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# Copper (II) complex of 1,10-phenanthroline and L-tyrosine with DNA oxidative cleavage activity in the gallic acid

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**Abstract** Copper (II) complex of formulation [Cu–Phen–Tyr]($H_2O$)]($ClO_4$) (Phen = 1,10-phenanthroline, L-Tyr = L-tyrosine), has been prepared, and their induced DNA oxidative cleavage activity studied. The complex binds to DNA by intercalation, as deduced from the absorption and fluorescence spectral data. Scatchard plots constructed from the absorption titration data gave binding constant $2.44 \times 10^4\ M^{-1}$ of base pairs. Extensive hypochromism, broadening, and red shifts in the absorption spectra were observed. Upon binding to DNA, the fluorescence from the DNA–ethidium bromide system was efficiently quenched by the copper (II) complex. Stern–Volmer quenching constant $0.61 \times 10^3\ M^{-1}$ obtained from the linear quenching plots. [Cu–Phen–Tyr] complex efficiently cleave the supercoiled DNA to its nicked circular form with gallic acid as biological reductant at appropriate complex concentration. The gallic acid as reductant could observably improve copper (II) complex to DNA damage. The pseudo-Michaelis–Menten kinetic parameters ($k_{cat}$, $K_M$) were calculated to be $1.32\ h^{-1}$ and $5.46 \times 10^{-5}\ M$ for [Cu–Phen–Tyr] complex. Mechanistic studies reveal the involvement of superoxide anions and hydroxyl radical ($HO^{\cdot}$) as the reactive species under an aerobic medium.

**Keywords** Copper (II) complexes · Gallic acid · DNA oxidative cleavage

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## Introduction

Molecules able to irreversibly modify nucleic acids have received considerable interest because of their potential applications as biological tools or chemotherapeutic agents. Transition metal complexes with their tunable coordination environments and versatile redox and spectral properties have been used for oxidative cleavage of DNA and as models for the chemotherapeutic agents. Typical examples of these DNA cleavers are iron-bleomycin, Fe(II)–EDTA, Mn(III)–porphyrin, nickel complexes, Co, Rh and Ru complexes of phenanthroline or bipyridine and $[Cu(Phen)_2]^{2+}$ (Pogozelski and Tullius 1998; Burrows and Muller 1998; Chen et al. 2001; Chifotides and Dunbar 2005; Pizarro and Sadler 2009). Moreover, Copper plus excess 1, 10-phenanthroline represent one of the most well studied families of DNA damaging agents, [Cu $(OP)_2$] in the presence of molecular oxygen and a reducing agent, acts as an effective DNAase on double stranded DNA (Sigman et al. 1979; Marshall et al. 1981). Copper complexes of 1,10-phenanthroline in the presence of auxiliary ligands and their derivatives are also widely used

chemical nucleases employed as footprinting reagents for determining ligand binding sites and recently also for therapeutic method (Sigman et al. 1993; Pitié et al. 2005). Amino acids, as auxiliary ligands, are the basic structural units of proteins. Copper ions and various amino acids are widely distributed in biological systems such as cells or body fluids, and some copper complexes of amino acids were reported to exhibit potent antitumor and artificial nuclease activity (Alemón-Medina et al. 2007).

Oxidative cleavage of DNA by metal complexes can be achieved in the presence of external reagents, while common reductant were ascorbic acid, mercaptopropionic acid (MPA), polyphenol and S(IV) complexes (Patwardhan and Cowan 2001; Jin and Cowan 2005; Lainé et al. 2004; Thyagarajan et al. 2006; Alipázaga et al. 2008). Recent report from our laboratory has shown that 1, 10-phenanthroline/L-threonine copper (II) complexes with chlorogenic acid as biological reductant can induce DNA oxidative damage (Wang et al. 2010). Gallic acid (GA), a naturally occurring plant phenol, was also found to be a strong antioxidant in emulsion or lipid systems (Hirose et al. 1993). However reactive oxygen species formed by the reactions of GA and $Cu^{2+}$ participates in the DNA damage (Kobayashi et al. 2004).

In this context, we focus our attention on the preparation of [Cu–Phen–Tyr] complex and the oxidative DNA cleavage with GA as biological reductant. The interaction has been investigated using a host of physical methods like electronic absorption spectroscopy, competitive ethidium bromide (EB) studies. The cleavage reactions have been assayed using agarose gel electrophoresis. The significant results revealed that the system of [Cu–phen–Tyr] complex mixed with GA for DNA damage is highly efficient.

