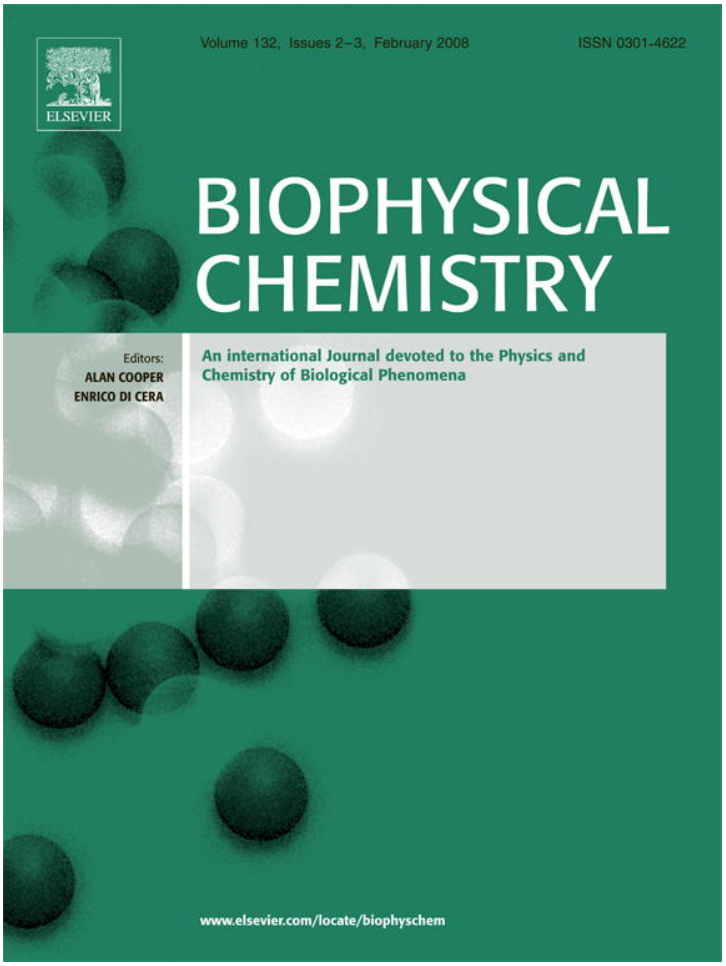

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Structural transition from the random coil to quadruplex of $AG_3(T_2AG_3)_3$ induced by Zn^{2+}

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Abstract

Structures of G-quadruplex DNAs can be typically stabilized by monovalent cations such as K^+, Na^+. Some divalent and trivalent cations, such as Sr^{2+}, Pb^{2+}, Tb^{3+} and Eu^{3+}, can also induce the formation of G-quadruplex DNA. Here we show that Zn^{2+} can induce the human telomeric sequence $AG_3(T_2AG_3)_3$ to fold the G-quadruplex structure by UV absorbance difference spectra and circular dichroism (CD) spectroscopy. At micromolar concentrations, the Zn^{2+}-induced changes in the UV absorbance difference spectra and CD spectra are the characteristics of antiparallel G-quadruplexes although the long wavelength CD maximum is around 285 nm rather than the typical value of 295 nm. The binding stoichometry of Zn^{2+} per one $AG_3(T_2AG_3)_3$ molecule is four. To our knowledge, the structural transition of human telomeric sequence induced by Zn^{2+} was observed for the first time.

Keywords: Zn^{2+}; G-quadruplexes; Spectroscopy; Structural transitions

1. Introduction

Metal ions are abundant in the body. DNAs are the major targets of cations in cells, their binding can affect the structures and conformations of DNAs significantly. For example, Ni^{2+} can induce a conformational transition from the natural right-handed B-form to left-handed Z-form in poly(A-T) [1], Zn^{2+}, Co^{2+}, Fe^{2+} and Ni^{2+} can incorporate into the helix of duplex DNA and replace the imino protons to form metal-DNA complexes [2,3]. These structural and conformational changes of DNA induced by metal ions play a key role in the different biological processes such as genes express, mutagenesis and carcinogenesis in living cells.

Guanine-rich DNA sequences have been found in telomeres, promoters of oncogenes and most growth control genes [4–7]. In the presence of a wide variety of monovalent cations, these sequences can form G-quadruplex structures built from the stacking of multiple coplanar G-tetrads [8–10], and some divalent cations, such as Pb^{2+} and Sr^{2+}, are also able to induce the thrombin aptamer to form G-quadruplexes [11,12].

