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Oxidative damage to DNA by 1,10-phenanthroline/L-threonine copper (II) complexes with chlorogenic acid

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Abstract The oxidative DNA damage by copper (II) complexes in the presence of chlorogenic acid was explored using agarose gel electrophoresis. The extent of pBR322 DNA damage was enhanced significantly with increasing concentration of [Cu-phen-Thr] complex and incubation time. A fluorescence quenching activity of calf thymus DNA–EB was observed more remarkably with chlorogenic acid than without chlorogenic acid. The fluorescence measurements suggested that [Cu-phen-Thr] complex not only can bind to DNA by intercalation but also can damage the double strand DNA in the presence of chlorogenic acid. Further, 8-hydroxy-2′-deoxyguanosine, a biomarker of DNA oxidative damage was determined by electrochemical method. The control experiments revealed that the structure of copper (II) complexes affected capability of complex to DNA damage. The planar structure copper (II) complex showed high efficiency to DNA damage. The chlorogenic acid as biological reductant could improve copper (II) complex to DNA damage. A mechanism on [Cu-phen-Thr] complex to DNA damage in the presence of chlorogenic acid was proposed.

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Abbreviations

phen	1,10-phenanthroline
Thr	L-Threonine
CGA	Chlorogenic acid
TBE	Tris-boracic acid-EDTA
EB	Ethidium bromide
8-Hydroxy-2′-deoxyguanosine	8-OHdG
•OH	Hydroxyl radical

Introduction

Oxidants with free-radical character were well-known instigators of DNA damage. The group includes certain activated antibiotics, metal complexes, redox-active metalloenzymes, oxygen metabolites, and molecules ionized by high-energy radiation. The sugar-phosphate backbone was highly vulnerable to attack. Abstraction of a hydrogen atom from deoxyribose produced a carbon-based sugar radical that could rearrange, culminating in scission of the nucleic acid strand. The heterocyclic bases of nucleic acids were more important sites of free radical-mediated alteration (Wolkenberg and Boger 2002). Especially, guanine was the most pregnable

site because it has the lowest oxidation potential of the four common DNA bases and its radical cation (formed by loss of an electron) was usually involved in oxidative reactions (Chifotides and Dunbar 2005; Sidorenko and Zharkov 2008). In duplex DNA, guanine radical cations react with water to form mainly 8-hydroxy-2′-deoxyguanosine (8-OHdG, a biomarker of DNA oxidative damage) (Liu and Schuster 2003). Significantly, these lesions, if unrepaired, were generally considered to be deleterious and could contribute to mutagenesis, carcinogenesis, aging, inherited disease, and cell death. The DNA-scission ability of different metallonucleases was largely determined by the reactivity of reactive species (ROS), which could stem from Fenton-type reaction in the complex system mixed with glutathione or ascorbic acid as reducer (Kelly et al. 2007; Silveira et al. 2008).

Synthetic nucleases have been developed in recent years and have been useful in the treatment of disease and in research as probes of macromolecular structure (Wang et al. 1991; Mancin et al. 2005; West and Marnett 2006; Pellestor et al. 2008).Complexes showing oxidative scission of DNA have the potential for footprinting and therapeutic application (Sasmal et al. 2008). Since Sigma discovered that copper ion complexed to 1,10-phenanthroline [Cu-$(phen)_2$] showed efficient scission activity to DNA in the presence of thiol and hydrogen peroxide (Sigman et al. 1979), copper complexes of 1,10-phenanthroline and its derivatives have drawn great interest. They exhibit numerous biological activities such as antitumor, antimicrobial activity (Katsarou et al. 2008). Moreover, considerable attention has been focused on the use of phenanthroline complexes as intercalating agents of DNA and as artificial nucleases (Sigman et al. 1993). Recently, a new type of ligand with two phenanthrolines bridged on their C_2 or C_3 carbon by a short flexible arm, namely Clip-phen, were prepared by Meunier and coworkers (Pitié et al. 2003), and its corresponding copper complexes cleaved DNA more efficiently than [Cu-$(phen)_2$] in the presence of reductants. Amino acids were the basic structural units of proteins, and the introduction of amino acids in the copper complex gave rise to potential selective interaction with DNA, which might result in the variation of nuclease activity of Cu–phen system (Alemón-Medina et al. 2007).