## Materials and methods

### Materials

[Cu–phen–Tyr] complex were synthesized according to literature (Patra et al. 2009). Gallic acid was from Acrōs, 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.

### UV absorption spectrum

Absorption spectrum was recorded on a U-3010 spectrophotometer (Hitachi, Japan). The UV absorbance at 260 and 280 nm of the CT-DNA solution in Tris–HCl buffer (10 mM, pH 7.4) gave a ratio of $\sim$1.9, indicating that the DNA was free of protein (Reichmann et al. 1954). The DNA concentration per nucleotide was determined by measuring the UV absorption at 260 nm, taking the molar absorption coefficient ($\xi_{260}$) of CT-DNA as 6600 $M^{-1}$ $cm^{-1}$ (Wolfe et al. 1987). The absorption titrations were performed by mixing various proportions of [Cu–Phen–Tyr] complex and DNA in Tris–HCl buffer (10 mM, pH 7.4) while maintaining the total volume of the solution constant (2 ml). This resulted in a series of solutions with varying concentrations of DNA but with a constant concentration of [Cu–Phen–Tyr] complex. The molar ratio of complex and DNA, R was monitored at 0, 0.13, 0.26, 0.53, 0.80, and 1.07. To correct the absorbance of DNA itself, equivalent amount of DNA was taken in the reference cell. The intrinsic binding constant of [Cu–Phen–Tyr] complex with calf thymus DNA was determined by absorption titrations. In the case of the former, the absorbance at 272 nm was recorded after each addition of CT-DNA. The intrinsic binding constant $K_b$ was calculated from the following Eq. 1 (Kumar and Asuncion 1993):

$$[DNA]/\Delta\xi_{aP} = [DNA]/\Delta\xi + 1/K_b\Delta\xi \quad (1)$$

$\Delta\xi_{ap} = (\xi_a - \xi_f)$, $\Delta\xi = (\xi_b - \xi_f)$, where $\xi_a$ is the extinction coefficient observed for the charge-transfer absorption band at a given [Cu–Phen–Tyr] complex concentration, $\xi_f$ is the extinction coefficient of the free complex, $\xi_b$ is the extinction coefficient of the complex when fully bound to DNA, respectively. The data were fitted to equation, with a slope equal to $1/\Delta\xi$ and a $y$-intercept equal to $1/\Delta\xi K_b$. $\xi_b$ was determined from $\Delta\xi$, and $K_b$ was obtained from the ratio of the slope to the $y$-intercept.

### Fluorescence spectrum

The assay was based on the fact that a highly fluorescent complex was formed between native

DNA and the intercalating agent 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). A serial TE buffer (10 mM Tris–HCl–1 mM EDTA, pH 7.4) containing CT-DNA ($2.0 \times 10^{-4}$ M), GA($5.0 \times 10^{-4}$ M), EB ($6.25 \times 10^{-5}$ M) and different concentration [Cu–Phen–Tyr] complex were incubated at 37°C for 60 min under aerobic condition. After incubation, the fluorescence spectrum was recorded. Another serial test solution only without GA, 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 600 nm. The quenching data were analyzed by the Stern–Volmer equation:

$$F_0/F - 1 = K_{sv}[Q] \quad (2)$$

where $[Q]$ is the molar concentration of [Cu–Phen–Tyr] complex, $F_0$ and $F$ are the fluorescence intensity in the absence and in the presence of [Cu–Phen–Tyr] complex, 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).

Data from the fluorescence titrations were also used to determine the binding constant of [Cu–Phen–Tyr] complex with EB–DNA. The solutions were excited at 510 nm, and the emission was monitored at 600 nm. The concentration of the free EB was determined using Eq. 3:

$$C_F = C_r(F/F_0 - P)/(1 - P) \quad (3)$$

where $C_T$ is the concentration of EB added, $C_F$ is the concentration of the free EB, and $F$ and $F_0$ are the fluorescence intensities in the presence and in the absence of [Cu–Phen–Tyr] complex, respectively. $P$ is the ratio of the observed fluorescence quantum yield of the bound EB to that of the free EB. The value of $P$ was obtained from a plot of $F/F_0$ versus 1/[[Cu–Phen–Tyr] complex] such that it is the limiting fluorescence yield given by the $y$-intercept. The amount of bound EB ($C_B$) at any concentration was equal to $C_T - C_F$. A plot of $r/C_F$ vs. $r$, where $r$ is equal to $C_B$/[[Cu–Phen–Tyr] complex], was constructed according to the modified Scatchard Eq. 4 given by McGhee and von Hippel (McGhee and von Hippel 1974). $K_i$ is the intrinsic binding constant and $n$ is the binding site size in base pairs.

