Interaction of several Ni(III) complexes of peptides with DNA; Analysis by DNA-fiber EPR

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ABSTRACT
EPR spectra of Ni(III) complexes of GGH, GHG, and GHK were obtained by in-situ oxidation of the Ni(II) complexes in DNA-pellet and on DNA-fibers. A species with an apical coordination of nitrogenous base of DNA was detected for Ni(III)GGH. Both GHG and GHK complexes showed the EPR signals of Ni(III) species when the ligand to metal ion ratio was 2:1. The complexes bind as Ni(III)(N6), Ni(III)(N5), and Ni(III)(N4) species on DNA.

INTRODUCTION
It has been reported that Ni(II) complexes of the peptides of the type Xaa-Xaa'-His, where Xaa and Xaa' represent appropriate amino acids, bind to a minor groove of double helical DNA and cleave the A/T-rich site in the presence of oxidizing agent.\(^1\)\(^2\) It has been also reported that the nickel complexes of some Schiff-bases and macro-cyclic base ligands lead to solvent exposed guanine oxidation.\(^3\)\(^4\) Despite of these biologically significant reactions of nickel complexes with DNA, the direct observation of the Ni(II) complexes on DNA has been quite rare because of the instability of the complexes in a physiological condition.

To delineate the binding structure of Ni(III) complexes on DNA, we have oxidized the Ni(II) complexes of GGH, GHG, and GHK in situ in DNA-pellet and on DNA-fibers. By this method, we could observe the EPR spectra of the unstable Ni(III) complexes on the DNA.

EXPERIMENTAL
The DNA-pellet and A-form DNA-fibers containing the Ni(II) complexes were prepared as described previously.\(^5\) The DNA-fibers were prepared by inserting small holes of a quartz fiber holder for EPR measurement and the inlet of the holes were wiped by a cotton wool wetted with an aqueous solution of (NH\(_4\))\(_2\)S\(_2\)O\(_8\) (100mM). The oxidant solution diffused through the gaps between the holes and the fibers and oxidized the Ni(II) complexes in the fibers. X-band EPR spectra were measured at different angles of \(\Phi\) between the DNA-fiber axis and the static magnetic field.

RESULTS AND DISCUSSION

Ni(III)GGH
The g values (\(g// = 2.261, g\perp = 2.014\)) of the species in frozen solution are typical of the case when the unpaired electron is in the \(dz^2\) orbital (Fig. 1(a)).\(^6\) Despite of these biologically significant reactions of nickel complexes with DNA, the direct observation of the Ni(III) species on DNA has been quite rare because of the instability of the complexes in a physiological condition.

To delineate the binding structure of Ni(III) complexes on DNA, we have oxidized the Ni(II) complexes of GGH, GHG, and GHK in situ in DNA-pellet and on DNA-fibers. By this method, we could observe the EPR spectra of the unstable Ni(III) complexes on the DNA.

The EPR spectra of the frozen solution of the Ni(III) complex in the presence of excess imidazole showed two \(g\perp\) signals (2.261 and 2.228) and a three-line hyperfine splitting on the \(g//\) signal due to an apical coordination of an imidazole molecule (Fig. 1(c)), indicating that a

Fig. 1. Structure of (a) Ni(II)GGH and (b) Ni(II)GHG (S=H), Ni(II)GHK (S=(CH\(_2\))\(_4\)NH\(_2\)), X = Cl\(^-\) or SO\(_4\)\(^{2-}\))

Fig. 2. EPR spectra of Ni(III)GGH (a) in frozen solution (10 mM Na\(_2\)SO\(_4\), pH 5.09), (b) in DNA-pellet, and (c) in frozen solution with excess imidazole at \(-150^\circ\)C.

