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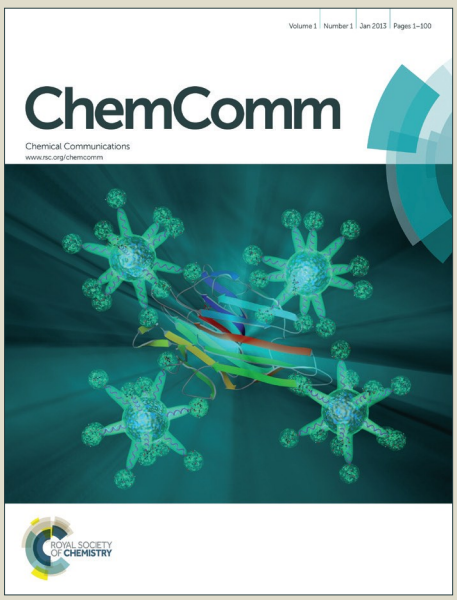

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Enantioselective Targeting Left-handed Z-G-Quadruplex

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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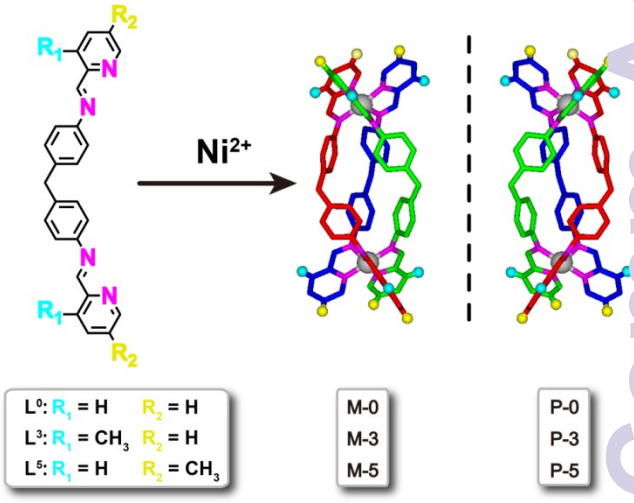
Herein we report the first example that M-enantiomer of a chiral metal complex can selectively stabilize left-handed G-quadruplex, but its P-enantiomer cannot. The interactions between the chiral metal complexes and left-handed G-quadruplex were evaluated by UV melting, circular dichroism, isothermal titration calorimetry, gel electrophoresis and NMR titrations.

DNA is polymorphic and has diverse conformations. Chiral DNA recognition has been the subject of increasing interest due to the fact that multitudinous chiral drugs have been applied in clinic annually and some of them have been demonstrated working directly on DNA.[1] However, previous studies on DNA chiral recognition are mostly focused on GC double-stranded DNA B-Z transition using a pair of chiral molecules.[2] As noncanonical DNA secondary structures, G-quadruplexes have been regarded as rising therapeutic targets in oncology since they are widespread in gene promoter regions and especially in telomeres, and substantial G-quadruplex targeting ligands were designed and proved can selectively interact with G-quadruplex.[3] Nevertheless, exploring chiral ligands that can target G-quadruplex is still at the primary stage. Besides, natural G-quadruplexes have long been observed only in right-handed form though their conformation is highly polymorphic.[4] However, most recently, Phan's group recently reported a left-handed G-quadruplex (Z-G4) formed by the G-rich sequence derived from AS1411 which showed effective antiproliferative activity against a number of cancer cells and was found to adopt multiple G4 conformations.[5] This wholly new structure awakened our interest since no one has studied the effect of G-quadruplex's chirality on chiral DNA recognition.

Certain G-rich DNA sequences can fold into unusual secondary structures with the help of metal cations, which named G-quadruplex.[6] It has been proposed that G-quadruplexes take part in several important biological processes, such as transcription, translation and telomere maintenance.[7] More importantly, a range of dramatic experiments confirmed that G-quadruplexes unambiguously exist in human genomic DNA.[8] Hence, numerous ligands have been developed to targeting G-quadruplexes for the purpose of regulating the biological processes G-quadruplexes involved.[9] A common feature of these ligands is that almost all of them containing polyaromatic hetercycles which facilitate their interactions with G-quartets through stacking on the end of G-quadruplex. In contrast to these organic ligands, few chiral metal ligands have been reported to interact with G-quadruplex.