$$r/C_F = K_i(1 - nr)[(1 - nr)/[1 - (n - 1)r]]^{n-1} \quad (4)$$

## Detection of plasmid pBR322 DNA damage by agarose gel electrophoresis

A typical reaction was carried out by mixing 0.5 μl of pBR322 DNA (0.125 μg, 4361 bp), GA($2.0 \times 10^{-4}$ M) and different concentrations of [Cu–Phen–Tyr] complex solution in TE buffer (10 mM Tris–HCl–1 mM EDTA, pH 7.4) to yield a total volume of 20 μl. After mixing, the sample was incubated at 37°C for 60 min. The reactions were quenched at appropriate time by the addition of loading buffer (0.25% bromphenol blue, 50% glycerol). Then, the solution was subjected to electrophoresis on 1.0% agarose gel in 1× TBE buffer (45 mM Tris–boracic acid/1 mM EDTA) at 100 V for 60 min. After electrophoresis, the Image-Master Video Documentation System (Ultra-Violet Products Ltd., UK) was assessed using labworks imaging. Densitometric calculations were made using the analysis method in UVP software. The intensities of supercoiled pBR322 DNA were corrected by a factor of 1.42 as a result of its lower staining capacity by EB (Bernadou et al. 1989). The decrease of form I was fitted to a single-exponential decay curve (pseudo-first-order kinetics) by use of Eq. 5a or b, where $y_0$ is the initial percentage of a form of DNA, $y$ is the percentage of a specific form of DNA at time $t$, $a$ is the percentage of uncleaved DNA, and $k_{obs}$ is the cleavage rate, or apparent rate constant, and $V_{max}$ is the maximal reaction velocity.

$$y = (y_0 - a)\exp(-k_{obs}x) + a \quad (5a)$$

$$y = (100 - y_0)(1 - \exp(-k_{obs}x)) \quad (5b)$$

$$k_{obs} = V_{max}[\text{complex}]/(K_M + [\text{complex}]) \quad (5c)$$

Further, based on the plots of $k_{obs}$ vs. concentrations of complex, the pseudo-Michaelis–Menten kinetic parameters were calculated. Careful optimization of electrophoretic and densitometric techniques led to clean pseudo-firstorder kinetics and allowed the determination of true Michaelis–Menten kinetic parameters by use of Eq. 5c; $k_{cat}$ is calculated as $V_{max}$/[complex], where concentration of complex = 200 μM (Sreedhara et al. 2000).

In the inhibition reactions, catalase (2 U/μl), SOD (2 U/μl), sodium azide (30 mM) and mannitol

(30 mM) was added to the solution containing pBR322 DNA (0.125 μg), GA ($2.0 \times 10^{-4}$ M) and [Cu–Phen–Ser] complex ($5 \times 10^{-4}$ M), respectively. The mixture was diluted with TE buffer (10 mM Tris–HCl–1 mM EDTA, pH 7.4) to a total volume of 20 μl. After the mixed solution was incubated at 37°C for 60 min, the sample was subjected for gel electrophoresis using the described procedures.

### Electrochemical experiments

All voltammetric measurements were performed with the CHI440A electrochemical analyzer (Shanghai Chenhua Apparatus, China). The three-electrode system used in this work contained a glassy carbon electrode (GCE, ∅3 mm), a platinum wire counter electrode and an Ag/AgCl (sat KCl) as reference electrode. Cyclic voltammogram (CV) was performed in a cell with 5 ml Tris–HCl buffer (0.1 mM, pH 7.4, 50 mM NaCl).

