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Specific Recognition of Human Telomeric G-Quadruplex DNA with Small Molecules and the Conformational Analysis by ESI Mass Spectrometry and Circular Dichroism Spectropolarimetry

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Abstract: Electrospray ionization mass spectrometry (ESI-MS) was utilized to investigate the binding affinity and stoichiometry of small molecules with human telomeric G-quadruplex DNA. The binding-affinity order obtained for the $(AGGGTT)_4$ quadruplex was: Tel01 > ImImImβDp ≫ PyPyPyγImImImβDp. The specific binding of Tel01 and PyPyPyγImImImβDp in one system consisting of human telomeric G-quadruplex and duplex DNA was observed directly for the first time. This revealed that PyPyPyγImImImβDp has a binding specificity for the duplex DNA, whereas Tel01 selectively recognizes the G-quadruplex DNA. Moreover, both ESI-MS and circular dichroism (CD) spectra indicated that Tel01 favored the formation and stabilization of the antiparallel G-quadruplex, and a structural transition of the $(AGGGTT)_4$ sequence from a coexistence of parallel and antiparallel G-quadruplexes to a parallel G-quadruplex induced by annealing.

Keywords: circular dichroism · DNA recognition · human telomeres · mass spectrometry · quadruplexes

Introduction

Telomeres are DNA–protein structures located at the ends of chromosomes. They contain guanine-rich DNA regions and protect chromosomes from damage and recombination. Although most telomeric DNA structures are double stranded, the 3′ end of telomeres is a single-stranded G-rich overhang.[1,2] Recent research indicated that the G-rich oligonucleotides can form the G-quadruplex in the presence of certain cations. G-quadruplex structures comprise stacked G-tetrads that are formed by cyclic Hoogsteen hydrogen bonds of four guanines (Figure 1A).[3,4] Results of NMR and X-ray diffraction studies, as well as gel and spectroscopic techniques, have shown that G-rich sequences can form intramolecular dimer and tetramer complexes in both parallel and antiparallel orientations.[5,6]

Telomeric DNA has several essential functions for genome integrity and the formation of the quadruplexes has been shown to decrease the activity of the enzyme telomerase, which is responsible for elongating telomeres.[7] The G-quadruplex DNA has became a potential target in the development of anticancer drugs, for which the factors affecting the formation and structures of G-quadruplexes could play an important role.[8,9]

Recently, electrospray ionization mass spectrometry (ESI-MS) has been used for bimolecular analysis,[10–18] for example, the analysis of complexes between drugs and DNA,[10–13,16] RNA,[19,20] and proteins.[18] ESI-MS has clear advantages over other methods such as NMR spectroscopy and X-ray diffraction: it is fast, requires minimal samples, provides direct-viewing information and allows simple data processing, especially in some complicated cases, such as mixtures of several different DNA molecules.

We have reported previously noncovalent complexes between polyamides and double-stranded oligonucleotides containing the $(TTAGGG)_n$ sequence.[12] In the current study, ESI-MS and circular dichroism (CD) spectroscopy were used to investigate the selectivity, binding affinity, and stoichiometry of small molecules with human telomeric G-quadruplex DNA, and the factors affecting the structures of the quadruplex. The target DNA sequence chosen was a 24-bp oligonucleotide, $(AGGGTT)_4$, which can fold itself into a unimolecular quadruplex. The binding molecules selected for the quadruplex DNA were the ImImImβDp and PyPyPyγImImImβDp polyamides (Figure 1B: Py = *N*-methylpyr-

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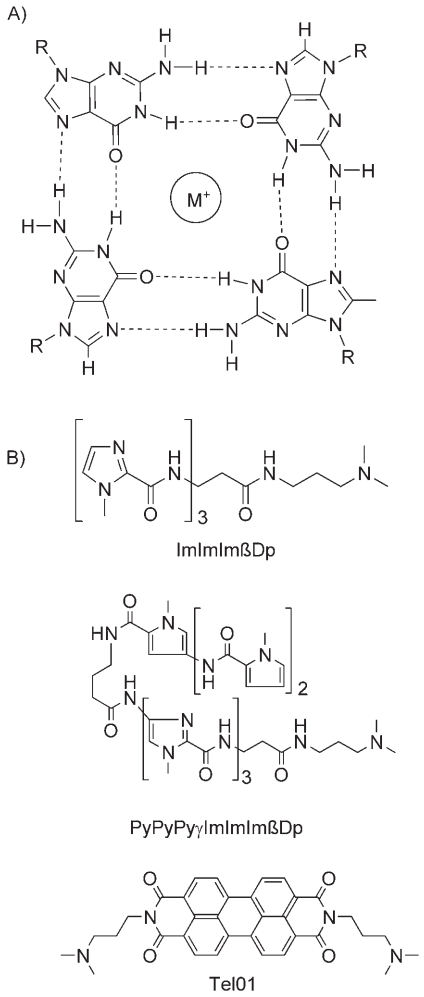

