

*Full Length Research Paper*

## Design of a new hairpin DNzyme: The activity controlled by TMPyP<sub>4</sub>

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**A new 10-23 hairpin DNzyme with a G-quadruplex structure stem was designed. The formation and stability of the G-quadruplex structure as the DNzyme stem in the absence or the presence of TMPyP<sub>4</sub> were investigated by UV-visible spectroscopy, circular dichroism (CD) and differential scanning calorimetry (DSC) methods, respectively. The results showed that the stability of this DNzyme can be enhanced greatly due to the interaction between TMPyP<sub>4</sub> and the DNzyme. The relationship between structural stability and activity of the DNzyme was studied by *in vitro* catalytic reaction. The activity of this DNzyme was regulated by the stability of DNzyme when TMPyP<sub>4</sub> was intercalated into G-quadruplex structure stem. The catalytic activity of the 10-23 hairpin DNzyme decreased and even inactivated due to the enhanced stability of G-quadruplex structure by TMPyP<sub>4</sub> molecules. This DNzyme is controllable to cleavage substrate and has some potential significance in gene therapy.**

**Key words:** 10-23 DNzyme, hairpin DNzyme, G-quadruplex structure, TMPyP<sub>4</sub>.

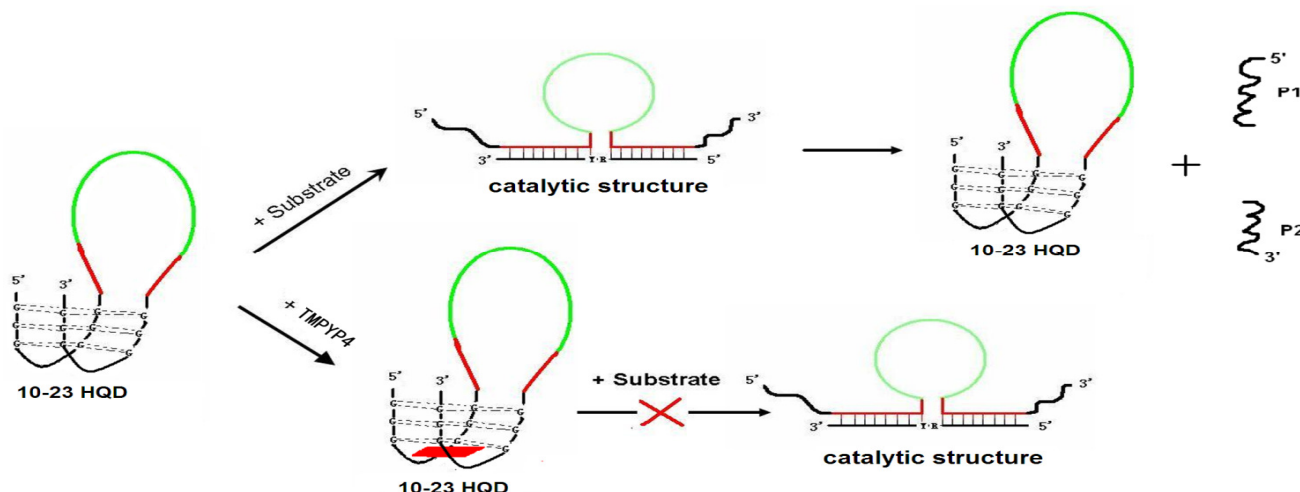
### INTRODUCTION

DNzyme is a DNA molecule with catalytic activity. In 1989, Joyce found the first DNzyme molecule by *in vitro* selection methodology (Joyce, 1989). With the development of gene therapy, DNzymes have attracted more and more attentions, (Chan and Khachigian, 2009; Tan et al., 2009; Dass et al., 2008; Benson et al., 2008), genetic analysis (Fu et al., 2009), biosensors (Liu et al., 2009; Willner et al., 2008) and nucleic acid enzyme tools (Baum and Silverman, 2008; Breaker, 2004). In 1998, Travascio found a new DNzyme with peroxidase activity (Travascio et al., 1998) for the first time, a G-quadruplex aptamerhemin complex was described as a novel DNzyme and the G-quadruplex aptamer enhanced the peroxidative activity of hemin in their study. Moreover, such a DNzyme with peroxidase activity was used to detect metal ions in solution, such as Ag<sup>+</sup> and Hg<sup>2+</sup> (Zhou

et al., 2010; Kong et al., 2009). In fact, the hemin could also influence the behavior of the G-quadruplex structure (Majhi and Shafer, 2006). Even though the G-quadruplex aptamer has its own catalytic activity, it can also be regulated by its cofactor such as TMPyP<sub>4</sub> (Wieland et al., 2006). Based on this, we designed a new hairpin DNzyme with controllable activity in this study.

10-23 DNzyme is one of the most widely known DNzymes. As a general DNzyme, 10-23 DNzyme can cleavage any target RNA (Santoro et al., 1997). The activity of 10-23 DNzyme can be affected by many factors, such as Mg<sup>2+</sup> (Yuan et al., 2007) and chemical modifications in the catalytic core (Zaborowska et al., 2002). It is necessary to stabilize 10-23 DNzymes against nucleolytic attack for *in vivo* applications and chemical modifications can be introduced into the binding arms to increase the stability but these may alter catalytic activity and increase cell toxicity (Abdelgany et al., 2007). The hairpin DNzyme is a new tool which exhibit efficient gene silencing effect in cells without chemical modification (Abdelgany et al., 2007). The aim of this

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**Figure 1.** Design of 10-23 HQD.