## Results and discussion

### Absorption studies

The electronic absorption spectra of [Cu–Phen–Tyr] complex in the presence of increasing amounts of CT-DNA showed strong decreases in the peak intensities (hypochromicity). The change in the absorbance of [Cu–Phen–Tyr] complex at 272 nm with increasing concentration of calf thymus DNA (CT-DNA) was used to construct the half-reciprocal plot shown in Fig. 1. A continuous decrease in the intensity of [Cu–Phen–Tyr] complex absorption was followed by saturation at high concentrations of DNA (inset in Fig. 1). The plot of the absorption titration data according to Eq. 1 gave a linear plot and resulted in an intrinsic binding constant ($K_b$) of $2.44 \times 10^6$ $M^{-1}$ in base pairs. Hypochromism was suggested to be due to a strong interaction between the electronic states of the intercalating chromophore and that of the DNA base (Barcelo et al. 1986). Since the strength of this electronic interaction is expected to decrease as the cube of the distance of separation between the chromophore and the DNA bases, the observed large hypochromism strongly suggests a close proximity of the aromatic chromophore of the ligand to the DNA bases. For example, intercalation of the aromatic

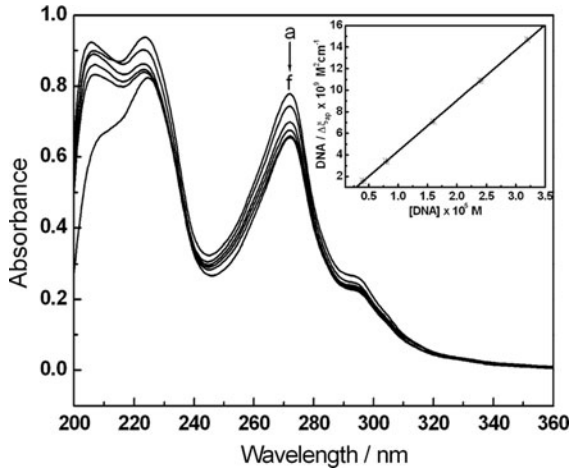



**Fig. 1** Absorption spectra of [Cu–phen–Tyr] complex ($2.0 \times 10^{-5}$ M) was measured upon addition of CT-DNA (R = 0, 0.13, 0.26, 0.53, 0.80, and 1.07). The inner plot of [DNA]/($\xi_a$-$\xi_f$) versus [DNA] shows the binding constant, $K_b = 2.44 \times 10^6$ $M^{-1}$

chromophore into the helix and strong overlap of the $\pi \rightarrow \pi^*$ states of the aromatic with the electronic states of the DNA bases are consistent with the observed spectral changes. In addition to the decrease in intensity, a small red shift was also observed in the spectra. These various spectral changes are consistent with the intercalation of [Cu–Phen–Tyr] complex into the DNA base stack.

### Fluorescence studies

The fluorescence intensity of EB-DNA decreases rapidly with increasing concentration of [Cu–Phen–Tyr] complex (Fig. 2 and the inset). The fluorescence quenching constant evaluated using the Stern–Volmer equation was $2.88 \times 10^3$ $M^{-1}$ (GA) and $0.61 \times 10^3$ $M^{-1}$ (without GA) of DNA phosphates. It indicated that the complex not only can bind to DNA but also can damage the double strand DNA in the presence of GA. Such feature was found in DNA interaction by the intercalative mode and the structure–activity relationship obtained from this experiment is in consistent with the result obtained from UV spectra. The quenching constants were comparable to the values reported for [Cu–Phen–Thr] complex ($K_{sv} = 15.63 \times 10^4$ $M^{-1}$). The result explained that [Cu–Phen–Tyr] complex has a stronger affinity than [Cu–Phen–Thr] complex (Wang et al. 2010), this resulted from L-tyrosine contained aromatic structure.

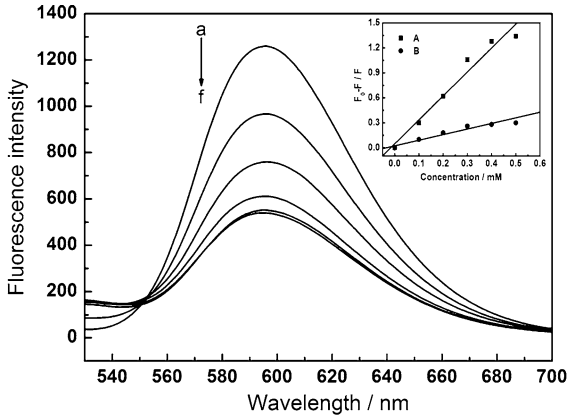



**Fig. 2** Extent of calf thymus DNA damage induced by different concentration of [Cu–phen–Tyr] complex (0, 50, 100, 200, 300, 400, 500 μM) in the presence of GA. The inset shows the Stern–Volmer plot of quenching of the fluorescence of CT-DNA–EB system by [Cu–phen–Tyr] complex (*A*, $K_{sv} = 2.88 \times 10^3\ M^{-1}$) in the presence and (*B*, $K_{sv} = 0.61 \times 10^3\ M^{-1}$) in the absence of GA. The fluorescence emission spectrum was exited at 510 nm

