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A human telomeric DNA-based chiral biosensor†

Lingyan Feng, Bailu Xu, Jinsong Ren, Chuanqi Zhao and Xiaogang Qu*

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Here we report an electrochemical DNA (E-DNA) chiral sensor that can distinguish zinc-finger like metallo-supramolecular enantiomers based on human telomeric G-quadruplex formation specifically induced by one of the enantiomers. The assay is easy to operate and reusable with an enantioselective recognition ratio higher than 5.

Nucleic acids are polymorphic and exist in a variety of unique sequences and structures that play key roles in many biological processes.[1] Human telomeres are composed of tandem repeats of the double-stranded DNA sequence (5-TTAGGG):(5-CCCTAA). The G-rich strand can form a four-stranded G-quadruplex consisting of G-quartets.[2] Since the substrate of telomerase is the 3′-single-stranded overhang of telomeric DNA, the formation and function of these G-quadruplexes are of intense current interest because of their roles in important biological processes, such as aging and cancer, and their potential as therapeutic targets for cancer.[3] Compounds that can stabilize the intramolecular DNA G-quadruplexes formed in the human telomeric sequence have been shown to inhibit the activity of telomerase and telomere maintenance. Chiral recognition of G-quadruplex can provide a novel strategy for the generation of G-quadruplex selective chiral binders.[4] Recently, our group reported that one of the enantiomers of a chiral metallo-supramolecular compound $[Ni_2L_3]Cl_4$ (for the structure of L see Fig. S1, ESI†), a tetracationic triple helical enantiomer consisting of three bis(pyridylimine) ligand L strands wrapped in a helical fashion about two nickel(II) centers, displayed chiral and steric selection in stabilizing human telomeric G-quadruplex under physiological salt concentrations. The chiral supramolecular complex has both small molecular chemical features and the large size of a zinc-finger-like DNA-binding motif; it can also convert single strand human telomeric DNA to a hybrid G-quadruplex structure.[5]

Chirality is of central importance in biomolecular recognition; consequently there is great interest in techniques for distinguishing enantiomers.[6] Various optical and magnetic spectroscopies, such as circular dichroism (CD) spectra, high performance liquid chromatography (HPLC), NMR, capillary electrophoresis, and X-ray diffraction (XRD), *etc.*, are now the principal tools for distinguishing chiral molecules, which either need special training to operate or prepare the high quality crystal samples for measurements, they are usually time-consuming and expensive.[7] As far as electrochemistry approaches to chiral sensing are concerned, there have been reports that predominantly rely on the use of matrices which contain enantioselective receptor structures (such as cyclodextrins and molecularly imprinted polymers) or the use of a reagent containing a redox active subunit to read out the enantiomeric composition of the mixture.[8] As a new kind of detection platform, electrochemical DNA (E-DNA) sensors have been described to date against targets as diverse as DNA, RNA, proteins, small molecules and nanotubes.[9] Target-binding induced "folding" of electrode-bound DNA sensors employ electrochemistry to monitor binding-induced changes and have been proved to be rapid (responding in seconds to minutes), sensitive (detecting sub-picomolar to micromolar concentrations), and reagentless.[10]

Here we report a proof-of-concept E-DNA sensor for distinguishing chiral molecules, on the basis of one enantiomer of a chiral G-quadruplex ligand that can selectively induce the human telomeric quadruplex formation with high sensitivity and selectivity while the other enantiomer cannot. To the best of our knowledge, this is the first demonstration of the E-DNA sensor that can distinguish chiral quadruplex-binding complexes. The detection limit of one of the enantiomers is as low as 5×10^{-8} M. Besides, the designed chiral sensor can also be easily regenerated through a simple distilled water rinse at room temperature. When challenging the same concentration of the different chiral complex, the enantioselective recognition ratio, defined as $(I_0 - I_{NiP})/(I_0 - I_{NiM})$, is higher than 5, which is comparable with most of the enantioselective sensors reported before.[8,11]

As shown in Scheme 1, the E-DNA sensor used in our studies consisted of a short single-stranded DNA containing a human telomeric G-rich DNA sequence as well as a flexible alkyl chain linker to ensure solvent exposure. The DNA sequence was modified with a redox-active ferrocene (Fc) at its 5′-terminus and covalently attached at its 3′-terminus to a gold electrode *via* a thiol–gold bond (Scheme 1, middle). The E-DNA sensor preparation, coverage, and characterization were performed as described previously.[9] A ferrocene redox peak pair was observed with the E-DNA-modified gold electrodes, and the peak currents were directly proportional to potential scan rates, consistent with a surface-confined electrochemical reaction (Fig. S2, ESI†). In the absence of a target ligand, the immobilized DNA was unfolded in