**Table 1.** Deoxyribozyme and substrate.

Name	Sequence
10-23 HQD	5'- <b>GGGTTAGGG</b> TGATTCAGGCTAGCTACAACGAGGTGGAG <b>GGGTTAGGG</b> -3'
SLD5	5'- <b>CCCGG</b> TGATTCAGGCTAGCTACAACGAGGTGGAG <b>CCGGG</b> -3'
SLD6	5'- <b>CCCGGG</b> TGATTCAGGCTAGCTACAACGAGGTGGAG <b>GGGCC</b> -3'
Substrate	5'-ACTCTCCACC <u>AU</u> GAATCACTC-3'

study was to design a new hairpin DNAzyme with the catalytic sequence of 10-23 DNAzyme whose activity can be regulated by the stability of G-quadruplex structure stem. Based on the basis of the mechanism of molecular beacons (Bourdoncle et al., 2006), we designed a new hairpin 10-23 DNAzyme with a stem composed of a G-quadruplex structure formed by two identical sequences d(GGGTTAGGG) and a loop which was composed of 15nt 10-23 DNAzyme active core sequence and 7nt substrate binding sequence at each side. We named this DNAzyme 10-23 HQD (10-23 Hairpin Quadruplex DNAzyme). We presented an idea that we changed the stability of the stem-loop structure of this new DNAzyme to control the catalytic activity of the DNAzyme. It has been confirmed by many other studies that as a well-known G-quadruplex structure binding ligand, TMPyP<sub>4</sub> (5, 10, 15, 20-tetrakis (1-methyl-4-pyridyl)-21H, 23H-porphine) can stabilize the G-quadruplex structure formed by human telomere sequence to inhibit telomerase (Dixon et al., 2005; Mikami et al., 2009). Moreover, the sequence forming a G-quadruplex structure in this study is similar to the human telomere sequence d(GGG(TTAGGG)<sub>3</sub>). Therefore, TMPyP<sub>4</sub> was selected to regulate the stability of the G-quadruplex structure stem of 10-23 HQD. The idea is shown in Figure 1. When no TMPyP<sub>4</sub> molecule interacts with 10-23 HQD, the stem-loop structure of the DNAzyme can be destroyed by the substrate binding sequence once bound with target RNA. When 10-23

HQD interacts with TMPyP<sub>4</sub> molecules, the stem-loop structure exhibits a higher stability, thus, the difficulty in destroying the stem-loop structure becomes larger. When the stem-loop structure of the DNAzyme is stable enough that cannot be destroyed by binding with target RNA, 10-23 HQD exhibits no catalytic activity to cleave target RNA at all. The study has some potential significance in gene therapy.

## MATERIALS AND METHODS

### Synthesis and purification of oligonucleotide sequences

The DNAzyme was synthesized by Sangon (Shanghai, CHINA). The oligonucleotides were purified with HPLC and used without further purification. The concentration of the oligonucleotides was estimated as described by the manufacturer provided by Sangon Corporation.

The substrate was synthesized by Takara (Dalian, CHINA) and used without further purification too. Based on the feature of the 10-23 DNAzyme, the two bases near the cleavage site were two RNA bases, A and U. The other bases in the substrate sequence were composed of deoxy-bases. The sequences of DNAzyme and substrate used in this study are shown in Table 1.

### Preparation of buffer and sample

Tris-HCl buffer (50 mM, pH = 7.5) was prepared from Tris-base and

HCl dissolved in 18 MΩ cm purity water (Millipore). Tris-HCl buffer was used in order to conserve the conformation and integrity of different nucleic acids.

In order to eliminate the secondary structure or aggregation state of DNAzyme sample, the DNAzyme sample was heated at 95°C for 5 min and cooled to room temperature in air.

TMPyP<sub>4</sub> porphyrin (5, 10, 15, 20-tetrakis(1-methyl-4-pyridyl)-21H,23H-porphine) was purchased from Aldrich and used without further purification. It was dissolved in 50 mM Tris-HCl buffer (pH = 7.5), which was prepared for the interaction of it with 10-23 HQD.

#### Stability assay of DNAzyme by differential scanning calorimetry (DSC)

The structural stability of DNA molecules corresponds to their melting temperature (T<sub>m</sub>). Differential scanning calorimetry (DSC) was used to investigate the melting temperature (T<sub>m</sub>) of 10-23 HQD, SLD5 and SLD6. The DNAzyme solution was prepared at a fixed concentration of 5×10<sup>-4</sup>M in 50 mM Tris-HCl buffer (pH= 7.5, 25°C) with 10 mM Mg<sup>2+</sup>. The DSC scans were performed from 10 to 100°C at a heating rate of 1°C /min. The DSC spectra were recorded on a VP-DSC micro calorimeter.