Chlorogenic acid (3-caffeoyl-D-quinic acid; CGA) was an ester formed between caffeic acid and quinic acid, and was a prominent plant polyphenol compound found in various agricultural products (Oszmianski and Lee 1990). CGA, like other catechol derivatives, was a excellent antioxidant, possessing powerful radical scavenging property in a variety of vitro model systems (Guglielmetti et al. 2008), because the one-electron oxidation product of CGA formed by the reaction with free radicals is rapidly broken down to further products that cannot generate any free radicals (Shibata et al. 1999). However, there was a report in the literature that CGA exhibited prooxidative property and induced dna damage under certain conditions as well as other catechol derivatives (Miura et al. 2000). Purportedly, the CGA and Cu (II)-mediated DNA damage was determined by the method of agarose gel electrophoresis (Zheng et al. 2008). Recent report from our laboratory have also shown that the autooxidative activity of CGA and damage to DNA (Yang et al. 2008).

Accordingly, the present work aimed at exploring the activity of copper (II) complexes and giving a better understanding of the mechanism of oxidative damage to DNA. The DNA damage was mediated by Cu (II) complexes in the presence of CGA as biological reductant and was detected using agarose gel electrophoresis and fluorescence spectrum. At the appropriate concentration and incubating time, DNA can be efficiently damaged. In the damage system, the hydroxyl radical was the most important reactive species. It attacked the most pregnable guanosine, thus 8-hydroxy-2′-deoxyguanosine can be determined by electrochemical method. The experimental results revealed that the system of [Cu-phen-Thr] complex mixed with CGA for DNA damage is highly efficient.

Materials and methods

Materials

The sources of materials used in this work as follows: Cu-$(phen)_2$, Cu-$(L\text{-}Thr)_2$ and [Cu-phen-Thr] complex were synthesized according to literature (Chikira et al. 2002). Chlorogenic acid was from Acros, EB and double stranded supercoiled plasmid pBR322 were from MBI. Catalase, superoxide dismutase (SOD) and calf thymus double-stranded DNA

(CT-DNA) from Sigma was used without further purification. All other chemicals were of analytical reagents grade. Milli-Q water was utilized to prepare all the solution.

Detection of plasmid pBR322 DNA damage by agarose gel electrophoresis

The pBR322 plasmid DNA strand breaks were measured by the conversion of supercoiled plasmid double-stranded DNA to open circular and linear forms (Kelly et al. 2007). Briefly, the pBR322 plasmid DNA was incubated with Cu (II) complexes and CGA at 37°C for 60 min. The 20 μl of mixture contained 0.125 μg pBR322 DNA, 5.0×10^{-4} M CGA, and different concentrations of Cu (II) complexes in 10.0 mM Tris–HCl-1 mM EDTA (pH 7.4). Following incubation, the samples were immediately loaded on a 1% agarose gel, and electrophoresed for 60 min. After electrophoresis, the gels were visualized and photographed with Image-Master Video Documentation System (Ultra-Violet Products Ltd., UK). Quantification of closed-circular and nicked DNA was performed using the UVP software and the results were shown in a bar graph.

Fluorescence measurements

A serial test solution containing 10.0 mM Tris–HCl buffer solution, 1.0 mM EDTA, 1.0×10^{-4} M CT-DNA, 5.0×10^{-4} M CGA, different concentration [Cu-phen-Thr] complex were incubated at 37°C for 60 min under aerobic condition. After incubation, a 5 μl of 7.5 mg/ml EB was added into every solution and the fluorescence spectrum was recorded. Another serial test solution only without CGA, other component same as above mention solution were treated with the same process. The fluorescence measure was performed on an F-4500 spectrofluorimeter (Hitachi, Japan) under excitation at 510 nm and emission at 610 nm. The quenching data were analyzed by the Stern–Volmer equation: $F_0/F - 1 = \underline{K}_{sv}\,[Q]$, where $[Q]$ is the molar concentration of the [Cu-phen-Thr] complex, F_0 and F are the fluorescence intensity in the absence and in the presence of [Cu-phen-Thr], respectively, and K_{sv} is the Stern–Volmer quenching constant. The loss of the fluorescence was used as a measure of DNA damage (Barcelo et al. 1986).

