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Polyethylenimine effectively induces, stabilizes, and regulates intramolecular G-quadruplexes

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ABSTRACT

The interaction between polyethylenimine (PEI) and telomeric DNA was studied. The results demonstrated that PEI could effectively induce, stabilize, and regulate intramolecular G-quadruplexes. The mechanism for these results has been discussed, and was estimated to be condensation effect of PEI.

In vertebrates, telomeric DNA contains a single-stranded 3′-end overhang with a simple repeat sequence, TTAGGG, which could form a stable structure named G-quadruplex through Hoogsteen hydrogen bonding.[1] G-quadruplex structure has been used as a target for anti-cancer drug design because it could inhibit telomerase activity which ties up with cellular immortalization and tumorigenesis.[2] Thus, molecules that could induce or stabilize G-quadruplex structures are thought to be telomerase inhibitors as well as potential anti-cancer drugs. Up to now, great efforts has been devoted to investigating aromatic molecules with extended planar structures because the extended planar structures enable these molecules to intercalate or stack into G-quadruplexes easily and enhance G-quadruplex stability.[3] However, little attention has been paid to polymers as G-quadruplex ligands, although they could be seen everywhere and show advantages in preparation, purification and chemical modification as well as stability.

PEI, a polycation, has been widely used in gene transfer system to interact with DNA, where PEI condenses DNA to form a compact structure, and packs DNA to shield DNA against non-specific interaction with other molecules.[4] In fact, intramolecular G-quadruplex structure is the compact structure of G-rich DNA sequences with a special topology. One may wonder what effect would be observed when PEI interacts with telomeric DNA or G-quadruplex structures. In this Letter, the interaction between telomeric DNA, G-quadruplex and PEI has been investigated by using various spectrometric methods.

Figure 1 showed the effect of PEI on the CD spectra of human telomeric DNA d($(T_2AG_3T_2AG_3)_4$) (abbreviated as H24). The spectrum of H24 without PEI present exhibited two negative bands at 236 and 278 nm, and a positive band at 256 nm, corresponding to the random conformation of H24.[5] After adding PEI, these three bands disappeared while two new positive bands at 270 and 300 nm and a negative band at 246 nm appeared, which was

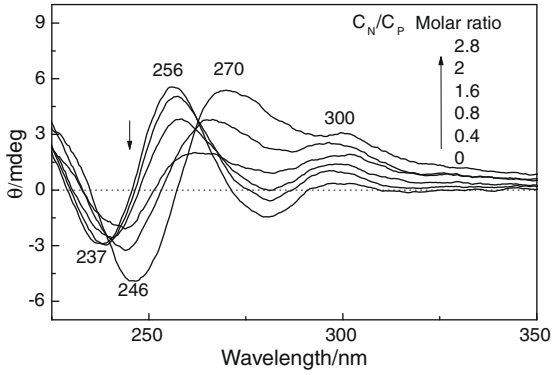

Figure 1. CD spectra of 4 μM H24 with different amount of PEI in Tris–HCl buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.4). The C_N/C_P ratio denotes the quotient of the nitrogen atoms of PEI to H24 phosphates.

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consistent with the CD spectral feature of the mixture of the parallel and antiparallel G-quadruplexes or mixed-type G-quadruplexes that had both parallel and antiparallel characteristics,[6] implying the formation of hybrid or mixed-type G-quadruplexes. Variant temperature absorption[7] (Fig. S1) and fluorescence resonant energy transfer (FRET)[8] (Fig. S2) spectra could further support this result. Moreover, the denaturation temperature of G-quadruplexes induced by PEI is concentration-independent (Fig. S3), implying the G-quadruplexes induced by PEI resulted from intramolecular folding of H24.