Differential scanning calorimetry (DSC) was used to investigate the effect of the interaction of TMPyP<sub>4</sub> on the melting temperature (T<sub>m</sub>) of 10-23 HQD. The DNAzyme solution was prepared at a fixed concentration of 5×10<sup>-4</sup>M in 50 mM Tris-HCl buffer (pH = 7.5, 25°C) with 10 mM Mg<sup>2+</sup>. TMPyP<sub>4</sub> was added at a series of molar ratios R(TMPyP<sub>4</sub>/10-23 HQD) of 0:1, 1:10, 1:1 and 10:1, respectively. The DSC scans were performed from 10 to 100°C at a heating rate of 1°C/min. The DSC spectra were recorded on a VP-DSC micro calorimeter.

#### Spectroscopic studies on the interaction between 10-23 HQD and TMPyP<sub>4</sub>

##### UV-vis spectroscopy

The binding mode between 10-23 HQD and TMPyP<sub>4</sub> was investigated by UV-vis spectroscopy. The concentration of TMPyP<sub>4</sub> was fixed at 5×10<sup>-6</sup> M in 50 mM Tris-HCl buffer (pH = 7.5, 25°C) with 10 mM Mg<sup>2+</sup>. The DNAzyme sample was added at a series of molar ratios R (TMPyP<sub>4</sub>/10-23 HQD) of 1:0, 10:1, 1:1 and 1:10, respectively. The spectra of the mixtures were recorded from 350 to 550 nm on a spectrometer (Shimadzu UV-2550).

##### Circular dichroism (CD)

The formation and change of the G-quadruplex structure stem of 10-23 HQD induced by TMPyP<sub>4</sub> were monitored via circular dichroism (CD) spectroscopy. Oligonucleotides used in this study were prepared by dissolving them in 50 mM Tris-HCl buffer (pH = 7.5, 25°C). The 10-23 HQD solution was prepared at a fixed concentration of 2×10<sup>-4</sup>M in 50 mM Tris-HCl buffer (pH = 7.5, 25°C) with 10 mM Mg<sup>2+</sup>. TMPyP<sub>4</sub> was added at a series of molar ratios R(TMPyP<sub>4</sub>/10-23 HQD) of 0:1, 1:10, 1:1 and 10:1, respectively. The spectra of the mixtures were recorded from 220 to 350 nm on a JASCO-810 spectropolarimeter and corrected against the background of Tris-HCl buffer.

#### Activity assays of DNAzymes

##### Radioactive labeling of substrate

For *in vitro* cleavage experiments, 2 μM of substrate was labeled by

250 μCi of (γ-<sup>32</sup>P) ATP in the presence of T4 polynucleotide kinase for 45 min at 37°C. Then, the substrate was precipitated from the solution by adding ethanol which was left overnight at -20°C. At last, it was dissolved in 18 MΩ.cm purity water (Millipore).

#### Determining the *in vitro* activities of DNAzymes

To analyze the catalytic activities of SLD5, SLD6 and 10-23 HQD, *in vitro* activity assays of DNAzyme under multiple-turnover conditions were carried out with 100 nM DNAzyme in the presence of 100 nM <sup>32</sup>P-labeled RNA substrate in 10 μl 50 mM Tris-HCl buffer (pH = 7.5) with 10 mM Mg<sup>2+</sup> at 37°C for 20 min. The reaction was stopped by adding 100 mM EDTA and 9 M urea. The substrate and cleaved products were separated on a 16% denaturing PAGE and visualized by autoradiography.

To analyze the catalytic activities of 10-23 HQD at different molar ratios R (TMPyP<sub>4</sub> / 10-23 HQD), DNAzyme *in vitro* activity assays under multiple-turnover conditions were carried out with 100 nM DNAzyme in the presence of 10 nM <sup>32</sup>P-labeled RNA substrate in 10 μl 50 mM Tris-HCl buffer (pH = 7.5) with 10 mM Mg<sup>2+</sup> at 37°C for 40 min. The reaction was stopped by adding 100 mM EDTA and 9 M urea. The substrate and cleavage products were separated on a 16% denaturing PAGE and visualized by autoradiography.