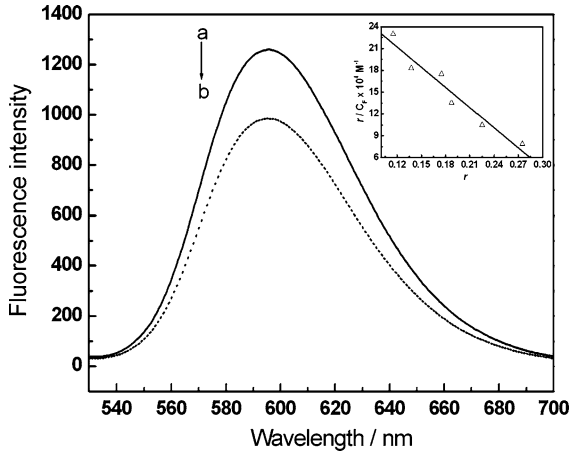



**Fig. 3** Normalized fluorescence spectra of CT-DNA-EB (*a*) in the absence and (*b*) in the presence of $2 \times 10^{-4}$ M [Cu–phen–Tyr] complex, with excitation at 510 nm. Inset: Scatchard plot of the fluorescence titration data, $K_i = 1.51 \times 10^6\ M^{-1}$of base pairs, $n = 5.8$ in base pairs

## Scatchard analysis of the fluorescence data

The fluorescence titration data were analyzed to construct the binding curves, and from these curves, binding constants as well as binding site sizes were estimated. A plot of $r/C_F$ vs. $r$ gave the binding curve, and best fit of the data to Eq. 4 resulted in a binding constant of $1.51 \times 10^6\ M^{-1}$of base pairs and a site size of 5.8 in base pairs (Fig. 3 and the inset). On the other hand, the absorption titration data gave an intrinsic binding constant ($K$) of $2.44 \times 10^6\ M^{-1}$, and the estimated value of $\xi_b$ was 21800 $M^{-1}\ cm^{-1}$, from these data. The binding constants obtained from the two methods are consistent, within the experimental error of the two different methods, and lend credibility to these measurements. The large binding constant observed for [Cu–Phen–Tyr] complex was indicative of the high affinity of the aromatic nucleus chromophore for the DNA base pairs. The binding parameter was comparable to the values reported for typical intercalators such as EB ($K_b$, $1.4 \times 10^6\ M^{-1}$ in 10 mM Tris–HCl/40 mM NaCl buffer, pH 7.9 (Lepecq and Paoletti 1967). These results indicate the complex has a very strong binding affinity to DNA.

## DNA cleavage

Supercoiled plasmid DNA cleavage by the Cu(II) complexes was studied in the presence of GA (Fig. 4a), and a time-dependent cleavage was observed (Fig. 4b). 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). We found that the supercoiled DNA was completely cleaved by [Cu–Phen–Tyr] complex after 1 h in the presence of GA. To the same Cu(II) complex concentration ($2.0 \times 10^{-4}$ M), DNA cleavage was not promoted by either [Cu–phen–Tyr] complex or GA alone (Fig. 4a, lanes 3 and 4). This result revealed that [Cu–phen–Tyr] complex was a potent DNA cleavage agent in the presence of GA as a reducing agent.

## Pseudo-Michaelis–Menten kinetics of DNA cleavage

A concentration-dependent cleavage was also observed (Fig. 5a). Reaction that leads to formation of open circular DNA (OC) from the supercoiled form (SC) over various concentrations of complex (50–300 μM) and constant DNA concentration was followed for for up to 60 min at 37°C. It is not an