Laboratory of Chemical Biology, Division of Biological Inorganic Chemistry, State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Graduate School of the Chinese Academy of Sciences, Chinese Academy of Sciences, Changchun, Jilin 130022, China. E-mail: xqu@ciac.jl.cn
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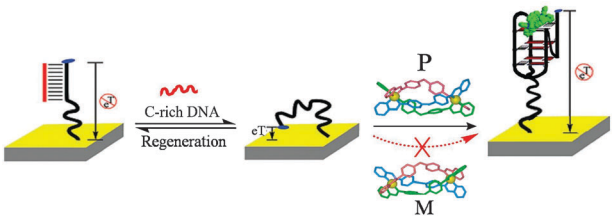

Scheme 1 Schematic representation of the human telomeric DNA-based electrochemical DNA (E-DNA) sensor.

Li^+ solution,[5,12] thereby allowing the attached Fc to collide with (or weakly bind to) the electrode and transfer electrons (Scheme 1, middle). One of the chiral G-quadruplex ligands, $[Ni_2L_3]^{4+}$-*P*, could selectively induce the hybrid quadruplex formation of the immobilized human telomeric DNA on the electrode surface, forcing the Fc tag away from the surface and inhibiting its electron transfer (Scheme 1, right). However, no evident signal decrease was observed upon another enantiomer of the supra-molecular cylinder, $[Ni_2L_3]^{4+}$-*M*, binding.[5] When the sensor was challenged with 5 μM $[Ni_2L_3]^{4+}$-*P* (10 mM Tris buffer containing 10 mM Li^+, pH 7.2), the Faradic current decreased dramatically (Fig. 1, left) and fell to ~20% of the original current. The presence of $[Ni_2L_3]^{4+}$-*P* could convert single strand human telomeric DNA to a hybrid G-quadruplex structure by external stacking and contacting with the lateral loops,[5a] as shown in CD spectra (Fig. S8, ESI†). This formed quadruplex structure would lift up the Fc tag away from the gold surface, thereby producing the suppressed signal.

The G-quadruplex DNA-based sensor exhibited a rapid response and a low detection limit in comparison to using highly expensive spectroscopic instruments and complicated sample preparation.[7,8] The conditions of the response time, the AC voltammograms (ACV) operating frequency, and the modified DNA concentration were detailedly optimized to maximize the sensor performance (Fig. S3–S5, ESI†). The *P*-enantiomer preferentially bound to the end of the G-quartet by external stacking at a 1 : 1 binding ratio.[5] The E-DNA sensor displayed hyperbolic binding with a binding constant, K_a, of $1.28 \pm 0.16 \times 10^7$ M^{-1}, this value was comparable with that determined by the fluorescent assay.[5a] The detection limit was about 5×10^{-8} M for $[Ni_2L_3]^{4+}$-*P*, and the dynamic range covered from 0.1 to 5 μM, as shown in Fig. 2. Because the quadruplex DNA in the E-DNA sensor was covalently attached to the sensor surface, this reagentless device was readily regenerated (Fig. 1A).

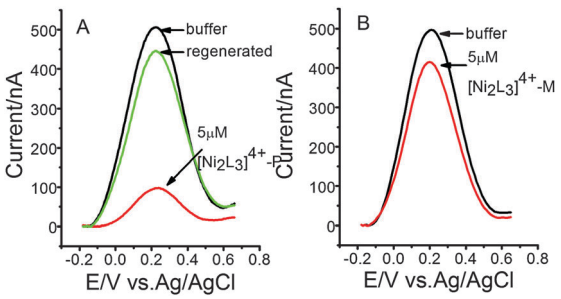

Fig. 1 ACV of the E-DNA sensor after incubation with 5 μM $[Ni_2L_3]^{4+}$-*P* (A, left) and $[Ni_2L_3]^{4+}$-*M* (B, right).

