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Hydrolytic cleavage of DNA by quercetin zinc(II) complex

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Abstract—Quercetin zinc(II) complex was investigated focusing on its hydrolytic activity toward DNA. The complex successfully promotes the cleavage of plasmid DNA, producing single and double DNA strand breaks. The amount of conversion of supercoiled form (SC) of plasmid to the nicked circular form (NC) depends on the concentration of the complex as well as the duration of incubation of the complex with DNA. The rate of conversion of SC to NC is 1.68×10^{-4} s^{-1} at pH 7.2 in the presence of 100 μM of the complex. The hydrolytic cleavage of DNA by the complex is supported by the evidence from free radical quenching, thiobarbituric acid-reactive substances (TBARS) assay, and T4 ligase ligation.

DNA is naturally stable polymer and the half life of DNA phosphate-diester bonds is reported to be approximately 130,000 years via spontaneous hydrolysis under physiological conditions.[1] Since DNA is very sensitive to oxidative cleavage, many studies on metallonucleases have been focused on molecules capable of cleaving DNA oxidatively.[2–4] Most of these molecules can just induce effective oxidative cleavage of DNA in the presence of UV light, a reducing agent or H_2O_2 as an additive.[2–7] However, in spite of their high efficiency and versatility, oxidative cleavage products are not readily amenable to further enzymatic manipulations, and thus have limited use in molecular biology. However, hydrolytic cleaving agents do not have these flaws. The synthetic metallohydrolases follow a mechanistic pathway targeted at the phosphate-diester bonds linking the nucleosides which leads to the formation of fragments like endonucleases.[8,9] So study on metallohydrolases capable of mimicking the function of these endonucleases has been paid more and more attention. Some of complex with transition metals actually exhibited the ability of hydrolytic cleavage of DNA.[10–12]

Quercetin (Que, 3,5,7,3′,4′-pentahydroxyflavone), a bioflavonoid widely distributed in fruits and vegetables, has been reported to exert multiple biological effects as an antioxidant and antitumor activity.[13–15] It has been reported that flavonoids have a protective effect on DNA damage induced by hydroxyl radical.[16] Quercetin can chelate metal ions to form metal complex that has higher antioxidation and antitumor activity than quercetin alone.[17,18] In this work, we demonstrate that quercetin zinc(II) complex could not protect DNA from damage, but promote the cleavage of plasmid DNA, producing single and double DNA strand breaks via a possible hydrolytic mechanism.

Quercetin Zn(II) complex was prepared according to the literature.[18] The possible structure model of the complex is shown in Figure 1. DNA cleavage was measured by the conversion of supercoiled pBR322 plasmid DNA to nicked circular and linear DNA forms under physiological conditions (37 °C, pH 7.2). The ability of the quercetin Zn(II) complex in inducing DNA cleavage has been studied by gel electrophoresis using supercoiled pBR 322 DNA in 50 mM Tris–HCl/50 mM NaCl buffer (pH 7.2) in the dark. Both quercetin (data not shown) and Zn^{2+} (Fig. 2, lane 2) alone induced little DNA cleavage. Figure 2 shows the results of the gel electrophoresis experiment carried out with supercoiled DNA cleavage induced by various concentrations of the complex. The complex scarcely catalyzes the cleavage of plasmid DNA both at 10 μM and at 25 μM. While increasing the concentration of the complex to 50 μM, the cleavage of plasmid DNA was observed obviously. At 100 μM, the increase in the amounts of nicked DNA was

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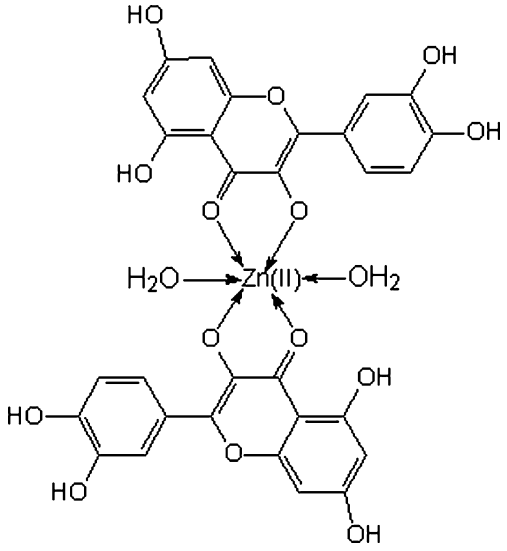

Figure 1. The possible structure model of quercetin zinc(II) complex.