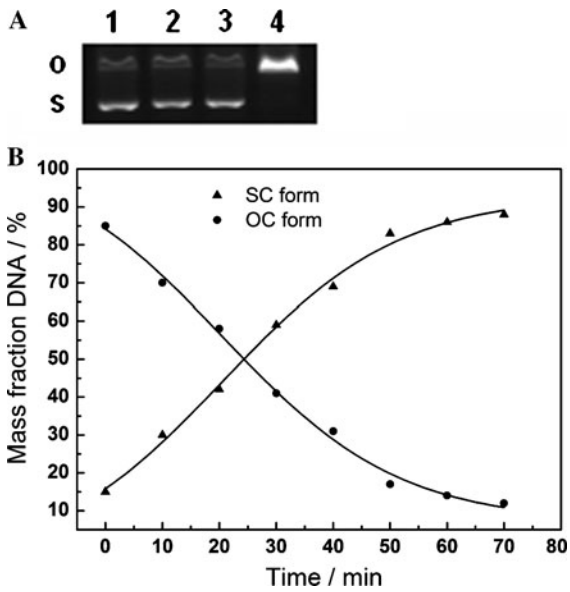



**Fig. 4** pBR322 DNA strand breakage was detected by agarose gel electrophoresis in 10 mM Tris–HCl–1 mM EDTA (pH 7.4). **a** Native DNA (*lane 1*); without complex (*lane 2*); in the absence of GA: [Cu–Phen–Tyr] complex (0.2 mM, *lane 3*); in the presence of GA: [Cu–Phen–Tyr] complex (0.2 mM, *lane 4*). **b** The pBR322 DNA was treated at 37°C with different time. Native DNA (*lane 1*); [Cu–Phen–Tyr] complex/GA = 200 μM/500 μM, incubating time for 0, 10, 20, 30, 40, 50, 60, 70 min

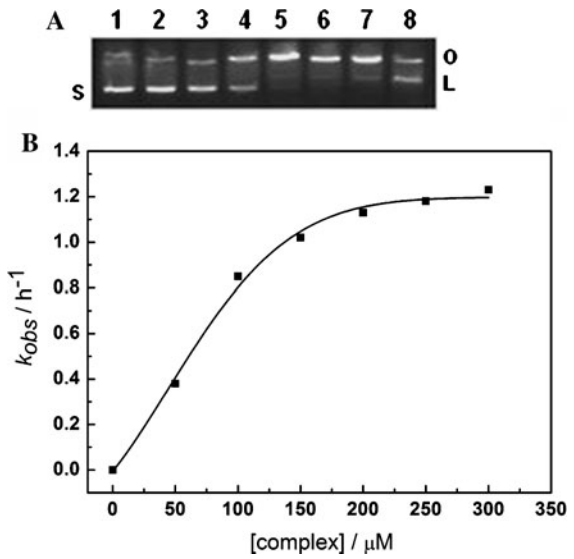



**Fig. 5** **a** Different concentrations of [Cu–Phen–Tyr] complex with 0.5 mM GA at 37°C for 1 h. Native DNA (*lane 1*); [Cu–Phen–Tyr] complex/GA: 0, 50, 100, 150, 200, 250, 300 μM/500 μM (*lanes 2–8*). **b** Saturation kinetics for the cleavage of plasmid DNA with 50–300 μM [Cu–Phen–Tyr] complex in the presence of 500 μM GA under conditions at 37°C, 10 mM Tris–HCl–1 mM EDTA, pH 7.40. The samples were incubated for 60 min, and the samples were run on a 1% agarose gel

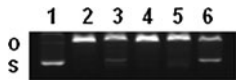



**Fig. 6** Effects of scavengers on DNA damage in the presence of 500 μM GA. Native DNA (*lane 1*); 200 μM [Cu–Phen–Tyr] complex (*lane 2*); 200 μM [Cu–Phen–Tyr] complex + SOD 2 U/μl (*lane 3*); 200 μM [Cu–Phen–Tyr] complex + catalase 2 U/μl (*lane 4*); 200 μM [Cu–Phen–Tyr] complex + 30 mM sodium azide (*lane 5*); 200 μM [Cu–Phen–Tyr] complex + 30 mM mannitol (*lane 6*)

easy task to control the reaction conditions, and extract good quality rate data from gels. However, the disappearance of SC with time followed pseudo-first-order kinetic profiles and can be well fitted with a single-exponential decay curve (Fig. 5b). Based on the plots of $k_{obs}$ versus concentration of complex, saturation kinetics of DNA cleavage was observed at high concentration of complexes. The pseudo Michaelis–Menten kinetic parameters ($k_{cat}$ and $K_M$) were calculated to be $1.32 \pm 0.03$ h$^{-1}$ ($R^2 = 0.991$) and $5.46 \times 10^{-5}$ M for [Cu–phen–Thr] complex. The obtained oxidative rate constant showed that [Cu–phen–Tyr] complex with GA have very high nuclease activity.

