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Communications

Oxidative DNA Cleavage by Zn(X-BDPA)$(NO_3)_2$ Complexes (X=F, H, and Me): Effect of Different Ligand Substituents

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Metal complexes have a large potential in biological and medicinal applications because of their cationic character, modularity, reactivity, redox chemistry, photoreactions and defined three dimensional structures. Particularly, the catalytic effects of various metal complexes on the nucleic acids cleavage has been a subject for an intensive study.[1-3] As a recent example, oxidative DNA cleavage by [M(2,2'-dipyridylamine)$_2$$(NO_3)_n$]$^{x+}$ (M = Cd, Cu, Ni and Zn, n = 1, 2, x = 0, 1) metal complexes has been reported.[4] The cleavage has been shown to depend on the nature of the central metal: Zn performed the fastest and Ni the slowest in double stranded DNA cleavage. Another report showed that (*N*,*N*'-ethylenediaminediacetato) M(II) complexes (M = Cu, Co, Ni, Zn) cleaved plasmid DNA in the presence of hydrogen peroxide. The Cu(II) complex was more efficient in DNA cleavage than the Zn and Ni complexes; the different amounts of OH radicals among the complexes were responsible for their different efficiencies.[5]

In this study, we report the cleavage of supercoiled pgem-7zf-nisin DNA (referred to as pgem DNA) by Zn(X-BDPA)$(NO_3)_2$ complexes (Figure 1).[6] As shown in the structure, the ligand possesses Me or F at the position of X. The presence of Me or F represents an electron donating or withdrawing group, respectively, resulting in a different electron density on the complex in each case (see Supporting Information for the syntheses and characterizations of three ligands and their metal complexes). Figure 2 depicts the time-dependent appearance of nicked circular and linear pgem DNA with respect to the incubation time. The amount of super coiled pgem DNA decreased with incubation time for all three complexes. After 40 minutes of incubation, no super coiled DNA was left for the Zn(X-BDPA)$(NO_3)_2$ complexes, where X represents H or Me. The appearance of linear DNA was also noted for the X = Me complex after 35 minutes of incubation. The band corresponding to linear DNA appeared after 50 minutes incubation for the complex with X = H.

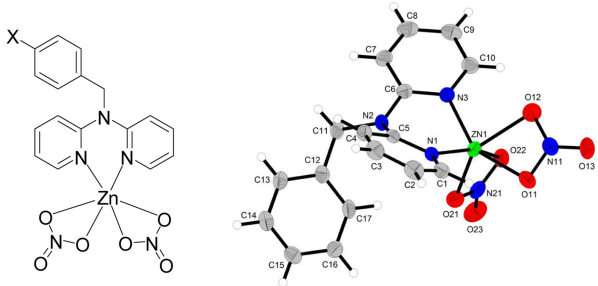

Figure 1. Chemical and crystal (**2**) structures of the Zn(X-BDPA)$(NO_3)_2$ complexes adopted in this study. X = F (**1**), H (**2**), and CH_3 (**3**).

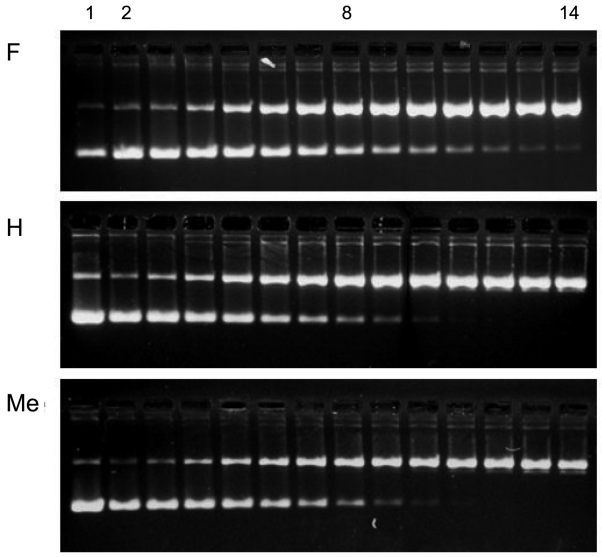

Figure 2. Appearance of nicked circular and linear pgem DNA in 1% agarose gel electrophoresis with respect to the incubation time. Lane 1: control (unreacted pgem DNA). Lane 2-14: incubation time increased from 0 to 60 minutes in increments of 5 minutes. Incubation solution contains 50 μM pgem DNA, 10 μM [Zn(X-BDPA)$(NO_3)_2$], 100 mM acscorbate, and 100 μM $CuCl_2$ in 5 mM cacodylate buffer, pH 7.

