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Spectroscopic Studies on the Interaction of Hemin with Human Telomeric G-Quadruplex DNA

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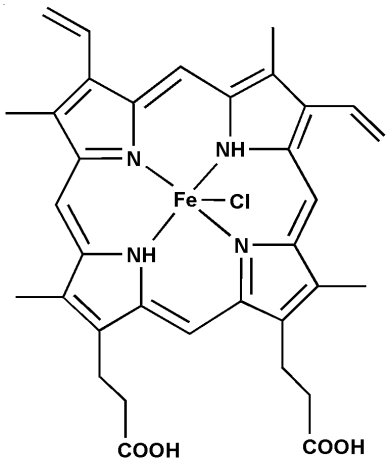
The interaction behaviours of hemin as a ligand binding with three types of human telomeric G-quadruplex DNA were examined by UV-visible and circular dichroism spectroscopic methods. The circular dichroism spectra of these three types of G-quadruplex DNA all showed major positive absorption peaks at *ca*. 290 nm with shoulder peaks at *ca*. 270 nm, along with a negative absorption peak at *ca*. 250 nm. The presence of hemin did not induce obvious circular dichroism spectral changes of these G-quadruplex DNA, which indicates that hemin is able to stabilize human telomeric antiparallel G-quadruplex DNA structure. For comparison, the addition of hemin induced obvious increment on the characteristic antiparallel circular dichroism absorption of the corresponding single-chain human telomeric sequences, which suggests that hemin may induce and stabilize the formation of antiparallel G-quadruplex DNA even in the absence of K^+. On the other hand, the addition of the three types of G-quadruplex DNA into the hemin solution with gradually increasing concentrations induced significant hypochromicity (31.86-47.26 %) along with 11-23 nm of bathochromicity on the UV-visible absorption of hemin. From this result, it is suggested that the high binding affinity of hemin to antiparallel G-quadruplex DNA is based on the intensive intercalative binding mode of hemin to antiparallel G-quadruplex DNA by the π-π stacking between the macrocyclic aromatic hemin and the guanine quartet.

Key Words: G-quadruplex DNA, Hemin, Telomeric DNA, Intercalative binding.

INTRODUCTION

Contributing to the potential for inhibiting telomerase and halting tumor cell proliferation, complexes which can bind and stabilize G-quadruplex DNA have attracted researcher's interests in recent years. Telomeres are special classes of functional DNA structures which located at the terminal regions of eukaryotic chromosomes[1]. Telomeric DNA play an important biological role in cell growth and genome stability, such as protecting chromosomes from nuclease attacks and loss information in processes cell divisions[2-4]. In human, telomeric DNA contains of tandem repeats of guanines rich sequence (TTAGGG), with a single strands in 3'-end overhang of 100-200 nucleotides[5], this sequence 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 (*e.g.*, potassium or sodium) and consequently form G-quadruplex DNA structures[6]. Previous study has been shown that ligand-induced quadruplex formation or stabilization of G-quadruplexes by the telomeric G-rich strand are able to inhibit the activity of telomerase[7,8]. Therefore, G-quadruplex DNA has become an important target for cancer chemotherapy. And finding or designing small molecules binding G-quadruplex may be an attractive strategy for the development on anticancer drugs[9].

In addition to heteroaromatic compounds as G-quadruplex DNA ligands, metal complexes as effective stabilizers of quadruplex DNA have raised attention in recent years[10]. Indeed, some of them have been proven to interact strongly with G-quadruplex DNA and exhibit high selectivity for G-quadruplex DNA[11]. Cationic metalloporphyrins, a class of G-quadruplex DNA binding molecules are able to bind different types of G-quadruplex DNA. Hemin (**Scheme-I**) formed as a cationic Fe(II) porphyrin with large aromatic planarity wide spreadly exists in biological system. It is apt to externally stack on the terminal G-tetrads of the G-quadruplexes[12,13]. On the other hand, hydrogen bonds between the carboxyl groups of hemin and the quadruplex phosphate backbone would enhance the interaction between the hemin and the quadruplex DNA[14]. The aim of this work was to investigate the hemin selective recognition of different human telomeric G-quadruplex DNA in the absence and presence of K^+ using UV-visible absorption and circular dichroism spectroscopic methods[15].