Several proto-oncogenes, including c-MYC, Bcl-2, c-kit, also contain guanine-rich DNA sequences, which have been proved to form G-quadruplexes with cations present.[9] Here, the DNA sequences of d($G_3TG_3TAG_3$ TG_3) (c-MYC), d($G_3CGCG_3AG_2A_2T_2G_3CG_3$) (Bcl-2), and d($AG_3AG_3CGCTG_3AG_2AG_3$) (c-kit) could also be induced to form G-quadruplexes by PEI under cations deficient conditions. All of their CD spectra showed a negative band at 243 nm and a positive band at 267 nm after PEI was added in (Fig. S4), which was consistent with the spectral feature of parallel G-quadruplexes,[10] indicating these sequences formed parallel G-quadruplexes.

Compared with the conventional G-quadruplex ligands, PEI has no extended planar aromatic structure, thus the formation of G-quadruplexes induced by PEI could not be through π–π interaction. However, PEI has its special characteristics as a polycation, for example easily forming molecular crowding condition, with positive charged amino groups, and condensation effect. These characteristics may be the factors for inducing G-quadruplex formation.

Molecular crowding has been proved to enable G-quadruplex formation.[11] For example, polyethylene glycol (PEG) could induce G-quadruplex formation because of a molecular crowding condition formed by excessive PEG (almost 40% (w/v)).[11] As a polymer, PEI maybe also caused a molecular crowding condition. However, the concentration of PEI was too low (several μM) herein to form a molecular crowding condition as that PEG did. Moreover, the solution viscosity only slightly changed after PEI with such low concentrations was added in (Fig. S5), also excluding the molecular crowding condition. Therefore, the molecular crowding could not account for the formation of G-quadruplexes here.

Electrostatic interaction is a generally used method for inducing G-quadruplexes.[12] So far, several Group 1 and 2 metal cations and NH_4^+ have been identified as inducing G-quadruplex formation. As a polycation, PEI could provide positive charges by its amino groups, which also probably compel G-quadruplex formation. Here, NH_4^+ with the same concentrations to that of the amino groups of PEI was used to mimick the positively charged amino groups of PEI. However, H24 with such low concentrations of NH_4^+ present still exhibited a CD spectral feature of random conformation (Fig. S6), meaning the electrostatic interaction between PEI and H24 could not result in the formation of G-quadruplex.

Moreover, spermine, a polyamine, that is similar to PEI in structure but is much shorter than PEI was also used to interact with H24. The results showed spermine could not effectively induce G-quadruplex formation (Fig. S7). Diethylene triamine, another polyamine much shorter than PEI, also had no capability of inducing G-quadruplex structures.[13] Based on above results, we expected that the macromolecular structures should play a key role in inducing G-quadruplex formation.

It has been well-known that condensation was a special effect of PEI on DNA as a macromolecule, which made DNA to form compact structures.[14] Based on this effect, PEI has been used to condense and pack DNA to shield DNA against non-specific interaction with blood components, extracellular matrix and untargeted cells in gene transfer system.[13] G-quadruplex just is a compact structure of DNA. Thus, it is possible for PEI condensing telomeric DNA H24 to form compact structures, which leads the guanines in DNA to be close-by thus facilitates intramolecular hydrogen bonding within DNA and final compels G-quadruplex structures formation.

The condensation effect could be supported by the interaction between PEI and the oligonucleotide d(T_2AG_3T) (abbreviated as H7). If condensation effect was the major factor for PEI inducing intramolecular G-quadruplexes formation, H7 G-quadruplexes shouldn't be induced, because these G-quadruplexes needed four chains of H7 bonding to each other simultaneously, rather than a single chain folding.[15] As expected, the result of the CD spectra showed that PEI could not induce G-quadruplex formation of H7, indirectly supporting the condensation effect of PEI for inducing intramolecular G-quadruplexes (Fig. S8).

Further, the absorption spectra of H24 with PEI also supported this mechanism (Fig. S9), where the changes in 230–600 nm region were attributed either to the change of extinction coefficient of nucleic bases as a result of structural alterations in DNA secondary structure or to the light scattering by micellar structures formed by DNA with PEI, since PEI has no any absorption band in this region.[16] A regular increase of absorbance with increasing [PEI] was observed, corresponding to the formation of a compact structure of H24 with PEI.