Human telomeric DNA contains highly repeated Guanine-rich (GGGTTA)*n* sequences [13]. In the presence of Na^+ and K^+, the telomeric $AG_3(T_2AG_3)_3$ sequence forms the folding intramolecular G-quadruplex structures [14,15]. Recently, Galezowska et al. reported that lanthanide ions Eu^{3+} and Tb^{3+} can also induce human telomeric $AG_3(T_2AG_3)_3$ DNA sequences to form G-quadruplex [16]. It has been shown that the formation of intramolecular G-quadruplex by the telomeric guanine-rich strand inhibits the activity of telomerase in cancer cells [17,18]. Therefore, G-quadruplexes in telomere have been

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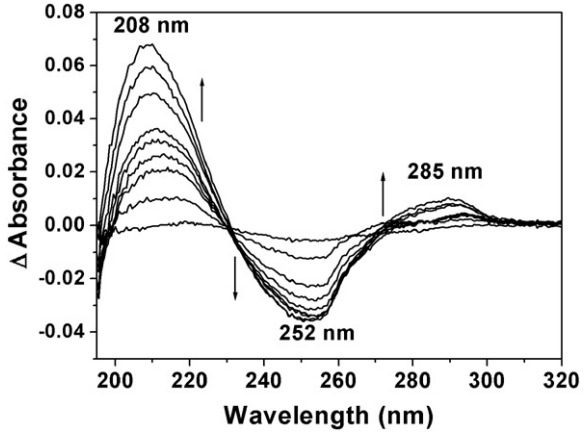

Fig. 1. UV absorbance difference spectra between $AG_3(T_2AG_3)_3$ and $AG_3(T_2AG_3)_3$–Zn^{2+} in 10 mM Tris–HCl buffer solution (pH 7.5). The concentration of $AG_3(T_2AG_3)_3$ is 3 μM, and the concentration of Zn^{2+} is 0, 3, 6, 9, 12, 15, 30, 75 and 100 μM according to the direction of arrows.

established a promising anticancer target, and ligand-induced stabilization of intramolecular telomeric G-quadruplex has become an attractive strategy for the development of novel anticancer drugs [19,20]. Here we are reporting that Zn^{2+} can also induce the structural transition of human telomeric $AG_3(T_2AG_3)_3$ sequence from random coil to G-quadruplex.

2. Materials and methods

2.1. Materials

The DNA oligonucleotide $AG_3(T_2AG_3)_3$ was purchased from the SBS Genetech Co., Ltd (China) in a PAGE purified form. Single-strand concentrations were determined by measuring the absorbance at 260 nm at a high temperature. Single-strand extinction coefficients were calculated from mononucleotide and dinucleotide data by a nearest-neighbor approximation method [21,22], and the molar extinction coefficient at 260 nm is 228,500 M^{-1} cm^{-1} for $AG_3(T_2AG_3)_3$. All measurements were performed in a buffer solution containing 10 mM Tris–HCl at pH 7.5. $ZnSO_4$ was used in all experiments.

2.2. UV spectroscopy

UV absorbance difference spectra were measured on a HP 8453 ChemStation with 1 cm-path-length quarter cell. Both reference and sample solutions contain 3 μM $AG_3(T_2AG_3)_3$ in 10 mM Tris–HCl at pH 7.5. The difference spectra are obtained by subtraction of the UV spectrum for $AG_3(T_2AG_3)_3$ in the absence of Zn^{2+} from that in the presence of Zn^{2+}.

2.3. Circular dichroism

CD experiments were performed at room temperature using a Jasco-820 spectropolarimeter. For each sample, at least three spectrum scans were accumulated over the wavelength range of 220–320 nm in a 1 cm-path length cell at a scanning rate of 50 nm/min. The concentration of oligonucleotide is 4 μM. The scan of the buffer (10 mM Tris–HCl) alone was subtracted from the average scan for each sample.

3. Results and discussion

3.1. Structural transition of $AG_3(T_2AG_3)_3$ induced by Zn^{2+}

UV absorption spectroscopy is usually used to examine the interactions of DNA with metal ions and small molecules. In order to get the detail information about effect of Zn^{2+} on the structure of $AG_3(T_2AG_3)_3$, we measured their UV absorbance difference spectra that are sensitive to the small changes of DNA structure and conformation, the results are shown in Fig. 1.

With the increasing Zn^{2+} concentration, the two positive peaks near 208 and 285 nm and a negative peak around 252 nm were observed. It was reported that the positive peak near 290 nm in the UV absorption spectra is the marker of G-quadruplex formation [23], and the difference spectrum exhibits a isobestic point around 280 nm whereas a net hyperchromism is observed upon G-quart formation at 285 nm or the longer wavelength [24,25]. According to these reports our results indicate that Zn^{2+} can induce the structural transition from the random coil to G-quadruplex of $AG_3(T_2AG_3)_3$.