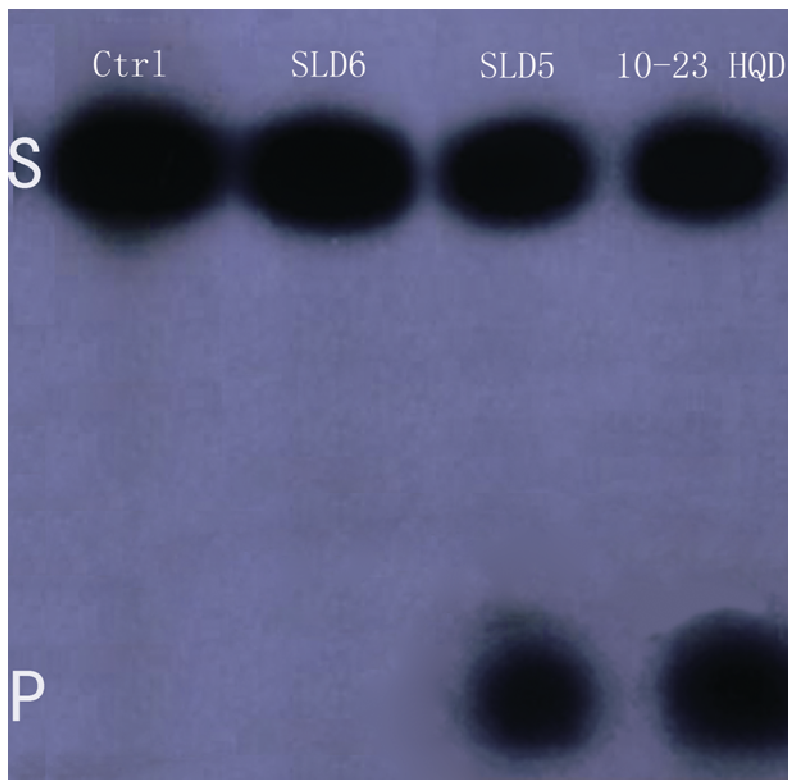
## RESULTS AND DISCUSSION

### Relationship between structural stability and catalytic activity of three hairpin DNAzymes

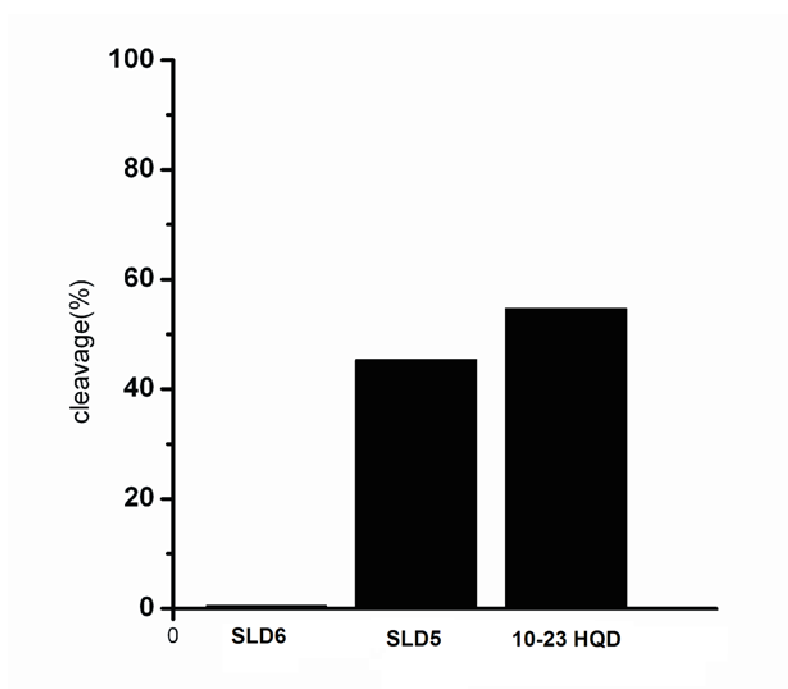
The stability of DNAzyme was described by the melting temperature (T<sub>m</sub>) of DNAzyme molecule. Based on the results in theory of the secondary structure and melting temperature (T<sub>m</sub>) of the hairpin DNAzymes by the Oligo-6.0 software and the web service ([www.Quadruplex.org](http://www.Quadruplex.org)), three hairpin DNAzymes (we named them SLD6, SLD5 and 10-23 HQD) as shown in Table1 and Figure 2a were designed to investigate the relationship between stem stability and activity of the hairpin DNAzymes; there was a little difference between the stem part of 10-23 HQD and each of two hairpin DNAzymes. The stems of the two other hairpin DNAzymes were composed of six G:C Watson-Crick base pairs (SLD6) and five G:C Watson-Crick base pairs (SLD5), respectively. 10-23 HQD and the two other hairpin DNAzymes had the same catalytic sequence.

The differential scanning calorimetry (DSC) measurement was employed to investigate their melting temperature (T<sub>m</sub>) in the experiment as shown in Figure 2b and the *in vitro* catalytic reaction was employed to investigate their catalytic activity; the reaction products were visualized autoradiographically by 16% denaturing PAGE as shown in Figure 3.

As shown in Figure 2, it was acquired that the melting temperature (T<sub>m</sub>) of 10-23 HQD, SLD5 and SLD6 were 34, 64 and 79°C, respectively in theory and 48, 57 and 61°C, respectively in the experiment. The experimental results were consistent with those in theory. As is shown in Figure 3 the rates of cleaving the same substrate for 10-23 HQD, SLD5 and SLD6 were 55, 47 and 0%,



**A**



**B**

**Figure 3.** (A) *In vitro* activity assays of 10-23 HQD, SLD5 and SLD6 DNAzyme activity; assays were carried out with 100 nM DNAzymes in the absence (Ctrl) or presence of 100 nM <sup>32</sup>P-labeled RNA substrate as indicated, the reaction product was visualized autoradiographically by 16% denaturing PAGE. S, Substrate; P, reaction product); (B) the rates of cleaving the same substrate for 10-23 HQD, SLD5 and SLD6.

respectively. Considering these results, the stability and catalytic activity of 10-23 HQD were compared with those of SLD6 and SLD5. The melting temperature of 10-23 HQD was the lowest among those of the three DNAzymes and that of SLD6 was the highest, while the catalytic activity of 10-23 HQD was the highest and SLD6 exhibited no catalytic activity. Based on the melting temperature ( $T_m$ ) and the rates of cleaving the same substrate for the three DNAzymes, it was confirmed that the hairpin DNAzymes in this study exhibited no catalytic activity to cleave target RNA if their stem-loop structures were so stable enough that they could not be destroyed by binding with target RNA and the stem-loop structures prevented their recognition with the target RNA. In other words, if the target RNA could not destroy the stem-loop structures of the DNAzymes, the DNAzymes could not form catalytic structure with the substrate, so it could not cleave the substrate into products.

