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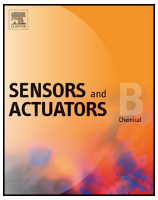

Supramolecular recognition of A-tracts DNA by calix[4]carbazole

Gang Li[c], Xiaoyu Song[d], Hui Yu[b], Chun Hu[c], Mingzhe Liu[c], Jie Cai[b], Liang Zhao[a,c], Yan Chen[a,c], Peng Yang[a,c,*]

[a] Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] Engineering Technology Research Center for Functional Textiles in Higher Education of Guang Dong Province, College of Textiles and Clothing, Wuyi University, Jiangmen 529020, China
[c] Key Laboratory of Structure-Based Drug Design and Discovery of Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China
[d] China-Japan Research Institute of Medical and Pharmaceutical Sciences, Shenyang Pharmaceutical University, Shenyang 110016, China

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ABSTRACT

Naturally occurring biomolecules such as transcription factors recognize specific sequences of DNA by binding to its major groove whereas the synthetic sequence-specific major-groove binders are rare. We herein report that a synthetic calix[4]carbazole could serve as the optical ligand of A-tracts (B*-form DNA) by binding to its major-groove, elucidated by UV-vis, fluorescence, CD and NMR titrations.

1. Introduction

DNA optical recognition by synthetic molecules has received long term interests to both chemists and biologists due to their potential therapeutic uses for treating cancers [1]. Phosphodiester backbone binding, base pair intercalation, minor and major grooves binding are main noncovalent interactive patterns for DNA ligands [2]. Among them, the ligands binding to grooves may be able to specifically recognize DNA because the size, the shape as well as the geometry of both major and minor grooves correlate elaborately with the sequences of DNA bases [2]. An elegant example of synthetic minor-groove binders is Dervan's polyamide, which could recognize various DNA sequences [3]. Hannon's metallo-supramolecular cylinder and Schrader's calix[4]arene dimers are the pioneering synthetic major groove binders [4,5].

Compared with the minor groove, the major groove possesses a larger and more complicated structure. The complexity of DNA major groove makes it difficult to create its structurally complementary synthetic ligands. As such, the construction of sequence-specific major-groove binding ligands remains challenging. By now, the most successful sequence-specific major groove binders are still natural molecules (e.g.the zinc finger, the leucine zipper motifs) and/or semi-synthetic natural molecules analogues (*e.g.*, PNA, neomycin derivatives) [6]. The synthetic major groove specific binders are rare.

To fit DNA major groove, the synthetic ligands should possess a large and specific conformation like those DNA-binding natural molecules. The structurally diverse macrocycles in the "molecular library" of supramolecular chemistry might be able to rival those DNA-binding natural molecules as far as their sizes, shapes and geometries are concerned. Our group recently report the syntheses of calix[*n*]carbazoles and their host-guest binding properties [7a–c]. Among those carbazolyl macrocycles, 2,7-dimethoxy-substituted calix[4]carbazole (compound **2**, Fig. 1) adopts 1,3-alternate conformation in water, which looks like a pair of perpendicular molecular tweezers [7b].

The tube-like inner space of this new class of macrocycle enables **2** to serve as the host for the molecular guests like BPF. We now intend to see its capability of serving as a "guest" to be accommodated by a "host". In other words, could its outer space (its size, shape and geometry, etc.) be recognized by a biomolecular host, *e.g.* DNA? As the above mentioned, **2** possesses a bis-tweezers' shape, this geometry is somewhat analogous to the double scissor geometry of the leucine zipper. As leucine zipper is the well-known sequence-specific DNA major groove binder, we wonder if the rela-

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* Corresponding author at: Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang 110016, China.
E-mail address: pengyangcn05@hotmail.com (P. Yang).

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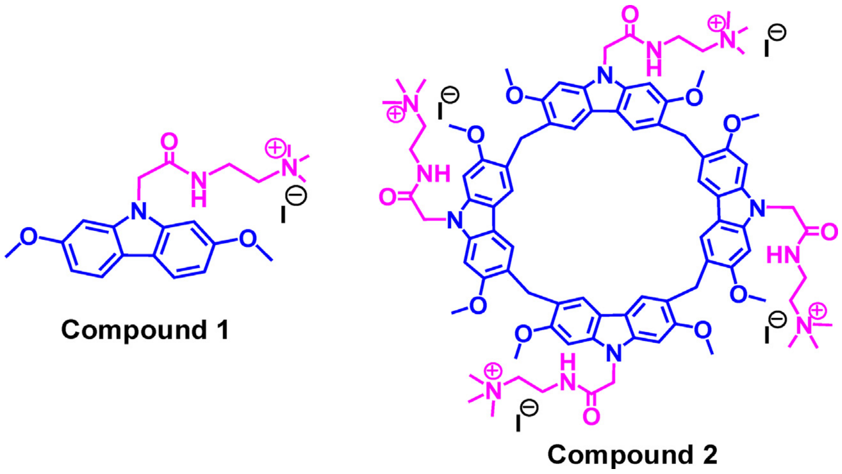

Fig. 1. Structures of **1** (control compound) and **2**.

tively large size, the unique shape and geometry of **2** might be able to fit and bind to the major groove of some specific DNA structures?