Electrochemical determination of 8-OHdG

Determination of 8-OHdG was similar to the described previously based on differential pulse voltammetry (Yang et al. 2008). All voltammetric measurements were performed with the CHI660A electrochemical analyzer (Shanghai Chenhua Apparatus, China). The three-electrode system used in this work contained a glassy carbon electrode (GCE, Φ3 mm), a platinum wire counter electrode and an Ag/AgCl (sat KCl) as reference electrode. For differential pulse voltammetry (DPV), a 2 mg/ml of DNA in 10 mM Tris–HCl (pH 7.4), was incubated with 1.0×10^{-3} M [Cu-phen-Thr] and 5.0×10^{-4} M solution of CGA at 37°C for 60 min. Then, some 20.0 μl solution containing different components was covered on surface of the treated GCE to dry in the desiccator for 6 h to form different working electrodes. These solutions were DNA, 8-hydroxy-2′-deoxyguanosine and incubation solution, respectively.

Results and discussion

DNA damage by [Cu-phen-Thr] complex and CGA

The ability of the complexes to mediate pBR322DNA damage was explored using agarose gel electrophoresis at physiological pH and temperature conditions. The mix solution containing [Cu-phen-Thr)] complex, DNA were incubated in the presence of CGA as a reducing agent for certain time. The electrophorogram of pBR322 DNA damage in presence of different concentrations of the [Cu-phen-Thr] complex incubated for 60 min were shown in Fig. 1a. The treated pBR322 DNA showed two bands on agarose gel electrophoresis. The foremost moving band corresponded to the native form of supercoiled circular DNA (abbreviated as SC) and the slower moving band was the open circular form (abbreviated as OC). Double stranded supercoiled structure of plasmid pBR322 DNA with a relatively high electrophoretic mobility is disrupted upon formation of strand breaks, resulting in an open-circle conformation with a reduced electrophoretic mobility in agarose (Rahman et al. 1989). From Fig. 1a, the pBR322 DNA damage could be observed in the

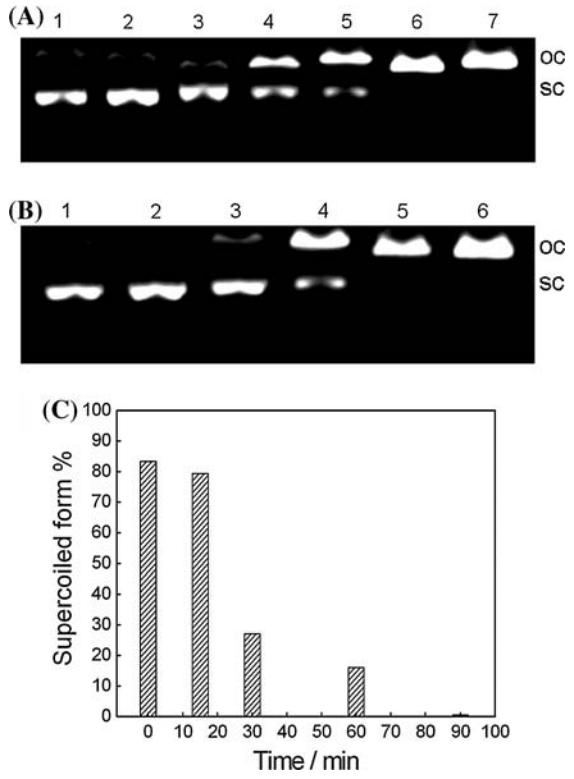


Fig. 1 pBR322 DNA strand breakage was detected by agarose gel electrophoresis. Supercoiled plasmid DNA (0.125 μg) was incubated with different concentrations of [Cu-phen-Thr] complex in 10 mM Tris–HCl-1 mM EDTA (pH 7.4) contained 0.5 mM CGA at 37°C for 1 h. (**a**) Native DNA (lane 1); [Cu-phen-Thr] complex/CGA: 0, 0.1, 0.3, 0.5, 1.0, 2.0 mM/0.5 mM (lanes 2–7). (**b**) The pBR322 DNA was treated at 37°C with different time. Native DNA (lane 1); [Cu-phen-Thr] complex/CGA = 2.0 mM/0.5 mM, incubating time for 0, 15, 30, 60, 90 min (lanes 2–6). (**c**) Scanning densitometry results of the gel picture shown in B