### Spectroscopic study on the interaction between 10-23 HQD and TMPyP<sub>4</sub>

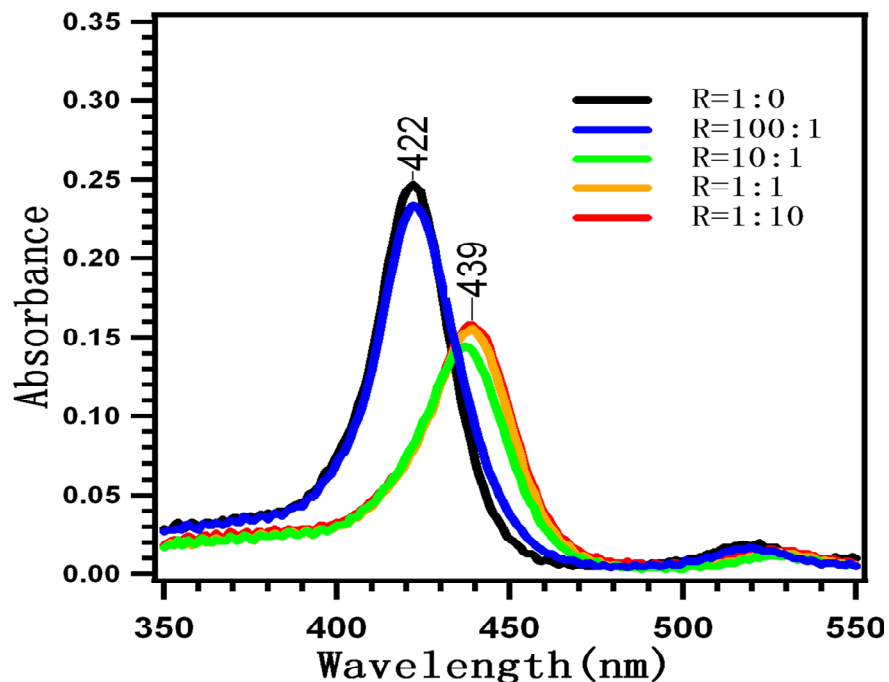
Figure 4 shows the UV-vis spectra and the circular dichroism (CD) spectra of the interaction between 10-23 HQD and TMPyP<sub>4</sub>. In order to determine the binding between TMPyP<sub>4</sub> and 10-23 HQD, the interaction of TMPyP<sub>4</sub> with the DNAzyme at different molar ratios were investigated by UV-vis absorption experiment. As shown in Figure 4a, with the molar rate changing from 1:0 to 1:10, the intensity of the Soret band of TMPyP<sub>4</sub> decreased from 0.25 to 0.15 and almost reduced 40%. There was a slightly red shift of 17nm from 422 to 439 nm. According to the earlier studies on the interaction between TMPyP<sub>4</sub> and G-quadruplex structure Wei et al. (2006), a hypochromicity more than 35% and a red shift of more than 17 nm of the Soret band show an intercalation binding mode. Therefore, the binding mode between TMPyP<sub>4</sub> and 10-23 HQD was intercalation binding mode. The results are in agreement with the result of Wei and Parkinson (Wei et al., 2009; Wei et al., 2006; Parkinson et al., 2007). Wei proposed the intercalation binding mode of TMPyP<sub>4</sub> to the G-quadruplex structure formed by the sequence d(AGGGG(TTAGGG)<sub>3</sub>) (Wei et al., 2009, 2006). Parkinson observed the binding mode in which TMPyP<sub>4</sub> interacted with the G-quadruplex structure from d(TAGGGTTAGGG) (Parkinson et al., 2007).

The circular dichroism (CD) spectrum was used to investigate the structure change of 10-23 HQD by adding TMPyP<sub>4</sub>. Figure 4b shows the CD spectra of 10-23 HQD in 50 mM Tris-HCl buffer with 10 mM Mg<sup>2+</sup> (pH = 7.5) at different molar ratios R (TMPyP<sub>4</sub>/ 10-23 HQD). The CD spectra of 10-23 HQD without binding TMPyP<sub>4</sub> showed a positive peak at 290 nm and a shoulder around 269 nm. From the reports by the other researchers (Xue et al., 2007), it was concluded that the stem structure of 10-23