On the other hand, another merit of **2** is its fluorescent nature. It is well established that DNA fluorescent ligand is powerful tools to *in vivo* detect DNA either qualitatively or even quantitatively, e.g. the DNA ratiometric probes [8]. Moreover, although many fluorescent sensors of various DNAs (*e.g.* duplex-, triplex- and quadruplex DNA) have been reported, the fluorescent ligand for A-tracts DNA is rare. A-tracts is the duplex DNA containing more than four consecutive adenine base pairs in length. The helical structure of A-tracts, often referred as B*-DNA, possesses a wider major groove, a narrower minor groove and a more propeller twisted base pairs than that of regular B-DNA [9]. It is known that A-tracts strongly affects nucleosome organization and regulates genome function [10]. However, the real intracellular role of A-tracts remains less understood, partially due to the lack of the specific ligand. Therefore, the exploration for its specific and fluorescent ligand is urgently needed.

Based on the above mentioned reasons and our long term interests in DNA ligands [7d–g], we then examined the A-tracts DNA binding property of **2** and found the positive result. We herein report our finding. To our knowledge, **2** is the first synthetic and optical ligand for A-tracts DNA, although its naturally occurring and semi-synthetic natural ligands have ever been developed [6g,h].

2. Experimental

2.1. General techniques

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Thin layer chromatography (TLC) analysis of reaction mixtures was performed on Dynamic adsorbents silica gel F-254 TLC plates. Column chromatography was performed on silica gel 200–300 mesh. Fluorescence emission spectra were obtained using Shimadzu RF-5301 PC Spectrofluorophotometer. UV–vis absorption spectra were obtained on Beijing purkinje TU-1810. CD spectra were recorded on Bio-logic MOS-450 instrument. Dynamic light scattering (DLS) measurements were performed on Brookhaven's ZetaPlus instrument. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded with Bruker Avance-III 600 spectrometers at 298 K. Chemical shifts were reported in units (ppm) and all coupling constants (J values) were reported in Hertz (Hz). High resolution mass spectra were obtained using Bruker micrOTOF-Q instrument with an ESI source.

For all the measurements, the solutions of compounds and DNA were freshly prepared before use. For UV-vis, fluorescence and ^{1}H NMR titrations, the stock solutions of target compound were prepared by dissolving them in DMSO (20 mM, 20 mM and 500 mM respectively). For UV–vis and fluorescence titrations, the final concentration of DMSO was less than 1%. For fluorescence titration, both excitation and emission slit widths were 3 nm, excitation wavelength was 329 nm. Before the spectra were recorded, the sample solutions were mixed for 2 min after each addition of DNA. All the experiments were repeated for 3 times at least.

More experimental details can be found in Supporting Information.

2.2. Double-stranded DNA preparation

The oligonucleotides (purified by HPLC) were purchased from GenScrip® (China). The two complementary strands were dissolved and mixed in PBS buffer (10 mM PBS buffer, 50 mM NaCl, 1 mM EDTA, pH 7.2), heated to 95 °C for 5 min and slowly cooled to room temperature to form the duplex. The volume of the solution was adjusted to a final concentration of 1.0 mM/duplex.

The structures of DNA, which were studied in this work:

A-Tracts (dA_{10}-dT_{10})	5′-dAdAdAdAdAdAdAdAdAdA-3′ 3′-dTdTdTdTdTdTdTdTdTdT-5′
G-Tracts (dG_{10}-dC_{10})	5′-dGdGdGdGdGdGdGdGdGdG-3′ 3′-dCdCdCdCdCdCdCdCdCdC-5′
dA_{10}	5′-dAdAdAdAdAdAdAdAdAdA-3′
dT_{10}	5′-dTdTdTdTdTdTdTdTdTdT-3′
dG_{10}	5′-dGdGdGdGdGdGdGdGdGdG-3′
dC_{10}	5′-dCdCdCdCdCdCdCdCdCdC-3′
d(ATATATATAT)$_2$	5′-dAdTdAdTdAdTdAdTdAdT-3′ 3′-dTdAdTdAdTdAdTdAdTdA-5′
d(GCGCGCGCGC)$_2$	5′-dGdCdGdCdGdCdGdCdGdC-3′ 3′-dCdGdCdGdCdGdCdGdCdG-5′

2.3. Synthesis

Compound **1** and **2** were prepared by following our previously developed methods[7] with slight modification. The detailed synthetic procedures and all the spectra for their characterizations had been listed in Supporting Information.

2.4. Determination of fluorescence quantum yield of compound 2

The quantum yield of compound **2** ($\Phi = 0.267$) in PBS buffered water/methanol (1:1) solution is measured by a relative comparison procedure using quinine as the standard ($\Phi = 0.54$ in 0.05 M sulfuric acid).