to clarify this point. Nevertheless, these results are quite consistent with the structure in the model shown in Fig. 1(b). The Ce–Ce bond length obtained for the Ce/DPP complexes shown in Figs. 2(d) and 2(e) is 0.352±0.005 nm.

In order to examine the electronic structure, we analyzed the valence of the Ce ions in the complex form by measuring the Ce L_3-XANES spectra. Double white lines on the spectral edges, which are due to the electronic structures related to $4f^1$ (+3) and $4f^0$ (+4), are clearly observed in the spectra. Figure 3(a) shows the spectrum obtained for a Ce/DPP sample (Ce 10 mM, DPP 5 mM). From the analysis of the ratio between these two peaks, the number of $4f$ electrons on
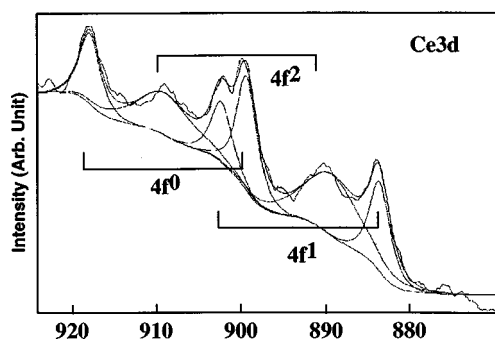
FIG. 4. XPS spectra for DPP, Ce(IV) without DPP and Ce/DPP complex samples.

FIG. 5. Ce $3d$ spectra obtained for a specimen of Ce/DPP$=2$.

Ce in the Ce/DPP complex was calculated to be 0.67 ± 0.02.

For comparison, a Ce L_3-XANES spectrum for the case of Ce(III) ions prepared from CeCl$_3$ is shown in Fig. 3(b). Only one peak exists and no measurable decrease in the valence, from $+3$ to $+2$, is observed in spite of the formation of the complexes. Similar results were obtained for other lanthanide (III) ions.

Figure 3(c) shows the Ce L_3-XANES spectrum obtained for the Ce(III)+Ce(IV) mixture. Since one Ce(III) peak and two peaks originating from Ce(IV) can be identified separately, the possibility that the two peaks in Fig. 3(a) come from the contamination of Ce(III) is ruled out. All the Ce(IV) ions retain their tetravalent states throughout the present analysis, as confirmed by potentiometric titration.[2] This result strongly suggests the formation of the hybrid orbital by Ce(IV) ions.

If the observed change in electronic charge on Ce(IV) ions plays an important role in DNA hydrolysis, the superiority of Ce(IV) over other lanthanide ions may be related to its high potential for withdrawing electrons from its counterpart in chemical reaction. This mechanism may stabilize the Ce/DPP structure, which is necessary for DNA hydrolysis.

In order to confirm the valence of Ce(IV) in the Ce/DPP complex, the Ce $3d$ structure was studied by XPS, which is related to the charge transfer of $4f$ electrons.[6,7] Figure 4 shows the Ce $3d$ spectra obtained for a sample of Ce/DPP $=2$. Three $3d$ signals exist, indicated by f^0, f^1, and f^2, which correspond to the three electronic states, namely, with 0, 1, and 2 $4f$ electrons, caused by the hybridization of the final states. Each of them has two components due to spin-orbital interaction. Since the transition between $4f^0$ and $4f^1$ in the final states is negligible, the electronic charge on Ce in Ce/DPP was calculated from the ratios of $f^0/(f^1+f^2)$, to be 0.69 ± 0.02. This value is in good agreement with that obtained by XANES analysis.

Another experiment was performed to examine the electronic structure. Figure 5 shows the spectra around the Fermi level obtained for DPP, the Ce(IV) hydroxide cluster without DPP and Ce/DPP complex samples. No density of states exists in the spectra around the Fermi level for the DPP and Ce(IV) hydroxide cluster samples. Since there exists a large O peak around 4 eV, it is difficult to see the valence electronic structure. However, the density of states is clearly observed in the spectrum around the Fermi level for the Ce/DPP complex. This result suggests the change in the
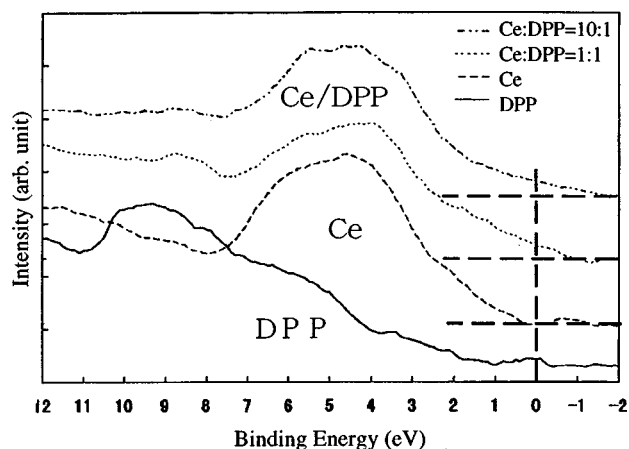
electronic structure of Ce(IV) ions through the formation of a complex with DPP, as consistent with the proposed model.

In the complex formation between Ce(IV) and DPP, hybrid orbitals are efficiently formed between the $4f$ orbitals of Ce(IV) and the phosphate residue, and significant charge transfer may occur through them. From resonant photoemission spectroscopy and XPS studies on some Ce(IV) compounds, a charge of 0.6–1.0 $4f$ electrons was confirmed to exist on Ce ions.[8,9] The large amount of charge transfer is induced by the formation of the hybrid orbitals caused by the characteristics of the spreading $4f$ orbitals in Ce. For example, in the case of CeO$_2$, a large amount of charge observed on Ce is caused by partial charge transfer from O $2p$ to $4f$ through the formation of a hybrid orbital between Ce $4f$ and O $2p$. Therefore, a similar electronic structure may be formed in the Ce(IV)/DPP complex. In contrast, lanthanide (III) ions bind to the phosphodiester linkage of DPP via electrostatic attraction, rather than the formation of covalent bonds. The $+3\rightarrow +2$ transition is thermodynamically unfavorable.

In conclusion, the formation of a Ce–Ce–DPP structure was confirmed by EXAFS in a Ce/DPP solution with a molar ratio higher than 2. The formation of $4f$ hybrid orbitals, as well as the charge transfer through them, is considered to be the reason for the superiority of the Ce(IV) ion in terms of its DNA hydrolytic activity compared to other lanthanide (III) ions.

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