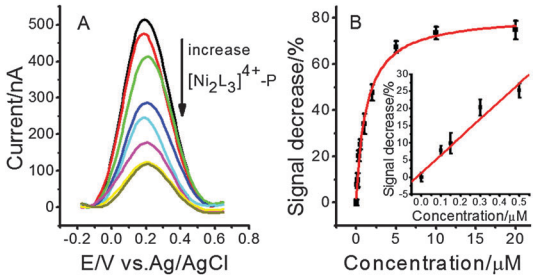

Fig. 2 (A) ACV of the E-DNA sensor with addition of $[Ni_2L_3]^{4+}$-*P* solution at concentrations of 0, 0.15, 0.3, 0.5, 1, 2, 5, 10 μM (from top to bottom). (B) $[Ni_2L_3]^{4+}$-*P* concentration dependence.

In order to confirm that the signal decrease was caused by the distance change of the redox reporter to the electrode, we performed the hybridization of the immobilized DNA with its complementary C-rich DNA (Scheme 1, left). In the presence of C-rich DNA, the signal gain of the Faradic current decreased to about 67.8%. Additionally, washing the electrode by ultrapure water for 30 s recovered ~95% of the original signal (Fig. S6, left, ESI†). However, no signal decrease was observed using dA_{22} DNA as a non-complementary control (Fig. S6, right, ESI†),[12b] indicating that the observed decrease was due to the Fc tag away from the electrode surface. The assumption was further supported by the electrochemical impedance spectroscopy (EIS) results (Fig. S7 and Table S1, ESI†), quantitative analysis of the EIS data showed significant increase in the electron-transfer resistance at the electrolyte/film interface $R_{e/f}$ (Fig. 3A), in accord with the decreased signal of ACV[9c] and the square-wave voltammetry (SWV) technique. We observed a critical frequency (f_{cr}) of approximately 13.8 Hz for $[Ni_2L_3]^{4+}$-*P versus* approximately 37.1 Hz for $[Ni_2L_3]^{4+}$-*M* (Fig. 3B). Since f_{cr} was linearly proportional to the electron transfer rate between Fc and electrode, a smaller f_{cr} represents slower electron transfer.[9e] In addition, the change in chemical environment, such as hydrophobicity, bulky structure of G-quadruplex around the redox Fc reporter, may also influence the signal decrease.[9f,g] All the above results indicated the "chain-flexibility" mechanism containing the expected length dependence of a reaction-limited process in which the overall rate was proportional to the equilibrium probability that the end of the oligonucleotide chain approached the surface.[9h]

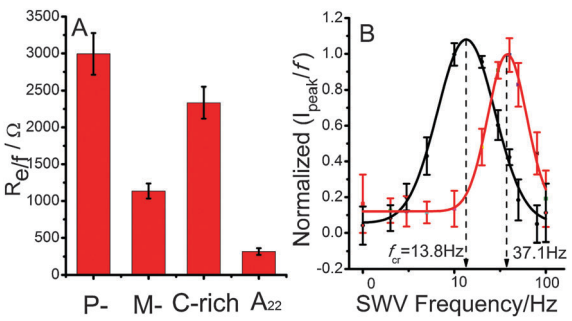

Fig. 3 (A) EIS of the E-DNA sensor after incubation with 5 μM $[Ni_2L_3]^{4+}$-*P*, $[Ni_2L_3]^{4+}$-*M*, 5 μM C-rich and A_{22} DNA solution. (B) Plot of critical frequencies (f_{cr}, defined as the frequency at which the maximal value of SWV frequency-normalized peak current (I_{peak}/f) was observed) obtained from SWV of $[Ni_2L_3]^{4+}$-*P* (black) $[Ni_2L_3]^{4+}$-*M* (red) binding. The frequency is displayed in a logarithmic scale.

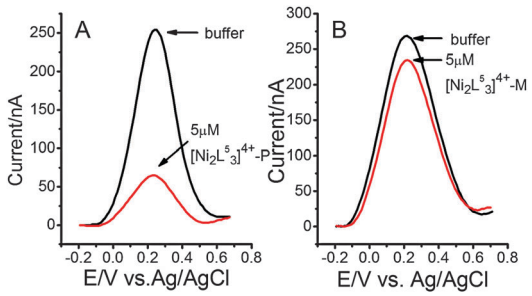

Fig. 4 ACVof the E-DNA sensor after incubation with 5 μM $[Ni_2L_3^5]^{4+}$-*P* (A, left) and $[Ni_2L_3^5]^{4+}$-*M* (B, right).