Figure 1. Schematic of a G-tetrad and typical binding molecules.

role; Im = *N*-methylimidazole; γ = γ-aminobutyric acid; β = β-alanine; Dp = *N*,*N*-dimethylpropyldiamine), which have good cell permeation and binding preference on base G,[21,22] and a perylene derivate (Tel01), containing a conjugate aromatic system in favor of π stacking with G-quartets.[23]

Results and Discussion

Binding affinity and stoichiometry of small molecules with human telomeric quadruplex DNA: Firstly, ESI-MS was utilized to investigate the binding affinity and stoichiometry between the human telomeric repeat sequence $(AGGGTT)_4$ ($M_w = 7575.0$) and small molecules, such as perylene derivate (assigned as Tel01), ImImImβDp (marked as III), and PyPyPyγImImImβDp (assigned as T6Y).

After adding the NH_4OAc buffer, the ESI-MS spectrum of the $(AGGGTT)_4$ oligonucleotides was recorded and revealed two main ions at *m*/*z* 1262 and 1514. The ion [quadruplex]$^{5-}$ (marked as Q^{5-}) at *m*/*z* 1514 could be distinguished easily from the ions with many adducts of single-stranded form (Figure 2), confirming the formation of the quadruplex

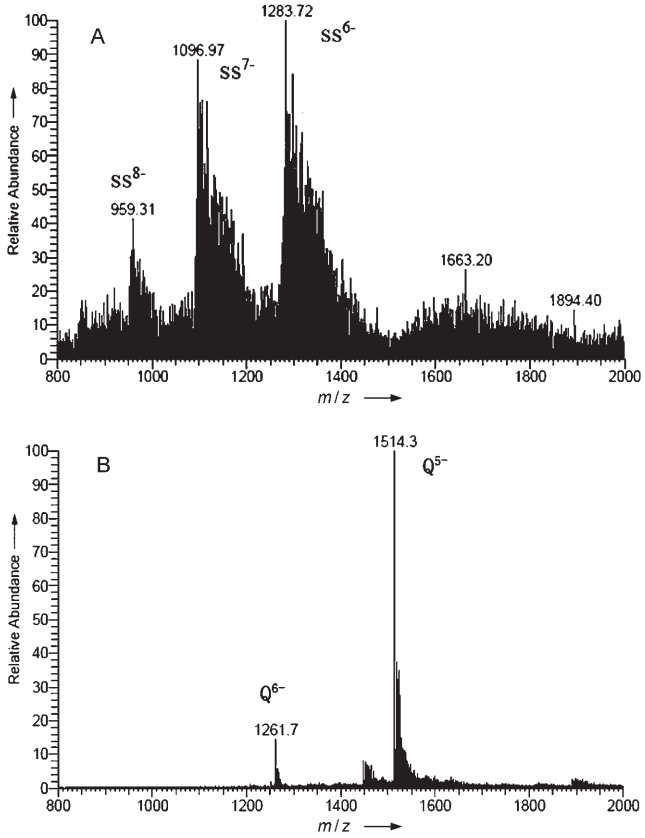

Figure 2. ESI-MS spectra of the oligonucleotides $(AGGGTT)_4$: A) In pure water; B) in NH_4OAc buffer.

upon addition of NH_4^+ ions. Because the Q^{5-} ion was the base peak, the 5− charge state was selected as the main target.