The EPR spectra of the frozen solution of the Ni(III) complex in the presence of excess imidazole showed two \(g\perp\) signals (2.261 and 2.228) and a three-line hyperfine splitting on the \(g//\) signal due to an apical coordination of an imidazole molecule (Fig. 1(c)), indicating that a
mono-imidazole adduct is in equilibrium with the Ni(III)GGH in the solution. It should be noted that the bis-imidazole adduct was not formed in the solution even in the presence of excess imidazole. As the $g_{\perp}$ and the $A_{\perp}$ values estimated from the two shoulders observed for the DNA-pellet are almost the same as those observed for the imidazole mono-adduct, the shoulders were assigned as the two of the three line hyperfine splitting due to a nitrogenous base.

$$g_\perp = 2.261$$
$$g_\parallel = 2.014$$

The EPR spectra of Ni(III)GGH on A-form DNA-fibers (Fig. 3) did not change conspicuously with the angle $\Phi$, though the ratio of the intensity of the $g_\perp$ and $g_\parallel$ signals decreased a little with $\Phi$. It has been reported that the Ni(II) and Cu(II) complexes bind in the minor groove of B-form DNA.\(^2^5\) The minor groove of the A-form DNA fibers is wide and shallow. So, if the Ni(II) complex was incorporated in the minor groove of the B-form DNA, the orientation of the $g_\parallel$ axis will be randomized by the conformational change of the DNA from B- to A-form. However, the shoulders observed for the DNA-pellet remained after such a conformational change.

It has been reported that guanine nucleobase oxidation takes place under higher ionic strength conditions.\(^2\) As the A-form DNA-fibers prepared in a low humid condition contain only a small amount of water, the fibers present a high ionic strength condition for the complex. Therefore, present result shows a possibility that such an oxidative reaction progressed by an axial coordination of a guanine N7. Increasing the temperature of the fibers resulted in an irreversible disappearance of the shoulders suggesting that the oxidative cleaving of the DNA took place in the fibers.

**Ni(III)GHG and Ni(III)GHK**

The oxidation of the Ni(II) complexes in a similar condition used for the GGH complex did not give any EPR signals from the corresponding Ni(III). However, when the ligand to metal ion ratio was increased to 2:1, relatively stable Ni(III) species were detected (Fig. S1). The three- and five-line hyperfine splitting observed at the $g_\parallel$ signals for the frozen solutions represent that Ni(III)(N\(_6\)) and Ni(III)(N\(_5\)) species were formed, respectively.

The Ni(III)(GHG)\(_2\) on A-form DNA-fibers at -150°C showed complicated and $\Phi$-independent EPR patterns indicating that several kinds of Ni(III) species, represented as Ni(III)(N\(_6\)), Ni(III)(N\(_5\)) and Ni(III)(N\(_4\)), are randomly oriented on the fibers (Fig. 4). The strong signals of $g_\perp = 2.224$ and $g_\parallel = 2.020$ are due to the Ni(III)(N\(_4\)) species which was produced by a dissociation of a coordinating nitrogen in the Ni(II)(N\(_5\)) species on the DNA. The rise of the temperature of the fibers to -20°C resulted in a disappearance of the broad signal around $g_\perp = 2.163$ and the hyperfine splitting of the $g_\parallel$ signals (Fig. S2). These results suggest that the Ni(III)(N\(_6\)) and Ni(III)(N\(_5\)) species were near the surface of the DNA and easily reduced to the Ni(II) species while the Ni(III)(N\(_4\)) was deeply involved in the hydrophobic environment. The EPR spectra of Ni(III)(GHK)\(_2\) on the A-form DNA-fibers showed only a signal of free radicals and a broad peaks around $g = 2.167$ (Fig. S3), suggesting that this complex was converted more rapidly to the Ni(II) complex.

![Fig. 3. EPR spectra of Ni(III)GGH on A-form DNA-fibers at -150°C](https://academic.oup.com/nass/article-abstract/2/1/195/1058535/0)

![Fig. 4. EPR spectra of Ni(III)GHG on A-form DNA-fibers at -150°C](https://academic.oup.com/nass/article-abstract/2/1/195/1058535/0)

Supporting data Figs. S1 – S3 are available on request as pdf-files from the author (chikira@chem.chuo-u.ac.jp).

**REFERENCES**