Scheme-I: Chemical structure of hemin

EXPERIMENTAL

Hemin was purchased from Sigma-Aldrich. Human telomeric sequences were purchased from Sangon Biotech (Shanghai) Co. Ltd. They were used as received without further purification unless noted specifically. The concentration of G-quadruplex DNA per nucleotide was determined spectrophotometrically by employing a molar absorptivity [2.285×10^5 mol(quadruplex)$^{-1}$ m^3 cm^{-1}] at 260 nm. The 1×10^{-4} M G-quadruplex DNA stock solution was stored at 4 ºC for no more than 5 days before use. UV-visible absorption spectral titration analyses were performed on a Perkin-Elmer Lambda 45 UV-visible spectrophotometer. CD spectra were recorded by using a JASCO J-810 automatic recording spectropolarimeter.

Spectroscopic studies on DNA interaction: Hemin was prepared as 2×10^{-3} M DMSO stock solutions for DNA binding studies. The stock solution was diluted to final working solution by TBS (*tris*-NaCl buffer solution, 5 mM *tris*, 100 mM KCl, pH was digitally adjusted to 7.35 by titration with hydrochloric acid with Sartorius professional meter). In UV-visible absorption spectrometry, the 2.5 mL working solution of hemin was constantly kept as 2×10^{-5} M. The G-quadruplex DNA stock solution was added into the working solution with increasing concentrations. After each addition, the solution was allowed to incubate for 8 min before the absorption spectrum was recorded. The intrinsic binding constant K_b was determined by the following equation: $[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_a-\varepsilon_f)+1/[K_b(\varepsilon_a-\varepsilon_f)]$, where [DNA] is the concentration of the G-quadruplex DNA, ε_a is the apparent absorption coefficient ($\varepsilon_a = A_{obsd}/[hemin]$), ε_f is the extinction coefficient for the free hemin and ε_b is the absorption coefficient of hemin fully bound to G-quadruplex DNA. The intrinsic binding comstant, K_b, of hemin are calculated as the ratio of the slope to the Y intercept by linear fitting of $[DNA]/(\varepsilon_a-\varepsilon_f)$ with [DNA] from the above equation. CD absorption spectrometry of G-quadruplex DNA in TBS of 4×10^{-6} M was measured under 100 nm/min scan rate in the wavelength range from 220 to 400 nm, in the absence and presence of each compound of 8×10^{-6} M, respectively. The added compound was pre-incubated with DNA for 1 h. The CD signal of TBS was subtracted in advance as the background. All the spectral analyses were operated under room temperature of 25 ºC and were based on repeated experiments.

RESULTS AND DISCUSSION

Circular dichroism is a powerful technique for monitoring G-quadruplex folding and the influence of a ligand when binding to a quadruplex structure[16]. Studies have revealed that different human telomeric sequences adopted diverse of G-quadruplex structure under K^+ rich conditions[17]. The human telomeric Htel-1, Htel-2 and Htel-3 sequences adopt an anti-parallel intramolecular G-quadruplexes Form-1, Form-2 and Form-3 in the presence of 100 mM K^+, respectively[18], as shown in Fig. 1(a), 1(b) and 1(c), in which their CD spectra all showed a positive absorption peak at *ca.* 290 nm with a shoulder peak at *ca*. 270 nm and a weak peak at *ca*. 250 nm. The maximum absorption peak at *ca*. 290 nm is characteristic of antiparallel G-quadruplex conformation. After addition of hemin into the human telomeric G-quadruplex DNA solution, slight decrease on the intensity of the CD absorption of the Htel-2 and Htel-3 quadruplexes can be observed. Comparatively, addition of hemin induced more significant spectral changes on Htel-1 quadruplex. However, the shapes of the characteristic absorption curves of all the three types of quadruplexes DNA remained unchanged, suggesting that the presence of K^+ can stabilize the human telomeric G-quadruplex structure and the interaction of G-quadruplex with hemin did not disturb its secondary structure.

