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The contribution of adenines in the catalytic core of 10-23 DNAzyme improved by the 6-amino group modifications

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ABSTRACT

In the catalytic core of 10-23 DNAzyme, its five adenine residues are moderate conservative, but with highly conserved functional groups like 6-amino group and 7-nitrogen atom. It is this critical conservation that these two groups could be modified for better contribution. With 2′-deoxyadenosine analogues, several functional groups were introduced at the 6-amino group of the five adenine residues. 3-Aminopropyl substituent at 6-amino group of A15 resulted in a five-fold increase of k_{obs}. More efficient DNAzymes are expected by delicate design of the linkage and the external functional groups for this 6-amino group of A15. With this modification approach, other functional groups or residues could be optimized for 10-23 DNAzyme.

The appearance of noncoding nucleic acids and their critical roles in modulating genetic process has initiated great interest in exploring more natural and unnatural functions and their practical applications,[1–4] such as antisense,[5] quadruplex,[6] CpG motifs,[7] ribozymes[8] and deoxyribozymes,[9] aptamers,[10] siRNAs[11] and miRNAs,[12] et al. In the efforts toward these purposes, understanding the structural basis and optimization of their functions is thus extremely required. Simple residue mutation on these functional nucleic acids, 10-23 DNAzyme[13,14] and 8-17 DNAzymes,[15,16] ribozymes,[17] TBA (thrombin-binding aptamer),[18,19] and siRNAs,[20] confirmed the residue conservation and the optimum selection results by nature and in vitro selection. It is rational that both the residues and the sequence composition contribute to the active conformation and functions by base stacking and hydrogen bonding. As revealed by NMR and X-ray analysis for TBA and its interaction with thrombin, its eight guanine residues form a quadruplex motif and the functional groups of the loop residues are responsible for the direct interactions with the active residues from thrombin.[21,22] Fluorescence method determined that pK_a shifts of nucleobases are prevalent in DNA and RNA tertiary structures, this kind of ionization led to activation of the functional groups of nucleobases as general acid/base catalysts.[23] Experimental evidences suggested that cytidine,[24] adenine,[25] and guanine[26] could act as general acid–base in ribozyme catalysis. Therefore, both base-stacking and the specific functional groups around the four nucleobases are equally important for a specific functional nucleic acid.[27] In the optimization of functional nucleic acids, nucleobase modification for an insight into the structural basis of nucleic acids has been extensively studied.[28] Especially, amino and imidazolyl groups are most often used as external groups for an activation of nucleic acids, and this kind of base modification has been transferred to nucleoside triphosphates used in in vitro selection, to find the optimum disposition of the active functional groups and more powerful nucleic acids.[29]

10-23 DNAzyme is an in vitro selected catalytic DNA molecule, its catalytic function is performed by a 15-mer catalytic core,[9a] in which every nucleobase is related to the reaction rate more or less, but all are unique at their specific positions (Scheme 1). Our studies revealed that the spacial location and functional groups of five dA residues are both critical for their contribution.[30] For example, 7-nitrogen atom of nine purine residues were critical for the catalytic activity, but its contribution at A9 could be optimized with 8-aza-7-deaza-2′-deoxyadenosine **1** and its 7-substituted derivative **2** (Scheme 2).[30] Especially, some of the highly conserved dG residues could be improved by its analogues. By this way, the functional groups could be regarded as the origin of mechanistic understanding and optimization of functional nucleic acids. In adenine, its 6-amino group is an important functional group, it was suggested to act as general acid or base in the

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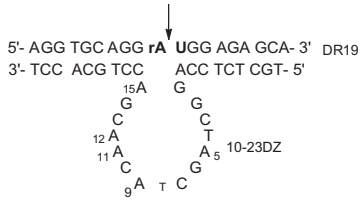


Scheme 1. 10-23 DNAzyme and its complementary DNA-RNA-DNA substrate DR19, in which the bold letters are the ribonucleotide residues as the cleavage site, as indicated by an arrow, and the adenine residues in the catalytic core are numbered.

catalytic reactions of ribozymes and form active conformation with other residues through hydrogen bonding.[25] Here, compound **3** (Scheme 2)[30b] was designed as probe to study the importance of the 6-amino group of five dA residues in the catalytic core of 10-23 DNAzyme, and it was further activated by amino and imidazolyl groups by compounds **4**–**6**. Another two compounds **7** and **8** (Scheme 2) were designed for the evaluation of the 6-amino group in the lead compound **1** and the active compound **2** for A9. All of them were the analogues of 2′-deoxyadenosine, by which the least interruption on the base occupation and stacking was kept.

