Spectroscopic studies on the binding properties of Zn-PP with various human telomeric G-quadruplex DNA and ct-DNA

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The binding property of Zn-PP with human telomeric G-quadruplex DNA as compared to binding with ct-DNA is examined by UV-vis, fluorescence and CD spectroscopic methods. UV-vis absorption spectral analysis in the presence of K+ shows that addition of three types of human telomeric G-quadruplex DNA (Hetel-G4, Hetel-1-G4, and Hetel-2-G4) in the Zn-PP solution induces moderate hypochromicity with intrinsic binding constants of $(3.13\pm0.45)x10^5$, $(1.47\pm0.12)x10^5$ and $(2.51\pm0.16)x10^5$ $M^{-1}$, respectively, while for ct-DNA, the $K_b$ value is $(9.94\pm0.32)x10^4$ $M^{-1}$. In the fluorescence emission spectral analysis, the addition of G-quadruplex DNA induces significant quenching of the fluorescence emission of Zn-PP with total quenching ratios of 26.31–36.84%, while the addition of ct-DNA under the same conditions induces only 17.33% of the quenching ratio of Zn-PP. Both the UV-vis and fluorescence spectral analysis indicate that Zn-PP exhibits higher binding affinity to human telomeric G-quadruplex DNA than to ct-DNA. In the CD spectral analysis, the interaction with Zn-PP does not disturb the characteristic absorption of G-quadruplex at 290 nm corresponding to its antiparallel conformation, and only slightly decreases the positive absorption at 270 nm as a shoulder peak. In comparison, the addition of Zn-PP induces significant spectral changes on the CD absorption of double helix ct-DNA, with a 22.2% increase on the positive peak absorption, along with a 9.63% decrease on the negative peak absorption, which suggests that the antiparallel structure of G-quadruplex is stable in the presence of Zn-PP.

Keywords: Bioinorganic chemistry, Zinc, DNA binding, G-quadruplex DNA, ct-DNA, Binding properties, Spectral studies, DNA interactive drugs

DNA is generally accepted as the primary intracellular target for many anticancer agents. However, the main problem for these conventional anticancer agents is their significant toxicity because of their nonspecific interaction with the duplex DNA. In order to improve the selectivity and reduce the side effects of DNA-interactive drugs, many researchers have focused on designing molecules targeting the specific DNA secondary structures, such as G-quadruplex DNA.

Telomeres are specialized class of functional DNA-protein structures. One of the most abundant sources of DNA sequences capable of forming G-quadruplex structures is found in the telomeres. Telomeric DNA plays an important biological role in protecting the chromosomes from nuclease attacks and losing information in the process of cell divisions. Telomeric DNA is composed of a repeated double-stranded [TTAGGG/CCCTAA]n sequence except in the 3’-terminal region, which consists of a single-stranded tandem [TTAGGG] repeated sequence over several hundred bases. The rich sequence (TTAGGG) formation of planar molecular G-tetrads by hydrogen-bonding interactions between the Watson-Crick edge and the Hoogsteen edge, the G-tetrad can be further stabilized by alkali metal cations and consequently form G-quadruplex DNA structures. Previous studies have shown that ligand-induced quadruplex formation or stabilization of G-quadruplexes by the telomeric G-rich strand can inhibit the activity of telomerase. Therefore, G-quadruplex DNA has become an important molecular target for developing new anticancer drugs.

In recent years, many researchers have focused on metal complexes as effective stabilizers of quadruplex DNA. Some of the metal complexes interact strongly with G-quadruplex DNA and exhibit high selectivity for G-quadruplex DNA. Zn-PP formed as a cationic Zn(II) porphyrin of large aromatic planarity exists widely in biological systems. It is suggested that Zn-PP prefers to bind with the terminal G-tetrads of the G-quadruplex DNA by π–π stacking. On the other hand, hydrogen bonds between the carboxyl groups of Zn-PP with the quadruplex phosphate backbone will enhance the interaction between the Zn-PP and the quadruplex DNA. Herein, we have studied the interactions between Zn-PP and different human telomeric G-quadruplex DNA as well as ct-DNA in the presence of K+ by UV-vis, fluorescence and CD spectroscopy.
Experimental

