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Short communication

Enantioselective Diels–Alder reactions using a G-triplex DNA-based catalyst

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ABSTRACT

In this study, it was found that the G-triplex DNA could be used as an enantioselective catalyst without further addition of ligands in Diels–Alder reactions when coordinated with copper ions, for the first time. The efficiency and selectivity of the catalyst were investigated. The kinetic measurements were made using ultraviolet (UV) light. The stability of the catalyst in aqueous buffer was confirmed by circular dichroism (CD) and nuclear magnetic resonance (NMR) spectra.

1. Introduction

Since the double-stranded DNA (dsDNA) has been recognized as a catalytic species and used in various key asymmetric reactions [1], DNA-based asymmetric catalysis has attracted the attention of many chemists. Recently, the concept was extended to G-quadruplex DNA sequences. Unlike dsDNA, the G-quadruplex, exhibiting polymorphous strand orientation and various loop sizes [2], constructs a semi-enclosed structure [3], providing a rigid and finite space for chiral transformation, which induced appreciable levels of enantioselectivity in Diels–Alder (D–A) reactions [4], confirming that the G-quadruplex structure could also act as a suitable chiral template [5].

Earlier research works reported that a DNA-based catalyst comprises a nonchiral ligand by the supramolecular and covalent anchoring strategies, which can chelate a transition metal ion. Therefore, the catalyzed reaction takes place in, or very close to, the DNA helix to allow the transfer of chirality of the DNA to the reaction [1c]. In the G-quadruplex, the G-tetrad is a square planar alignment of four guanines connected by cyclic Hoogsteen hydrogen bonds [6] and plays a very important role in recognizing planar aromatic ligands through π–π stacking [4] mainly, which brings the reactants into, or very close to, the G-quadruplex host [7].

Compared with the G-quadruplex DNA, the G-triplex, intensely studied recently [8], seems to be more attractive because of its controversial but noncanonical structure and biological functions [9]. This structure was first characterized as an intermediate formation of the G-quadruplex, but a stable conformation [6b]. Similarly to the G-quadruplex, the G-triplex is stabilized by Hoogsteen-like hydrogen bonds [10]. Because of the G-quadruplex's role and function in the enantioselective D–A reaction, we hypothesized that the G-triplex structure would also exert asymmetric induction in D–A reactions to some extent if similar structural and electrical requirements are met. Furthermore, the G-triplex has not yet found significant application in the development of asymmetric catalysis. Our discovery of asymmetric induction of Cu^{2+}/G-triplex DNA complex may allow the G-triplex motif to be used for the construction of novel and comparatively inexpensive chiral catalysts.

2. Experimental

2.1. Materials and methods

DNA oligodeoxynucleotides 5′-GGTTGGTGTGG-3′ (ODN-**1**) were purchased from Invitrogen, whose strand concentrations were determined by measuring the absorbance at 260 nm using the extinction coefficient values. All the other solvents and reactants were purchased from Shanghai Chemical Reagent Co., Ltd. of Chinese Medicine Group.

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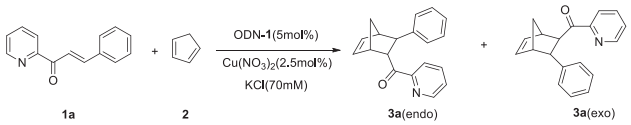
Table 1
Diels–Alder reaction catalyzed by G-triplex based catalyst. All data are the average of two experiments.



Entry	Catalyst	Conversion [%][a]	*endo*/*exo*[a]	*ee* [%][b]
1	None	9	85:15	0
2	Cu^{2+}	66	90:10	0
3	ODN-**1**	13	90:10	−14
4	ODN-**1** + Cu^{2+}	99	91:9	−51

[a] Determined for the crude product by HPLC analysis on a chiral stationary phase; results are reproducible within ±5%.
[b] Determined for the *endo* isomer by HPLC analysis on a chiral stationary phase; results are reproducible within ±5%.

2.2. General procedure

An aqueous solution of ODN-**1** (5′-GGTTGGTGTGG-3′, 25-μM final concentration) was added to a 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (2 mL, 20 mM, pH = 7.0) containing KCl (70 mM) and KH_2PO_4 (10 mM). After stirring for 30 min below 5 °C, a solution of $Cu(NO_3)_2$ (25-μM final concentration) was added. Then, aza-chalcone **1** in CH_3CN (20 μL of a 0.1-M solution) was added. The reaction was initiated by the addition of freshly distilled cyclopentadiene **2** (15 μL), and the mixture was stirred for 24 h at 4 °C, and then extracted with diethyl ether (3 × 6 mL). After drying the extracted mixture with anhydrous Na_2SO_4 and removing the solvent, the crude products were directly analyzed by ^1H nuclear magnetic resonance (NMR) spectroscopy, and then through high-performance liquid chromatography (HPLC) on a chiral stationary phase. The conversions of the crude product were determined by HPLC (only for **3a**).

3. Results and discussion

In this study, G-triplex DNA (ODN-**1**, 5′-GGTTGGTGTGG-3′) was used as a catalyst scaffold in D–A reactions. Because of the G-triplex, DNA also becomes water-soluble. Water was used as the major solvent because it is environmentally friendly and inexpensive [11]. First, a model D–A reaction between aza-chalcone (**1a**) and cyclopentadiene (**2**) was chosen as the model substrate to explore the catalytic performance of ODN-**1**. As expected, ODN-**1** promoted the D–A reaction, affording the *endo* isomer **3a** in a 14% enantiomeric excess (*ee*) albeit with low conversion (Table 1).

