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Chiral Resolution Capabilities of DNA Oligonucleotides

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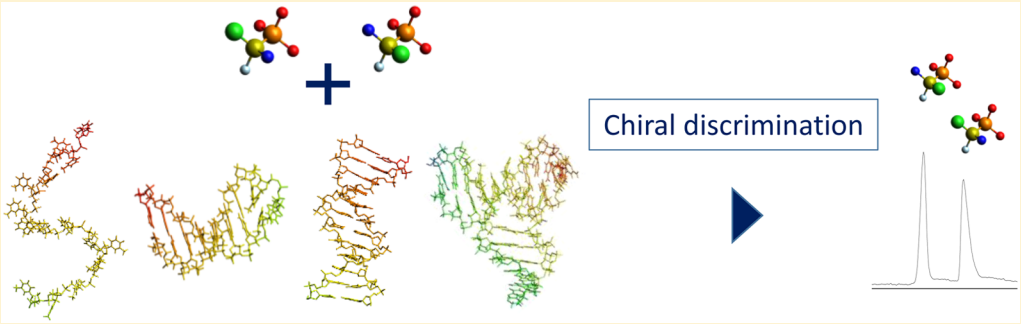
Supporting Information



ABSTRACT: Herein, we studied the chiral resolution properties of a repertoire of arbitrarily chosen DNA oligonucleotides (ON). Ten oligonucleotidic sequences characterized by diverse base compositions, sizes, and structural features, ranging from secondary structure-free homo-oligonucleotides to duplex, hairpin, and three-way junction architectures, were investigated as potential chiral selectors. Their enantioselective features were assessed by using ONs as running buffer additives in partial-filling capillary electrophoresis. It was shown that all the screened sequences displayed enantiodiscrimination capabilities toward small aromatic compounds. Under (sub)millimolar DNA concentration conditions, the combination of only three oligonucleotidic sequences provided the chiral resolution of around 20 racemates, including drugs, illegal drugs, amino-acids, and nucleosides. This work represents the first demonstration of such analyte selectivity spectrum for nucleic acid-based chiral separation tools.

Chirality is an exquisite feature of nucleic acids originating from both their backbone sugar units and their secondary/tertiary topologies. Moreover, during the last 3 decades, a huge number of studies have demonstrated not only the enantioselective ability of the double helix as well as other organized architectures toward chiral ligands[1] but also some chiral recognition properties at the molecular level.[2] These stereoselective features have attracted considerable attention in numerous areas: to induce asymmetric synthesis,[3] to create chiral ligands probing specific sequences/structures or modulating biological processes,[4] and to construct functional mirror-image structures.[5]

The importance of the strict control of the enantiomer purity for bioactive substances has generated an enormous demand for the development of efficient enantioselective methodologies at the analytical level. In this context, high-resolution separation technologies such as chromatography and capillary electrophoresis (CE) constitute the most powerful methods through the use of a variety of surface-immobilized or free chiral selectors.[6] To date, naturally occurring species (and their derivatives), ranging from polymers including polysaccharides[7] and proteins[8] to macrocycles/low-molecular weight molecules such as cylodextrins,[9] glycopeptide antibiotics,[10] and cinchona alkaloids,[11] represent the dominant classes of commercially available chiral selectors.

In contrast, the exploitation of nucleic acids in the chiral separation field has yet received much less attention. Indeed, only a few examples of enantioseparations based on DNA/RNA molecules have been reported. These earlier approaches were typically developed in a peculiar rather than a general perspective in such a way that they were either dedicated to chiral ligands of modest application interest or restricted to a narrow analyte selectivity range.[12−24] Thus, there is an urgent need to systematically reconsider the stereoselective properties of nucleic acids within the chiral separation framework.