All chemical reagents were commercially available and received without further purification, unless noted specifically. Calf thymus DNA (ct-DNA), Zn-PP (Zn(II) complex of porphyrin) and Tris were purchased from Sigma. Htel (AGGGTTAGGGTTAGGG), Htel-1 (TAGGGTTAGGGTTAGGG) and Htel-2 (TAGGGTTAGGGTTAGGTT) were purchased from Sangon Biotech Co. Ltd. (Shanghai). All the oligonucleotides were purified by HPLC by the manufacturers. Fluorescence spectra were obtained on a Shimadzu RF-5301/PC spectrofluorophotometer, CD spectra were recorded on Jasco J-810 spectropolarimeter and the UV-vis spectra were recorded on Perkin Elmer Lambda 45 UV-visible spectrophotometer.

For the spectroscopic studies, Zn-PP was dissolved in DMSO to prepare a stock solution of 2.0 mM. Tris-KCl buffer solution (10 mM Tris, 100 mM KCl, adjusted to pH = 7.40 with hydrochloric acid) was prepared using doubly distilled water. Ct-DNA was dissolved in Tris-KCl buffer solution to prepare the stock solution (2.0 mM). G-quadruplex DNA was dissolved in Tris-KCl buffer solution to prepare the stock solution of 100 µM. The calf thymus DNA and G-quadruplex DNA stock solution were stored at 4 °C for no more than 5 days before use.

For UV–vis absorption spectral analysis, working solution of the Zn-PP was kept constant at 8.0 µM. The ct-DNA or G-quadruplex DNA stock solutions were added until saturation was achieved, wherein the G-quadruplex DNA was added with [G4-DNA]/[compound] ratios ranging from 0.1 to 1.0 at every 0.1 interval, and, the [ct-DNA]/[compound] ratios ranged from 2.0 to 20 at every 2.0 interval. The fluorescence emission spectra were recorded under slit width as 15 nm/15 nm with excitation wave length (Ex) as 410 nm. The concentration of Zn-PP was kept at 4 µM for the working solutions. The G-quadruplex DNA was added with increasing [G4-DNA]/[compound] ratios ranging from 0.02 to 0.2 at every 0.02 interval, while the [ct-DNA]/[compound] ratios ranged from 0.2 to 4.0 at every 0.4 interval. The CD absorption spectra of DNA were measured in Tris-KCl buffer solution at a scan rate of 100 nm/min in the wavelength range of 200–400 nm. The concentration of Zn-PP was kept at 10 mM as working solutions. The concentrations of G-quadruplex DNA and ct-DNA were maintained at 5 mM and 1 mM, with [G4-DNA]/[Zn-PP] = 0.5:1 and [ct-DNA]/[Zn-PP] = 0.1:1, respectively. All the spectroscopic experiments were performed at room temperature.

The intrinsic binding constant, $K_b$, was determined by the following equation: $([DNA]/(ε_a−ε_b)) = ([DNA]/ (ε_c−ε_b)+1/(K_b(ε_c−ε_b)))^{15}$, where [DNA] is the total concentration of the different types of DNA, $ε_a$ is the apparent absorption coefficient (which is lower than that of the three types of DNA), $ε_c$ is the extinction coefficient for the free Zn-PP, and $ε_b$ is the absorption coefficient of Zn-PP fully bound to DNA. $K_b$ was calculated as the ratio of the slope to the Y intercept by linear fitting of $([DNA]/(ε_a−ε_b))$ versus [DNA] plot from the above equation.

Results and discussion

It has been well documented that the intercalative π-π stacking of the aromatic rings of small molecules with DNA base pairs can induce a significant influence on the transition dipoles of the molecules and usually leads to significant hypochromicity$^{20}$. The UV-vis absorption spectra of Zn-PP bound with G-quadruplex DNA or ct-DNA are shown in Fig. 1. In the presence of K$^+$ (100 mM), the addition of different types of DNA into the working solution of Zn-PP induced significant hypochromicities on the characteristic peak absorption of Zn-PP at around 400 nm. With increasing [DNA]/[Zn-PP] ratios in the range from 1:1 to 10:1, hypochromic ratios of 13–17% for the peak absorbance of Zn-PP at 400 nm were achieved, which suggests the intercalative binding mode between Zn-PP and DNA.