presence of [Cu-phen-Thr] complex and CGA. However, DNA damage was not observed on electrophorogram in the absence of [Cu-phen-Thr] complex (see Fig. 1a, lane 2). The amount of DNA damage markedly was enhanced with increasing the concentration of [Cu-phen-Thr] complex (Fig. 1a, lanes 3–7). The scission efficiency of [Cu-phen-Thr] complex reached about 100% at a concentration of 1.0×10^{-3} M in converting SC to OC (see Fig. 1a, lane 6), which indicated that [Cu-phen-Thr] complex was a potent DNA cleavage agent in the presence of CGA as a reducing agent. The influence of incubation time on DNA damage also was examined and the electrophorogram of pBR322 DNA damage varying with incubation time was shown in Fig. 1c. With elongating reaction time, converting efficiency of SC to OC was improved (see Fig. 1b, lane 3–6).

In order to describe the extent of pBR322 DNA damage, the percentage of net DNA scission was calculated by the following equation: $f_s = [SC]/(0.8[OC] + [SC]$ (Fresco et al. 1995). The amount of supercoiled DNA was quantified by densitometry. The influence of incubation time on the percentage of net DNA scission was shown in Fig. 1c. This result implied that the conversion of SC to OC trended to completion when incubation time exceeded 90 min in our experiment.

Control experiments

In order to explore the mechanism of cooperative effects on pBR322DNA damage by [Cu-phen-Thr] complex in the presence of CGA, a series of control experiments were set up. For example, copper chloride, Cu-(L-Thr)$_2$, Cu-(phen)$_2$ were used to instead of [Cu-phen-Thr] complex. The obtained electrophorogram was shown in Fig. 2a. When the

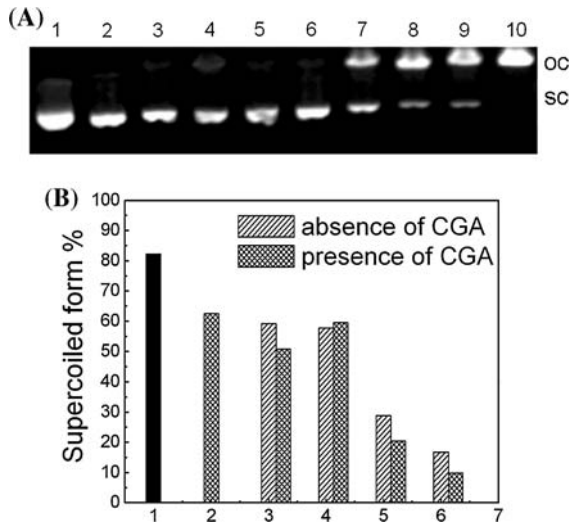


Fig. 2 Agarose gel electrophorogram of pBR322 DNA was incubated with different Cu (II) complexes in the absence and presence of 0.5 mM CGA in 10 mM Tris–HCl-1 mM EDTA (pH 7.4) at 37°C for 1 h. (**a**) Native DNA (lane 1); without complex (lane 2); in the absence of CGA: Cu^{2+} (lane 3), Cu-(L-Thr)$_2$ (lane 5), Cu-(phen)$_2$ (lane 7) and [Cu-phen-Thr] complex (lane 9); in the presence of CGA: Cu^{2+} (lane 4), Cu-(L-Thr)$_2$ (lane 6), Cu-(phen)$_2$ (lane 8) and [Cu-phen-Thr] complex (lane 10). (**b**) Scanning densitometry results of the gel picture shown in A

Cu^{2+} ion was instead of [Cu-phen-Thr] complex, a slight DNA breakage from SC to OC was observed on electrophorogram (lane 4). This result manifested that Cu^{2+} ion could cause pBR322DNA damage weakly in the presence of CGA. The [Cu-phen-Thr] complex (lane 10) could cause pBR322 DNA damage more significantly than Cu-$(phen)_2$ (lane 8) did in the presence of CGA. To the same Cu^{2+} ion or Cu (II) complexes, the extent of DNA damage was stronger in the presence of CGA than in the absence of CGA (lanes 3, 7, 9). This result revealed that CGA as a reducing agent could improve pBR322 DNA damage by Cu^{2+} ion or Cu (II) complex. However, the Cu-$(L\text{-}Thr)_2$ did not cause any DNA strand breakage both in absence of CGA and in the presence of CGA.