Scheme 1. Structures of the chiral supramolecular complex $[Ni_2L_3^0]^{4+}$ and its two methyl-substituted derivatives, $[Ni_2L_3^3]^{4+}$ and $[Ni_2L_3^5]^{4+}$.

Chiral metal complexes as sort of metal complexes can provide unique molecular probes for DNA owing to their asymmetric size and shape.[10] More recently, our group reported that the enantiomers of a chiral metal complex-

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

$[Ni_2L_3^0]^{4+}$ (Scheme 1)- show chiral recognition to human telomeric G-quadruplex DNA (Tel22).[11] Thereinto, the P-enantiomer can stabilizes both antiparallel and hybrid G-quadruplex and convert the antiparallel form into hybrid form whilst the M-enantiomer shows no preference for antiparallel G-quadruplex and a weaker preference for hybrid G-quadruplex compared with the P-enantiomer. This indicates the chirality of chiral metal complex plays key roles in G-quadruplex recognition. However, there is no report to show whether the chirality of G-quadruplex influences chiral DNA recognition. Herein, we report that the M-enantiomer of $[Ni_2L_3^0]^{4+}$ shows chiral selectivity to left-handed G-quadruplex but not its P-enantiomer, indicating that the chirality of G-quadruplex plays an important role in chiral DNA recognition.

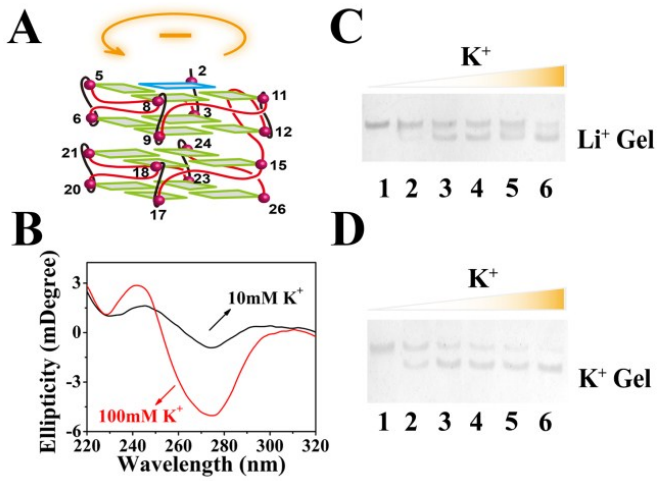

Fig. 1 (A) Structure of the left handed G-quadruplex. Anti and syn guanine bases are colored in green and blue, respectively. (B) CD spectra of Z-G4 under different concentration of K^+ in 10mM Tris buffer (pH 7.2). Native gel electrophoretic analysis of Z-G4 in Li^+ (C) and K^+ (D) containing gel. The samples in different lane were prepared with different cations conditions: lane 1, 100 mM Li^+; lane 2, 90 mM Li^+ and 10 mM K^+; lane 3, 70 mM Li^+ and 30 mM K^+; lane 4, 50 mM Li^+ and 50 mM K^+; lane 5, 30 mM Li^+ and 70 mM K^+; lane 6, 100 mM K^+. Gels were run in TB buffer containing 10 mM LiCl or KCl at 4 °C.

First, circular dichroism (CD) spectrum was used to characterize the secondary structure of Z-G4 under different K^+ concentration (Fig. 1B). Obviously, both of the spectra display a negative band at 275 nm and a positive band around 250 nm, indicating the formation of left-handed G-quadruplex,[5] and the band intensity was increased as K^+ concentration increased. Native gel electrophoretic analysis (PAGE) was employed to further investigate the structure of Z-G4. As shown in Fig. 1C, in Li^+ containing gel, the Z-G4 sequence form a homogeneous band with just Li^+ added. Since Li^+ cannot induce the formation of G-quadruplex, this band should be unstructured single stranded Z-G4. With addition of K^+, however, a new band which moved faster than the unstructured one was observed, suggesting the formation of G-quadruplex, and the new band content was increased obviously with the increase of K^+ concentration. We also performed the PAGE experiments in K^+ containing gel, the results were in accord with the one obtained from Li^+ containing gel (Fig. 1D). Therefore, both C[illegible] and PAGE experiment showed that Z-G4 sequences forme[illegible] left-handed G-quadruplex under K^+ containing buffer, and 10[illegible] mM K^+ could induce almost all Z-G4 sequences fold into C[illegible] quadruplex under our experimental condition.