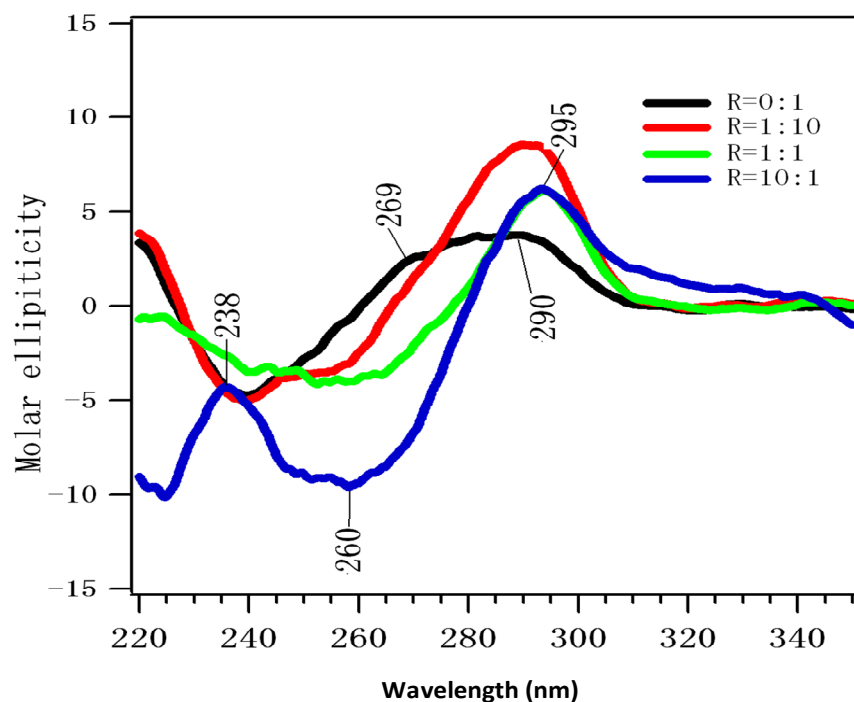
HQD had a hybrid type G-quadruplex with mixed parallel/anti-parallel strands. With the addition of TMPyP<sub>4</sub>, the band at 290 nm shifted to 295 nm and the change of this band was smaller, while the shoulder at 269 nm decreased. Meanwhile, a positive band at 238 nm and a negative band at 260 nm were observed at a molar rate of R=10. The circular dichroism (CD) spectra have been used to investigate the G-quadruplex structure by many researchers recently, Xue et al. (2007) proposed the complete structural conversion to a parallel-stranded G-quadruplex induced by PEG; Paramasivan investigated the effects of sequence, cations, chemical modification and ligand binding on quadruplex structure by CD spectra (Paramasivan et al., 2007); Lim discovered that the structure of the human telomere in K<sup>+</sup> solution was a basket type G-quadruplex with only two G-tetrad layers (Lim et al., 2009). Based on these studies (Xue et al., 2007; Paramasivan et al., 2007; Lim et al., 2009), the anti-parallel structure showed such a character of its CD spectra, that is, there was a negative peak near 260 nm and a positive peak near 295 nm, while the parallel structure shows such a character of a negative peak near 240 nm followed by a positive peak near 265 nm. It was concluded that the G-quadruplex structure stem of 10-23 HQD DNAzyme changed from hybrid type to the anti-parallel type due to the interaction of 10-23 HQD with TMPyP<sub>4</sub>. This result is consistent with the conclusion from other studies, that is, the human telomere sequence tends to form anti-parallel quadruplex structure (Paramasivan et al., 2007).

### Effect of the interaction of 10-23 HQD with TMPyP<sub>4</sub> on the structural stability

The structural stability of the DNAzyme was described by the melting temperature ( $T_m$ ) of DNAzyme. To investigate the effect of the interaction of it with TMPyP<sub>4</sub> on the melting temperature ( $T_m$ ) of 10-23 HQD, differential scanning calorimetry (DSC) was employed. Figure 5 shows the DSC spectra of 10-23 HQD in 50 mM Tris-HCl buffer with 10 mM Mg<sup>2+</sup> (pH = 7.5) at different molar ratios R (TMPyP<sub>4</sub>/ 10-23 HQD). It was obviously observed that the melting temperature ( $T_m$ ) was increased greatly with the addition of TMPyP<sub>4</sub>. The melting temperature ( $T_m$ ) of 10-23 HQD was 48°C. As the interaction between 10-23 HQD and TMPyP<sub>4</sub> took place, the melting temperature ( $T_m$ ) of the DNAzyme was enhanced obviously. When the molar rate R changed into 10:1, the melting temperature ( $T_m$ ) of 10-23 HQD reached 81°C. Based on the result of the UV-vis absorption experiment and the circular dichroism (CD) measurement, because the binding ability of porphyrin with G-quadruplex structure is more stronger than that with duplex DNA (Dixon et al., 2007) and the TMPyP<sub>4</sub> selectively interacted into the G-quadruplex structure stem of 10-23 HQD, it was concluded that the stability of 10-23