All the nucleoside analogues were converted to their corresponding phosphoramidites for solid-phase synthesis of the modified DNAzymes, in which each of the five dA residues were replaced by the nucleosides **1**–**8**, respectively. All the DNAzymes were purified by denaturing electrophoresis and characterized by MALDI-TOF or ESI MS (Supplementary materials, Table S1). Under single-turnover conditions, the observed rate constants of the DNAzymes (2 μM) were measured against the DNA-RNA-DNA substrate **DR19** (20 nM) (Scheme 1), in the presence of 2 mM Mg^{2+} (in the buffer: 50 mM Tris-HCl, pH 7.5, 37 °C), by which the effect of the modification on the catalytic core was evaluated, without consideration of the binding process of the DNAzyme with the substrate, and the leaving step of the cleaved products.[30,31]

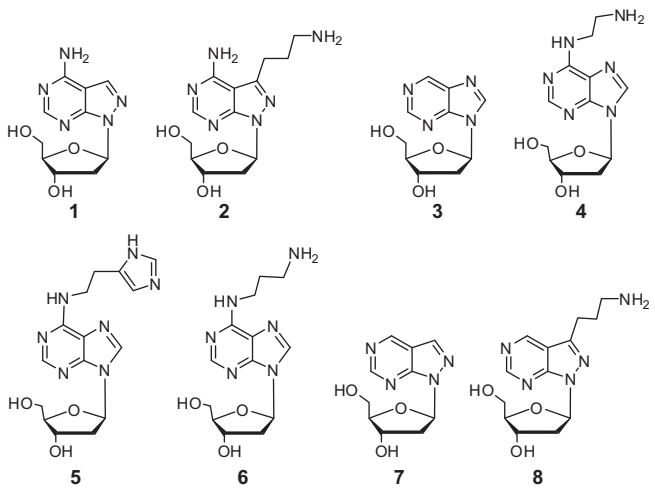
Under the reaction conditions, the T_m results indicated that the 6-amino substituents had little effect on the base stacking of the DNAzyme-substrate complexes (Table S2). CD spectra showed little changes of the amplitude and location of the positive lobe (Supplementary materials). Therefore, this kind of modifications remained the stable complex formation without significant change of the whole conformation characteristic of B-DNA duplex (Fig. S1), ensuring the DNAzyme-substrate complex formation.

Firstly, the conservation of the 6-amino group of five adenine residues (A5, A9, A11, A12 and A15) was studied by the deletion of the 6-amino group with compound **3**. Each replacement at these positions led to a change of the catalytic activity. The negative effect at A5, A9, A11 and A12 was observed for the modified DNAzymes (**DZ-A5-3**, **DZ-A9-3**, **DZ-A11-3**, and **DZ-A12-3**) compared to the unmodified **DZ01** (Table 1). But for A15, this deletion resulted in a positive effect on the catalytic reaction (**DZ-A15-3** vs **DZ01**), the interaction of the 6-amino group of A15 with other residues seems to be unfavorable for the catalytic reaction. These results demonstrated that the 6-amino group of all adenines was closely related to the activity, no matter whether they contribute to the activity by constructing the catalytic conformation or take part in the catalytic reaction directly (Fig. 1).

Based on these observations, compounds **4** and **5** were designed for the possibility of improving the effect of the 6-amino group, in which the 6-amino group was partially substituted with 2-aminoethyl group or imidazolylethyl group, respectively. By this design, the partial hydrogen-bonding ability of 6-amino group was kept, and the extra amino and imidazolyl groups were introduced for more interactions and more influence on the catalytic conformation.

When 2-aminoethyl group was introduced to 6-amino group by compound **4**, it was not accommodated at A5 and A12, it meant that 6-amino group of A5 and A12 is very conservative and uniquely needed. Its effect was similar to that of 2′-deoxyadenosine at A9 (**DZ-Z9-4**), A11 (**DZ-A11-4**), and A15 (**DZ-A15-4**). When compared to compound **3** with 6-hydrogen atom, the 6-substituent led to a significant increase at A9 (**DZ-A9-4**). At A15, although 6-(2-aminoethyl)amino group of compound **4** (**DZ-A15-4**) was not as good as a hydrogen atom of compound **3** (**DZ-A15-3**), its effect was still positive when compared with parent **DZ01**. These results demonstrated that 6-amino group at A9 and A15 could be further activated, either with a different functional group or a change of the linkage for the amino group, for a possible better 6-substitttuent.