The binding affinity and stoichiometry were studied by mixing the quadruplex $(AGGGTT)_4$ with the three molecules in different molar ratios, ranging from 1:1 to 1:8 (Figure 3 shows the spectra resulting from a 1:2 molar ratio). The abundance ratio of [complex]$^{5-}$ to [quadruplex]$^{5-}$ was used to evaluate the binding affinities. In the case of ImImImβDp, the base peak was the Q^{5-} ion, and the 1:1, 1:2, and 1:3 complex ions had relative abundances of 73, 27, and 18%, respectively. We also found the 1:4 complex ion of ImImImβDp at higher molar ratios. For Tel01, the 1:2 complex ion [Q+2Tel01]$^{5-}$ was the base peak in the spectrum, and the Q^{5-} ion almost disappeared, whereas the 1:1 and 1:3 complexes were minor peaks. As the Tel01 concentration increased, the signal of the quadruplex (*m*/*z* 1514) decreased dramatically, strikingly faster than that of ImImImβDp. Moreover, the 1:2 complex ion remained as the base peak over the course of the increasing molar ratio, which suggested that Tel01 had a preference for the 1:2 binding stoichiometry. However, in the case of PyPyPyγImImImβDp, no complex ion could be observed at the highest molar ratio. As a result, the complex ions of Tel01 and ImImImβDp in the spectra indicated that they were suitable binders for human telomeric quadruplex DNA. In contrast,

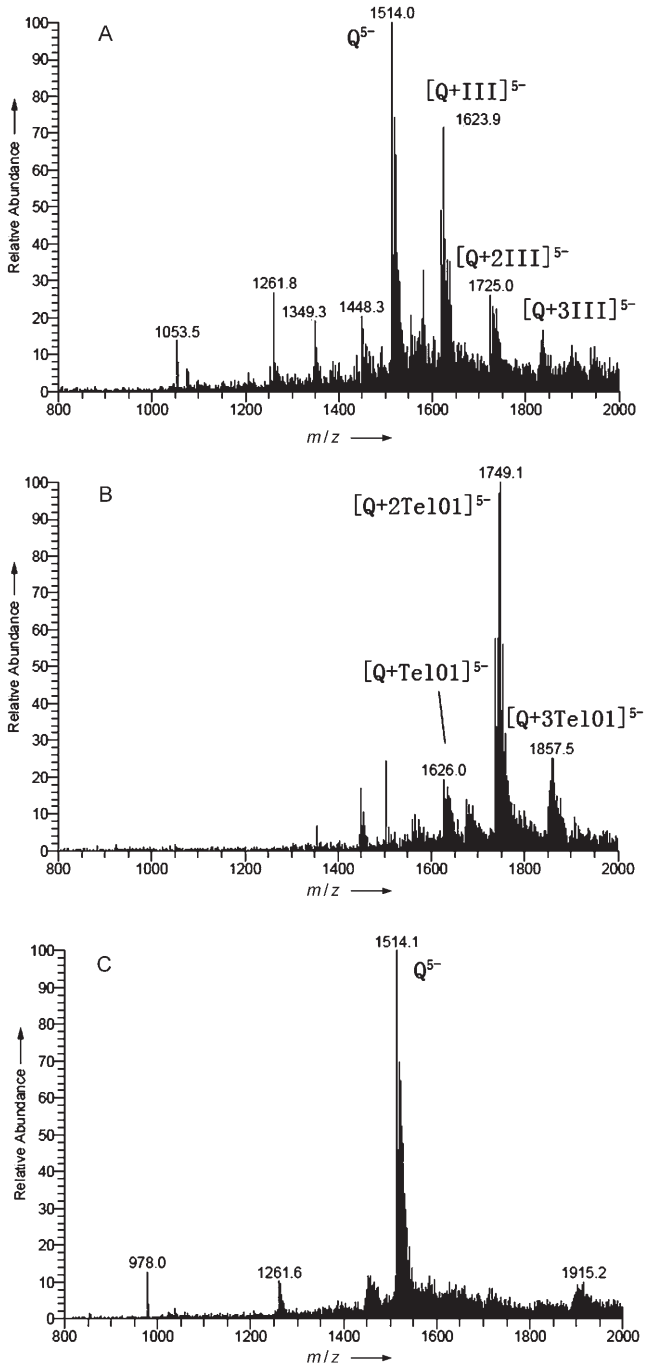

Figure 3. ESI-MS spectra of the binding molecule (A: ImImImβDp, B: Tel01, C: PyPyPyγImImImβDp) with $(AGGGTT)_4$ in a 1:2 molar ratio.

