DNA binding and cleavage activity of quercetin nickel(II) complex

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The interaction of a quercetin nickel(II) complex with DNA was investigated using UV-vis spectra, fluorescence measurements, viscosity measurements, agarose gel electrophoresis and thiobarbituric acid-reactive substances assay. The results indicate that the quercetin nickel(II) complex can intercalate into the stacked base pairs of DNA, and compete with the strong intercalator ethidium bromide for the intercalative binding sites with Stern–Volmer quenching constant $K_{sq} = 1.0$. 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 DNA to the nicked circular form (NC) depends on the concentration of the complex, ionic strength and the duration of incubation of the complex with DNA. The maximum rate of conversion of the supercoiled form to the nicked circular form at pH 7.2 in the presence of 100 μM of the complex is found to be 0.76×10^{-4} s^{-1}. The hydrolytic cleavage of DNA by the complex was supported by the evidence from free radical quenching and thiobarbituric acid-reactive substances assay.

Introduction

In recent years, many researches[1–3] have been focused on the interaction of small molecules with DNA. DNA is generally the primary intracellular target of anticancer drugs, so the interaction between small molecules and DNA can cause DNA damage in cancer cells, inhibiting the growth of cancer cells and resulting in cell death or apoptosis.[4–5] Small molecules can interact with DNA through the following three non-covalent modes: intercalation, groove binding and external static electronic effects. Among these interactions, intercalation is one of the most important DNA binding modes that is related to the antitumor activity of the compound. Recently, there has been a great interest on the binding of transition metal complexes with DNA, owing to their possible applications as new cancer therapeutic agents and their photochemical properties that make them potential probes of DNA structure and conformation.[6–8] Moreover, transition metal complexes have been widely exploited for metallohydrolases capable of mimicking the function of these endonucleases.[9–13] So it is essential to develop synthetic, sequence-selective DNA binding and cleavage agents for DNA itself and for new potential DNA targeting antitumor drugs.

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.[14–16] Quercetin can chelate metal ions to form metal complexes that have better antioxidation and antitumor activity than quercetin alone.[17–18] However, little research has been devoted to DNA-binding properties and DNA cleavage mechanism of the quercetin nickel(II) complex.

In this study, we investigated the mode of DNA binding and DNA cleavage activity of the quercetin nickel(II) complex. We demonstrate that the quercetin nickel(II) complex could be bound to DNA *via* an intercalation mode and could promote the cleavage of plasmid DNA *via* a hydrolytic pathway.

Experimental

Materials

All chemicals and reagents were purchased from commercial sources and were used without further purification. The plasmid pBR322 DNA was purchased from TaKaRa Biotechnology Co. Ltd. (Japan). Quercetin, calf thymus DNA (CT DNA), catalase and ethidium bromide (EB) were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Nickel(II) chloride ($NiCl_2 \cdot 6H_2O$) was from Guangfu Fine Chemicals, Tianjing (China). 2-Thiobarbituric acid (TBA) was from Sinopharm Chemical Reagent Co. Ltd., Shanghai (China). Agarose (molecular biology grade) was from Oxoid Limited Basingstoke, Hampshire (England). The Tris-HCl buffer solution was prepared with triple distilled water.

Preparation of quercetin nickel(II) complexes

Quercetin nickel(II) complex was prepared according to literature.[9] The solid quercetin·$2H_2O$ (2 mM) was dissolved into 60 mL of ethanol. Then the pH of the solution was adjusted to 6–7 with EtONa. After 5 min, $NiCl_2 \cdot 6H_2O$ (1 mM) was added to the above mixture. After being stirred and heated to reflux for 5 h at 60 °C, the reaction mixture was cooled to room temperature and poured into H_2O. The pale yellow precipitate, which formed immediately, was set aside for 48 h, filtered and washed thrice with 1 : 3 EtOH–H_2O. The solid product was dried under vacuum for 48 h at room temperature. Yield: 72%. Anal. Found: $Ni(Que)_2(H_2O)_2$: C, 51.2%; H, 3.7%; Ni, 8.2%. IR (KBr) cm^{-1}: ν_{max} = 3450–3098 (b, m), 1631 (s), 1392 (s), 1263 (s), 657 (w), 623 (w), 399 (m).

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UV (EtOH) nm: λ_{max} = 424 (12151), 261 (31983). Attempts to grow single crystals suitable for crystal structure determination were unsuccessful. These data indicated that stoichiometric ratio (Ni : Que) of $Ni(Que)_2(H_2O)_2$ was 1 : 2 and quercetin could chelate nickel(II) *via* 3-OH and 4-oxo groups. The possible structure model of the complex is shown in Fig. 1.