The results above have indicated that PEI was prone to make telomeric DNA to form a compact structure, G-quadruplex. Actually, the denaturation of G-quadruplexes is the process of G-quadruplexes becoming extended random structures. Thus it could be expected that PEI would also show effectivity in stabilizing G-quadruplex structures. As expected, PEI could effectively stabilize kinds of G-quadruplexes formed in the presence of potassium (Table 1). However, the efficiency of PEI stabilizing G-quadruplexes was different, which depended on the structures of G-quadruplexes and the length of the sequences.

The G-quadruplex formed by d($(T_2AG_3)_2$) (H12) was an intermolecular G-quadruplex composed of two DNA strands,[17] and that formed by H24 was an intramolecular G-quadruplex. The melting temperature of H24 G-quadruplexes increased by PEI was obviously higher than that of H12 G-quadruplexes, indicating PEI stabilizing intramolecular G-quadruplexes was more effective than that intermolecular G-quadruplexes.

The sequences with the similar length, H24, Bcl-2 and c-kit, have been known to form intramolecular G-quadruplexes under potassium conditions.[9] The topologies of their G-quadruplex structures were different, and H24 G-quadruplexes were the mixture of antiparallel and parallel structures,[18] Bcl-2 G-quadruplexes were mixed-type structures,[9] and c-kit G-quadruplexes were parallel structures (Fig. S10). The results demonstrated that PEI stabilizing parallel G-quadruplexes formed by c-kit was more effective than that of the mixture and mixed-type G-quadruplexes. Moreover, c-MYC also formed parallel G-quadruplex structures (Fig. S10).[19] However, the melting temperature of c-MYC increased by PEI was much lower than that of c-kit, which is probably due to the short length of c-MYC sequences.

All of the results above have demonstrated that PEI effectively induced and stabilized G-quadruplexes through condensation effect. However, it should be noted that the topologies of

Table 1
The melting temperature of different G-quadruplexes without (T_{m0}) and with (T_{m1}) PEI ($C_N/C_P = 2$) presence

Name	T_{m0} (°C)	T_{m2} (°C)	ΔT_m (°C)
H12	56	78	22
H24	48	85	37
Bcl-2	58	95	37
c-kit	52	95	43
c-MYC	80	93	13

G-quadruplexes were different, which decided the size and the charge distribution of their molecular structures.[20] Therefore, it is probable that these G-quadruplexes readjusted their structures to adapt the around conditions under the condensation and the package of PEI. The structural transformation of antiparallel and mixed-type G-quadruplexes with PEI present has been proved by the following experiments.

The oligonucleotide $d(AG_3(T_2AG_3)_3)$ (H22) formed antiparallel G-quadruplexes under Na^+ conditions,[21] whose CD spectrum showed a major positive band centered at 295 nm, a negative band at 263 nm, and a minor positive band at 245 nm (Fig. 2A).[22] After PEI was added in, the CD spectrum of H22 was greatly changed, and showed two positive bands at 265 and 295 nm and a negative band at 243 nm. The new CD spectrum was just in accordance with the CD spectral characteristics of hybrid/mixed-type G-quadruplexes, suggesting antiparallel G-quadruplexes were converted to parallel or mixed-type G-quadruplexes.[6]

The oligonucleotide $d(T_2G_3(T_2AG_3)_3A)$ (H24A) has been reported to form mixed-type G-quadruplex under K^+ conditions.[23] Its CD spectrum showed two positive bands at 267 and 285 nm, and a negative band at 240 nm (Fig. 2B). With PEI present, the CD spectrum of H24A was similar to that of parallel G-quadruplexes, which had a minor negative band centered at 242 nm and a major positive band at 264 nm,[24] indicating the transition from mixed-type to parallel G-quadruplexes of H24A.