The intrinsic binding constants, $K_b$, for G-quadruplex DNA and ct-DNA were calculated and found to be (3.13±0.45)×10$^5$, (1.47±0.12)×10$^5$ and (2.51±0.16)×10$^5$ M$^{-1}$ for Htel-2-G4, Htel-1-G4 and Htel-2-G2, respectively, while for ct-DNA, the binding constant was found to be (9.94±0.32)×10$^5$ M$^{-1}$, which is lower than that of the three types of G4-DNA (Table 1).

From the UV–vis spectral analysis, all the antiparallel G-quadruplex form DNA (including Htel-G4, Htel-1-G4 and Htel-2-G4) exhibited higher binding affinities to Zn-PP than to the double helix ct-DNA. This may be ascribed to the more expanded planar structure of G-quadruplex than ct-DNA, which exerts better π-π stacking with Zn-PP. However, the proposed binding mode of Zn-PP to all the four types of DNA was intercalative binding.

In aqueous solution, the fluorescence emission of small molecules can be quenched by polyanionic
nucleotide\textsuperscript{21}, and a collisional quenching mode is usually considered. The collisional quenching mode can be caused by the electronic transitions from the excited state ($\pi^*$ orbital) of the fluorescent molecule to the ground state ($\pi$ orbital) of the quencher (such as nucleic acid). This is based on the $\pi-\pi$ stacking between the aromatic groups of fluorescent molecules and base pairs of DNA, which is generally attributed to an intercalative binding mode of Zn-PP with DNA\textsuperscript{22}.

Zn-PP solution gave the characteristic fluorescence emission at around 589 nm under excitation wavelength of 410 nm. The quenching effects on the fluorescence emission intensity of Zn-PP in the presence of three types of G-quadruplex DNA are shown in Fig. 2, in which the values of $I/I_0$ ratios are compared under the same concentration. The spectral changes of Zn-PP in the presence of ct-DNA are shown in Fig. 3. It was found that the gradual addition of Htel-G\textsubscript{4}, Htel-1-G\textsubscript{4} or Htel-2-G\textsubscript{4} DNA caused significant fluorescence quenching on the peak emission of Zn-PP. With [DNA]/[Zn-PP] ratios increasing from 0.02 up to 0.20, quenching ratio of 32.04\%, 36.84\% and 26.31\% were achieved respectively by Htel-G\textsubscript{4}, Htel-1-G\textsubscript{4} or Htel-2-G\textsubscript{4} DNA. In comparison, on addition of four equivalents of ct-DNA to Zn-PP, only 17.33\% quenching ratio was achieved. This result further suggests that Zn-PP binds to the human telomeric G-quadruplex DNA preferentially than to ct-DNA, which is consistent with the result from the above UV-vis spectral analysis.

\textbf{Table 1 – Intrinsic binding constants and UV-vis absorption changes of Zn-PP on addition of ct-DNA and human telomeric G-quadruplex DNA}

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>[Zn-PP] ($\mu$M)</th>
<th>$K_b$ ($M^{-1}$)</th>
<th>Hypochromicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ct-DNA</td>
<td>9.6</td>
<td>(9.94±0.32)$\times10^4$</td>
<td>16.52</td>
</tr>
<tr>
<td>Htel-G\textsubscript{4}</td>
<td>8.0</td>
<td>(3.13±0.45)$\times10^5$</td>
<td>13.78</td>
</tr>
<tr>
<td>Htel-1-G\textsubscript{4}</td>
<td>8.0</td>
<td>(1.47±0.12)$\times10^5$</td>
<td>14.40</td>
</tr>
<tr>
<td>Htel-2-G\textsubscript{4}</td>
<td>8.0</td>
<td>(2.51±0.16)$\times10^5$</td>
<td>16.96</td>
</tr>
</tbody>
</table>