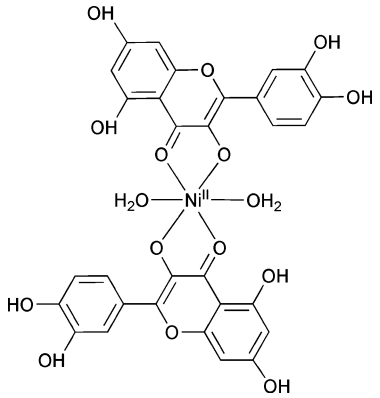

Fig. 1 The possible structure of quercetin nickel(II) complex.

DNA-binding measurements

All the experiments involving the interaction of compound with CT DNA were conducted in Tris buffer (0.01 M Tris–HCl/50 mM NaCl, pH 7.2). The purity of the DNA was determined by monitoring the value A_{260}/A_{280} about 1.8–1.9 : 1, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm.

UV-vis spectra were measured on a Lambda 900 UV/Vis/NIR Spectrometer (Perkin–Elmer) in 0.01M Tris buffer. Spectroscopic titrations were carried out at room temperature to determine the binding affinity between DNA and quercetin metal complex. Initially, the solutions (2000 μL) of the blank buffer and $Ni(Que)_2(H_2O)_2$ complex sample (10 μM) were placed in the reference and sample cuvettes (1 cm path length), respectively, and then the first spectrum was recorded in the range of 260–440 nm. During the titration, an aliquot (20 μL) of buffered DNA solution (concentration of 1 mM in base pairs) was added to each cuvette to eliminate the absorption from DNA itself, and the solutions were mixed by repeated inversion. After the solutions had been mixed for 10 min, the absorption spectra were recorded. The titration processes were repeated until there was no change in the spectra for four titrations at least, indicating that binding saturation had been achieved. During the spectrophotometric titration, the changes in the metal complex concentration are negligible.

Fluorescence measurements were made using Perkin–Elmer LS-50B fluorescence spectrophotometer with a slit width 5 nm for the excitation and emission beams. Fluorescence titrations were carried out by adding increasing amounts of CT DNA directly into the cell containing the solution of $Ni(Que)_2(H_2O)_2$ complex (c = 30 μM, 0.01 M Tris buffer, pH 7.2). The concentration range of the DNA was 0–60 μM bp. Emission spectra were recorded in the region 330–370 nm using an excitation wavelength of 312 nm. Fluorescence quenching study was conducted by adding increasing amounts of $Ni(Que)_2(H_2O)_2$ complex (0–120 μM) directly into the EB-DNA system (c_{EB} = 5 μM, c_{DNA} = 100 μM bp, 0.01 M Tris buffer, pH 7.2). Emission spectra were recorded in the region 520–650 nm using an excitation wavelength of 500 nm. All measurements were performed at 25 °C.

Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 30.0 ± 0.1 °C in a thermostatic bath. Flow time was measured with a digital stopwatch and each sample was measured three times and an average flow time was calculated. Data are presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio,[19] where η is the viscosity of DNA in the presence of the complex and η_0 is the viscosity of DNA in the absence of the complex. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t > 100 s) corrected for the flow time of buffer alone (t_0), $\eta = t - t_0$.

DNA cleavage studies

DNA cleavage was measured by the conversion of supercoiled pBR322 plasmid DNA to nicked circular and linear DNA forms. Supercoiled pBR322 plasmid DNA (0.25 μg per reaction) in Tris–HCl buffer (50 mM) with 50 mM NaCl (pH 7.2) was treated with the indicated amount of $Ni(Que)_2(H_2O)_2$ complex, followed by dilution with the Tris–HCl buffer to a total volume of 10 μL. The samples were incubated for 1 h at 37 °C. After the reaction had been stopped by addition of 1/10 volume of the loading buffer (0.25% bromophenol blue, 40% sucrose, 0.25% xylene cyanole and 200 mM EDTA), the samples were loaded on 1% neutral agarose gel containing 40 mM Tris/acetate and 1 mM EDTA (TAE buffer, pH 8.0), and were subjected to electrophoresis in a horizontal slab gel apparatus and 1 × TAE buffer, which was performed at 75 V for 1.5 h. The gel was stained with a solution of 0.5 μg mL^{-1} ethidium bromide for 30 min, followed by destaining in water. Agarose gel electrophoresis of plasmid DNA was visualized by photographing the fluorescence of intercalated ethidium bromide under a UV illuminator. The cleavage efficiency was measured by determining the ability of the complexes to convert the supercoiled DNA (SC) to nicked circular form (NC) and linear form (L). After electrophoresis, the proportion of DNA in each fraction was estimated quantitatively from the intensity of the bands using Glyko BandScan software.

To study the mechanism of the DNA cleavage reaction performed by $Ni(Que)_2(H_2O)_2$ complex, different scavengers or reactive oxygen intermediates such as dimethyl sulfoxide (DMSO) (0.4 M), glycerol (0.4 M), mannitol (0.2 M) catalase (15 units), and oxidant hydrogen peroxide (50 μM) were added to reaction mixtures, respectively. Samples were treated as described above.