For comparison, the interactions between the three human telomeric sequences (single-chain) (Htel-1, Htel-2 and Htel-3) and hemin in the absence of K^+ were also investigated. As shown in Fig. 1(d), 1(e) and 1(f), the CD spectra of the Htel-1, Htel-2 and Htel-3 all showed two positive absorption peaks at *ca.* 260 nm and *ca*. 290 nm, which is indicative of a mixture of antiparallel/parallel G-quadruplex conformations[19]. To Htel-1 and Htel-2, the major peak is at *ca*. 260 nm and the minor peak is at *ca.* 290 nm, while Htel-3 showed the major peak at *ca*. 290 nm and the minor peak at *ca*. 250 nm, which suggests that Htel-3 exhibited more characteristic of the anti-parallel G-quadruplex conformation[17]. Upon addition of 2 equiv. of hemin to three types of nucleotide solutions, respectively, dramatic changes on their CD spectra can be observed [Fig. 1(d), 1(e) and 1(f)], which is much more significant than those of G-quadruplexes as shown in Fig. 1(a), 1(b) and 1(c). The addition of hemin caused the increase on absorption of the antiparallel G-quadruplex at 290 nm, along with the decrease on absorption of the parallel G-quadruplex at 260 nm. It may be explained as hemin can effectively induce the single-chain human telomeric nucleotide sequence to form anti-parallel G-quadruplex conformation even in the absence of K^+.

The binding mode of hemin with Form-1, Form-2 and Form-3 G-quadruplex DNA by UV-visible absorption spectral titration analyses were further carried out to investigate the action mechanism of hemin to induce the formation of anti-parallel G-quadruplex. UV-visible absorption spectra were recorded in the 220-550 nm range by gradual addition of different types of G-quadruplex DNA samples in aliquots sequentially to hemin solution. As shown in Fig. 2, the hemin showed the maximum absorbance at *ca.* 380 nm, corresponding to its characteristic π-π^* electronic transition of macrocyclic aromatic structure. By addition of three types of G-quadruplex DNA into the hemin solution, respectively, the characteristic