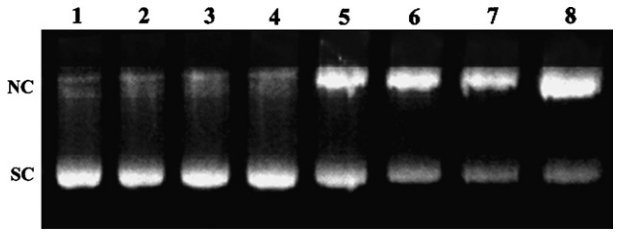

Figure 2. Agarose gel (1%) of pBR322 (0.25 μg) incubated for 1.5 h at 37 °C and pH 7.2 (50 mM Tris–HCl) with increasing complex concentrations: lane 1, DNA control; lane 2, Zn^{2+} 100 μM; lanes 3–8, Zn(II) complex 10, 25, 50, 100, 200, and 400 μM, respectively.

observed to be associated with the increase of reaction time. 81% and 92% nicked DNA was observed when the reaction time was 2.5 and 4 h, respectively (Fig. 3). Increasing the incubation beyond 6 h led to the appearance of linear DNA (data not shown).

Figure 4 shows the extent of DNA cleavage by quercetin Zn(II) complex with reaction time. The decrease in SC DNA and the formation of NC DNA with time show the expected exponential nature of the curves. The plot of ln (% SC DNA) versus time is linear, which confirms the process to be pseudo-first-order. The rate constant k_1 is obtained (1.68×10^{-4} s^{-1}), using a complex concentration of 100 μM (Fig. 4, inset). The rate of DNA

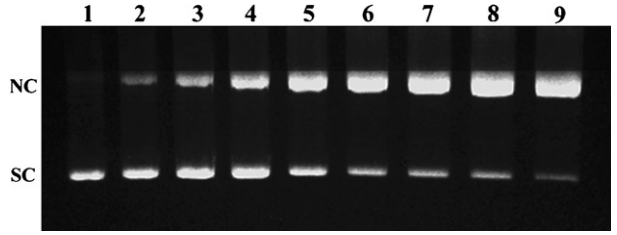

Figure 3. Agarose gel (1%) of pBR322 (0.25 μg) at 37 °C and pH 7.2 (50 mM Tris–HCl) with 100 μM Zn(II) complex with increasing reaction time: lane 1, DNA control; lanes 2–9, 15, 30, 60, 90, 120, 150, 180, and 240 min, respectively.

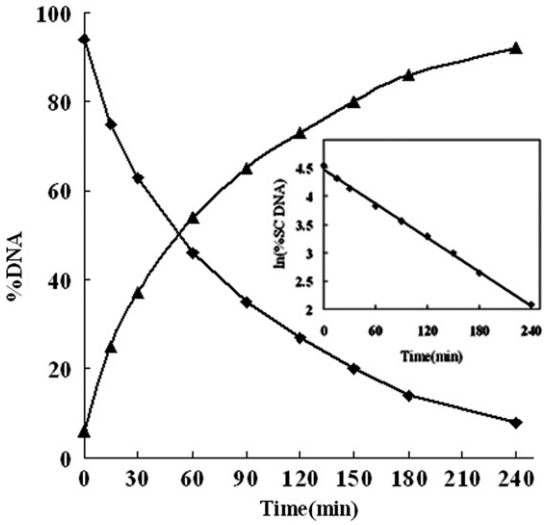

Figure 4. Disappearance of supercoiled (SC) and formation of nicked circular (NC) forms of pBR322 DNA in the presence of Zn(II) complex (100 μM) with incubation time (pH 7.2, temperature 37 °C). Inset: plot of ln (% SC form) versus time for a complex concentration of 100 μM.

hydrolysis by quercetin Zn(II) complex is similar to some reported complexes at the concentration of 100 μM, such as ternary amino acid Schiff-base copper(II) complex ($k_1 = 4.72 \times 10^{-4}$ s^{-1})[19] and dinuclear biphenylene bridged copper(II) complex ($k_1 = 8.90 \times 10^{-4}$ s^{-1}).[20]

To investigate the mechanism of DNA cleavage promoted by quercetin Zn(II) complex, hydroxyl radical scavengers (0.4 M DMSO, glycerol, and 0.2 M mannitol), catalase (15 U), and oxidant (50 μM H_2O_2) were introduced to the system. As shown in Figure 5, no evident inhibition of DNA cleavage was observed in the presence of scavengers, which suggested that hydroxyl radical and hydrogen peroxide might not occur in the reaction. And 50 mM hydrogen peroxide could not promote the cleavage of DNA induced by the complex. Therefore, DNA cleavage promoted by the complex might not occur via an oxidative pathway but takes place probably via a hydrolytic pathway.

