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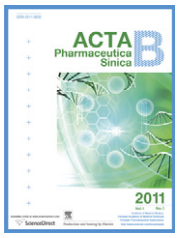

ORIGINAL ARTICLE

Catalytic cleavage activities of 10–23 DNAzyme analogs functionalized with an amino group in its catalytic core

Qi Wang[a], Di Zhang[b], Maosheng Cheng[b], Junlin He[a,*], Keliang Liu[a,*]

[a]*Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China*
[b]*School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China*

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KEY WORDS

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8-Aza-7-deaza-2′-deoxy-adenosine;
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Chemical modification

Abstract Functionalization of the catalytic loop of 10–23 DNAzyme with an amino group was performed by incorporation of 7-(3-aminopropyl)-8-aza-7-deaza-2′-deoxyadenosine in different single positions. Among the nine modified positions in the catalytic loop, A9 is the unique position with positive contribution by such modification. These results indicated that more efficient deoxyribozymes remain to be explored by introduction of exogenous functional groups in an appropriate position in the catalytic loop of 10–23 DNAzyme, such as the combination of 7-functional group substituted 8-aza-7-deaza-2′-deoxyadenosine analogs and A9 position.

*Corresponding authors. Tel.: +6 10 68169363, +6 10 68237761.
E-mail addresses: hejunlin@yahoo.com (Junlin He), keliangliu55@126.com, keliangliu@yahoo.com (Keliang Liu).

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1. Introduction

10–23 DNAzyme, an unnatural DNA enzyme obtained by *in vitro* selection from a DNA reservoir, is a sequence-specific RNA-cleaving DNA molecule[1,2]. Compared with ribozymes, its high chemical and biological stability and convenient synthesis seem to be more in favor of therapeutic and other biotechnological applications. Indeed, many efforts have been made for its applications, including the suppression of specific disease-related genes[3–16], nucleic acid structural and functional analysis, and other applications like DNA nanomachine[17–24]. Improvement of its intrinsic characteristics, such as cleavage site preferences, cleavage efficiencies, and high-level Mg^{2+}-dependence, as well as nuclease stability and efficient delivery for its optimal activities has been the focuses of many researches[25–34]. Some DNAyzmes with protein-like functional groups were selected *in vitro*, which could conduct RNA-cleavage reaction with M^{2+}-independence[29,34–37]. 10–23 DNAyzme analogs with M^{2+}-independence was obtained, but with a sacrifice of cleavage efficiency. Because an insight into the catalytic mechanism of the DNAzyme is not yet available[38,39], new random selections and chemical modifications for more efficient variants are still currently necessary[40–44]. As we have reported, screening with 7-substituted 8-aza-7-deaza-2′-deoxyadenosine derivatives in the positions of five dA residues (A5, A9, A11, A12, and A15) led to the finding that A9 is the right position for 7-substituted 8-aza-7-deaza-deoxyadenosine analogs, by which N8 atom, 7-subustituted phenethyl, 3-hydroxypropyl and 3-aminopropyl were introduced and positive effect on cleavage rate was always observed (Fig. 1)[45]. Especially, replacement of 7-(3-aminopropyl)-8-aza-7-deaza-2′-deoxyadenosine (**1**) at A9 position promised a 12-fold increase of cleavage rate. We postulated that the location of the amino group by this modification contributed to a most favorable catalytic conformation. In this study, the effect of this compound in other four non-dA positions were explored, with the aim to evaluate an appropriate position for the positive role of the extra amino group of compound **1** in the catalytic core of 10–23 DNAzyme.

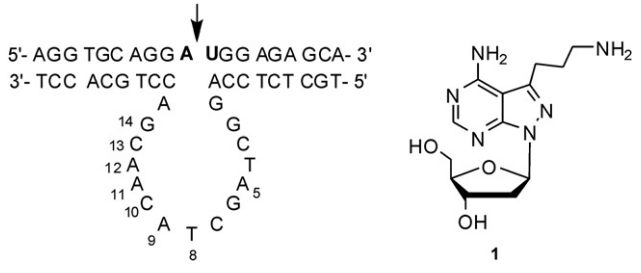

Figure 1 Left: the complex of 10–23 DNAzyme and its DNA–RNA–DNA substrate. Bold letters represent the RNA residues in the substrate. The arrow denotes the cleavage site on the substrate. Right: compound **1**.