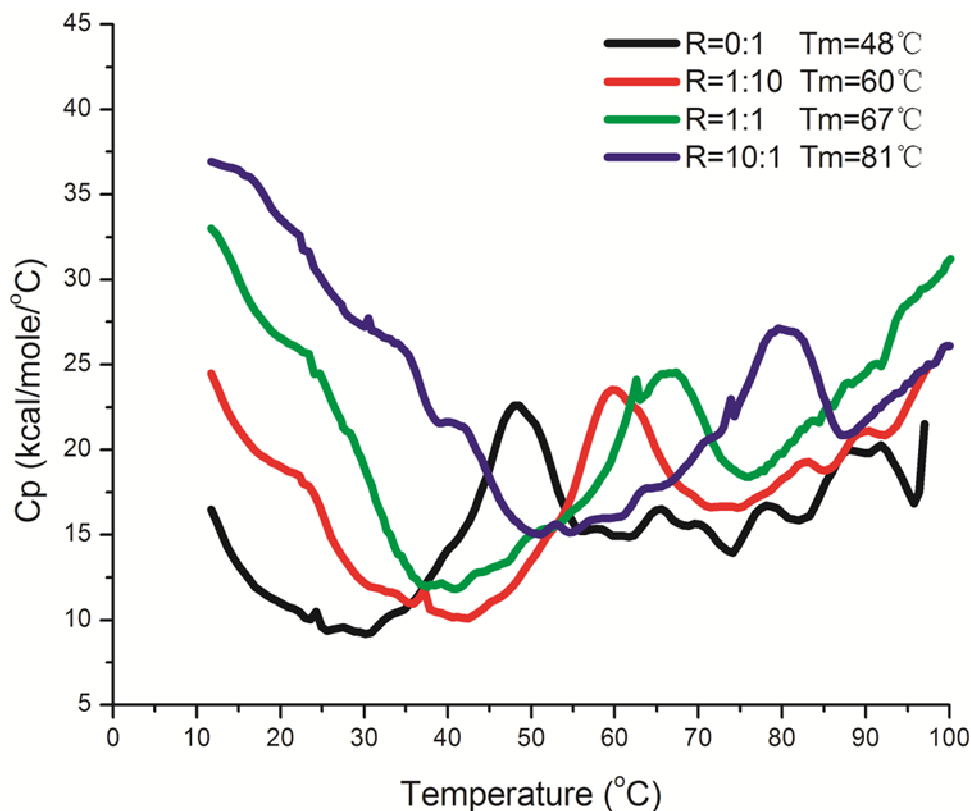
A



**Figure 4.** (A) UV-vis spectra of TMPyP<sub>4</sub> at a series of molar ratios R (TMPyP<sub>4</sub>/ 10-23 HQD) of 1:0, 100:1, 10:1, 1:1 and 1:10, respectively; (b) the CD spectra of 10-23 HQD at a series of molar ratios R (TMPyP<sub>4</sub>/ 10-23 HQD) of 0:1, 1:10, 1:1 and 10:1, respectively.

HQD was enhanced due to TMPyP<sub>4</sub> intercalated into the G-quadruplex stem of this DNAzyme. It is consistent with the result of the study by Ishikawa and Fujii, that is, the

binding of G-quadruplex structure with TMPyP<sub>4</sub> can enhance the stability of G-quadruplex structure (Ishikawa et al., 2008).



**Figure 5.** DSC spectra of 10-23 HQD at a series of molar ratios R (TMPyP<sub>4</sub>/ 10-23 HQD) of 0:1, 1:10, 1:1 and 10:1, respectively.

### Effect of the interaction of 10-23 HQD with TMPyP<sub>4</sub> on the catalytic activity

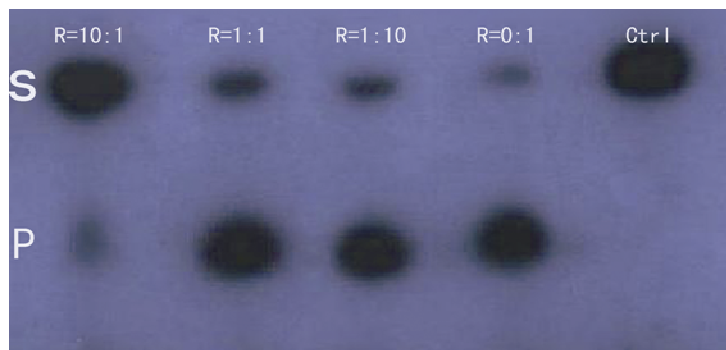
In order to examine the effect of the binding of 10-23 HQD with TMPyP<sub>4</sub> on the catalytic activity, *in vitro* DNAzyme activity assays were carried out with 10-23 HQD at a series of molar ratios R (TMPyP<sub>4</sub>/ 10-23 HQD) of 0:1, 1:10, 1:1 and 10:1, respectively. As shown in Figure 6, under the catalysis of 10-23 HQD without binding TMPyP<sub>4</sub>, the substrate was almost completely converted into products under multiple-turnover conditions. When the molar rates (TMPyP<sub>4</sub>/10-23 HQD) was at 1:10 and 1:1, it took place in slight changes in the percentage of the substrate catalyzed into product compared with no TMPyP<sub>4</sub> binding 10-23 HQD. However, when the molar rate (TMPyP<sub>4</sub>/10-23 HQD) reached 10:1, the 10-23 HQD almost exhibited no catalytic activity at all. The reason was that TMPyP<sub>4</sub> intercalated into G-quadruplex structure stem of this DNAzyme greatly enhanced the stability of the stem-loop structure of 10-23 HQD, thus prevented its recognition with the target RNA. As a result, the binding between 10-23 HQD and the target RNA could not destroy the G-quadruplex structure stem of the DNAzyme and the DNAzyme containing stem-loop structure could not form catalytic structure with the substrate, so the DNAzyme could not cleave the

substrate into product.