PyPyPyγImImImβDp showed a poor binding affinity. Therefore, the binding-affinity order of the three small molecules was indicated to be: Tel01 > ImImImβDp ≫ PyPyPyγImImImβDp.

Binding specificity of small molecules with human telomeric DNA: Considering that both duplex and G-quadruplex DNA should exist in human telomeres, and to simulate the telomeric system in vivo, the quadruplex DNA $(AGGGTT)_4$ (Q) and the duplex DNA d(TTAGGGTTAGGG/CCCTAACCCTAA) (D) were mixed and both peaks (*m*/*z* 1514 (Q^{5-}) and 1457 (D^{5-})) were revealed in the ESI-MS spectrum. A competition study for the duplex and the quadruplex DNA with a binding molecule was performed to determine directly the binding specificity (Figure 4). In the case of ImImImβDp, the ESI-MS spectrum indicated that this small molecule bound both duplex and quadruplex DNA and had no binding specificity. On the other hand, PyPyPyγImImImβDp had a clear preference to bind with the duplex DNA ($[D+T6Y]^{5-}$, *m*/*z* 1653), with no complex ion for the quadruplex, and the corresponding duplex peak (D^{5-}, *m*/*z* 1457) dramatically decreased. In the case of Tel01, it only bound with the quadruplex DNA ($[Q+Tel01]^{5-}$, *m*/*z* 1626; $[Q+2Tel01]^{5-}$, *m*/*z* 1738). The relevant quadruplex ion (Q^{5-}, *m*/*z* 1514) was completely consumed, and no complex of the duplex was observed.

These results suggest that PyPyPyγImImImβDp has a binding specificity for the duplex DNA, while Tel01 selectively recognizes the quadruplex DNA. This is consistent with the aforementioned binding affinities. Because the binding specificities were observed in one system that compares binding to duplex and quadruplex DNA, the biological significance of this study is tremendous and provides a powerful tool to screen the precursors of binding-site-specific anticancer drugs. Moreover, this is the first directly observed specific binding of small molecules in one system of duplex and quadruplex DNA, and ESI-MS provides a unique way to investigate the respective binding among a mixture of several oligonucleotides.

Effect of binding molecules on the conformation of $(AGGGTT)_4$: Firstly, the effect of binding molecules on the conformation of $(AGGGTT)_4$ was investigated by ESI-MS. As shown in Figure 2A, the mass spectrum of $(AGGGTT)_4$ in pure water revealed three main peaks with many adduct ions at *m*/*z* 1283, 1097, and 959, corresponding to 6−, 7−, and 8− charge states, respectively. In contrast, after adding the binder Tel01, the 7− and 8− ions of $(AGGGTT)_4$ almost disappeared, while the main peaks were the 6− charge state (Q^{6-}) of the oligonucleotides and the complex ion ($[Q+Tel01]^{6-}$) (Figure 5, *m*/*z* 1286 and 1373, respectively).

Because the conditions of the solution remained constant, Tel01 was the only factor inducing the decrease of adducts and charges. By considering the binding affinity and specificity of Tel01 with the quadruplex DNA, we can assume that Tel01 caused the transformation of the structure of $(AGGGTT)_4$ from a single-stranded conformation to a quadruplex. Because the quadruplex structure is more convergent than the single-stranded motif, the surface area of the single strand is larger than that of the quadruplex, suggesting more charges and adducts with the single strand. The result also indicated that Tel01 favored the formation and stabilization of the quadruplex. Furthermore, a CD titration assay was performed to confirm the results from ESI-MS. The conformational analysis of G-quadruplex