When an imidazolyl group was introduced by compound **5**, a different profile of the position-dependent effect was observed. A5, A9 and A12 seem to be more appropriate for the imidazolyl



Scheme 2. 2′-Deoxyadenosine analogues for 6-amino group modification in the catalytic core of 10-23 DNAzyme.

Table 1
The observed rate constants of modified 10-23 DNAzymes with nucleoside analogues **3**–**6** under single-turnover conditions

Name	Catalytic core residues	k_{obs} (min^{-1}) ($\times 10^{-3}$)
DZ01	5′-d(GGC TAG CTA CAA CGA)-3′	5.1 ± 0.7
DZ-A5-3	5′-d(GGC T**3**G CTA CAA CGA)-3′	0.78 ± 0.06
DZ-A9-3	5′-d(GGC TAG CT**3**CAA CGA)-3′	0.30 ± 0.01
DZ-A11-3	5′-d(GGC TAG CTA C**3**A CGA)-3′	0.37 ± 0.06
DZ-A12-3	5′-d(GGC TAG CTA CA**3**CGA)-3′	0.90 ± 0.14
DZ-A15-3	5′-d(GGC TAG CTA CAA CG**3**)-3′	12.7 ± 0.5
DZ-A5-4	5′-d(GGC T**4**G CTA CAA CGA)-3′	0.4 ± 0.1
DZ-A9-4	5′-d(GGC TAG CT**4**CAA CGA)-3′	6.0 ± 0.5
DZ-A11-4	5′-d(GGC TAG CTA C**4**A CGA)-3′	4.6 ± 0.3
DZ-A12-4	5′-d(GGC TAG CTA CA**4**CGA)-3′	0.36 ± 0.06
DZ-A15-4	5′-d(GGC TAG CTA CAA CG**4**)-3′	8.50 ± 0.06
DZ-A5-5	5′-d(GGC T**5**G CTA CAA CGA)-3′	8.9 ± 0.5
DZ-A9-5	5′-d(GGC TAG CT**5** CAA CGA)-3′	8.9 ± 0.5
DZ-A11-5	5′-d(GGC TAG CTA C**5**A CGA)-3′	1.40 ± 0.01
DZ-A12-5	5′-d(GGC TAG CTA CA**5**CGA)-3′	1.30 ± 0.05
DZ-A15-5	5′-d(GGC TAG CTA CAA CG**5**)-3′	1.40 ± 0.01
DZ-A5-6	5′-d(GGC T**6**G CTA CAA CGA)-3′	nd[a]
DZ-A9-6	5′-d(GGC TAG CT**6**CAA CGA)-3′	11.8 ± 0.7
DZ-A11-6	5′-d(GGC TAG CTA C**6**A CGA)-3′	3.4 ± 0.3
DZ-A12-6	5′-d(GGC TAG CTA CA**6**CGA)-3′	nd[a]
DZ-A15-6	5′-d(GGC TAG CTA CAA CG**6**)-3′	26.0 ± 0.6

[a] nd: no significant reaction was observed under present conditions.