Then, we studied the chiral selectivity of M-0 and P-0 (chemical structure shown in Scheme 1) to Z-G4 through U[illegible] melting experiment. Clearly, in buffer containing 100 mM K^+, only M-0 can stabilizes Z-G4 whilst P-0 cannot (Fig. 2A). Th[illegible] result is intriguing since previously we have reported that the ability of M-0 on stabilize human telomeric G-quadruplex (right-handed G-quadruplex) is much weaker than that of P-[illegible] in K^+ containing buffer (Fig. S2). The different chiral selectivi[illegible] of the enantiomer to different chiral G-quadruplex imply th[illegible] chirality of G-quadruplex also plays a role in the chira[illegible] recognition though Z-G4 is not the mirror image of huma[illegible] telomeric G-quadruplex. Next, CD titration was used [illegible] characterize the effect of M-0 and P-0 on the conformation o[illegible] Z-G4. Obviously, both of the enantiomers did not change [illegible] conformation of Z-G4 (Fig. 2B and 2C). We also performed isothermal titration calorimetry (ITC) experiments (Fig. 2D) [illegible] measure the binding affinity of M-0 with Z-G4. A 1:1 bindi[illegible] ratio was observed for M-0 binding with Z-G4, and the binding constant between M-0 and Z-G4 was estimated to b[illegible] $3.5(\pm0.3)\times10^6$ M^{-1}. However, the binding constant between P-0 and Z-G4 was estimated to be $2.3(\pm0.4)\times10^4$ M^{-1} (Fig. S4 an[illegible] Table S1).

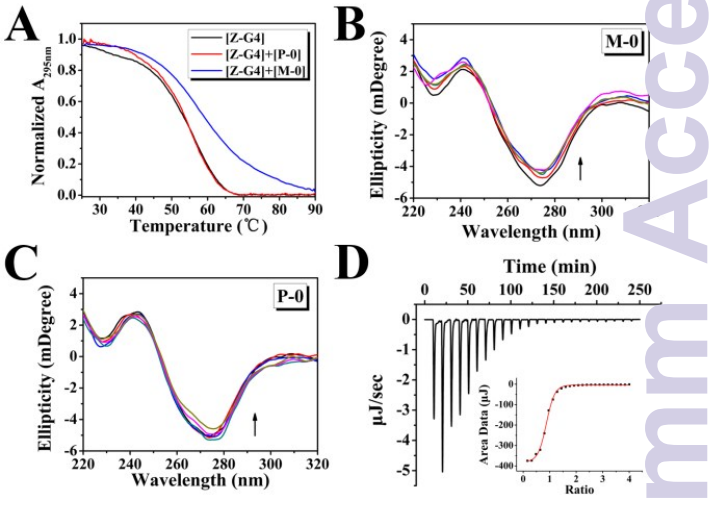

Fig. 2 (A) UV melting spectra of Z-G4 in the absence and presence of P-0/M-0. CD titration of Z-G4 with P-0 (B) and M-0 (C) in 10 mM Tris buffer (pH 7.2) containin[illegible] 100 mM KCl. The concentration of P-0/M-0 was varied from 0 μM to 6 μM. (D) ITC spec[illegible] for the titration of M-0 into the solution of Z-G4. Inset figure represent the corresponding normalized heat signals versus molar ratio.

