Probing DNA-Cleavage Efficiencies of Copper(II) Complexes: A Computational Perspective

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ABSTRACT: Theoretical studies on DNA-cleavage efficiencies of Cu(II) complexes 1−3 were carried out using density functional theory (DFT). The optimized Cu(II) complexes were allowed to bind to glutathiones (GSH) and ascorbic acids (VC) by the docking program so that corresponding docking structures can be obtained. To predict DNA-cleavage efficiencies, the docking structures of Cu(II) complexes with GSH and VC were further optimized by DFT. The activation energies of electrons from GSH to complexes, the redox potentials of these complexes, and binding energies of these complexes with GSH and VC were calculated. The efficiencies of complexes cleaving DNA were predicted and found to be in agreement with the experimental results. Finally, three occupied molecular orbitals of docking structures (GSH−complexes) were analyzed, and the DNA-cleavage abilities of complexes were also explained by the electron distribution on the three occupied orbitals. This work has important implications understanding the DNA-cleavage mechanism of Cu(II) complexes, which might be helpful for designing novel anticancer Cu(II) complexes for the future.

1. INTRODUCTION

Interactions of DNA with metal complexes have been widely investigated in the bioinorganic chemistry area. Since some metal complexes show high cytotoxicity, and thus they have been developed as metal-based drugs. As we all know, cancer, a malignant tumor, threatens human lives. The metal anticancer complexes, e.g., cisplatin, show anticancer activity and have been utilized to treat various forms of cancer, such as liver, ovarian, and lung cancers. However, their side effects, for example, myelosuppression, leukopenia, etc., cause damage to the patient’s body. Therefore, it is an imminent task for scientists to develop other metal anticancer drugs to kill cancer cells safely.

Copper, as a natural constituent of cells, participates in cupreins and multiple enzyme compositions in the body. Thus, the copper element plays key roles in living organisms. Since copper complexes can cleave DNA effectively, copper anticancer drugs might be promising alternatives to platinum-based drugs. Research shows that Cu(II) complexes induce DNA damage via the generated reactive oxygen species (ROS), such as singlet oxygen (\(1O_2\)), superoxide anion radical (\(O_2^-\)), hydroxyl radical (\(OH^\cdot\)), etc., and the change of Cu(II) to Cu(I) may be the main cause of ROS generation. A lot of glutathiones (GSH) and ascorbic acids (VC), as good reductants, reside in the body. As Cu(II) complexes are near DNA, Cu(II) is reduced to Cu(I) by GSH or VC around DNA. Such a process may cause much ROS generation, resulting in DNA-cleavage. The DNA-cleavage degree relies on the quantity of ROS, which is very important for cleaving DNA. Thus, how to predict the quantity of ROS and how to explain the reason for generation of ROS are key problems to be resolved.

To solve the problems mentioned above, Cu(II) complexes, i.e., CuL1Cl2, CuL2Cl2, and CuL3Cl2 (L1 = 4′-(3-methoxyphenyl)-2,2′:6′:2″-terpyridine, L2 = 4′-(4-methoxyphenyl)-2,2′:6′:2″-terpyridine, and L3 = 4′-(3,5-dimethoxyphenyl)-2,2′:6′:2″-terpyridine), were selected to carry out a density functional theory (DFT) study. Meanwhile, to facilitate the calculations, CuL1Cl2, CuL2Cl2, and CuL3Cl2 were replaced with \([CuL_1]^{2+}\) (1), \([CuL_2]^{2+}\) (2), and \([CuL_3]^{2+}\) (3), respectively. The structures of the studied Cu(II) complexes 1−3, GSH, and VC are shown in Figure 1.