In this work, we aimed to assess the chiral resolution ability of a repertoire of arbitrarily chosen oligonucleotides (ONs). Several DNA sequences with distinct base compositions, lengths, and structural characteristics, ranging from secondary structure-free homo-oligonucleotides to structured motifs such as duplex, small, and large hairpins and three-way junction, were evaluated as potential chiral selectors in CE (Scheme 1a). CE possesses many attractive features such as homogeneous format, high separation efficiency, and very low volume sample requirements (in the nanoliter range). The CE experiments were carried out using the capillary partial filling mode[25] (see the Supporting Information for experimental details and optimization experiments). Briefly, a discrete plug of ON was introduced into a bare fused silica capillary of 25 μm inner diameter. At a running buffer pH of 7.5 (ionic strength ~160 mM) and under normal polarity conditions, ON moved very

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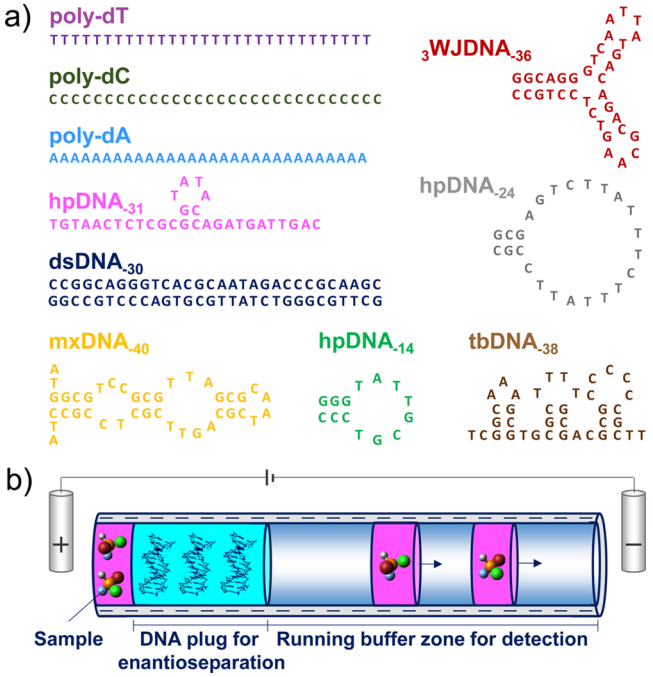
Scheme 1. (a) Sequences of Arbitrarily Chosen ONs Screened in the Present Work[a] and (b) Schematic illustration of the CE-Based Chiral Separation Procedure Using Oligonucleotides As Running Buffer Additives under Capillary Partial Filling Mode



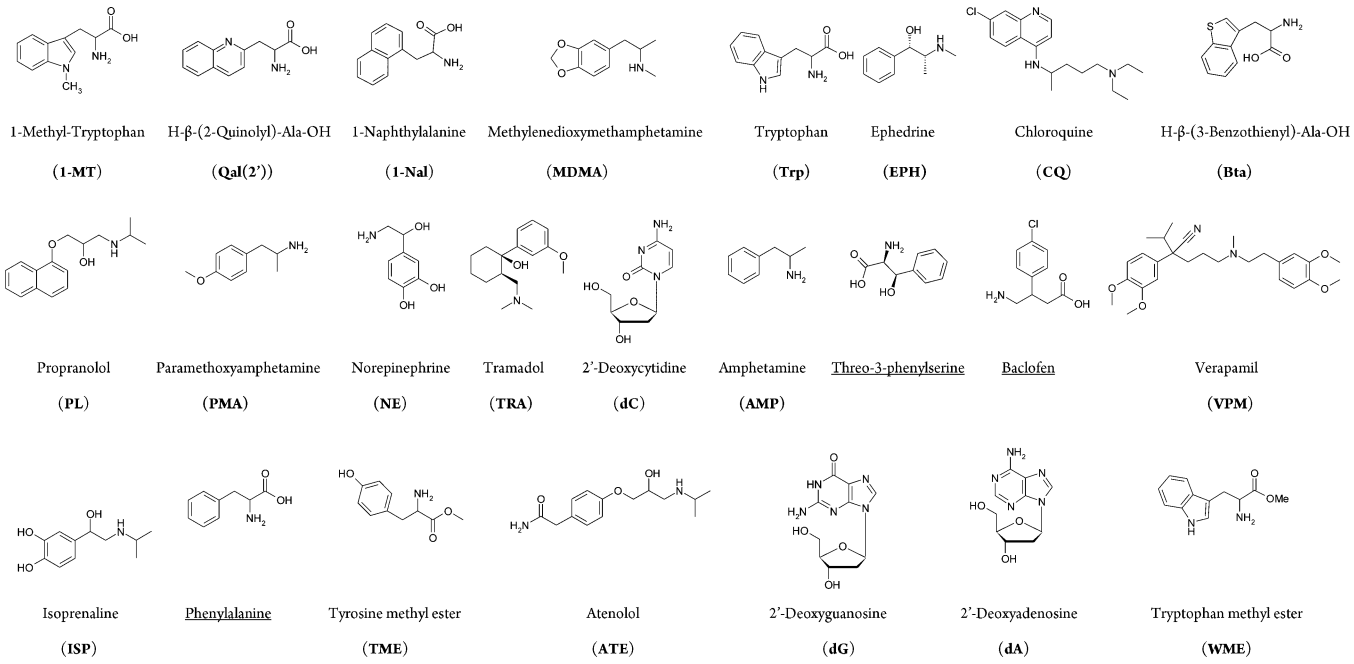
[a]ON secondary structures were predicted by using the Zuker mfold Web server (http://mfold.rna.albany.edu/?q=mfold/DNA-Folding-Form) for the temperature and buffer conditions of the electrophoresis experiments.