To further demonstrate the E-DNA sensor as an alternative efficient electrochemical method to distinguish chiral compounds, two other derivative enantiomers of $[Ni_2L_3^5]^{4+}$-*P*/-*M* complexes were synthesized, which were di-metallo helicates with alkyl substituents at the bis(pyridylimine) ligand backbone (the ligand L^5 structures and the CD spectra of chiral complexes are shown in Fig. S1, ESI†). The E-DNA sensor responded quite differently after incubation with $[Ni_2L_3^5]^{4+}$-*P*/-*M* complexes (Fig. 4). The phenomenon was the same as the pair of original $[Ni_2L_3]^{4+}$-*P*/-*M* enantiomers, indicating that derivative $[Ni_2L_3^5]^{4+}$-*P* could also cause the DNA structure transition and force the Fc tag away from the electrode surface, while $[Ni_2L_3^5]^{4+}$-*M* could not (Fig. S9, ESI†). The value of $(I_0 - I_{NiP})/(I_0 - I_{NiM})$ is ~5.4 for this pair of enantiomers at the concentration of 5 μM. The results of $[Ni_2L_3]^{4+}$-*P*/*M* and $[Ni_2L_3^5]^{4+}$-*P*/-*M* enantiomers further demonstrated that the E-DNA sensor could be used for distinguishing enantiomers based on DNA structural transition selectively induced by one of the enantiomers.

The specific target binding-induced conformational change could be used to distinguish $[Ni_2L_3]^{4+}$-*P* among *P*-, *M*-enantiomers and two other DNA-binding molecules, TMPyP4 (a cationic porphyrin) and thiazole orange (TO). TMPyP4 stabilized human G-quadruplex with good selectivity over single-stranded DNA but could not induce quadruplex formation in Li^+ solution.[13] TO was a useful fluorescence probe for labeling various DNA structures (single strand, duplex, triplex or quadruplex) with poor selectivity (Fig. S8 and S10, ESI†).[14] The contrasting results obtained from four different complexes are shown and carefully discussed in Fig. S11 (ESI†), which demonstrated the selectivity of E-DNA assay. The current decrease was just observed when the E-DNA sensor was incubated in $[Ni_2L_3]^{4+}$-*P* solution. The enantioselective recognition ratio of $[Ni_2L_3]^{4+}$-*P*/-*M* would be as high as 5 at the same concentration of 2 μM. Such a large difference in electrochemical signal change made the E-DNA sensor practically useful for the enantioselective recognition of the metallo-supramolecular complex.

In summary, we report here a novel, low-cost E-DNA chiral sensor that can distinguish chiral quadruplex ligands. In contrast to NMR and other spectroscopy instruments, this electrochemical sensor is economic, sensitive, easy to prepare and operate, and suitable for automation and field analysis. Since the electrochemical response is caused by the DNA structural transition induced by specific enantiomer binding, our work would shed light on the design of novel E-DNA chiral sensors.