2. RESULTS AND DISCUSSION

2.1. Redox Potentials of Complexes. The computed redox potentials of Cu(II) complexes 1−3 are given in Table 1.
It can be seen that the oxidation potentials of Cu(II) complexes 1–3 are 2.650, 2.598, and 2.621 V, respectively, significantly higher than those of many reported ruthenium complexes, e.g., [Ru(phen)$_3$]$^{2+}$ (1.27 V) and [Ru(bpy)$_2$mitatp]$^{2+}$ (1.30 V). As is well known, although both Cu(II) complexes and Ru(II) complexes damage DNA by the generated ROS, their DNA-cleavage mechanisms are significantly different. In the presence of light, the excited Ru(II) complexes cause generation of a large amount of ROS, resulting in DNA damage, whereas Cu(II) complexes cleave DNA via ROS without light irradiation. The primary reason may be differences between the oxidation potentials of metal complexes and reduction potential of DNA. For Ru(II) complexes, the small differences result in electrons moving with a slow speed from DNA to complexes. Only by light irradiation, the electron transfer (ET) speed can be accelerated, and thus these radical ions may be produced. Subsequently, the radical ions react with molecules around, such as O$_2$, H$_2$O, etc., causing generation of a large amount of ROS. Conversely, the big differences in Cu(II) complexes result in electrons moving with a high speed without light irradiation, that is, whenever Cu(II) complexes come in contact with DNA, fast-moving electrons from DNA to Cu(II) complexes may cause much ROS generation. The above view has been proved in experiments, i.e., fast-moving electrons can result in ROS generation. The ET speed between Cu(II) complexes and reduction molecules, e.g., GSH and VC, is the key point for cleaving DNA.

### 2.2. Binding Energies

The computed binding energies of Cu(II) complexes 1–3 with GSH and VC are given in Table 2. It can be seen that the binding energies of Cu(II) complexes 1–3 with GSH are $-249.0$, $-235.6$, and $-314.1$ kJ·mol$^{-1}$, respectively. After the basis set superposition error (BSSE) is corrected in vacuum, the binding energies of complexes 1–3 with GSH are $-167.7$, $-164.2$, and $-221.6$ kJ·mol$^{-1}$, respectively. Similarly, after corrections, the VC-binding energies of complexes 1–3 are $-141.6$, $-83.5$, and $-137.8$ kJ·mol$^{-1}$, respectively. It is quite obvious that the binding energies of complexes 1–3 with GSH are much stronger than those with VC. Such a result indicates that Cu(II) complexes 1–3 will show priority to bind to GSH. Hence, the interaction between Cu(II) complexes and GSH may be the cause of generation of a large amount of ROS.

### 2.3. DNA-Cleavage Efficiencies of Complexes

The computed $E_{\text{red}}$ of GSH is $-0.615$ V, and thus we obtained $\Delta G^0$ of Cu(II) complexes 1–3 by eq 4 (see the section Theory and Computational Methods). According to eq 3, the ET activation energies of complexes were calculated and are given in Table 3. It can be seen that ET activation energies of Cu(II) complexes 1–3 are $1913.7$, $3282.7$, and $1641.2$ kJ·mol$^{-1}$, respectively. This indicates that ET occurs much more easily between complex 3 and GSH relative to complexes 1 and 2. It means that ET between complex 3 and GSH gains the highest speed, whereas that between complex 2 and GSH is the slowest. The section “Calculations of Redox Potentials” above shows that fast-moving electrons lead to much active particle generation. Thus, we presumed that the interaction of complex 3 with GSH resulted in the most active particle generation, and thus, the ability of complex 3 toward cleaving DNA is the strongest. By contrast, the ability of complex 2 toward cleaving DNA is the weakest. Therefore, we predict theoretically that the trend in DNA-cleavage efficiencies ($\phi$) of Cu(II) complexes 1–3 is $\phi(3) > \phi(1) > \phi(2)$, in agreement with the experimental data. This further proves that the interaction between Cu(II) complexes and GSH is the cause of the large amount of ROS.