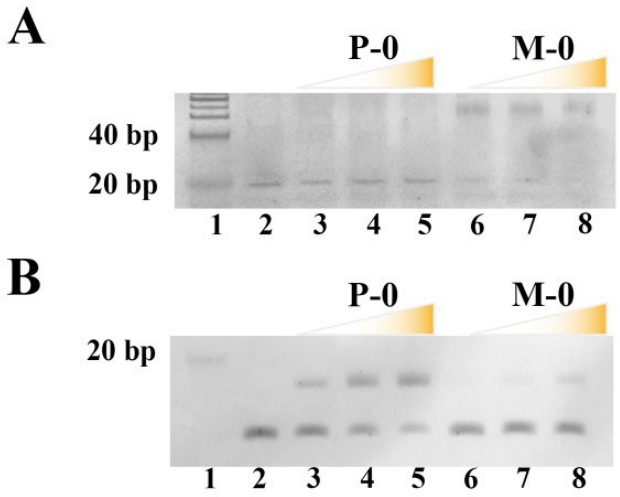

Fig. 3 Native gel electrophoretic analysis of (A) Z-G4 and (B) Tel22 in the presence of different concentrations of NiM and NiP: lane 1, DNA marker; lane 2, DNA alone; lanes 3-5, DNA with Ni-P at ratios of 2:1,1:1, and 2:3, respectively; lanes 6-8, DNA with Ni-M at ratios of 2:1, 1:1, and 2:3, respectively. Gels were run in TB buffer containing 10 mM KCl at 4 °C.

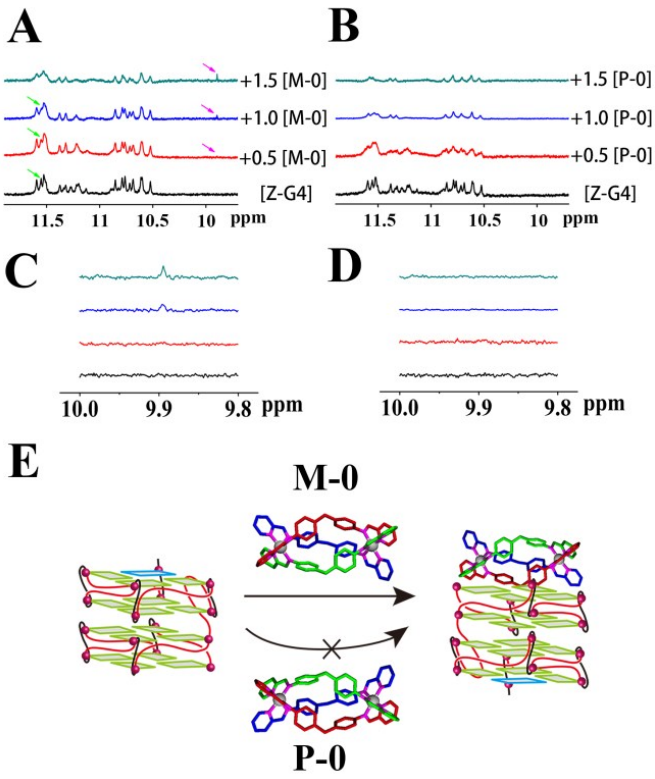

Fig. 4 ^{1}H NMR titration of Z-G4 with M-0 (A) and P-0 (B) at various [Ligand]/[Z-G4] ratios. The green arrow points to the imino proton peak corresponding to G17 whilst the red arrow points to the new peak. (C) Locally amplified ^{1}H NMR spectra in (A) centered at 9.9 ppm. (D) Locally amplified ^{1}H NMR spectra in (B) centered at 9.9 ppm. The profiles were measured in 10 mM Tris-100 mM KCl buffer containing 10% D_2O. (C) Schematic illustration of chiral metal complex selective recognition of Z-G4.

Furthermore, the chiral selectivity was confirmed by PAGE experiment. In the presence of the M-0, a new band moved slower than Z-G4 was observed owing to the formation of M-0/Z-G4 complex (Fig. 3A). In contrast, no new band was observed for Z-G4 mixed with P-0, indicating the weaker interaction between P-0 and Z-G4. For comparison, we also performed the PAGE experiment on human telomeric G-quadruplex. As we have previously reported, both P-0 and M-0 can form a retarded band with human telomeric G-quadruplex where the band intensity of P-0-Tel22 is stronger than that of M-0-Tel22 (Fig. 3B).[11a] Hence, PAGE experiments also demonstrate that M-0 binds selectively to Z-G4 and chiral recognition of G-quadruplex is related to the chirality of G-quadruplex.