The selectivity of PEI for G-quadruplexes versus duplexes has also been test. The thermal stabilization induced by PEI was greatly affected after the similar amounts of duplexes to that of G-quadruplexes were added in, and the melting temperature of G-quadruplexes was changed from 85 °C to 60 °C after duplexes were added in (Fig. S11). Though PEI could still stabilize G-quadruplexes in the presence of duplexes, the melting temperature of G-quadruplexes has lost 25 °C compared with that duplexes were absent. The results indicated PEI has a relatively weak selectivity for G-quadruplexes versus duplexes.

In summary, we herein reported an unusual ligand, PEI, effectively inducing, stabilizing, and regulating intramolecular G-quadruplex structures. The mechanism was discussed, and probably the condensation effect of PEI plays a key role. This result was quietly different from other reports where G-quadruplex formation was mainly dependent on the interaction of DNA sequences with metal cations or planar aromatic ligands, and only intramolecular G-quadruplexes could be specifically induced and stabilized while intermolecular G-quadruplex could not be influenced obviously. Additionally, PEI also affected the G-quadruplex structure formed under Na^+ or K^+ conditions, and converted antiparallel to parallel or mixed-type, and mixed-type to parallel G-quadruplex structures.

These unique features of polymers were significant for anti-cancer drugs filtration targeting G-quadruplexes. Up to now, almost all the G-quadruplex ligands used have extended planar aromatic structure, which facilitates ligands to stack onto or insert into G-quadruplexes.[3] Compared with these ligands, PEI exhibits similar abilities in inducing, stabilizing and regulating G-quadruplexes. Though there is some further work needs to do in order to improve the selectivity of these polycations for G-quadruplexes versus duplexes, this work may provide a new sight in finding G-quadruplex ligands.

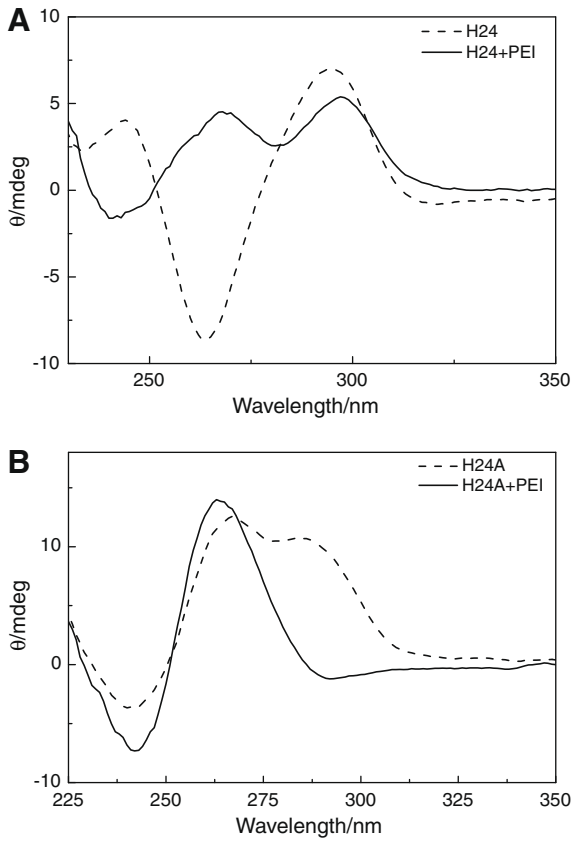

Figure 2. CD spectra of (A) (3.78 μM) H22 in 10 mM Na-phosphate buffer (10 mM NaH_2PO_4/Na_2HPO_4, 1 mM EDTA, pH 7.0), and (B) (3.6 μM) H24A in 10 mM K-phosphate buffer (10 mM K_2HPO_4/KH_2PO_4, 1 mM EDTA, pH 7.0) with PEI absent or present ($C_N/C_P = 2.5$).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.082.

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