### 2.4. Molecular Orbital Analysis

On the basis of the optimized docking structures of complexes 1–3 with GSH in the ground state, three occupied molecular orbitals (HOMO, Table 2. Calculated Binding Energies, $\Delta E$ (BSSE Uncorrected), and $\Delta E^{\text{sp}}$ (BSSE Corrected) of Complexes 1–3 with GSH and VC (All Energies in kJ·mol$^{-1}$)

| comp. | $E_{\text{GSH(VC)-complex}}$ | $E_{\text{GSH(VC)}}$ | $E_{\text{complex}}$ | $\Delta E$ | $\Delta E^{\text{sp}}$ |
|-------|-----------------|-----------------|-----------------|---------|-----------------|
| 1$^a$ | $-10852579.3$ | $-3693862.3$ | $-7162944.1$ | $-249.0$ | $-167.7$ |
| 2$^a$ | $-10852580.8$ | $-369397.3$ | $-7162947.9$ | $-235.6$ | $-164.2$ |
| 3$^a$ | $-11153348.4$ | $-369397.4$ | $-7463639.9$ | $-314.1$ | $-221.6$ |
| 1$^b$ | $-8961063.9$ | $-1797909.6$ | $-7162948.1$ | $-206.2$ | $-141.6$ |
| 2$^b$ | $-8961015.5$ | $-1797924.3$ | $-7162956.6$ | $-134.6$ | $-83.5$ |
| 3$^b$ | $-9261766.3$ | $-1797917.4$ | $-7463647.7$ | $-201.2$ | $-137.8$ |

$^a$Expresses complexes with GSH. $^b$Expresses complexes with VC.
the quantity of ET from GSH to complex molecular orbitals have electrons on complex when, according to HOMO and HOMO-1, two occupied complexes and GSH is the cause of much ROS generation. Therefore, we predict that the order of DNA-cleavage abilities of Cu(II) complexes is more than that to complex 3. CONCLUSIONS

In this work, the DNA-cleavage properties of Cu(II) complexes 1–3 were studied by theoretical calculations. The binding energies indicate that Cu(II) complexes show priority to bind to GSH and the interaction between Cu(II) complexes and GSH is the cause of much ROS generation. Finally, the DNA-cleavage efficiencies of complexes 1–3 were predicted accurately by the electron-transfer activation energies. Moreover, the DNA-cleavage abilities of Cu(II) complexes 1–3 were also explained by the electron distribution on the three occupied molecular orbitals.

4. THEORY AND COMPUTATIONAL METHODS

4.1. Optimizations of Complexes. Our previous work shows that the basis set 6-31G(d) is suitable for the calculation of the copper atom, so complexes 1–3, GSH, and VC in the ground state were optimized at the B3LYP/6-31G(d) level in aqueous solution using the conductor-like polarizable calculation model (CPCM). The minimum energy states were confirmed by frequency computations.

4.2. Calculation of the Redox Potential. Based on the optimized geometries of complexes, Gibbs free energies were obtained, which were used as the Gibbs free energies in vacuo. In addition, based on the optimized molecular geometries, the Gibbs free energies in aqueous solution were obtained by single point computations with the SMD model at the B3LYP/6-31G(d) level. By our reported computational method, the redox potentials of complexes 1–3 were computed.

4.3. Molecular Docking and Optimization. Obtained structures of complexes 1–3 were further docked into GSH and VC using the Dock6.0 program. The box size, grid space, energy cutoff distance, and maximum orientation were set as 30 Å, 0.3 Å, 9999 Å, and 150 000, respectively. The other parameters for docking were set to default values. The obtained docking geometries of complexes with GSH and VC were further optimized at the level of B3LYP/6-31G(d) in aqueous solution with the CPCM model. Meanwhile, the minimum energy states were also confirmed by frequency computations.

4.4. Calculation of Binding Energies. The binding energies involve weak interactions of complexes with GSH and VC, which are difficult to be computed accurately by the DFT method. Considering dispersion interactions, the DFT-D3
method can deal with this problem effectively. To obtain accurate binding energies, the docking geometries of complexes with GSH and VC were further optimized at the B3LYP-D3/6-31G(d) level in aqueous solution with the CPCM model. The binding energies between complexes and GSH and VC were calculated in aqueous solution at the same level of theory. Taking GSH as an example, the calculations of binding energies were expressed by eq 1

$$\Delta E = E_{\text{GSH-complex}} - E_{\text{GSH}} - E_{\text{complex}}$$  \hspace{1cm} (1)$$

where \(\Delta E\) is the binding energy of the complex with GSH and \(E_{\text{GSH}}\), \(E_{\text{complex}}\), and \(E_{\text{GSH-complex}}\) are the energies of the GSH complex, the complex and the optimized docking structure (GSH–complex) in aqueous solution, respectively. Meanwhile, considering BSSE, the computed \(\Delta E\) values were corrected by the counterpoise method. Since the Gaussian 09 program does not support the BSSE correction in aqueous solution, in this work, the BSSE was corrected only in vacuum.