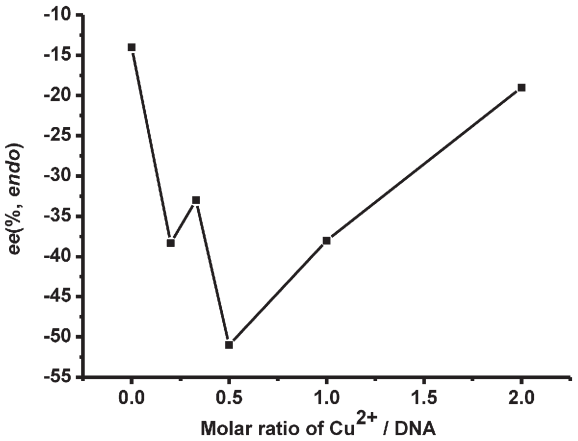


Fig. 1. The correlation of the enantioselectivity for the D–A reaction between the molar ratio of Cu^{2+}/ODN-**1** and *ee* value. The conversions are all over 95% except that 13% conversion (Cu^{2+}/ODN-**1** = 0).

This observation supported our hypothesis that ODN-**1** possesses asymmetric induction in the D–A reaction. In order to further improve the enantioselectivity and conversion of the reaction, $Cu(NO_3)_2$ was introduced into the system.

Interestingly, under these reaction conditions, the ODN-**1**–Cu^{2+} complex increased the yield with full conversion (Table 1, entries 4 and 2) together with enhanced *endo*/*exo* ratio (90:10 to 93:7). The enantioselectivity (51% *ee*) of the product **3a** (*endo*) was also significantly improved. These results demonstrated that the ODN-**1**–Cu^{2+} complex could be used as an efficient catalyst for asymmetric D–A reaction.

In order to confirm that ODN-**1** forms a G-triplex and further coordinates with Cu^{2+} under these reaction conditions, we analyzed the circular dichroism (CD) spectrum of ODN-**1** in aqueous buffer. Limongelli reported that the G-triplex is stable in a buffer containing 70-mM KCl, 10-mM KH_2PO_4, and 0.2-mM ethylenediaminetetraacetic acid (EDTA) [10]. The primary CD signals of the G-triplex were positive peaks at 253 and 289 nm. The CD spectra of ODN-**1** were obtained in water, MOPS (pH = 7.0) buffer containing 70-mM KCl and 10-mM KH_2PO_4, and MOPS buffer containing only 10-mM KH_2PO_4 (Fig. S1). EDTA was not added to the buffer because of its possibility to chelate with the copper ions. Strong signals were observed at 253 and 289 nm when ODN-**1** was in the buffer containing 70-mM KCl and 10-mM KH_2PO_4 (Fig. S1). This result confirmed that the G-triplex structure would not form in the absence of EDTA and that 10-mM KH_2PO_4 would affect the formation of the G-triplex. The results suggested that the G-triplex forms only in high concentrations of potassium chloride. When copper ions were added to the buffer, the CD signals did not change appreciably, producing typical peaks at 289, 253, and 265 nm, indicating the formation of G-triplex structures (Fig. S1). Furthermore, ^1H NMR spectroscopy was performed to confirm the formation of G-triplex when Cu^{2+} ions were added. As reported in the literature [10], the signals in the 11.0–12.5-ppm region of the ^1H NMR spectrum confirmed the presence of four well-defined exchangeable protons, which is typical of DNA structures with Hoogsteen hydrogen bonds, validating the formation of the G-triplex structure. Hence, all the experiments were

Table 2
Kinetic parameters of ODN-**1**, Cu^{2+} and ODN-**1**–Cu^{2+}.[a]

Entry	Catalyst	k_{app} [$M^{-1}S^{-1}$][b]	k_{rel}[c]
1	None	$(2.5 \pm 0.5) \times 10^{-3}$	1.0
2	ODN-**1**	$(3.2 \pm 0.4) \times 10^{-3}$	1.3
3	Cu^{2+}	$(1.4 \pm 0.2) \times 10^{-2}$	5.6
4	ODN-**1** + Cu^{2+}	$(2.5 \pm 0.9) \times 10^{-2}$	10.0

[a] D–A reactions of **2** (1 mM) and **1a** at fixed concentrations (10, 15, 25, 35, and 50 mM) were executed without catalyst and with ODN-**1** (25 μM), Cu^{2+} (25 μM) and ODN-**1**–Cu^{2+} (ODN-**1** (25 μM) and Cu^{2+} (25 μM)) catalysts. All of the reactions were performed in MOPS buffer (20 mM pH = 6.6) containing 70 mM KCl and 10 mM KH_2PO_4 at 298 K.
[b] The apparent second-order rate constant (k_{app}) was estimated from the initial rates ($k_{app} = V_{init} / ([\mathbf{1a}]_0 \cdot [\mathbf{2}]_0)$).
[c] Rate acceleration (k_{rel}) was calculated by the ratio of $k_{appcatalyst}/k_{appuncatalyzed}$, in which $k_{appuncatalyzed}$ is the apparent second-order rate constant in the absence of the catalyst.