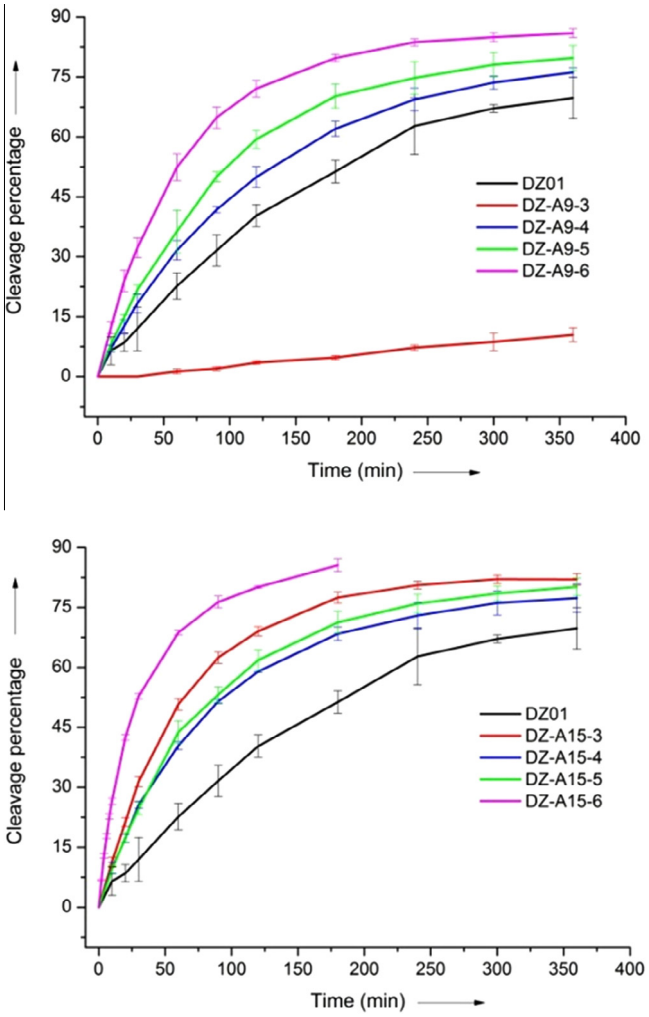


Figure 1. A comparison of compounds **3**–**6** at A9 and A15 for a positive effect on the catalytic reaction of 10-23 DNAzyme measured under single-turnover conditions, DNAzyme (2 μM) and the substrate (20 nM) were mixed in the buffer (50 mM Tris-HCl, pH 7.5, 2 mM Mg^{2+}, 37 °C), and monitored at certain time points. The samples were separated with denaturing PAGE (20%) and analyzed with PhospholImager.

group than hydrogen atom and 2-aminoethyl groups, but its effect is just as 6-amino group of natural 2′-deoxyadenosine. While at A11 and A15, further negative effect produced by this substitution. The different size and hydrogen bonding ability of the 6-positioned extra amino and imidazolyl group induced different position-dependent effect.

Compound **6** was designed for improving the effect of 6-substituted external amino group of compound **4** at A9 and A15. Compared with compound **4**, the effect of the extra amino group was strengthened by the one-carbon longer linkage (Table 1). Compared to parent **DZ01**, 2-fold and 5-fold increase of k_{obs} was observed for **DZ-A9-6** and **DZ-A15-6**, respectively. While, further negative effect was observed for the conservative A5 and A12, except for A11 with the similar result (Fig. 1).

Compound **1** is the lead compound for A9 modification and compound **2** gives a 9-fold increase of k_{obs}.[30] With compounds **7** and **8**, the importance of their 6-amino group was investigated. As listed in Table 2, the evaluation results indicated that the 6-amino group was highly needed for their positive effect. It was suggested that the 6-amino group and 8-N atom and 7-substituents in compounds **1** and **2** are cooperative in inducing a favorable catalytic conformation, and further improvement could be expected

Table 2
The observed rate constants of modified 10-23 DNAzymes with nucleoside analogues **1**, **2**, **7**, and **8** under single-turnover conditions

Name	Catalytic core residues	k_{obs} (min^{-1}) ($\times 10^{-3}$)
DZ-A9-1	5′-d(GGC TAG CT**1**CAA CGA)-3′	8.9 ± 0.8
DZ-A9-2	5′-d(GGC TAG CT**2**CAA CGA)-3′	45 ± 4
DZ-A9-7	5′-d(GGC TAG CT**7**CAA CGA)-3′	nd
DZ-A9-8	5′-d(GGC TAG CT**8**CAA CGA)-3′	6.90 ± 0.25
DZ-A5-7	5′-d(GGC T**7**G CTA CAA CGA)-3′	nd[a]
DZ-A11-7	5′-d(GGC TAG CTA C**7**A CGA)-3′	nd
DZ-A12-7	5′-d(GGC TAG CTA CA**7**CGA)-3′	9.8 ± 1.1
DZ-A15-7	5′-d(GGC TAG CTA CAA CG**7**)-3′	1.3 ± 0.1
DZ-A5-8	5′-d(GGC T**8**G CTA CAA CGA)-3′	<0.0001
DZ-A11-8	5′-d(GGC TAG CTA C**8**A CGA)-3′	0.30 ± 0.01
DZ-A12-8	5′-d(GGC TAG CTA CA**8**CGA)-3′	0.80 ± 0.01
DZ-A15-8	5′-d(GGC TAG CTA CAA CG**8**)-3′	0.90 ± 0.01

[a] nd: no significant reaction was observed under present conditions.

only with the 7-substituent in this context, either its functional group or the linkage could be optimized. At other four positions (A5, A11, A12 and A15), these modifications always led to significant negative effect, with only one exception for **DZ-A12-7**, in which the effect of compound **7** is similar to that of 2′-deoxyadenosine.

From this research, we learnt that in the catalytic core of 10-23 DNAzyme, two residues A9 and A15 could be improved by its 6-amino substituent and further optimized by changing the extra functional group and the linkage.

With this kind of chemical modification on single functional groups of canonical residues in functional nucleic acids, the specific functional group could be easily identified for activation with protein-like functional groups, as an approach for optimization of functional nucleic acids. By this way, a large space for chemical modification of functional nucleic acids was created.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.07.076.

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