Thiobarbituric acid-reactive substances (TBARS) assay

Each sample containing 0.5 mM CT DNA and 100 μM $Ni(Que)_2(H_2O)_2$ complex in 50 mM phosphate buffer (pH 7.2) was incubated at 37 °C for 24 h in a total volume of 2 mL. After incubation, samples were treated with 2 mL of 1% (w/v) solution of 2-thiobarbituric acid in 50 mM NaOH and 2 mL of glacial acetic acid, and were incubated at 100 °C for 30 min. After cooling, the absorbance at 532 nm was measured. Blanks contained all components except the complex. The control group contained $[Fe(EDTA)]^{2-}$ (100 μM) and hydrogen peroxide (10 mM) instead of the complex.

Results and discussion

DNA binding properties

The binding of $Ni(Que)_2(H_2O)_2$ complex with CT DNA was measured using absorption, fluorescence spectrophotometric titrations and viscosity measurements.

Electronic absorption spectral studies. The binding of the complex to DNA was characterized classically through absorption titration. A complex bound to DNA through intercalation is characterized by the change in absorbance (hypochromism) and red shift in wavelength, due to the intercalative mode involving a stacking interaction between the aromatic chromophore and the DNA base pairs. The electronic absorption spectra of $Ni(Que)_2(H_2O)_2$ complex exhibited broad absorption bands in the region 260–440 nm, typical for transitions between the π-electronic energy levels of the quercetin skeleton. The electronic spectra of the complex in the presence and absence of DNA were monitored over the wavelength range of 260–440 nm, as shown in Fig. 2. With increasing concentration of CT DNA (0–40 μM), a considerable drop in the absorptivity (64% hypochromicity) at about 270 nm and a substantial red-shift ($\Delta\lambda = 6.6$ nm) was observed. The hypochromicity suggests the complex may bind to DNA by intercalation mode, due to a strong interaction between the electronic states of the intercalating chromophore and those of the DNA bases.

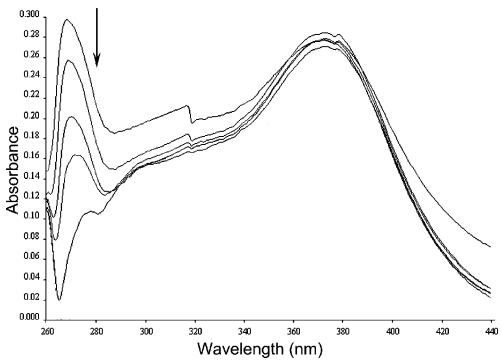

Fig. 2 Absorption spectra of quercetin nickel(II) complex (10 μM) in 0.01 M Tris-HCl buffer at pH 7.2, in the absence and presence of increasing amounts of CT DNA (from top to bottom, 0–40 μM bp). The arrow shows the intensity decreased with increasing amounts of DNA.

Fluorescence spectral studies. The additional evidence for intercalation into the DNA was obtained from fluorescence spectral studies. The fluorescence spectra of $Ni(Que)_2(H_2O)_2$ complex, exhibiting a broad emission band in the range 340–360 nm, were monitored at a fixed concentration of 30 μM. Enhanced fluorescence without wavelength shift was observed (Fig. 3), when CT DNA was added into the complex solution. The result suggests that the stronger enhancement of fluorescence intensity for the complex may be largely due to the interaction between adjacent base pairs of CT DNA and the complex. Furthermore, the binding of the complex to the DNA helix could decrease the collisional frequency of solvent molecules with the complex, which usually leads to emission enhancement of the complex. The result also agrees with those observed for other intercalators.[1,7–8] So the complex may intercalate into the adjacent base pairs of CT DNA.

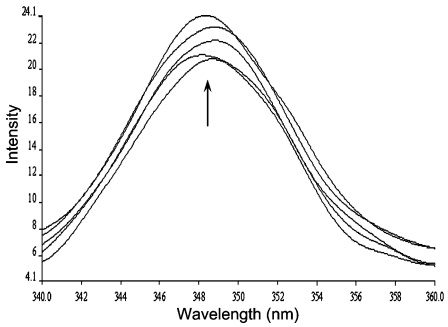

Fig. 3 The change of emission fluorescence spectra of quercetin nickel(II) complex (30 μM) in 0.01 M Tris-HCl buffer at pH 7.2, in the absence and presence of increasing amounts of CT DNA (from bottom to top, 0–60 μM bp), $\lambda_{ex} = 312$ nm. The arrow shows the intensity increased with increasing amounts of DNA.