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Notes and references

1 (*a*) J. L. Mergny and C. Helene, *Nat. Med.*, 1998, **4**, 1366–1367; (*b*) G. Song and J. Ren, *Chem. Commun.*, 2010, **46**, 7283–7294.
2 (*a*) T. Cech, *Cell (Cambridge, Mass.)*, 2004, **116**, 273–279; (*b*) D. Yang and K. Okamoto, *Future Med. Chem.*, 2010, **2**, 619–646.
3 (*a*) M. A. Blasco, *Nat. Rev. Genet.*, 2005, **6**, 611–622; (*b*) X. Li, Y. Peng, J. Ren and X. Qu, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 19658–19663.
4 (*a*) W. Wilson and H. Sugiyama, *Chem. Biol.*, 2007, **2**, 589–594; (*b*) M. Hannon, *Chem. Soc. Rev.*, 2007, **36**, 280–295; (*c*) Y. Xu, Y. Zhang, H. Sugiyama, T. Umano, H. Osuga and K. Tanaka, *J. Am. Chem. Soc.*, 2004, **126**, 6566–6567.
5 (*a*) H. Yu, X. Wang, M. Fu, J. Ren and X. Qu, *Nucleic Acids Res.*, 2008, **36**, 5695–5703; (*b*) H. Yu, C. Zhao, Y. Chen, M. Fu, J. Ren and X. Qu, *J. Med. Chem.*, 2010, **53**, 492–498; (*c*) C. Zhao, J. Geng, L. Feng, J. Ren and X. Qu, *Chem.–Eur. J.*, 2011, **17**, 8209–8215.
6 (*a*) J. Sadlej, J. Dobrowolski and J. Rode, *Chem. Soc. Rev.*, 2010, **39**, 1478–1488; (*b*) X. Qu, J. Trent, I. Fokt, W. Priebe and J. Chaires, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 12032–12037; (*c*) W. Wei, K. Qu, J. Ren and X. Qu, *Chem. Sci.*, 2011, **2**, 2050–2056.
7 (*a*) M. Bieri, C. Gautier and T. Burgi, *Phys. Chem. Chem. Phys.*, 2007, **9**, 671–685; (*b*) T. Tokunaga, M. Okamoto, K. Tanaka, C. Tode and M. Sugiura, *Anal. Chem.*, 2010, **82**, 4293–4297.
8 (*a*) P. Shagaldian and U. Pieles, *Sensors*, 2006, **6**, 593–615; (*b*) M. Trojanowicz and M. Kaniewskaa, *Electroanalysis*, 2009, **21**, 229–238; (*c*) L. Challier, F. Mavré, J. Moreau, C. Fave, B. Schöllhorn, D. Marchal, E. Peyrin, V. Noël and B. Limoges, *Anal. Chem.*, 2012, **84**, 5415–5420.
9 (*a*) C. Fan, K. Plaxco and A. Heeger, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 9134–9137; (*b*) Y. Xiao, X. Qu, K. W. Plaxco and A. J. Heeger, *J. Am. Chem. Soc.*, 2007, **129**, 11896–11897; (*c*) A. Radi, J. Sanchez, E. Baldrich and C. O'Sullivan, *J. Am. Chem. Soc.*, 2006, **128**, 117–124; (*d*) Y. H. Peng, X. H. Wang, Y. Xiao, L. Y. Feng, C. Zhao, J. S. Ren and X. G. Qu, *J. Am. Chem. Soc.*, 2009, **131**, 13813–13818; (*e*) K. Hsieh, R. White, B. Ferguson, K. Plaxco, Y. Xiao and H. Soh, *Angew. Chem., Int. Ed.*, 2011, **50**, 11176–11180; (*f*) J. Liu, M. Paddon-Row and J. Gooding, *J. Phys. Chem. B*, 2004, **108**, 8460–8466; (*g*) S. Delaney and J. Barton, *Biochemistry*, 2003, **42**, 14159–14165; (*h*) T. Uzama, R. Cheng, R. White, D. Makarov and K. W. Plaxco, *J. Am. Chem. Soc.*, 2010, **132**, 16120–16126.
10 (*a*) A. A. Lubin and K. W. Plaxco, *Acc. Chem. Res.*, 2010, **43**, 496–505; (*b*) A. Lubin and K. W. Plaxco, *Acc. Chem. Res.*, 2010, **43**, 631–641; (*c*) L. Feng, C. Zhao, Y. Xiao, L. Wu, J. Ren and X. Qu, *Chem. Commun.*, 2012, **48**, 6900–6902.
11 (*a*) H.-L. Liu, Q. Peng, Y.-D. Wu, D. Chen, X.-L. Hou, M. Sabat and L. Pu, *Angew. Chem., Int. Ed.*, 2010, **49**, 602–606; (*b*) F. Han, L. Chi, X. Liang, S. Ji, S. Liu, F. Zhou, Y. Wu, K. Han, J. Zhao and T. D. James, *J. Org. Chem.*, 2009, **74**, 1333–1336; (*c*) M. A. Vorotyntsev, L. I. Daikhin and M. D. Levi, *J. Electroanal. Chem.*, 1994, **364**, 37–49.
12 (*a*) C. Chen, C. Zhao, X. Yang, J. Ren and X. Qu, *Adv. Mater.*, 2010, **22**, 389–393; (*b*) C. Zhao, Y. Song, J. Ren and X. Qu, *Biomaterials*, 2009, **30**, 1739–1745.
13 F. Han, R. Wheelhouse and L. Hurley, *J. Am. Chem. Soc.*, 1999, **121**, 3561–3570.
14 J. Nygren, N. Svanvik and M. Kubista, *Biopolymers*, 1998, **46**, 39–51.