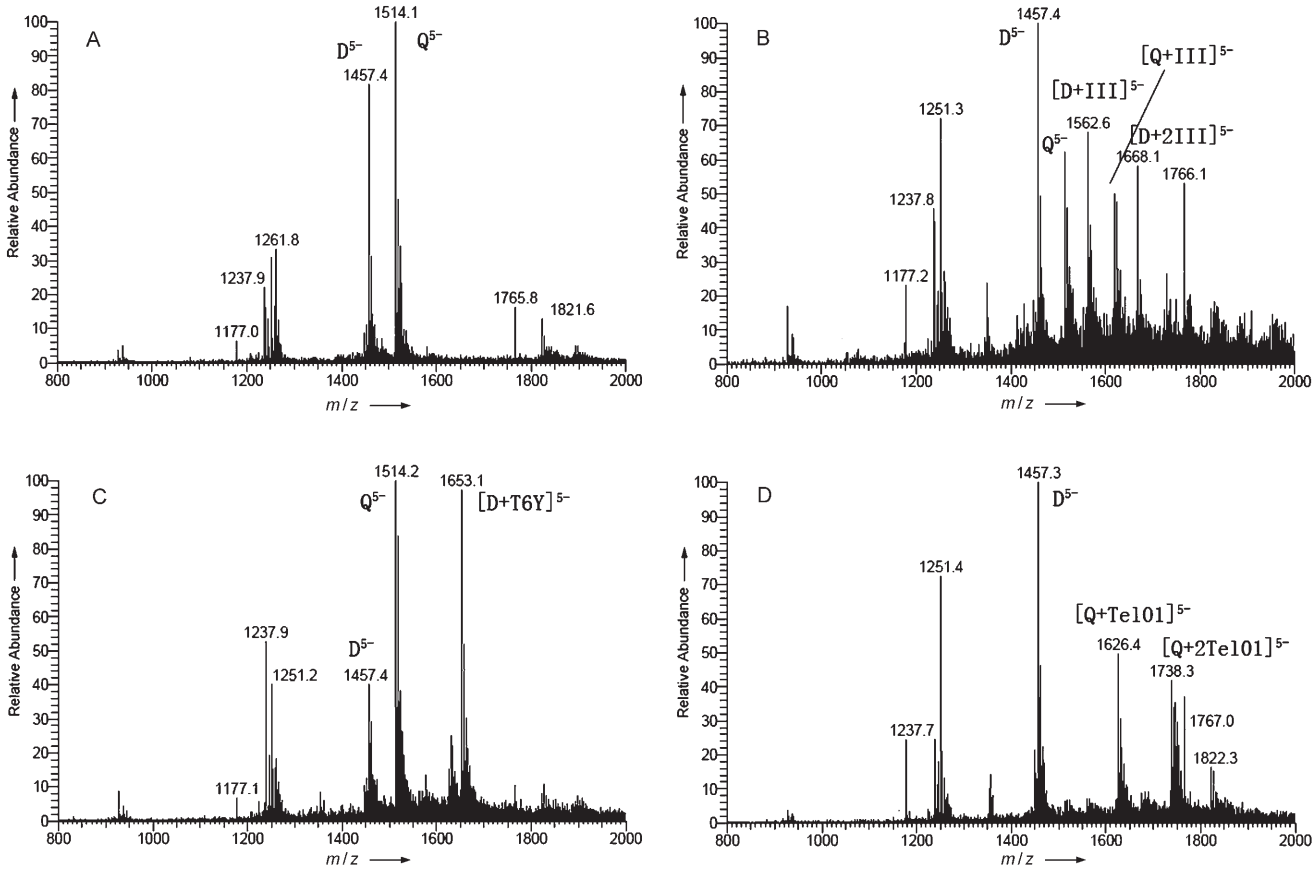

Figure 4. ESI-MS spectra of specific binding for certain molecules with $(AGGGTT)_4$ and d(TTAGGGTTAGGG/CCCTAACCCTAA): A) D:Q = 1:1; B) D:Q:III = 1:1:2; C) D:Q:T6Y = 1:1:2; D) D:Q:Tel01 = 1:1:2.

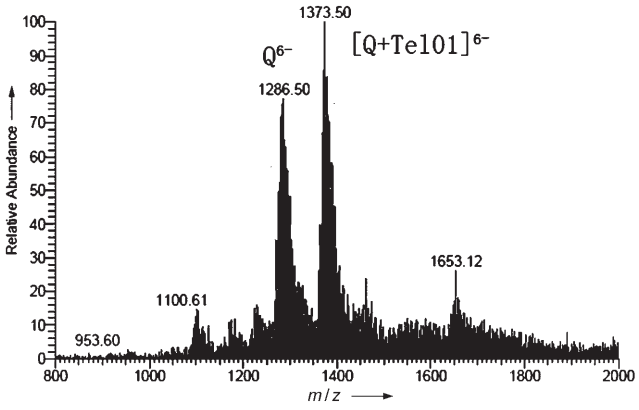

Figure 5. ESI-MS spectrum of Tel01 with $(AGGGTT)_4$ in pure water.