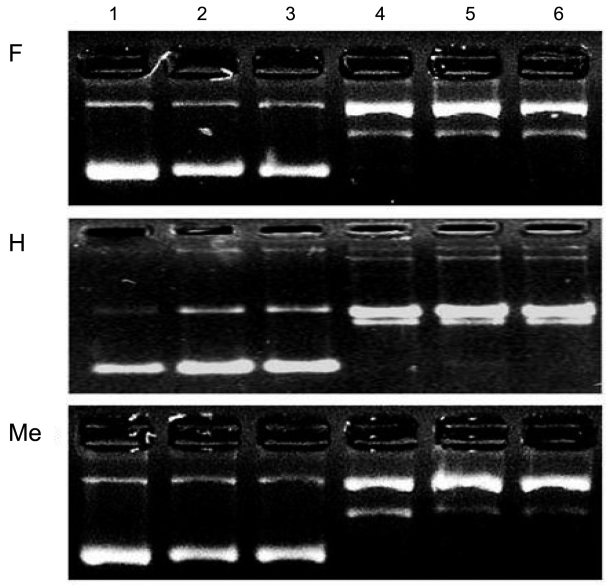

Figure 3. Effects of various scavengers for reactive oxygen species. Lane 1: unreacted pgem DNA as standard. Lane 2, in the presence of 1 mM tiron; lane 3: 5 mM sodium azide; lane 4: 0.125 U/μL catalase; lane 5: 50 mM DMSO; lane 6: in the absence of a scavenger. The reaction conditions were the same as in Figure 2.

These results suggested that the reaction time to transform the super coiled pgem DNA to circular form were similar for the X = Me and X = H complexes, while the transformation from circular to linear form was faster for the X = Me complex. The catalytic effect of the X = Me complex on the cleavage of super coiled pgem DNA is similar or slightly more efficient than that of the X = H complex. On the other hand, the X = F complex exhibited the slowest reaction for the cleavage of pgem DNA: even after 60 minutes of incubation, super coiled DNA still remained. The band corresponding to linear DNA was not observable even after 60 minutes of incubation. These results clearly demonstrate that the substituent on the BDPA ligand of the complex affects the rate of DNA cleavage. When an electron withdrawing group was attached at the *para*-position of the benzene ring, the catalytic effect was less pronounced compared to the case with an electron donating group. It is noteworthy, at this point, that the cleavage of pgem DNA in the absence of Zn complex was negligible although Cu^{2+} ion has been known to produce reactive oxygen species in the condition adopted in this study.[4]

The cleavage of pgem DNA occurs either by oxidation or by a hydrolysis mechanism.[7] The possibilities of these two mechanisms were tested by comparing the cleavage efficiencies in an N_2 environment. When the incubation mixtures were bubbled with N_2 for 30 minutes before the reaction started, the efficiency of the cleavage of pgem DNA was significantly decreased or totally suppressed (data not shown), suggesting that the cleavage of pgem DNA by the Zn(X-BDPA)$(NO_3)_2$ complexes occurred *via* the oxidation process. Figure 3 depicts the effects of various scavengers for reactive oxygen species on the pgem DNA cleavage. Tiron and sodium azide were shown to be very effective for the inhibition of DNA cleavage in the cases of all three complexes. Considering that tiron and sodium azide are effective scavengers for super oxide radicals ($O_2^{-}\cdot$) and singlet oxygen ($^1O_2$), respectively, the result indicates that neither of these reactive oxygen species contribute to the cleavage reaction. In contrast, catalase and DMSO did not suppress any cleavage activity of the metal complexes, suggesting that hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH) are involved in the cleavage of pgem DNA. Although the generation of superoxide radicals by transferring an electron from the Fe^{2+} ion to the coordinated molecular oxygen has been reported,[7,8] this type of reaction cannot occur in the current case. The oxidation of Zn(II) to Zn(III) is not likely to occur and even if the molecular oxygen is coordinated to Zn, this does not explain the different levels of activity induced by the different substituents in the benzene ring, as they are too far away from the central metal to affect its oxidation state. Thus, the detailed mechanism of the effect of the electron donating and withdrawing groups of the periphery group at the ligand is a subject for future study.

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