4.5. Calculations of Electron-Transfer Activation Energies. At present, many nonadiabatic electron transfer (ET) processes are usually dealt with the theory of the Marcus semi-classical model, and the rate constant of the ET reaction can be computed by eq 2

$$k_e = \frac{4\pi^2}{h} H_{\text{DA}}^2 \exp\left[-\frac{1}{4\lambda^2 RT}\right]$$  \hspace{1cm} (2)$$

where \(h\) is Planck’s constant, \(H_{\text{DA}}\) is the ET matrix element, \(T\) is the temperature, \(\lambda\) is the reorganization energy, and \(E_c\) is the electron-transfer activation energy. The \(E_c\) can be computed by eq 3

$$E_c = \left(\Delta G^0 + \lambda^2\right) / 4\lambda$$  \hspace{1cm} (3)$$

where \(\Delta G^0\) is the standard Gibbs free energy change of the ET reaction. According to reaction (5), \(\Delta G^0\) can be computed by eq 4

$$\Delta G^0 = -nF(E_{\text{ox}} - E_{\text{red}})$$  \hspace{1cm} (4)$$

where \(n\) is the number of ET, \(F\) is the Faraday constant, \(E_{\text{ox}}\) is the oxidation potential of the Cu(II) complex, and \(E_{\text{red}}\) is the reduction potential of GSH in this work.

4.6. Calculation of the Reorganization Energy \(\lambda\). Cu(II) complexes reduce to Cu(I) complexes when they come in contact with reduction compounds, such as GSH and VC. It means that the Cu(II) complex can easily get an electron taking \([\text{CuL}]^{2+}\) and GSH as example, the ET process can be represented by the following reaction

$$[\text{CuL}]^{2+} + \text{GSH} \rightarrow [\text{CuL}]^{+} + \text{GSH}^+$$  \hspace{1cm} (5)$$

In reaction (5), electrons transfer from GSH to the Cu(II) complex with a high speed. After the ET, the reorganization energy \((\lambda)\) can be computed from GSH (\(\lambda_1\)) and Cu(II) complex \((\lambda_2)\), i.e.,

$$\lambda = \frac{\lambda_1 + \lambda_2}{2}$$  \hspace{1cm} (6)$$

To calculate \(\lambda\), the optimized complex and GSH can be substituted in the place of the \([\text{CuL}]^{2+}\) and GSH in reaction (5), respectively. Similarly, the complex and GSH of the optimized docking structure can be substituted in the place of the \([\text{CuL}]^{2+}\) and GSH in reaction (5), respectively. We obtain the total energy \(E(GSH)\) at its optimized structure, and the total energy \(E(GSH^+)\) at its structure in the optimized docking structure. Besides, we calculate the energy \(E'(GSH)\) at the structure of GSH and the energy \(E'(GSH^+)\) at the structure of GSH. Thus, \(\lambda_1\) is calculated by

$$\lambda_1 = E'(GSH) + E'(GSH^+) - E(GSH) - E(GSH^+)$$  \hspace{1cm} (7)$$

Similarly, \(\lambda_2\) is calculated by

$$\lambda_2 = E'[\text{CuL}]^{2+} + E'[\text{CuL}]^{+} - E[\text{CuL}]^{2+} - E[\text{CuL}]^{+}$$  \hspace{1cm} (8)$$

we obtained \(\lambda\) via eqs 6–8.

All the calculations were performed using the Gaussian 09 program package.

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Notes

The authors declare no competing financial interest.

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