The oxidative DNA-damage induced by metallonucleases often proceeds via redox cycles between different oxidation states of the metal ions. Therefore, the redox potential is a useful index for the evaluation of the scission ability. The formal potential, $E_{1/2}$, was determined as the average of the peak potentials Epc and Epa. Cyclic voltammetric experiments were performed to measure Epc and Epa of the selected compound. The values of $E_{1/2}$ obtained were in the following order: Cu^{2+} (0.0065 V) > Cu-$(phen)_2$ (−0.045 V) > Cu-$(L\text{-}Thr)_2$ (−0.047 V) > [Cu-phen-Thr] complex (−0.098 V). Although the most effective complex is the one with the highest redox potential (Hirohama et al. 2005), ligands play a key role in the damage effect. The planar structure such as phen can strengthen the DNA binding potential of metallonuclease by intercalation (Wang et al. 2004). The favorable effect of the planar group was evident and the results pointed out that the DNA damage promotion by CGA and [Cu-phen-Thr] complex-induced systems were specific, due to 1,10-phenanthroline having planar heterocyclic bases structure (Dülger et al. 2000). Moreover, it's capable of DNA-intercalation/binding in the presence of auxiliary ligands. The selection of L-threonine as a second ligand in the ternary [Cu-phen-Thr] complex may enhance the affinity of the complex towards DNA because of the formation of hydrogen bonds between the hydroxyl group of L-threonine and DNA double helix and increase the biocompatibility of the complex (Harda et al. 1996). The percentage of net DNA scission by different Cu^{2+} ion or Cu (II) complex in the presence and absence of CGA were shown in Fig. 2b.

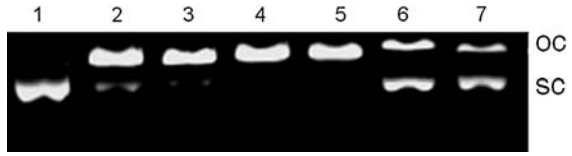


Fig. 3 Effects of scavengers on DNA damage in the presence of 0.5 mM CGA. Native DNA (lane 1); 1 mM [Cu-phen-Thr] complex (lane 2); 1 mM [Cu-phen-Thr] complex + SOD 2 U/μl (lane 3); 1 mM [Cu-phen-Thr] complex + catalase 2 U/μl (lane 4); 1 mM [Cu-phen-Thr] complex + 30 mM sodium azide (lane 5); 1 mM [Cu-phen-Thr] complex + 30 mM mannitol (lane 6); 1 mM [Cu-phen-Thr] complex + 30 mM DMSO (lane 7)

Effects of scavengers in the damage of pBR322DNA

To further examine the essential roles of [Cu-phen-Thr] complex and CGA in process of DNA damage, several active oxygen species scavengers were added into the reaction systems. The obtained agarose gel electrophorogram of pBR322 DNA was shown in Fig. 3. Transition metal ions in a variety of ligand environments could play a key role in the redox cycle to form primary and secondary reactive oxygen species (ROS). Most reactive intermediates were produced in an aerobic environment or in the presence of co-oxidants (Jiang et al. 2007). The sequential reduction of molecular oxygen can generate a group of ROS such as superoxide anion ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH). The •OH radical was an extremely strong and highly diffusible oxidant with redox potential of 2.8 V, which can mediate DNA damage by adding to double bonds of DNA bases or abstracting hydrogen atoms from sugar moiety (Marnett et al. 2003).

As shown in Fig. 3, the scavengers of hydroxyl radical-mannitol and dimethyl sulphoxide (DMSO) exhibited inhibition on DNA scission markedly (lanes 6, 7). Whereas, SOD (capable of catalyzing the reduction of superoxide anions to hydrogen peroxide) and catalase (catalyses conversion of hydrogen peroxide to water and molecular oxygen) did not exhibit significant inhibition effect (lanes 3, 4), so did the scavenger of singlet oxygen-sodium azide (lane 5). Considered as the control experiments, [Cu-phen-Thr] complex may firstly interact with DNA by intercalation to form Cu (II)-DNA species, which then be reduced to Cu (I)-DNA by reductant with the generation of hydroxyl radical. The hydroxyl radical

attacked DNA and thus caused the DNA strands scission availably.