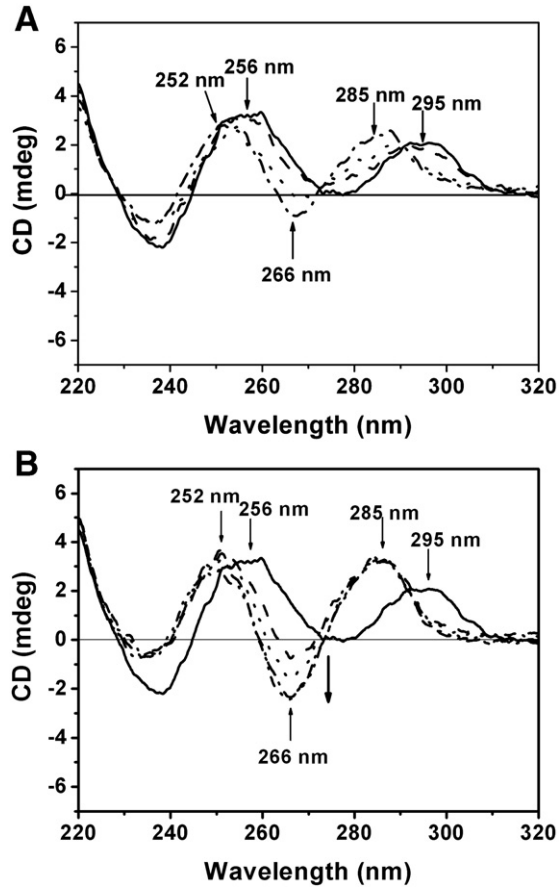

Fig. 2. CD spectra of $AG_3(T_2AG_3)_3$ in the different concentrations of Zn^{2+}. The concentration of $AG_3(T_2AG_3)_3$ is 4 μM, and the concentration of Zn^{2+} is 0 (solid), 3 (dash), 9 (dot) and 15 μM (dash dot) (A) or 0 (solid),15 (dash), 30 (dot), 45 (dash dot), 100 (dash dot dot) (B).

Based on the results obtained by UV absorbance difference spectra, we performed a CD study of $AG_3(T_2AG_3)_3$ in the presence of different concentrations of Zn^{2+}. CD spectra are sensitive to the structural and conformational changes of G-quadruplexes, antiparallel G-quadruplex presents the positive peak near 295 nm and negative peak near 265 nm, although parallel G-quadruplex shows the positive and negative peaks around 265 and 240 nm, respectively [26,27].

As shown in Fig. 2, the CD spectrum of $AG_3(T_2AG_3)_3$ exhibits two positive peaks at 295 and 256 nm in the absence of Zn^{2+}. With the increasing Zn^{2+} concentration from 3 to 15 μM, the positive peaks at 256 and 295 nm are blue shifted to 252 and 285 nm, respectively, and a new negative peak at 266 nm is observed (Fig. 2A). When the Zn^{2+} concentration is increased gradually from 15 to 45 μM, the two positive peaks at 252 and 285 nm are almost unchanged although the intensity of negative peak at 266 nm is increased. Continue to increase the concentration of Zn^{2+} from 45 to 100 μM, both intensity and shift of the peak at 266 nm fail to change (Fig. 2B). Based on these results, the Zn^{2+} induced change of most obvious note is the negative peak near 266 nm along with the shift of an already existing peak near 295 to 285 nm.

To further understand the conformational transition of $AG_3(T_2AG_3)_3$ upon addition of Zn^{2+}, the CD spectra of $AG_3(T_2AG_3)_3$ in the presence of either 100 μM Zn^{2+} or 100 mM Na^+ were compared (Fig. 3). The shapes of two CD spectra are similar to each other, and there are one negative peak and two positive peaks, whereas the positions and intensities of these peaks are distinctly different. It is characterized by two strong positive bands at 246 and 295 nm and one negative peak at 266 nm in the presence of 100 mM NaCl, which is a typical anti-parallel G-quadruplex DNA, whereas the CD spectra containing Zn^{2+} posses two positive bands at 252 and 285 and one negative band at 266 nm. The amplitude of CD intensity induced by Zn^{2+} is also lower than that induced by Na^+.