To understand the interaction pattern of the complex with DNA more clearly, a fluorometric competitive binding experiment was carried out using ethidium bromide (EB) as a probe that shows no apparent emission intensity in buffer solution because of solvent quenching. However, ethidium bromide emits intense fluorescent light in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. A competitive binding of a small molecule to CT DNA could result in the displacement of EB or quenching of the bound EB by the compound decreasing its emission intensity. It was previously reported that the enhanced fluorescence could be quenched by the addition of another molecules.[1,13] The emission spectra of EB bound to DNA in the absence and in the presence of $Ni(Que)_2(H_2O)_2$ complex are given in Fig. 4. The emission band at 587 nm of the DNA-EB system decreased in intensity with the increasing concentration of the complex, and an equal absorption point appeared at 543.2 nm (data not shown). It has been reported that some small organic molecules interact with DNA by intercalation when the concentration ratio of them to DNA (c_M/c_{DNA}) is less than 100 and the fluorescence intensity of EB-DNA system is decreased by 50%.[1,20] The inset in Fig. 4 shows the quenching extent has reached up to 52% when $c_{complex}/c_{DNA} = 1.2$. The changes observed here are

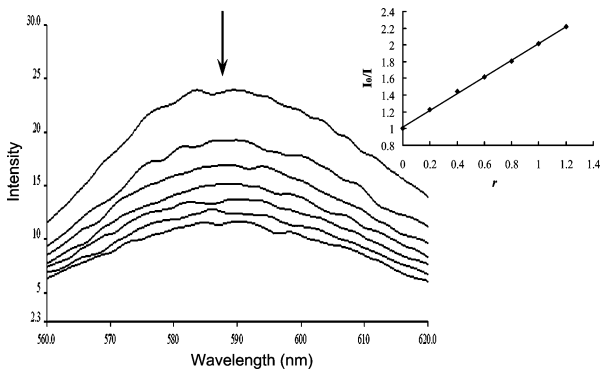

Fig. 4 Fluorescence spectra of the binding of EB to DNA in 0.01 M Tris-HCl buffer at pH 7.2, in the absence and presence of increasing amounts of quercetin nickel(II) complex (from top to bottom, 0–120 μM), $\lambda_{ex} = 500$ nm, $c_{EB} = 5$ μM, $c_{DNA} = 100$ μM bp. The arrow shows the intensity decreased with increasing concentration of the complex. The inset is Stern–Volmer quenching plots of DNA–EB system by the complex. $r = c_{complex}/c_{DNA}$.

often characteristic of intercalation. Moreover, this phenomenon indicates that $Ni(Que)_2(H_2O)_2$ complex competes with EB in binding to DNA.

According to the classical Stern–Volmer equation:[21]

$$I_0/I = 1 + K_{sq}r \quad (1)$$

where I_0 and I represent the fluorescence intensities in the absence and presence of the complex, respectively, and r is the concentration ratio of the complex to DNA. K_{sq} is a linear Stern–Volmer quenching constant dependent on the ratio of the bound concentration of EB to the concentration of DNA (Fig. 4, inset). The K_{sq} value is obtained as the slope of I_0/I *versus* r linear plot. From the inset in Fig. 4, the K_{sq} value for $Ni(Que)_2(H_2O)_2$ complex is 1.0, which suggests that the interaction of the complex with DNA is strong.[22] It may be due to the complex interacting with DNA through intercalation binding, so releasing some free EB from the DNA–EB complex,[23] which is consistent with the above absorption spectral results.

Viscometric studies. To investigate further the DNA-binding mode of $Ni(Que)_2(H_2O)_2$ complex, viscosity measurements on solutions of CT DNA incubated with the complex were performed. It is well known that intercalative DNA binding would cause elongation of DNA polymer by effecting separation of DNA base pairs, resulting in an increase in viscosity. In contrast, a partial or non-classical intercalation of the ligand could bend or kink DNA resulting in a decrease in its effective length with a concomitant decrease in its viscosity.[24–25]

The relative specific viscosity (η/η_0) of DNA generally reflects the increase in contour length associated with separation of DNA base pairs caused by intercalation. Fig. 5 shows the increase in the relative specific viscosity of DNA when $Ni(Que)_2(H_2O)_2$ complex is added into CT DNA solution. So the results demonstrate that the complex binds to DNA by the intercalation mode, which is consistent with the above absorption and fluorescence spectral results.

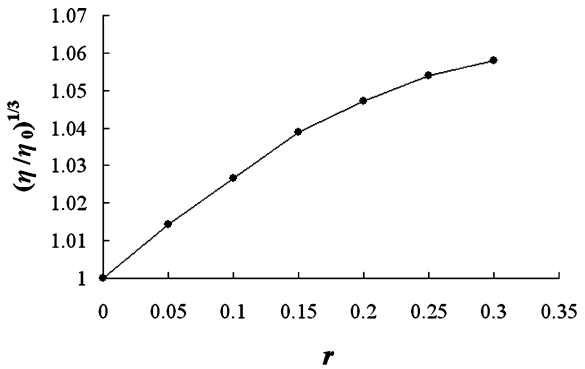

Fig. 5 Effect of increasing amounts of quercetin nickel(II) complex on the relative viscosity of CT DNA in 0.01 M Tris-HCl buffer (pH 7.2) at 30 ± 0.1 °C. $c_{DNA} = 50$ μM, $r = c_{complex}/c_{DNA}$.