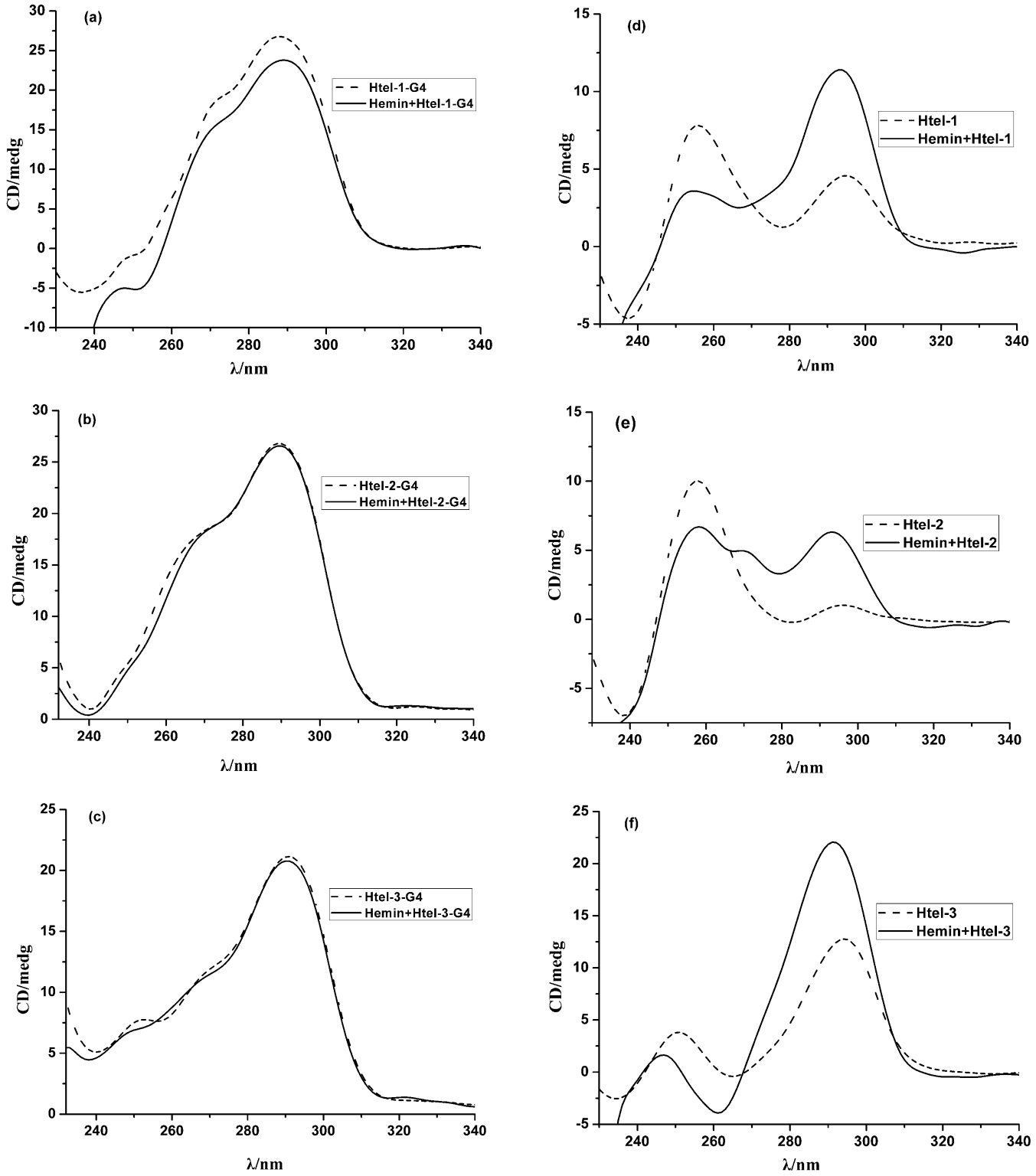


Fig. 1. CD spectra of human telomeric sequences G-quadruplexes in the presence of 100 mM K^+ upon addition of hemin: (a) Htel-1-G4; (b) Htel-2-G4; (c) Htel-3-G4. CD spectra of single-chain human telomeric sequences in the absence of K^+ upon addition of hemin: (d) Htel-1; (e) Htel-2; (f) Htel-3

absorption of hemin at 380 nm all significantly decreased on intensities (hypochromicity) along with various red-shifts (bathochromicity). Interaction of hemin with Form-1 G-quadruplex DNA caused a 43.5 % hypochromicity with a 13 nm bathochromicity. Addition of Form-2 G-quadruplex DNA to hemin solution under same condition led to a 47.3 % hypochromicity with 11 nm bathochromicity. While addition of Form-3 G-quadruplex DNA to hemin solution led to a 31.9% hypochromicity with 23 nm bathochromicity. The resulted data are summarized in Table-1. The significant hypochromicities and bathochromicities on hemin caused by the addition of G-quadruplex DNA are characteristic of the intercalative binding mode of hemin with the G-quadruplex DNA[20].