structures indicated that an antiparallel G-quadruplex structure had a positive peak near 295 nm and a negative peak near 265 nm in the CD spectra, whereas a parallel G-quadruplex structure had positive and negative peaks near 260 and 240 nm, respectively.[24] According to this information, the structural type of a G-quadruplex can be determined by CD measurement. Figure 6 shows the CD spectra of 25 μM $(AGGGTT)_4$ with various concentrations of Tel01 (from 0–50 μM) in a pure water solution.

The CD spectrum obtained in the absence of Tel01 in water had weak positive and negative peaks near 260 and 240 nm, respectively. This indicated that a small quantity of parallel G-quadruplex structure existed in the pure water. As the concentration of Tel01 increased, it induced the positive and negative CD intensity near 295 and 265 nm, indicat-

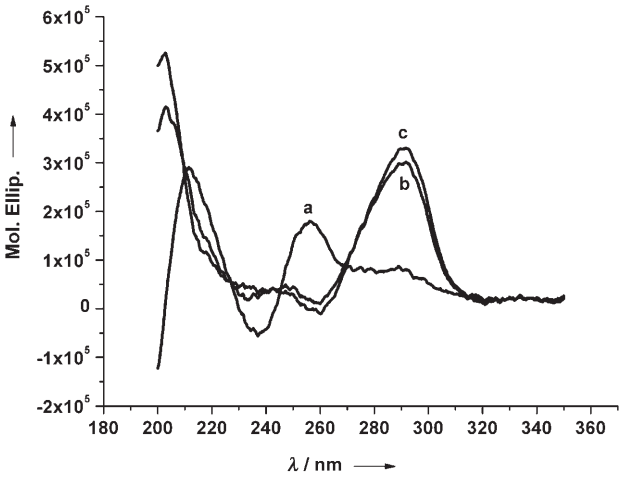

Figure 6. CD spectra of 25-μM $(AGGGTT)_4$ with various concentrations of Tel01 in pure water: a) 0-μM Tel01; b) 25-μM Tel01; c) 50-μM Tel01.

ing that the conformation of $(AGGGTT)_4$ had altered into an antiparallel G-quadruplex. Furthermore, the intensity of the positive peak after addition of Tel01 was much higher than that before the titration, suggesting more G-quadruplex structures induced by Tel01. These results led to the conclusion that Tel01 was of advantage to the formation and stability of the quadruplex structure, consistent with the results by ESI-MS, and Tel01 had a tendency to bind with an antiparallel G-quadruplex.

Effect of annealing on the conformation of $(AGGGTT)_4$: Figure 7 shows the ESI-MS spectra of ImImImβDp and Tel01 binding with $(AGGGTT)_4$ in a 1:1 molar ratio before and after annealing. Panels A and C show that before annealing, the complex ions of ImImImβDp and Tel01 reveal abundances of 24 and 100% (*m*/*z* 1619 and 1626, respectively), and the relative abundance ratios ($[Q+binder]^{5-}/Q^{5-}$) were 0.24 and 1.16, respectively. Panels B and D show that after annealing, the abundances of $[Q+III]^{5-}$ and $[Q+Tel01]^{5-}$ were 32 and 6%, respectively, and the relative abundance ratios ($[Q+binder]^{5-}/Q^{5-}$) were 0.32 and 0.06, respectively. Comparison of the ESI-MS spectra before and after annealing shows that the relative abundance ratio of Tel01 decreases dramatically, indicating a change of $(AGGGTT)_4$ conformation.

Moreover, the CD spectra before annealing revealed positive peaks near 260 and 295 nm, as shown in Figure 8, which indicated that the $(AGGGTT)_4$ sequence in NH_4OAc contained both parallel and antiparallel G-quadruplexes before annealing. After annealing, the CD intensity of the positive peak near 260 nm increased and that of the peak near 295 nm reduced, instead, a rising negative peak near 240 nm occurred. This reveals the structural transformation from a coexistence of parallel and antiparallel G-quadruplexes to a parallel G-quadruplex.