slowly toward the anodic side while injected analytes migrated in the opposite direction through the nucleic acid separation zone and reached the detector (Scheme 1b). This format allowed eliminating the UV-absorbing DNA interferences and reducing the consumption of ONs. In addition, sequential injections of multiple analytes can be performed with the same ON plug. Before CE analysis, the conductivity of the DNA sample was adjusted by the running buffer in such a way that the conductivities in all the capillary zones are approximately the same. This limited the concentration Kohlrausch adjustment which can cause the dilution of the DNA sample in the capillary during the run.[26]

A total of 24 chiral species were tested for enantiodiscrimination (Scheme 2). We chose a racemate collection containing diverse classes of small aromatic compounds of pharmacological or biological relevance, including drugs, illegal drugs, amino-acids and derivatives, and nucleosides. Unlike most works dealing with the chiral recognition features of DNA,[1] we did not include in the screening both the metal complexes and the common intercalators and groove binders (except chloroquine that is a weak intercalating agent).[27] Anionic compounds were not considered in the present study because of their charge-to-mass ratio close to that of ONs. This would lead to a small electrophoretic mobility difference between free and DNA-bound ligand then preventing enantiomeric separation under the present CE conditions. It should be noted however that the previously described drag-tag strategy could overcome this pitfall by modulating the ON mobility.[18]

In the first stage of this work, we spontaneously focused our attention on simple single-stranded sequences lacking any secondary structure, i.e., homopolymeric DNA. These included **poly dA**, **poly dC**, and **poly dT** sequences of 30-nt length. Because of its propensity to form high-order G-quadruplex structures,[28] poly dG was not investigated. As shown in Figure 1, a plug of 1.6 mM **poly dT** provided partial or complete enantiomeric separation for eight chiral substances (Table S1 in the Supporting Information). Enantioselective properties were also observed with **poly dA** and **poly dC** but to a lesser extent.

Scheme 2. Names, Abbreviations, And Chemical Structures of the Tested Chiral Compounds[a]



[a]Underlined racemates were not resolved by the 10 screened DNA sequences.

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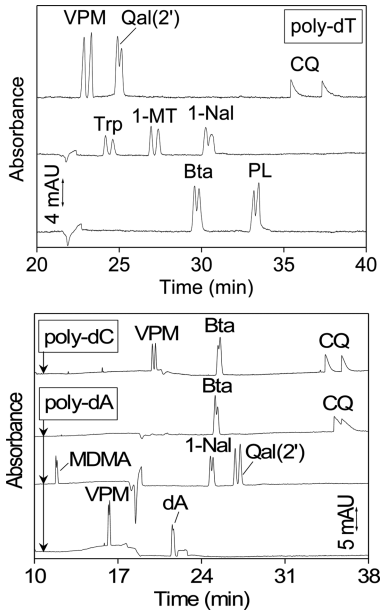

Figure 1. Electropherograms for the chiral resolution of 10 racemates by using **poly dT** (top), **poly dA** and **poly dC** (bottom) as running buffer additives. Experimental conditions: [DNA] at ~1.6 mM. T = 10 °C. DNA injection volume ~190 nL. Other conditions are in the Supporting Information.