DNA cleavage activity

The double-stranded plasmid pBR322 exists in a compact supercoiled conformation (SC). Upon formation of strand breaks, the supercoiled form of DNA is disrupted into the nicked circular form (NC) and the linear form. If one strand is cleaved, the supercoiled form will relax to produce a nicked circular form. If both strands are cleaved, a linear form will be produced. Relatively fast migration is observed for supercoiled form when the plasmid DNA is subjected to electrophoresis. The nicked circular form migrates slowly and the linear form migrates between SC and NC.[26] Hence, DNA strand breaks were quantified by measuring the transformation of the supercoiled form into nicked circular and linear forms.

Effects of the concentration of the complex and ionic strength on DNA cleavage. The ability of the $Ni(Que)_2(H_2O)_2$ complexes to induce DNA cleavage was studied by gel electrophoresis using supercoiled pBR 322 DNA in 50 mM Tris-HCl/50 mM NaCl buffer (pH 7.2). Both quercetin (data not shown) and Ni^{2+} (Fig. 6, lane 2) alone induced little DNA cleavage. Fig. 6 shows the results of the gel electrophoresis experiment carried out with supercoiled DNA cleavage induced by various concentrations of the complex. The complex scarcely catalyzed the cleavage of plasmid DNA both at 10 μM and at 25 μM. When the concentration of the complex was up to 50 μM, the cleavage of plasmid DNA was observed obviously. And the increase in the amounts of nicked DNA was observed associated with the increase of the concentration of the complex.

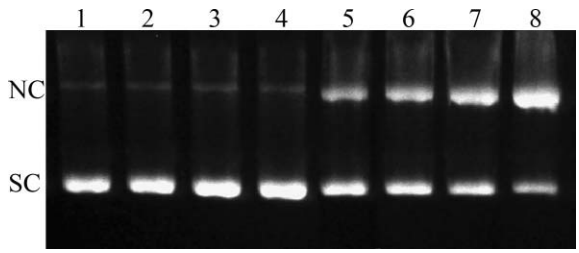

Fig. 6 Agarose gel (1%) of pBR322 (0.25 μg) incubated for 1.5 h at 37 °C and pH 7.2 (50 mM Tris-HCl) with increasing complex concentrations. Lane 1: DNA control; lane 2: Ni^{2+} 100 μM; lane 3–8: $Ni(Que)_2(H_2O)_2$ complex 10, 25, 50, 100, 200, 400 μM, respectively.

The effects of ionic strength on the double-strand DNA cleavage by adding NaCl were studied. Fig. 7 shows that the process of cleavage was sensitive to the change of ionic strength. When the concentration of NaCl was not more than 50 mM, the ionic strength could promote DNA cleavage. However, the extent of DNA cleavage became obviously weakened when the ionic strength from 50 to 800 mM. The results suggest that electrostatic interactions contribute to the DNA cleavage.

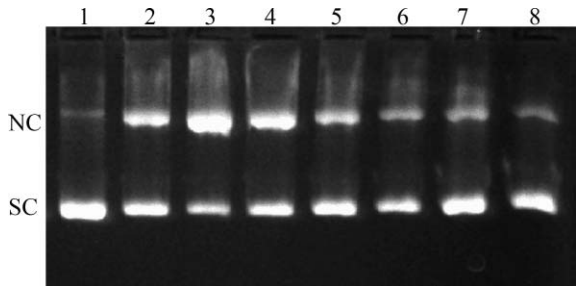

Fig. 7 Agarose gel (1%) of pBR322 DNA incubated for 1.5 h at 37 °C and pH 7.2 with 100 μM quercetin nickel(II) complexes for increasing NaCl concentrations. Lane 1: DNA control; lane 2–8: NaCl concentration was 10, 25, 50, 100, 200, 400, 800 mM, respectively.

Effects of time on DNA cleavage. The time dependence of the reaction was carried out in the presence and the absence of the complex, and pBR322 DNA was incubated with 100 μM complex in 50 mM Tris-HCl buffer at 37 °C for 15–240 min, respectively (Fig. 8). The increase in the amounts of nicked DNA was observed to be associated with the increase of reaction time. The amounts of nicked DNA were 57.5% and 65.3% when the reaction time was 3 h and 4 h, respectively. Increasing the incubation beyond 8 h led to the appearance of linear DNA (data not shown).

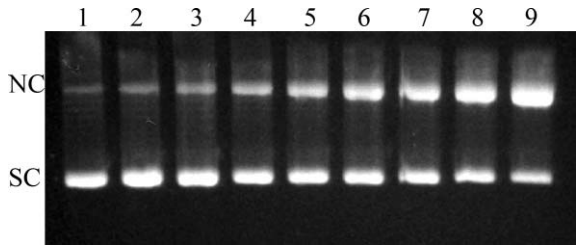

Fig. 8 Agarose gel (1%) of pBR322 (0.25 μg) at 37 °C and pH 7.2 (50 mM Tris-HCl) with 100 μM quercetin nickel(II) complex for increasing reaction time. Lane 1: DNA control; lane 2–9: 15, 30, 60, 90, 120, 150, 180, 240 min, respectively.