## Effects of scavengers

In order to clarify the DNA cleavage mechanism, several active oxygen species scavengers were added into the reaction systems. As shown in Fig. 6, the scavengers of hydroxyl radical–mannitol exhibited inhibition on DNA scission markedly (lane 6). Likewise, SOD (capable of catalyzing the reduction of superoxide anions to hydrogen peroxide) and catalase (catalyses conversion of hydrogen peroxide to water and molecular oxygen) exhibit weaker inhibition effect (lanes 3, 4), so did the scavenger of singlet oxygen-sodium azide (lane 5). Considered as the above experiments, [Cu–phen–Tyr] 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.

## Considerations on DNA damage mechanism

The intrinsic property of Cu(II) ion played an important role in the scission ability of metallonucleases. The oxidative DNA cleavage induced by [Cu–Phen–Tyr] complex proceeded via redox cycles between different oxidation states of the copper ion. Spectroscopic studies on [Cu–Phen–Tyr] complex interaction with DNA revealed the intercalation model resulted from the ligand has a planar structure. Moreover, the introduction of tyrosine in [Cu–Phen–Tyr] complex gave rise to potential selective interaction with DNA and increased the biocompatibility of the complex. Additionally, in order to demonstrate the involvement of oxygen in the process of DNA damage, [Cu–phen–Thr] complex mixed with GA and DNA were incubated under anaerobic conditions. The result showed that the signal of DNA damage almost disappeared (Fig. 7, line 3). So, oxygen plays an important role in the process of oxidative DNA damage. Likewise, influence of potential inhibitors also indicated that oxygen could play an important role in the process of oxidative DNA damage. DNA strand scission may be caused by $^{\cdot}OH$ generated from *Fenton* reaction of Cu(II) complexes with biological reductant under aerobic conditions. Electrochemical behaviours of [Cu–Phen–Tyr] complex and GA explained that a redox reaction occured between them (Fig. 8). In this process, [Cu–Phen–Tyr] complex may first interact with DNA by intercalation to form Cu(II)–DNA species, which then can further catalyze the initial step of GA oxidation, that is, GA was oxidized to a semiquinone radical anion with the formation of Cu(I)–DNA. The semiquinone radical anion would reduce molecular $O_2$ to superoxide anion concomitant with the formation of oxidized GA (*ortho*–quinone). Furthermore, Cu(I) reduced superoxide anion to hydrogen peroxide with

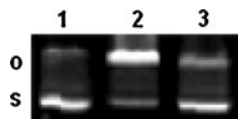



**Fig. 7** The role of oxygen in the process of oxidative DNA damage in the presence of 500 μM GA. Native DNA (*lane 1*); 200 μM [Cu–Phen–Tyr] complex (*lane 2*); 200 μM [Cu–Phen–Tyr] complex solution was deoxygenated for 30 min (*line 3*)