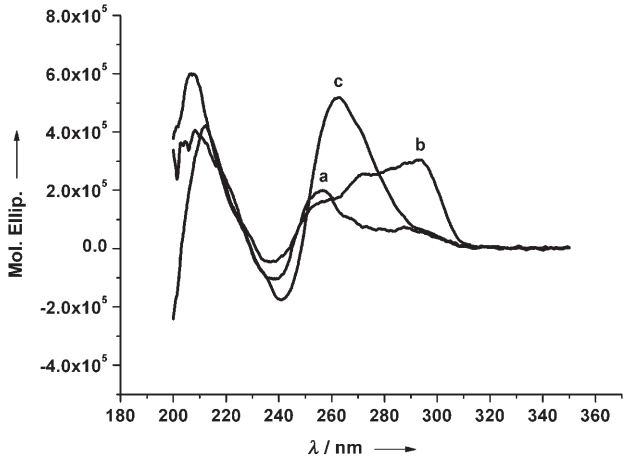

Figure 8. CD spectra of the effects of annealing on the conformation of $(AGGGTT)_4$: a) in pure water; b) in NH_4OAc buffer without annealing; c) in NH_4OAc buffer with annealing.

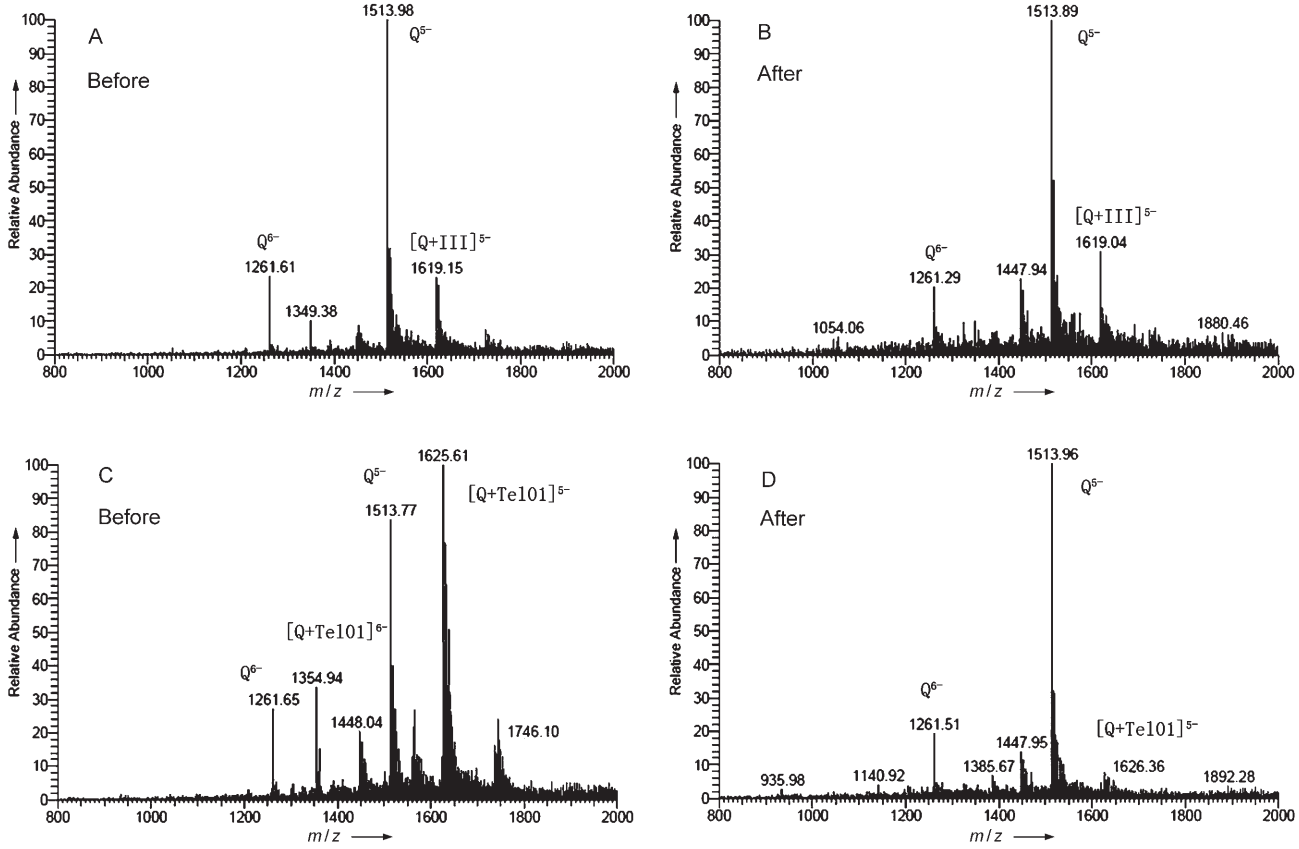

Figure 7. ESI-MS spectra of binding molecules with $(AGGGTT)_4$ before and after annealing: A) Q:III = 1:1, before annealing; B) Q:III = 1:1, after annealing; C) Q:Tel01 = 1:1, before annealing; D) Q:Tel01 = 1:1, after annealing.

From the results of both MS and CD spectra we can conclude that annealing induced the structural transition of the $(AGGGTT)_4$ sequence from a coexistence of parallel and antiparallel G-quadruplexes to a parallel G-quadruplex. Considering the conclusion described above, that Tel01 tends to bind with an antiparallel G-quadruplex, it is clear that the parallel conformation of the quadruplex after annealing is unfavorable for the binding of Tel01.

Conclusion

Electrospray ionization mass spectrometry was utilized to investigate the binding affinity and stoichiometry of small molecules with human telomeric G-quadruplex DNA. Furthermore, the specific binding of certain molecules in one system consisting of both quadruplex and duplex DNA was observed directly for the first time. By using ESI-MS and CD spectra, the factors affecting the conformation of $(AGGGTT)_4$ quadruplex, such as molecular binding and DNA annealing, could be summarized. This research, using ESI-MS and CD spectroscopy to study the specific binding and the conformation of quadruplex DNA, provides a useful tool to further investigate biological phenomena of interactions between small molecules and human telomeric DNA.

Experimental Section

DNA: Single-stranded oligonucleotides were purchased from Augct (Beijing, China) and oligonucleotides were directly dissolved in deionized water. For duplex DNA, two complementary single-stranded oligonucleotides were mixed in equimolar proportions, annealed at 90 °C, and cooled slowly to RT (over 4 hr) to allow formation of the duplex. For quadruplex DNA, annealing was according to the assay. Desalting was performed three times by using Microcon filters (Amicon, Beverly, MA, USA). The resulting DNA stock solution was 500 μM.