Fig. 9 shows the extent of DNA cleavage by $Ni(Que)_2(H_2O)_2$ complex with reaction time. The decrease in SC and the formation of NC of DNA with time shows 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 (0.76×10^{-4} s^{-1}), using a complex concentration of 100 μM (Fig. 9, inset).

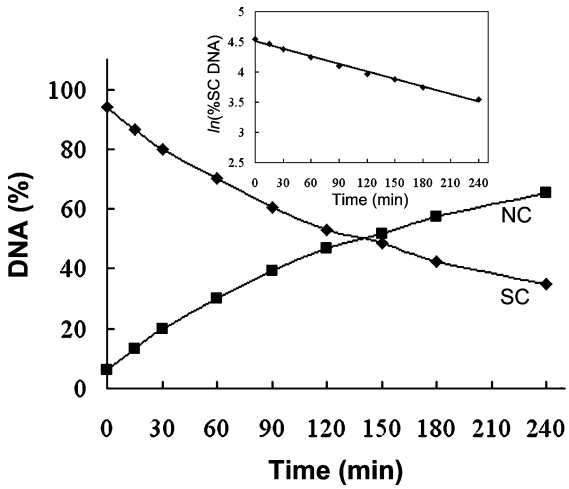

Fig. 9 Disappearance of supercoiled (SC) and formation of nicked circular (NC) forms of pBR322 DNA in the presence of quercetin nickel(II) complex (100 μM) with incubation time (pH 7.2, Temperature 37 °C). Inset: Plot of ln (%SC-DNA) *versus* time for the complex concentration of 100 μM.

Mechanism of DNA cleavage. To investigate the mechanism of DNA cleavage promoted by $Ni(Que)_2(H_2O)_2$ complex, hydroxyl radical scavengers (0.4 M DMSO and glycerol), catalase (15 units), and oxidant (50 μM H_2O_2) were introduced to the system. As shown in Fig. 10, no evident inhibition of DNA cleavage was observed in the presence of scavengers, which suggested that

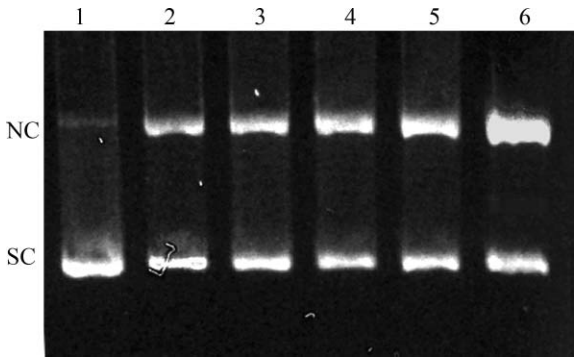

Fig. 10 Agarose gel (1%) of pBR322 (0.25 μg) incubated for 1.5 h at 37 °C and pH 7.2 (50 mM Tris-HCl) with 100 μM quercetin nickel(II) complex and different scavengers or H_2O_2. Lane 1: DNA control; lane 2: complex control lane 3: DMSO (0.4 M); lane 4: glycerol (0.4 M); lane 5: hydrogen peroxide (50 μM); lane 6: catalase (15 units).

hydroxyl radical and hydrogen peroxide might not occur in the reaction. And 50 μM 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 the complex may increase the formation of reactive oxygen species (ROS), we analyzed the influence of the complex on the oxidative damage of CT DNA by measuring the formation of 2-thiobarbituric acid reacting species (TBARS). It can be seen from Fig. 11 that the absorbance of a sample treated by complexes is very small compared with that of a sample treated by $[Fe(EDTA)]^{2-}/H_2O_2$. It suggested that the deoxyribose ring in DNA skeleton was not cut by ROS and oxidative cleavage of DNA did not occur.

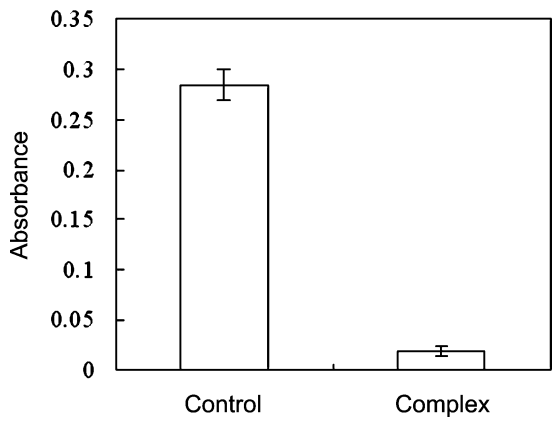

Fig. 11 The formation of 2-thiobarbituric acid reacting species (TBARS). Control: $[Fe(EDTA)]^{2-}/H_2O_2$. Each value represents the mean ± SD of three experiments.