Quadruplex exhibits an unusual dependence on specific metal ions for their formation and stabilization. Both K^+ and Na^+ are known to stabilize effectively many quadruplex structures with the former typically being more potent than the latter, because the size of K^+ ion is more suitable for

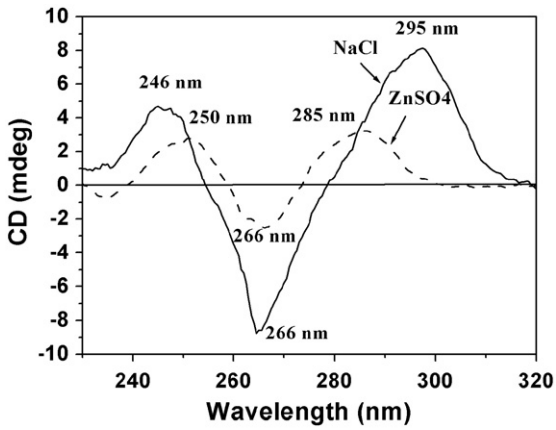

Fig. 3. Comparisons of CD spectra of $AG_3(T_2AG_3)_3$ in the presence of either 100 μM Zn^{2+} or 100 mM Na^+.

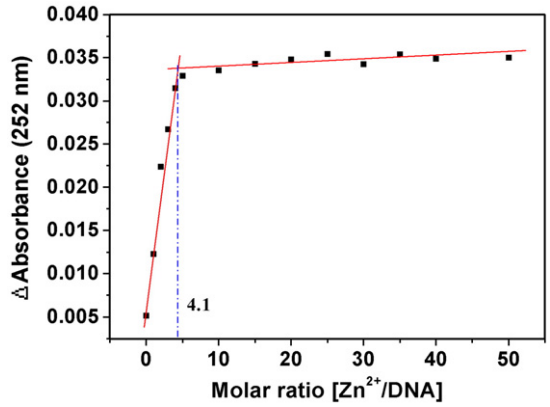

Fig. 4. Plot of UV absorbance change at 252 nm against the ratio of Zn^{2+} to $AG_3(T_2AG_3)_3$.

the G-quart than that of Na^+ ion [28]. According to the CD results, the stability of the Zn^{2+} ion form is less than that of the Na^+ ion form, which is also due to the different ion radius between Zn^{2+} and Na^+, the radius of Zn^{2+} ion is less than that of Na^+, which is 0.74 and 0.98 Å for Zn^{2+} and Na^+, respectively [29].

The difference of the peak positions between Zn^{2+}–$AG_3(T_2AG_3)_3$ and Na^+–$AG_3(T_2AG_3)_3$ may come from their different coordination environment. It is well known that Na^+ is coordinated with the oxygen of the C6═O group of $AG_3(T_2AG_3)_3$ [30], but Zn^{2+} is easy to coordinate with both N atom and O atom[2,29], although the exact structure of $AG_3(T_2AG_3)_3$ in the presence of Zn^{2+} needs to be ascertained by other methods.

CD signal at 260–265 nm reflects the G–G base stacking [31,32]. The CD intensity near 260 nm is useful for the detection of the structural transition between antiparallel and parallel G-quadruplexes because the CD spectra of typical parallel and antiparallel G-quadruplexes show a large positive band and a large negative band near 260 nm, respectively, which concludes that the enhanced magnitude of the negative band at 266 nm is the result of antiparallel character of $AG_3(T_2AG_3)_3$ in the presence of Zn^{2+}.

On the basis of these results, it is reasonable to conclude that Zn^{2+} binding to $AG_3(T_2AG_3)_3$ induces a structural transition of $AG_3(T_2AG_3)_3$ from the random coil to antiparallel G-quadruplex.

3.2. The binding stoichiometric ratio of Zn^{2+} to $AG_3(T_2AG_3)_3$

To further get the numbers of Zn^{2+} binding to $AG_3(T_2AG_3)_3$, a plot of absorbance at 252 nm upon the addition of Zn^{2+} against the molar ratio of Zn^{2+} to $AG_3(T_2AG_3)_3$ was obtained, as shown in Fig. 4. The results of the curve fitting show that four Zn^{2+} ions bind to $AG_3(T_2AG_3)_3$ molecule during the structural transition. The same fitting result was obtained by CD spectra using the values of 266 and 285 nm, respectively (data not shown).

The coordination number of Zn^{2+} with ligands is four and its configuration coordinated to DNA is the tetrahedral geometry

instead of square planar [2], we speculate that two Zn^{2+} ions occupy the two intervals between the three G-quartets, other two Zn^{2+} ions reside in the two loop regions at the two ends of G-quadruplex, respectively.

4. Conclusions

In summary, we reported that Zn^{2+} can induce the human telomeric sequence $AG_3(T_2AG_3)_3$ to fold the intramolecular anti-parallel G-quadruplex structure, and four Zn^{2+} ions are necessary for the formation of G-quadruplex DNA. The much lower concentration used than that for sodium suggests some kinds of specific complexation, and the more detail studies are underway.

Acknowledgements

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