The interactions between the enantiomers and Z-G4 were further studied by ^{1}H-NMR titration experiment (Fig. 4A and 4B). The ^{1}H-NMR spectrum of Z-G4 shows 16 sharp peaks between 10.4 and 11.6 ppm, indicating the formation of Z-G4 which is in line with previous report.[5] With addition of M-0, the imino proton peak corresponding to G17 is shifted obviously upfield and not broaden, indicative of a well-defined stacking of M-0 over G17.[3i, 6d] Due to the fact that the NMR spectrum of Z-G4 could not be assigned into free and bound signal after incubating with M-0 (Fig. S5),[12] and in view of the size and shape of M-0 (length ~18 Å, diameter ~8 Å) and Z-G4, we proposed that M-0 stacking at the bottom of Z-G4 G-quadruplex (Fig. 4E). It should be mentioned that Phan's group have reported that thymine (T16, T19, T22 and T25) in the loops of G-quadruplex also establish hydrogen bond with adjacent guanine bases. These structural arrangements constitute a sizable cavity (diameter ~9 Å, data was estimated from the Protein Data Bank) which may provide a matched platform for the stacking of M-0.[5, 13] Besides, a 1:1 binding ratio of M-0 and Z-G4 can also be obtained from the NMR titrations. In the case of P-0, however, all of the imino proton peaks are broaden, which corresponds to a weak and non-specific interaction between P-0 and Z-G4 G-quadruplex.[3i, 6d] It should be pointed out that the detailed information of M-0 selective binding to Z-G4 is not known and awaits further structural biology studies.

Generally speaking, the G-quartet located in the core of G-quadruplex provides an ideal platform for the stacking of ligands. However, the platform cannot explain the chiral selectivity of M-0 to Z-G4 over P-0. Then, the chiral selectivity must originate from other factors. As we have previously reported, loop sequence of G-quadruplex DNA significantly impacted on the chiral DNA recognition and it did not reverse the chiral recognition capability of the enantiomer to G-quadruplex.[11d] So we speculate that it is the chiral environment provided by the loops of G-quadruplex resulted in the chiral selectivity.

Next we studied $[Ni_2L_3{}^0]^{4+}$ derivatives interaction with Z-G4 G-quadruplex. We have previously studied the interaction between the methyl-substituted chiral metal complexes ($[Ni_2L_3{}^3]^{4+}$ and $[Ni_2L_3{}^5]^{4+}$, shown in Scheme 1) and human telomeric G-quadruplex and found ligand modification indeed have an effect on the chiral DNA recognition.[11e] Here the interactions between $[Ni_2L_3{}^3]^{4+}$/$[Ni_2L_3{}^5]^{4+}$ and Z-G4 were also compared. UV melting experiments were used to evaluate the effect of the four enantiomers (M-3, P-3, M-5, P-5) on the

stability of Z-G4 G-quadruplex. As shown in Fig. S6, none of the enantiomers show effect on the T_m of Z-G4 G-quadruplex under our experimental condition (Table S2). Besides, CD spectrum was chosen to study the effect of the enantiomers on the conformation of Z-G4 (Fig. S7). Since methyl substitution can result in the variation in size and shape of the chiral metal complex, we speculate that the methyl substituted $[Ni_2L_3^3]^{4+}$/$[Ni_2L_3^5]^{4+}$ may disturb their stacking to the left-handed Z-G4, which leads to the weak selectivity of $[Ni_2L_3^3]^{4+}$/$[Ni_2L_3^5]^{4+}$. Also, no remarkable conformational changes were observed upon the enantiomers binding to Z-G4. Hence, UV melting experiments and CD titration experiments demonstrate M-3/P-3/M-5/P-5 did not affect the stability and conformation of Z-G4. This further indicated that substituent groups is important for chiral DNA recognition.[11e]

In summary, DNA chiral recognition is essential because conversions of the chirality and diverse conformations of DNA are involved in a series of important life events. In this report, our results clearly demonstrate that the M-enantiomer of a chiral metal complex binds selectively to the left-handed Z-G4 G-quadruplex. As far as we know, this is the first example of a ligand that binds to left-handed G-quadruplex. This work opens up a new avenue for chiral recognition of left-handed G-quadruplex and will promote design and synthesis of ligand selectively targeting this unique DNA structure.

This work was supported by the 973 Project (2011CB936004,2012CB720602), and NSFC (21210002, 21431007, 21533008).

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