According to the experimental results above, we suppose simply that the mechanism of DNA cleavage induced by $Ni(Que)_2(H_2O)_2$ complex should be a cooperative process of the nickel cation and the ligand molecules (quercetin or H_2O), involved in the binding of the complex with DNA. The sketch of the hydrolysis mechanism is depicted in Fig. 12. The complex intercalating into DNA base pairs could promote the direct coordination between nickel cation and the negatively charged oxygen in the phosphodiester backbone of DNA. The newly formed metal–oxygen bonds possibly include

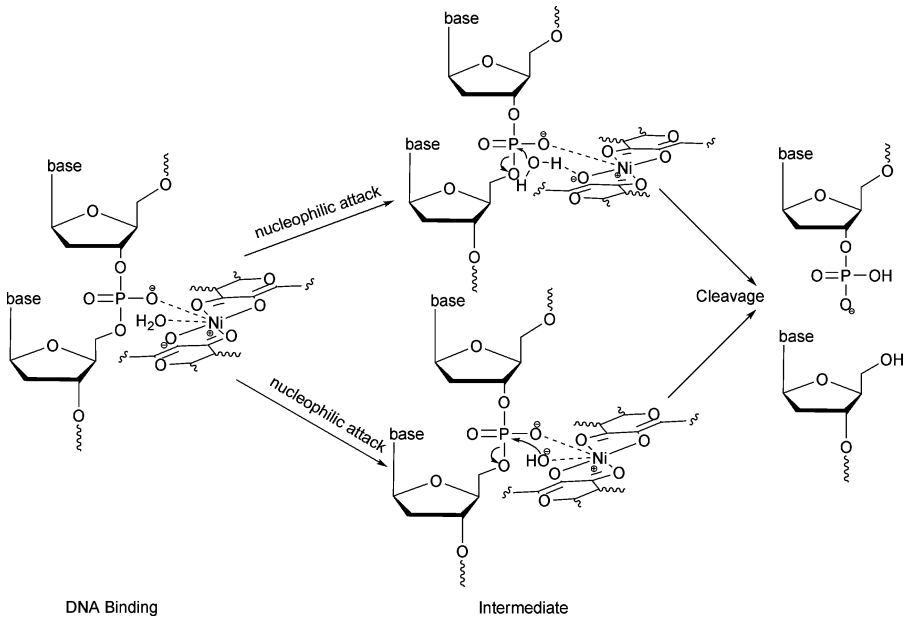

Fig. 12 Proposed mechanism of hydrolytic cleavage of DNA by quercetin nickel(II) complex.

electrostatic interactions which enhance the electrophilicity of the phosphorus, so the phosphorus becomes active enough to be target of nucleophilic attack. There are two possible nucleophilic species in the complex–DNA system. One is a water molecule linked to the proximate hydroxyl oxygen of the quercetin ligand. The hydroxyl oxygen of the quercetin ligand, acting as a Lewis base, pulls a proton from the water molecule to promote its attack the phosphorus atom. The other is coordination water linked to the nickel cation in the complex. The aquo-hydroxo form of the complex is formed when a proton is removed from one of the metal-coordinated water molecules in the complex. Then, the activated phosphorus atom could be nucleophilically attacked by these two active nucleophilic species, resulting in the formation of a pentacoordinated intermediate. Finally, one of the P–O ester bonds of the phosphodiester in the DNA backbone is broken by the intramolecular charge delivery, resulting in the DNA cleavage. However, the real hydrolysis mechanism needs to be confirmed through further detailed research.

Conclusion

In the present study, the DNA-binding properties of $Ni(Que)_2(H_2O)_2$ complex have been examined by absorption spectroscopy, fluorescence spectroscopy and viscosity measurements. Evidence is presented that $Ni(Que)_2(H_2O)_2$ complex could interact with DNA *via* intercalation mode. Furthermore, in the EB competition fluorescence assay, the Stern–Volmer quenching constant for this complex, K_{sq}, is 1.0, illustrating that this complex could strongly bind to DNA as an intercalator competing with EB. DNA cleavage of the complex has been studied by agarose gel electrophoresis and TBARS assay. It was demonstrated that the complex can effectively promote the cleavage of plasmid DNA at physiological pH and temperature *via* a hydrolytic pathway. Kinetic data of DNA cleavage promoted by the complex (100 μM) under physiological conditions give an observed rate constant k_1 of 0.76×10^{-4} s^{-1}, which shows a 10^7-fold rate acceleration over that of uncatalyzed supercoiled DNA. DNA cleavage activity of $Ni(Que)_2(H_2O)_2$ complex may be explained by the mechanism proposed for DNA hydrolysis that a pentacoordinated intermediate could be formed due to the active nucleophilic species nucleophilically attacking phosphorus atom in the phosphodiester backbone of DNA.