2. Results and discussion

The oligodeoxynucleotides were synthesized with phosphoramidite chemistry. The phosphoramidite of compound **1** was synthesized according to the published procedure[45]. The oligodeoxynucleotides were purified by preparative denaturing 20% polyacrylamide gel electrophoresis (containing 7 M urea) and desalted with SEP-PAK cartridges. Characterization was performed with MALDI-TOF MS (Table 1). In the 10–23 DNAzyme analogs, compound **1** was incorporated into four positions, instead of T8, C10, C13 and G14, namely, **DZ-1-8**, **DZ-1-10**, **DZ-1-13** and **DZ-1-14** were obtained, respectively.

In order to demonstrate whether these modified DNAzymes could bind the substrate as 10–23 DNAzyme, the T_m of the complexes of these modified DNAzymes with the substrate was evaluated under the conditions for the cleavage reaction (50 mM Tris–HCl, 2 mM Mg^{2+})[45], with the chimeric DNA–RNA–DNA substrate replaced by its corresponding full-DNA substrate D19, 5′-d (AGG TGC AGG ATG GAG AGC A)-3′ to avoid any cleavage reactions conducted by DNAzyme itself and nucleases in the environment. All the complexes have a T_m of 51 °C (Table 1), very close to that of the complex of 10–23 DNAzyme and D19 (52 °C). It meant that the chemical modifications on the catalytic core could not result in changes on the thermal stability of the complexes. Furthermore, the temperature of all the cleavage reactions was set at 37 °C, much lower than the T_m of DNAyzme-substrate complexes. Therefore, it was suggested that the changes of cleavage behavior of the modified DNAzymes could be attributed to the influence of the chemical modifications on the cleavage step, instead of the thermal stability of the complex. The cleavage reaction of these DNAzymes on the ^{32}P-labled target DNA–RNA–DNA substrate was evaluated under single-turnover conditions.[45]

It has been demonstrated that compound **1** at A9 position of 10–23 DNAzyme contributed a 12-fold increase of cleavage rate, **DZ-1–9** with k_{obs} of 0.0037 min^{-1} [45]. **DZ-1–9** is the fastest DNAzyme in the screening of five dA residues with compound **1** in the catalytic core of 10–23 DNAzyme. Instead of these five dA positions, other residues in the catalytic core were substituted by compound **1** to explore the possible positive role of the extra amino group. Firstly, a position shift of compound **1** around A9 was conducted. When compound **1** in **DZ-1–9** was shifted to its 5′-residue position T8 (**DZ-1–8**), as shown in Fig. 2, **DZ-1–8** could also conduct the reaction like the parent 10–23 DNAzyme, but with a slower cleavage rate (0.0020 ± 0.0002/min) (Table 2). This replacement resulted in moderate loss of activity, compared with 10–23 DNAzyme, which was not so consistent with previous observation that T8 is the least conserved residue, any other canonical residue or even modified residue replacement results in little effect on the cleavage of 10–23 DNAzyme[40,41]. On the other hand, the replacement of compound **1** at its 3′-positioned C10 led to a further decrease of the reaction rate, as

Table 1 MALDI-TOF MS data of 10–23 DNAzyme analogs.

DNAzyme	Sequences (5′–3′)	MW (calcd.)	MW (found)
DZ-1-8	d(tgc tct cca GGC TAG C**1**A CAA CGA cct gca cct)	10,060.5	10,062.5
DZ-1-10	d(tgc tct cca GGC TAG CTA **1**AA CGA cct gca cct)	10,075.6	10,077.4
DZ-1-13	d(tgc tct cca GGC TAG CTA CAA **1**GA cct gca cct)	10,075.6	10,077.0
DZ-1-14	d(tgc tct cca GGC TAG CTA CAA C**1**A cct gca cct)	10,035.6	10,036.6