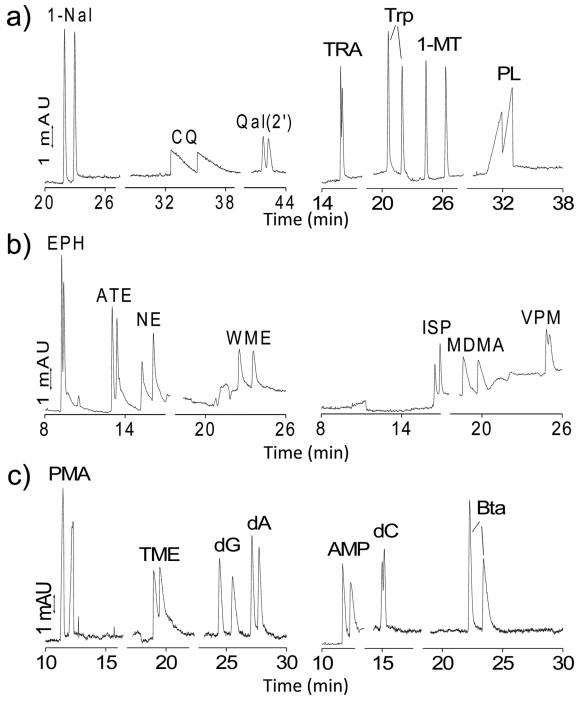

Figure 2. Electropherograms for the chiral resolution of 21 racemates by using (a) **hpDNA**$_{-24}$, (b) **hpDNA**$_{-31}$, and (c) $_3$**WJDNA**$_{-36}$ as running buffer additives. Experimental conditions are in the Supporting Information.

Interestingly, chloroquine enantiomers were separated at least in part in all cases (for example, with a high resolution of 4.2 for **poly dT**), although no typical intercalation mode was possible with such unstructured DNA molecules.

Encouraged by these findings, we subsequently concentrated our efforts on the analysis of the chiral discrimination ability of ONs characterized by higher diversity and complexity (Scheme 1). We deliberately screened arbitrary ONs with a variety of sequence compositions and lengths, consisting of different degrees of structuration. All the DNA species displayed enantiodifferentiation capabilities, with an amount of resolved probes ranging from seven for **dsDNA**$_{-30}$ to 15 for **hpDNA**$_{-31}$ (Table S1 in the Supporting Information). It was of interest to mention that resolution higher than 1.5 was reported for the three-quarters of the separated enantiomers. Not less importantly, as high as 21 enantiomer couples were separated with only 3 ON sequences (**hpDNA**$_{-31}$, $_3$**WJDNA**$_{-36}$, and **hpDNA**$_{-24}$, Figure 2). These resolved compounds are either cationic carrying one phenyl group or contain two aromatic cycles with no net or one positive charge (Scheme 2). This suggests that electrostatic, $\pi-\pi$ interactions, and hydrophobic effect may constitute driving forces for the enantiodifferentiation. The three chiral selectors demonstrate some complementary selectivity patterns. For example, propanolol and chloroquine were partly resolved with **hp-DNA**$_{-24}$ whereas no enantiomer separation was reached with $_3$**WJDNA**$_{-36}$ and **hpDNA**$_{-31}$. Amphetamine was well-resolved solely by using $_3$**WJDNA**$_{-36}$. The separation of enantiomers of isoprenaline, norepinephrine, and atenolol was observed only with the most broadly applicable **hpDNA**$_{-31}$ chiral selector.