Fig. 1 – UV-vis absorption spectra of Zn-PP in 10 mM Tris and 100 mM K\textsuperscript{+} in the absence and presence of ct-DNA and human telomeric G-quadruplex DNA. ([Zn-PP] = 8 $\mu$M; [ct-DNA]/[Zn-PP] = 2.0–20.0; [G\textsubscript{4}-DNA]/[Zn-PP] = 0.1–1.0; pH = 7.4. (a) Zn-PP + ct-DNA; (b) Zn-PP + Htel-G\textsubscript{4}; (c) Zn-PP + Htel-1-G\textsubscript{4}; (d) Zn-PP + Htel-2-G\textsubscript{4}.}
Fig. 2 – Fluorescence emission spectra of Zn-PP in the absence and presence of human telomeric G-quadruplex DNA in 100 mM K+ solution. ([Zn-PP] = 4 μM; [G4-DNA]/[Zn-PP] = 0.02–0.2; pH = 7.4; [1, Htel-G4, 2, Htel-1-G4, 3, Htel-2-G4]).

Fig. 3 – Fluorescence emission spectra of Zn-PP in the absence and presence of ct-DNA in 100 mM K+ solution. ([Zn-PP] = 4 μM; [ct-DNA]/[Zn-PP] = 0.2–4.0).

Fig. 4 – CD spectra of between ct-DNA and human telomeric G-quadruplex DNA in the presence of K+ on addition of Zn-PP. ([Zn-PP] = 10 mM): (a) [ct-DNA] = 1 mM; (b) [Htel-G4] = 5 mM; (c) [Htel-1-G4] = 5 mM; (d) [Htel-2-G4] = 5 mM.
Circular dichroism (CD) is a useful technique to assess whether the nucleic acids undergo conformational changes as a result of complex formation or changes in environmental conditions, including monitoring the folding of G-quadruplex and the influence of a ligand when binding to a quadruplex structure.

As shown in Fig. 4, the CD spectra of ct-DNA exhibits a positive absorption peak at 274 nm and a negative absorption peak at 246 nm due to π-π base stacking and right-hand helicity, respectively, which is consistent with the characteristic B conformation of DNA. The CD spectra of Htel-G₄, Htel-1-G₄ and Htel-2-G₄ show a negative peak at 240 nm and two positive peaks at about 270 nm and 290 nm, which is indicative of a mixture of antiparallel and parallel G-quadruplex conformations, and the antiparallel G-quadruplex is the main fraction.

The addition of two equivalents of Zn-PP to G-quadruplex DNA solution induced different CD spectral changes as compared to that of ct-DNA. The interaction with Zn-PP did not disturb the characteristic absorption of G-quadruplex at 290 nm corresponding to its antiparallel form, and only slightly decreased the positive absorption at 270 nm as a shoulder peak. This suggests that the antiparallel structure of G-quadruplex remains stable in the presence of Zn-PP. Comparatively, the addition of Zn-PP induced significant spectral changes on the CD absorption of double helix ct-DNA, with 22.2% increment on the positive peak absorption, along with a 9.63% decrement on the negative peak absorption. However, the more significant spectral changes of ct-DNA than of G-quadruplex DNA induced by Zn-PP should not be regarded as the higher binding affinity of Zn-PP to ct-DNA as compared to G-quadruplex DNA. Since CD spectrum is sensitive to the conformational changes of DNA, the significant spectral changes of ct-DNA suggests that the B-form double helix ct-DNA tends to transform under the interaction with Zn-PP, while the antiparallel G-quadruplex form DNA is more stable.

In summary, the interaction mechanism of Zn-PP with three types of human telomeric G-quadruplex DNA as well as ct-DNA was studied by UV-vis, fluorescence and CD spectroscopic methods. The results indicate that Zn-PP exhibits higher binding affinity with human telomeric G-quadruplex DNA than to ct-DNA. This may be explained as due to the more expanded planar aromatic structure of G-quadruplex DNA as compared to ct-DNA, which facilitates its π-π stacking with Zn-PP. Also, the antiparallel G-quadruplex form of DNA is more stable than the double helix form of DNA in the presence of Zn-PP.

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