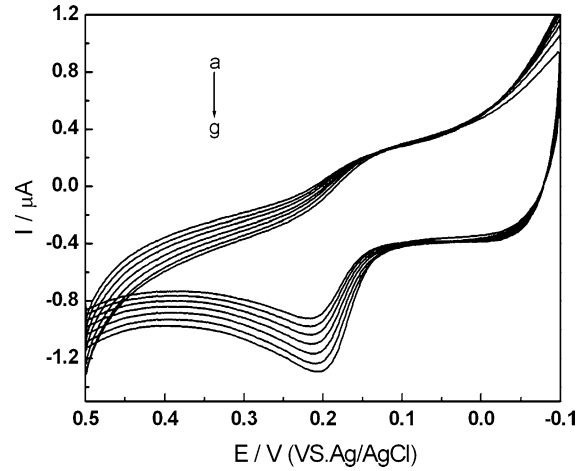
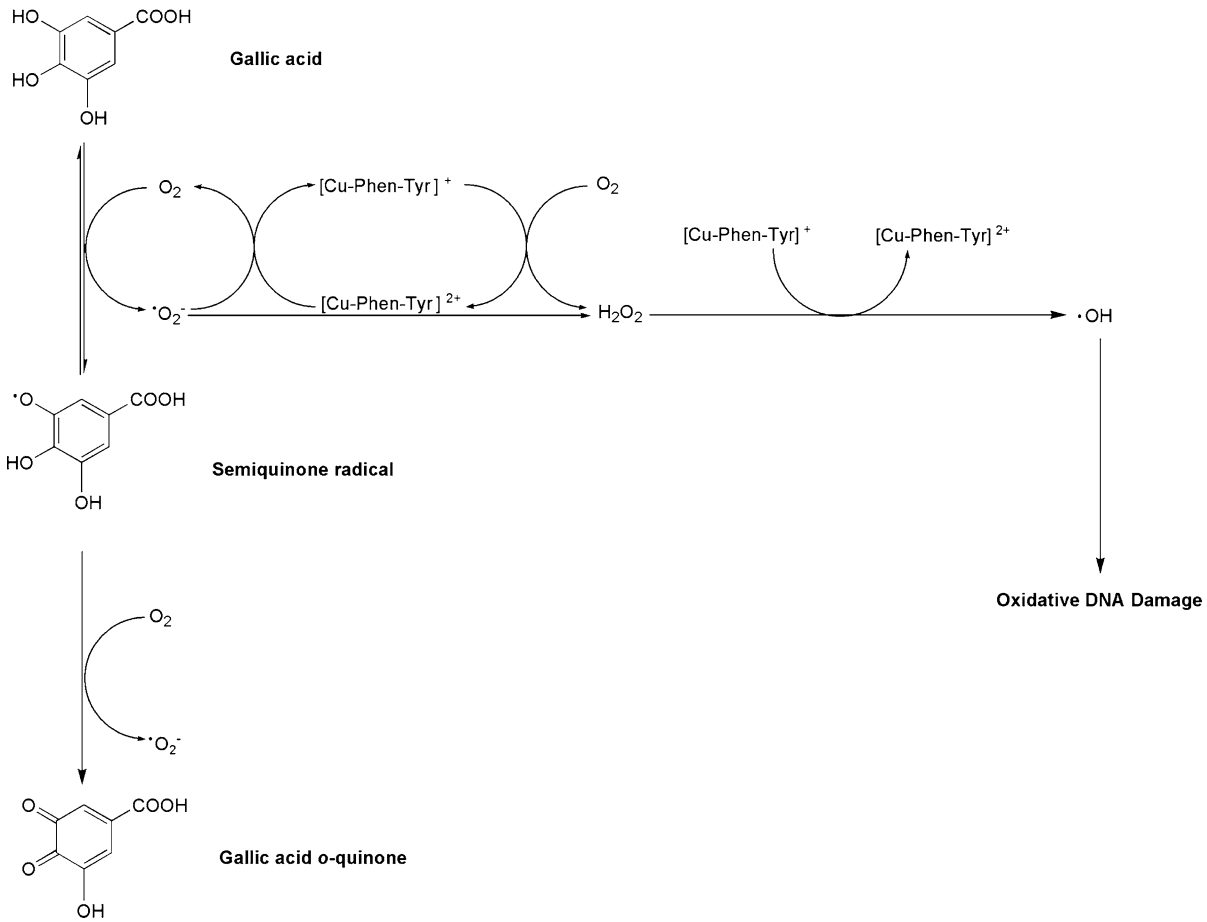



**Fig. 8** Cyclic voltammograms of 100 μM gallic acid in 0.10 M 0.1 M Tris–HCl buffer (pH 7.4) in the presence of (*a*) 0, (*b*) 0.2, (*c*) 0.4, (*d*) 0.6, (*e*) 0.8, (*f*) 1.0, (*g*) 1.2($\times$ $10^{-3}$ M) [Cu–Phen–Tyr] complex. Scan rate: 50 mV $s^{-1}$

the formation of Cu(II). Cu(II) could interact with hydrogen peroxide, generating a highly reactive hydroxyl radical. Protection of DNA from Cu(II) complex-mediated strand breaks by SOD and mannitol, provided evidence for the hypothesis that hydroxyl radicals produced from hydrogen peroxide by *Fenton* reaction. The hydroxyl radicals attacked DNA and thus caused the DNA strands scission. We concluded that the GA-mediated Cu(II)/Cu(I) redox cycle played a principal role in the generation of reactive oxygen species causing oxidative DNA. Based on the above, this mechanism of DNA oxidative scission has been conjectured that the following scheme was displayed (Scheme 1).

## Conclusions

The [Cu–phen–Tyr] complex exhibited very high DNA affinity and nuclease activity in the presence of GA as a biological reductant. The planar structure copper (II) 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 GA. This study may help in developing rationally engineered DNA cleavers in which anticancer drugs are combined with copper phenanthroline complexes.




**Scheme 1** Possible mechanism of DNA oxidative damage induced by [Cu–phen–Tyr] complex in the presence of GA

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