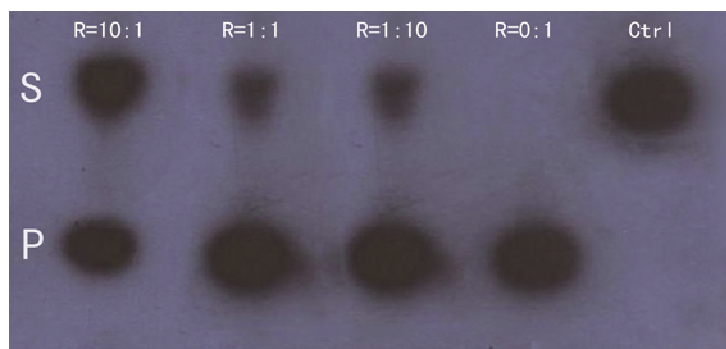
From Figure 6b, it was observed that the percentage of the substrate catalyzed into product by SLD5 decreased also with the addition of TMPyP<sub>4</sub>, which was because the TMPyP<sub>4</sub> could be intercalated into duplex structure stem and enhanced the stem-loop structure stability of SLD5. However, compared with 10-23 HQD, the catalytic activity of SLD5 decreased more slightly. According to the study by Dixon et al. (2007), the porphyrin molecule had 10000-fold selectivity with G-quadruplex structure ligand over duplex DNA. Therefore, SLD5 was affected slightly by interaction with TMPyP<sub>4</sub> than 10-23 HQD.

### Conclusion

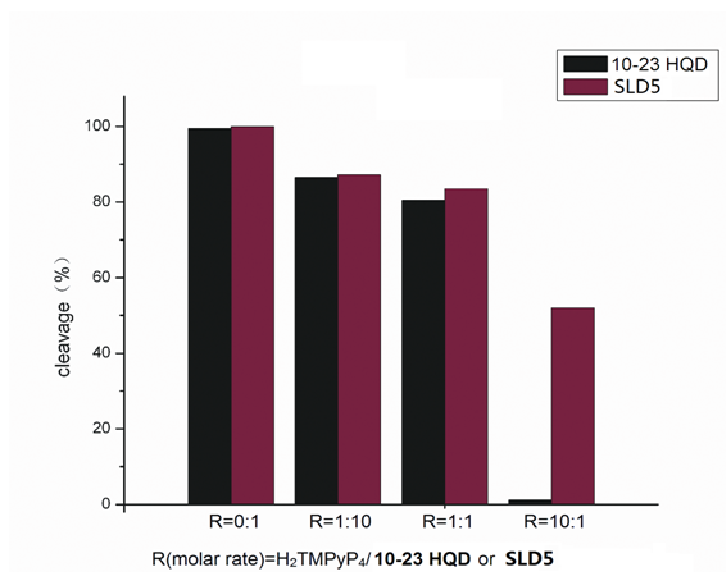
TMPyP<sub>4</sub> could interact with 10-23 HQD strongly and the binding mode was intercalation binding mode. Moreover, the intercalation binding enhanced the stability of stem-loop structure of this DNAzyme greatly, thus prevented its recognition with target RNA. In other words, target RNA could not destroy the stem-loop structure of the DNAzyme and 10-23 HQD could not form catalytic structure with the substrate, so it could not cleave the substrate into product. When TMPyP<sub>4</sub> intercalated into the G-quadruplex structure stem of 10-23 HQD, the



**a**



**b**



**Figure 6.** *In vitro* activity assays of 10-23 HQD. (a) DNAzyme activity assays were carried out with 100 nM 10-23 HQD at a series of molar ratios R(TMPyP<sub>4</sub>/ 10-23 HQD) of 0:1, 1:10, 1:1 and 10:1, respectively in the absence (Ctrl) or presence of 10 nM <sup>32</sup>P-labeled RNA substrate; as indicated, the reaction product was visualized autoradiographically by 16% denaturing PAGE; S, Substrate; P, reaction product; (b) DNAzyme activity assays were carried out with 100 nM SLD5 at a series of molar ratio R(TMPyP<sub>4</sub>/ SLD5) of 0:1, 1:10, 1:1 and 10:1, respectively in the absence (sub) or presence of 10 nM <sup>32</sup>P-labeled RNA substrate; as indicated, the reaction product was visualized autoradiographically by 16% denaturing PAGE; S, Substrate; P, reaction product; (c) cleavage rate for 10-23 HQD or SLD5 at a series of molar ratio R(TMPyP<sub>4</sub>/ 10-23 HQD or SLD5) of 0:1, 1:10, 1:1 and 10:1, respectively.



DNAzyme exhibited great changes in the catalytic activity; due to the increased stability, the catalytic activity of this DNAzyme was decreased and even inactivated. This DNAzyme was controllable to the cleavage substrate and had some potential significance in the gene therapy.

## ACKNOWLEDGEMENT

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