Fluorescence quenching activity of [Cu-phen-Thr] complex and CGA by CT-DNA

Copper-dependent oxidative DNA damage can also be quantitatively measured by the ethidium bromide-binding assay. The assay was based on the fact that a highly fluorescent complex was formed between native DNA and the intercalating agent ethidium bromide (EB). Several forms of DNA lesions, including strand scission, base oxidation, and base liberation, were believed to contribute to the loss of fluorescence (Milne et al. 1993). Using CT-DNA as a model, DNA damage by [Cu-phen-Thr] complex was explored with fluorescence method. A series of experiment with increasing concentration of [Cu-phen-Thr] complex in the presence of fixed concentration of CGA were carried out. The fluorescence emission spectrum was given in Fig. 4. When the concentration of the complex was increased in the presence of CGA ($K_{sv} = 15.63 \times 10^4\ M^{-1}$), fluorescence intensity of CT-DNA-EB decreased more remarkably than without CGA ($K_{sv} = 7.72 \times 10^4\ M^{-1}$). It indicated that the complex not only can bind to DNA but also can damage the double strand DNA in the presence of CGA. Such feature was found in DNA interaction by the intercalative mode and the structure–activity relationship obtained from this experiment is in consistent with that obtained from the strand breakage of pBR322 DNA by gel electrophoresis mentioned above.

Determination of 8-OHdG in CT-DNA treated with CGA and [Cu-phen-Thr]

Considering deoxyguanosine (dG) as the easiest site to oxidate the four nucleosides, its primary oxidation product, 8-OHdG has been analysed deeply (Cadet et al. 1999). Here, it was determined by electrochemical method as a potential biomarker of oxidative DNA damage. The solution of [Cu-phen-Thr] complex with CT-DNA was incubated and was not incubated in the presence of CGA, and then a 20.0 μl solution was covered on the treated GCE surface to form different working electrodes. The DPV curve of the prepared working electrode in 0.10 M acetate buffer solution (ABS, pH 6.0) was recorded as shown in Fig. 5. Before incubated (curve c), there were three oxidation peaks occurring on the voltammogram,

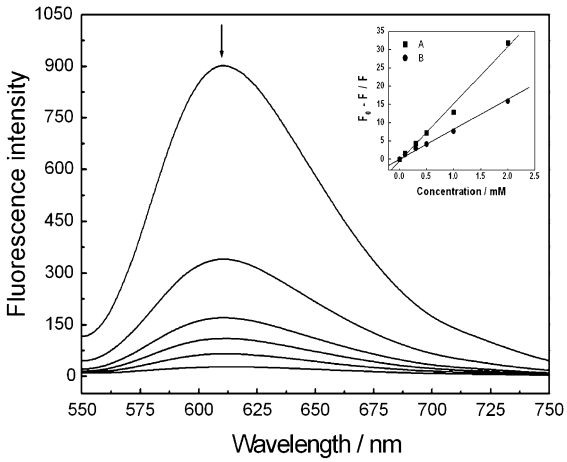


Fig. 4 Extent of calf thymus DNA damage induced by different concentration of [Cu-phen-Thr] complex (0, 0.1, 0.3, 0.5, 1, 2 mM) in the presence of CGA. The inset shows the Stern–Volmer plot of quenching of the fluorescence of CT-DNA-EB system by [Cu-phen-Thr] complex in the presence (**a**, $K_{sv} = 15.63 \times 10^4\ M^{-1}$) and absence (**b**, $K_{sv} = 7.72 \times 10^4\ M^{-1}$) of CGA. The fluorescence emission spectrum was exited at 510 nm