In order to determine whether the presence of quercetin zinc(II) complex may increase the formation of ROS, we

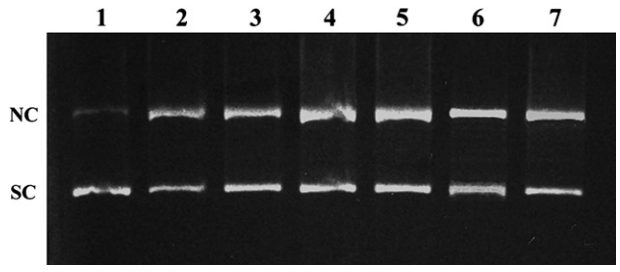

Figure 5. Agarose gel (1%) of pBR322 (0.25 μg) incubated for 1.5 h at 37 °C and pH 7.2 (50 mM Tris–HCl) with 100 μM Zn(II) complex and different scavengers or H_2O_2: lane 1, DNA control; lane 2, complex control; lane 3, DMSO (0.4 M); lane 4, glycerol (0.4 M); lane 5, mannitol (0.2 M); lane 6, catalase (15 U); lane 7, hydrogen peroxide (50 μM).

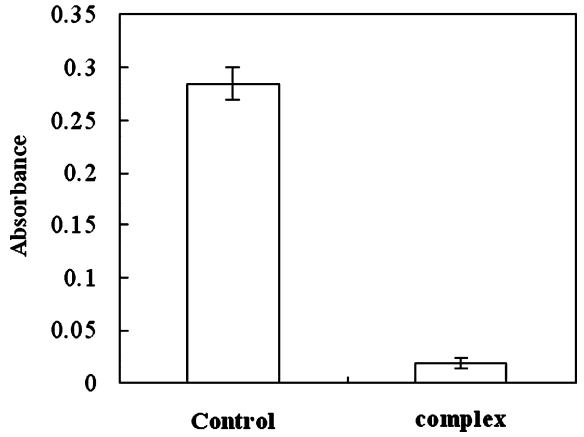

Figure 6. The formation of 2-thiobarbituric acid reacting species (TBARS). Control: $[Fe(EDTA)]^{2-}/H_2O_2$. Each value represents mean ± SE of three experiments.

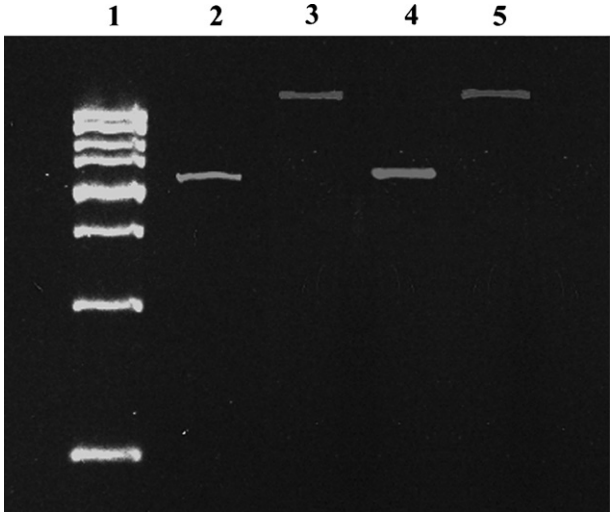

Figure 7. Agarose gel (1%) for ligation of pBR322 DNA linearized by Zn(II) complex. lane 1, DNA markers; lanes 2 and 3, pBR322 DNA linearized by Zn(II) complex without and with T4 DNA ligase; lanes 4 and 5, pBR322 DNA linearized by *Eco*RI without and with T4 DNA ligase.

analyzed the influence of the complex on the oxidative damage of calf thymus DNA (CT DNA) by measuring the formation of 2-thiobarbituric acid reacting species (TBARS). It can be seen from Figure 6 that the absorbance of sample treated by complex is very small compared with that of sample treated by $[Fe(EDTA)]^{2-}/H_2O_2$. It suggested that the desoxyribose ring in DNA skeleton was not cut by ROS and oxidative cleavage of DNA did not occur.

Further experiments support this assumption. It is well known that in DNA hydrolytic cleavage 3′-OH and 5′-OPO_3 (5′-OH and 3′-OPO_3) fragments remain intact and that these fragments can be enzymatically ligated and end-labeled.[21] Figure 7 shows that the linear DNA fragments cleaved by quercetin Zn(II) complex can be religated by T4 ligase just like the linear DNA mediated by *Eco*RI. Hence, this result implied that the process of DNA cleavage by the complex occurs via a hydrolytic path.

In conclusion, evidence is presented that quercetin zinc(II) complex is very efficient in promoting the cleavage of plasmid DNA via a hydrolytic path in the absence of any added cofactors in this work. The rate of conversion of SC to NC is $1.68 \times 10^{-4}\ s^{-1}$ at the complex concentration of 100 μM. The specific DNA cleavage mechanism and the potential of the complex as artificial nuclease and footprinting agent are under investigation.

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