We further established the usefulness of the ON-based chiral selectors by reporting the straightforward manipulation of the enantiomer migration order. As most of selectors used in CE are naturally occurring compounds available in only one chiral configuration (cylodextrins, proteins, glycopeptide antibiotics, for example), the reversal of the enantiomer migration order commonly requires either their substitution by chiral selectors with opposite enantiomer affinity features or the careful control of the experimental conditions.[29] Herein, we were able to apply the mirror-image strategy by using ONs composed entirely of L-nucleosides (L-ONs).[30] Figure S13 in the Supporting Information shows the separation of 1-methyl-tryptophan enantiomers obtained using **L-hpDNA-14** as the electrolyte additive. A reversed migration order of enantiomers was observed relative to the parent **hpDNA-14**. As expected from the principle of chiral inversion, **L-hpDNA-14** recognizes the mirror-image of the most **hpDNA-14** interacting enantiomer with the same binding features. This mirror-image approach allows also designing a chiral selector resistant to nuclease digestion for potential applications in biological conditions.[30,31]

The repeatability study was performed by injecting a test mixture of Trp, 1-MT, and Bta racemates (n = 6) with **tbDNA**$_{-38}$ as the running buffer additive. The % RSD values for the intraday repeatability of the relative migration time, chiral resolution, and peak area were in the range of 0.4−0.6, 2.7−4.8, and 3.9−14.4, respectively.

As examples of chiral separation were reported for all the screened DNA molecules, it would seem that the enantiodiscrimination ability is a common denominator of ONs as long as sufficiently high concentrations (typically milli- or submillimolar) are used. Indeed, when ON in the micromolar concentration range was employed, no separation was possible whatever the sequence (see Figure S14 in the Supporting Information for $_3$**WJDNA**$_{-36}$ as a representative example). In that sense, ONs are close to chiral selectors such as cylodextrins and glycopeptide antibiotics for which millimolar concentration in the electrolyte is generally needed for achieving successful chiral separation. As the optimal concentration of a chiral selector depends inversely on its affinity for enantiomers,[32] the binding of ONs to enantiomers is expected to be of low

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magnitude (i.e., apparent K_d in the millimolar range). The understanding of the chiral recognition mechanism of polymer-based chiral selectors is commonly a very difficult task due to the lack of precise information on the macromolecule structure and the complexity of binding modes.[33,34] This should be particularly true for the various ON chiral selectors described here. Small ligands such as tested chiral analytes could weakly associate with DNA through multiple mechanisms and interaction sites as a function of its nature.[1,35−38] As an additional complicating factor, enantioseparations in CE rely not only on the thermodynamic selectivity of the chiral recognition but also possibly on the mobility differences of diastereoisomeric complexes.[29] Thus, structural, computational, and further electrophoretic studies are needed to gain insight into the mechanistic aspects of the different ON-based enantiomeric separations.

In summary, the present work demonstrates that very diverse ONs, even in their simplest unstructured forms, all display chiral resolution capabilities toward chiral aromatic compounds. This opens up new prospects for developing nucleic acid-based enantioseparation tools of wide applicability. In this study, several pharmacologically relevant compounds were preliminarily resolved (Scheme 2) and we may envisage much more cases of enantiodiscrimination in the medicinal field since many drugs are aromatic and possess basic functions.[39,40]

One peculiarity of ONs as chiral templates is related to the fact that their base and backbone nature, overall structure, chiral configuration, and functionalization can be systematically varied in a reliable way. On one side, the possibilities to generate an almost unlimited arsenal of chiral agents as well as to readily reverse the migration order of enantiomers constitute significant advantages over most of the available chiral selectors. Conversely, the identification of a reduced set of practical, broadly applicable selectors is expected to be laborious and challenging. In this context, the screening of small libraries derived from the best sequences reported here constitutes the most obvious way to investigate in future works. Along lines resembling to those of many derivatized chiral selectors,[6,7] a promising increase in the molecular space diversity could be realized by the incorporation within these chiral templates of a variety of commercially available base/backbone chemical substituents.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and optimization as noted in text. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01252.

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Notes

The authors declare no competing financial interest.

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