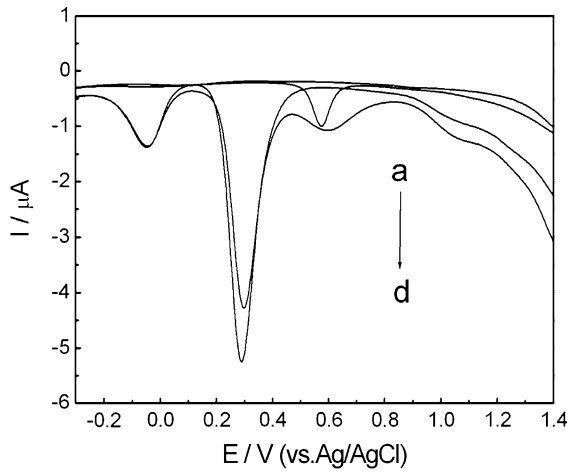


Fig. 5 DPV curves obtained in 0.10 M ABS (pH 6.0) with different solutions preparing electrodes: (**a**) CT-DNA solution, (**b**) 8-hydroxy-2′-deoxyguanosine solution, (**c**) in the presence of CGA, solution containing [Cu-phen-Thr] complex and CT-DNA was not incubated, (**d**) in the presence of CGA, solution containing [Cu-phen-Thr] complex and CT-DNA was incubated at 37°C for 1 h. Effects of scavengers on DNA damage induced by 1 mM [Cu-phen-Thr] complex in the presence of 0.5 mM CGA

$E_{pa1} = -0.05$ V, $E_{pa2} = +0.29$ V and $E_{pa3} = 1.05$ V, respectively. The former oxidation peak attributed to [Cu-phen-Thr] complex, the second peak was resulted from chlorogenic acid and the last peak may be caused by chlorogenic acid o-quinone. While the solution was incubated at 37°C for 60 min, a new oxidation peak ($E_{pa} = +0.58$ V) appeared at curve d. It was neither contributed to the direct oxidation of double strand DNA bases nor originated from the oxidative peak of CGA and [Cu-phen-Thr] complex. Thus, we speculated that this oxidation peak was possibly due to the oxidation peak of 8-OHdG that occurred after DNA was damaged. In order to enable the identification of the hypothesis, a control experiment was performed using a GCE covered with 8-OHdG film. There was an oxidation peak appeared at $E_{pa} = +0.58$ V on the voltammogram (shown in Fig. 5, curve b). This oxidation peak corresponded to oxidation of 8-OHdG on electrode in 0.10 M ABS (pH 6.0). Hence, it was confirmed that the oxidation peak at $E_{pa} = +0.58$ V (shown in Fig. 5, curve d) was oxidation peak of double strand DNA damage product. The results revealed that the hydroxyl radical attacked to double strand DNA preferentially at guanosine site, leading to oxidative damage and formation of 8-OHdG.

Considerations on DNA damage mechanism

The intrinsic property of Cu (II) played an important role in the scission ability of metallonucleases. The oxidative DNA-cleavage induced by metallonucleases often proceeds via redox cycles between different oxidation states of the metal ions. The DNA-cleavage ability of a series of Cu-based nucleases highly depends on the Cu (II)/Cu (I) redox potential. Moreover, some structural features are also important to metallonuclease activity. Ligand planarity has major impact on the mechanism of DNA-cleavage, the planar structure such as phen can strengthen the DNA binding potential of metallonuclease by intercalation. The damage ability of Cu (II) complexes is as follows: [Cu-phen-Thr] > Cu-$(phen)_2$ > Cu-$(L\text{-}Thr)_2$, which suggested the larger planar structure enhanced the DNA-damage efficiency. The former ligand contains a planar group while the last contains a non-planar one. As above, the introduction of L-threonine in the [Cu-phen-Thr] complex gives rise to potential selective interaction with DNA and

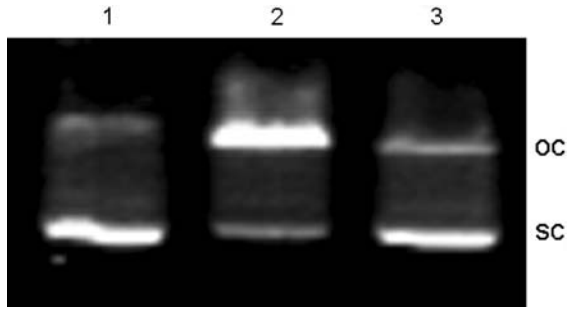


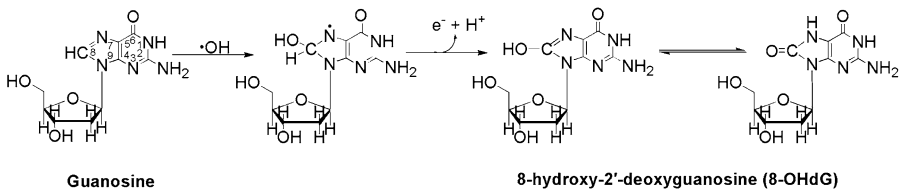
Fig. 6 The role of oxygen in the process of oxidative DNA damage in the presence of 0.5 mM CGA. Native DNA (lane 1); 1 mM [Cu-phen-Thr] complex (lane 2); 1 mM [Cu-phen-Thr] complex solution was deoxygenated by N_2 for 20 min (line 3)