In conclusion, we have determined that the intercalation binding modes of $Ni(Que)_2(H_2O)_2$ complex with DNA and the hydrolytic cleavage of DNA by the complex. Bioflavonoid, derived from a variety of natural resources, can basically chelate metal ions to form metal complexes, which could be a new source of artificial metallonucleases, even antitumor agents. The selective DNA binding mechanism, the potential of the complexes as artificial nucleases and cytotoxicity activity of the complex are under investigation.

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Notes and references

1 Y. M. Song, Q. Wu, P. J. Yang, N. N. Luan, L. F. Wang and Y. M. Liu, *J. Inorg. Biochem.*, 2006, **100**, 1685.
2 M. Kožurková, D. Sabolová, L. Janovec, J. Mikeš, J. Kovaľ, J. Ungvarský, M. Stefanisinová, P. Fedorocko, P. Kristian and J. Imrich, *Bioorg. Med. Chem.*, 2008, **16**, 3976.
3 C.-P. Tan, J. Liu, L.-M. Chen, S. Shi and L.-N. Ji, *J. Inorg. Biochem.*, 2008, **102**, 1644–1653.
4 G. Zuber, J.C. Jr. Quada and S. M. Hecht, *J. Am. Chem. Soc.*, 1998, **120**, 9368.
5 S. M. Hecht, *J. Nat. Prod.*, 2000, **63**, 158.
6 C. Metcalfe and J. A. Thomas, *Chem. Soc. Rev.*, 2003, **32**, 215.

7 A. Silvestri, G. Barone, G. Ruisi, M. T. Lo Giudice and S. Tumminello, *J. Inorg. Biochem.*, 2004, **98**, 589.
8 M. Navarro, E. J. Cisneros-Fajardo, A. Sierralta, M. Fernández-Mestre, P. Silva, D. Arrieche and E. Marchán, *JBIC, J. Biol. Inorg. Chem.*, 2003, **8**, 401.
9 J. Tan, B.-C. Wang and L.-C. Zhu, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1197.
10 P. Nordell and P. Lincoln, *J. Am. Chem. Soc.*, 2005, **127**, 9670.
11 J. Tan, B.-C. Wang and L.-C. Zhu, *Colloids Surf., B*, 2007, **55**, 149–152.
12 A. N. R. Pattubala, N. Munirathinam and R. C. Akhil, *Eur. J. Inorg. Chem.*, 2004, **2007**, 1440.
13 N. Ademir, T. Hernán, H. Rosmari, H. Adolfo, Jr., S. Bruno and S. Juliet, *Inorg. Chem. Commun.*, 2001, **4**, 388.
14 A. Nègre-Salvayre and R. Salvayre, *Free Radical Biol. Med.*, 1992, **12**, 101.
15 I. B. Afanasev, A. I. Dorozhko, A. V. Brodskii, V. A. Kostyuk and A. I. Potapovitch, *Biochem. Pharmacol.*, 1989, **38**, 1763.
16 I. Morel, G. Lescout, P. Cogrel, O. Sergent, N. Posdeloup, P. Brissot, P. Cillard and J. Cillard, *Biochem. Pharmacol.*, 1993, **45**, 13.
17 J. Zhou, L.-F. Wang, J.-Y. Wang and N. Tang, *Transition Met. Chem.*, 2001, **26**, 57.
18 J. Tan, B.-C. Wang and L.-C. Zhu, *Bioorg. Med. Chem.*, 2009, **17**, 614.
19 G. Cohen and H. Eisenberg, *Biopolymers*, 1969, **8**, 45.
20 W.-Y. Li, S.-T. Zhu and X.-W. He, *Acta Chim. Sinica*, 2002, **60**, 105.
21 J. R. Lakowicz and G. Weber, *Biochemistry*, 1973, **12**, 4161.
22 J. Liu, T.-X. Zhang, T.-B. Lu, L.-H. Qu, H. Zhou, Q.-L. Zhang and L.-N. Ji, *J. Inorg. Biochem.*, 2002, **91**, 269.
23 D. L. Boger, B. E. Fink, S. R. Brunette, W. C. Tse and M. P. Hedrick, *J. Am. Chem. Soc.*, 2001, **123**, 5878.
24 S. Satyanarayana, J. C. Dabrowiak and J. B. Chaires, *Biochemistry*, 1992, **31**, 9319.
25 T. Hirohama, Y. Kuranuki, E. Ebina, T. Sugizaki, H. Arii, M. Chikira, P. T. Selvi and M. Palaniandavar, *J. Inorg. Biochem.*, 2005, **99**, 1205.
26 Y. Masataka, H. Miyako, N. Makoto and M. Keiko, *Mol. Genet. Metab.*, 1999, **68**, 468.