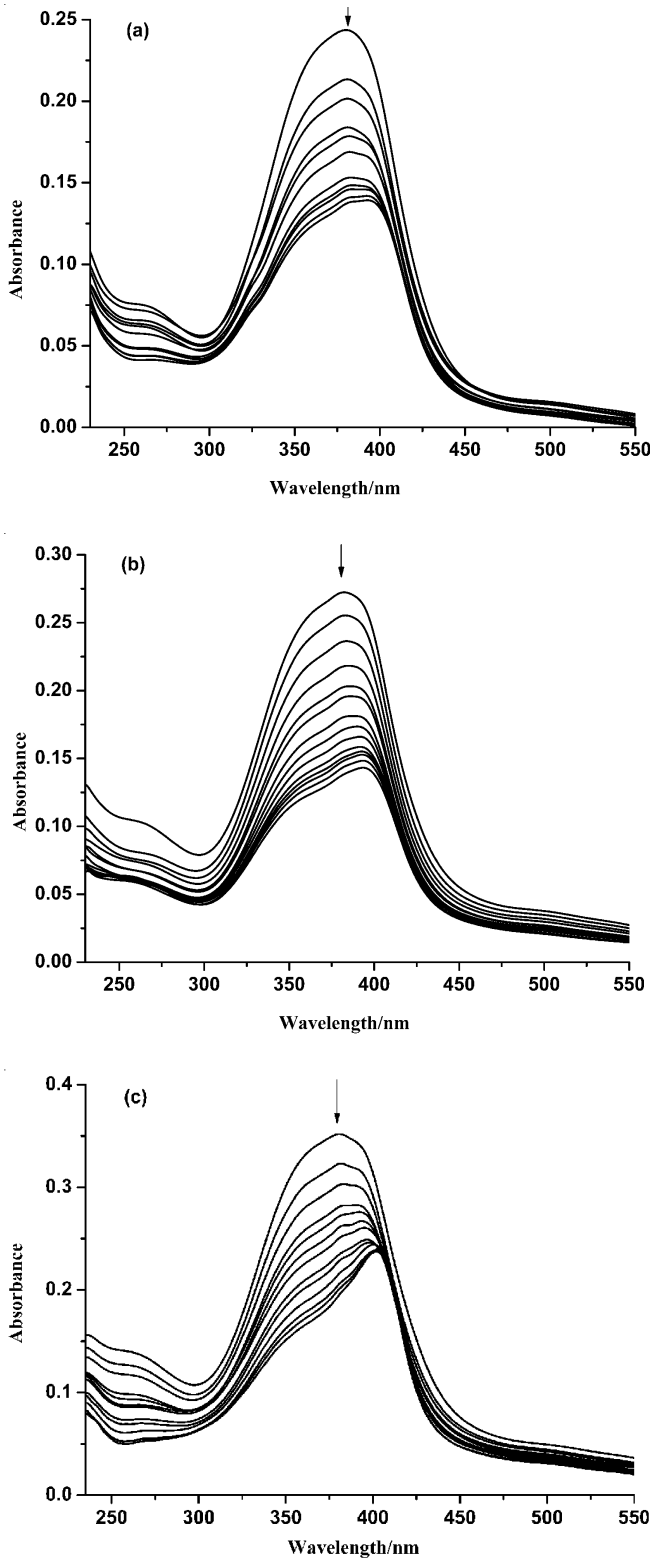


Fig. 2. UV-visible absorption spectra of hemin in 100 mM K^+ solution in the absence and presence of human telomeric G-quadruplex DNA (10 μM *tris*-HCl, pH 7.4). (A) Form-1, (B) Form-2, (C) Form-3

The intrinsic binding constants (K_b) of hemin to different types of G-quadruplex DNA were also evaluated from the results of UV-visible titration analyses by using the classic linear fitting equation[21], as summarized also in Table-1. It indicates that hemin showed moderate binding intensity with Form-3 quadruplex DNA with K_b value of 4.89×10^5 mol^{-1} L and was comparatively weaker than those of hemin with Form-1 and Form-2 quadruplex DNA, with binding constants (K_b) of 2.81×10^6 and 1.82×10^6 mol^{-1} L, which are 5.7 folds and 3.7 folds higher than that of Form-3, respectively. Nevertheless, these results strongly suggested that the intensive intercalative binding mode of hemin to the three types of G-quadruplex DNA should contribute to the formation and stabilization of antiparallel G-quadruplex DNA by the π-π stacking between the macrocyclic aromatic hemin and the guanine quartet.

TABLE-1
BINDING CONSTANTS AND SPECTRAL CHANGES OF HEMIN UPON ADDITION OF DIFFERENT HUMAN TELOMERIC QUADRUPLEX DNA

G-quadruplex	K_b [M^{-1} cm^{-1}]	Hypochromicity	Bathochromicity
Form-1	2.81×10^6	43.54 %	13 nm
Form-2	1.82×10^6	47.26 %	11 nm
Form-3	4.89×10^5	31.86 %	23 nm

Conclusion

The interactions of hemin and human telomeric G-quadruplex DNA are studied by CD and UV-visible spectroscopic analyses. The comparison on the changes on the CD spectra of G-quadruplex DNA and the corresponding single-chain telomeric sequences clearly show that hemin is able to induce the formation of antiparallel G-quadruplex structure and stabilize the G-quadruplex DNA. UV-visible absorption spectral analyses were further carried out to discuss the action mechanism of hemin to form and stabilize the antiparallel G-quadruplex DNA. The hemin exhibits characteristic intercalative binding affinities to all the three types of G-quadruplex DNA, with intrinsic binding constants, K_b, of 4.89×10^5 mol^{-1} L, 2.81×10^6 and 1.82×10^6 mol^{-1} L, respectively, which should be ascribed to the π-π^* electronic transition based on the π-π stacking between the macrocyclic aromatic hemin and the guanine quartet.

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