Synthesis: A haloform reaction was used to synthesize polyamide ImImImβDp and PyPyPyγImImImβDp (yields 65 and 58%, respectively), and the purification and characterization of polyamides was performed according to our previous paper.[22] The perylene derivative Tel01 was synthesized by the refluxed mixture of 3,4,9,10-perylenetetracarboxylic dianhydride and 3-(dimethylamino)propylamine (Dp) (yield 86%).

Mixing and binding assays: Oligonucleotides were stocked in deionized water. Binding molecules were dissolved in a mixture of methanol/water (50:50, v/v) at a concentration of 500 μM. Each 2.0-μL DNA solution was mixed with 2.0–16 μL of binder solutions, and then diluted with 20:80 (v/v) methanol/100-mM ammonium acetate to 40 μL (resulting in 25 μM for each DNA). Methanol was added to obtain a good spray.[10,11]

Mass spectrometry: ESI-MS spectra were obtained in the negative-ion mode by using a Finnigan LCQ Deca XP Plus ion trap mass spectrometer (San Jose, CA). The direct infusion flow rate was 2 μL min^{-1}. The electrospray source conditions were spray voltage of 2.0–2.5 kV and capillary temperature of 100 °C, and a double sheath gas to ensure efficient desolvation. Data were collected and analyzed by using the Xcalibur software developed by ThermoFinnigan, and ten scans were averaged for each spectrum.

Circular dichroism: The CD spectra of DNA oligonucleotides were measured by using a J-810 spectropolarimeter (JASCO, Japan) with a 0.1-cm path-length quartz cell at 25 °C. The CD spectrum was obtained by taking the average of three scans made at 0.1-nm intervals from 200 to 350 nm. The total concentrations of all DNA samples were between 10 and 25 μM.

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