increases the biocompatibility of the complex, which may result in the variation of nuclease activity of Cu–phen system. These differences lead to different binding mode with DNA and scission capability of the three Cu (II) complexes to DNA. Additionally, in order to demonstrate the involvement of oxygen in the process of DNA damage, [Cu-phen-Thr] complex mixed with CGA and DNA were incubated under anaerobic conditions. The result showed that the signal of DNA damage almost disappeared (Fig. 6 line 3). So, oxygen plays an important role in the process of oxidative DNA damage. DNA strand scission may be caused by •OH generated from the Fenton reaction of Cu (II) complexes with biological reductant under aerobic conditions. In this process, that Cu (II) complex was reduced to Cu (I) complex by CGA and the re-oxidation of Cu (I) complex to Cu (II) complex was accompanied by the formation of hydroxyl radical. It mediated DNA damage may involve the following two steps: the addition of •OH to the C-8 of guanine first and then the subsequent loss of an electron plus proton from the intermediate to form 8-OHdG (shown in Scheme 1). The mechanism of oxidative scission of DNA by [Cu-phen-Thr] complex with CGA can be conjectured based on the above (shown in Scheme 2).

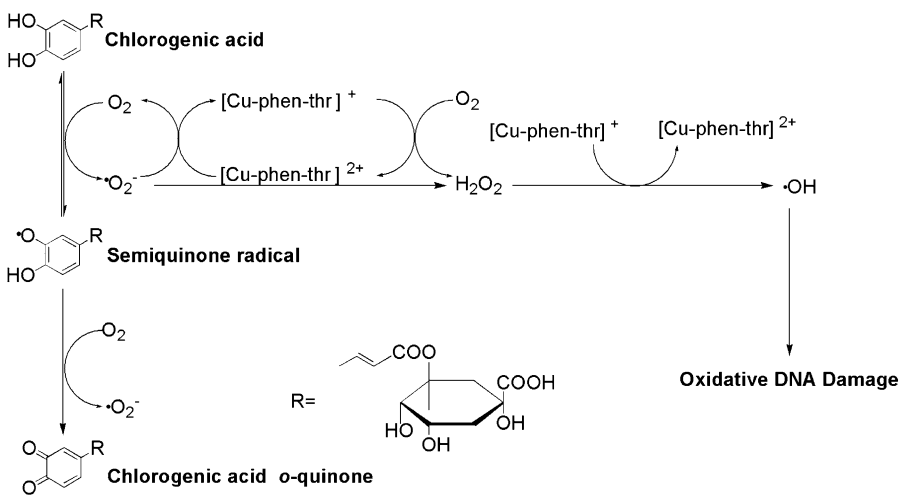
Conclusions

The [Cu-phen-Thr] complex could induce DNA damage in the presence of CGA as a biological reductant. 8-Hydroxy-2′-deoxyguanosine as a

Scheme 1 Possible mechanism to explain hydroxyl free radical mediated 8-OHdG formation



Scheme 2 Possible mechanism of oxidative DNA damage induced by [Cu-phen-Thr] complex in the presence of CGA



potential biomarker of oxidative DNA damage was determined by electrochemical method. The extent of DNA damage was enhanced markedly with increasing concentration of copper (II) complex and incubation time. The structure of copper (II) complexes affected capability of complex inducing DNA damage. The planar structure copper (II) complex showed high efficiency to damage. The [Cu-phen-Thr] complex could intercalate into double strand DNA and a hydroxyl radical as the active species was generated in process of reaction in the presence of CGA. Then hydroxyl radical interacted with double strand DNA preferentially at guanosine site, leading to oxidative damage and formation of 8-hydroxy-2′-deoxyguanosine.

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