Backbone Modification Promotes Peroxidase Activity of G-Quadruplex-Based DNAzyme

Cong Li,[†[a]] Ling Zhu,[†[a]] Zhi Zhu,[†[a]] Hao Fu,[[a]] Gareth Jenkins,[[a][b]] Chunming Wang,[[a]] Yuan Zou,[[a]] Xin Lu,[[a]] Chaoyong James Yang[*[a]]

[a] State Key Laboratory of Physical Chemistry of Solid Surfaces, Key Laboratory of Chemical Biology of Fujian Province, Key Laboratory of Analytical Science, Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005 China
[b] Institute of Biomedical Engineering, Imperial College London, South Kensington, London SW7 2AZ, UK

EXPERIMENTAL SECTION

1. Instruments and Reagents

The DNA sequences were synthesized on an ABI 3400 DNA synthesizer. All of the oligos were purified by an Agilent (Santa Clara, CA) 1100 series HPLC system on a reverse-phase C18 column. Hemin and diammonium salt of 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma-Aldrich Co. (St. Louis, MO). Dimethylsulfoxide (DMSO), Triton X-100 and hydrogen peroxide were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). MALDI-TOF mass spectra of modified sequences were given in Supporting Information (Fig. S1), indicated successful synthesis of the modified-DNA.

2. Kinetic Measurements of Peroxidase Reactions

Peroxidase activities of hemin and hemin-G-quadruplex DNAzymes were measured in the ABTS-H_2O_2 system by surveying the appearance of the ABTS radical anion ($ABTS^{\bullet+}$) at 414 nm.[1] The $\Delta\varepsilon$ value used was 36000 $M^{-1}cm^{-1}$. Kinetics experiments were all performed in 96 well microplates using a SpectraMax M5 (Molecular Devices) at room temperature. The reactions included hemin-G-quadruplex complexes (concentration ratio of 2:1) or hemin alone, ABTS, and H_2O_2 in buffer with 25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 20 mM KCl (or without), 0.03% Triton X-100, and 1% DMSO. The samples were prepared as follows. All the DNA sequences were heated to 95 °C for 5 min in working buffer, cooled quickly on ice for 5 min, and placed at room temperature for 30 min. Then hemin was added to the mixtures by incubating at room temperature for another 3 h. Before the peroxidase-catalyzed reactions started, the ABTS was

added to the equilibrated mixture with intensive mixing. Final concentrations of DNA and hemin were 2 μM and 1 μM respectively. ABTS and H_2O_2 both were 2 mM. Upon the addition of H_2O_2, the oxidation of ABTS began. The absorbance at 414 nm increased over time. The initial rates (V_{obs}) were obtained by calculating the slope of the linear portion of the increase in absorbance during the first 20 s. Based on the Michaelis-Menten equation, kinetic parameters (K_m and V_{max}) were obtained by calculating the initial rates using different concentrations of H_2O_2 (0-10 mM).

3. Circular Dichroism Spectra

The oligonucleotides were dissolved in sodium phosphate buffer (PB buffer, 0.1 M, pH 8.0) with or without 20 mM KCl, heated to 95 °C for 5 min, cooled quickly on ice for 5 min, and placed at room temperature for 3 h. DNA-hemin concentration ratio was 1:1. CD spectra were recorded on a Jasco J-810 circular dichroism spectropolarimeter (JASCO Ltd.) at a rate of 500 nm/min using 400 μL of solution in 1 cm fused quartz cells with a bandwidth of 2 nm. Spectra were collected between 320-220 nm and a buffer baseline was subtracted from each sample spectrum. Measurements were taken at certain concentrations (1-2 μM) at room temperature. Final spectra were normalized to have zero ellipticity at 320 nm. The addition of hemin to G-quadruplex did not have significant influences on quadruplex structures (Fig. S3).

4. Thermal Denaturation

Melting curve analysis is employed to assess the structural stability of G-quadruplex. UV melting curves of G-quadruplexes in the presence of 20 mM KCl in sodium phosphate buffer (PB buffer, 0.1 M, pH 8.0) were monitored at 295 nm for absorbance signals using a UV-Vis spectrometer (Agilent 8453, USA). Concentrations of quadruplex were 4 μM, 10 μM and 20 μM. The samples were heated from 20 to 95 °C at a rate of 2 °C/min. Each sample was measured three times.

For all the sequences, except 2-OMe-RNA and PS-DNA modified PS2.M showed increased Tm value with increasing concentration, T_m value at 295 nm was almost independent of concentration, which suggests the formation of inter-molecular structures of 2-OMe-RNA and PS-DNA modified PS2.M and intra-molecular structures of the others (Table S2).[2]

5. Thermal Differential Spectra

For each oligonucleotide sample (2 μM), an UV spectrum was recorded above and below its melting temperature (T_m) in 0.1 M sodium phosphate buffer with 20 mM KCl. The difference between the UV spectrum at high temperature (95°C) and the UV spectrum at low temperature

(25°C) is defined as the thermal difference spectrum and represents the spectral difference between the unfolded and the folded form.

Thermal difference spectra (TDS) indicated that these oligos (except LNA candidates) possessed quadruplex structure: two major positive peaks at ~240 and ~270 nm and one negative peak at 295 nm (Fig. S4).[3]

6. Measurement of Binding Property of G-quadruplex to Hemin

The ability of G-quadruplexes and their modified sequences to form complexes with hemin was investigated by UV-Vis spectrometric analysis in 40KT buffer described by Travascio et al (50 mM 2-morpholinoethanesulfonic acid hydrate (MES) pH 6.5, 100 mM Tris acetate, 40 mM potassium acetate, 1% DMSO, 0.05% Triton X-100).[4] The ssDNA were heated at 95 °C for 5 min in the buffer and then cooled quickly on ice for 5 min. Each solution was then prepared to the final concentration with 0.5 μM hemin and incubated for 2 h at room temperature. The spectra were obtained using Spectro UV-2550 (SHIMADZU, Japan). The dissociation constant (K_d) for the DNA-hemin complexes was determined by plotting the changes in absorbance of hemin at 404 nm against varying concentrations of G-quadruplex. The plot was fitted to the following equation. [5]

$$[DNA]_0 = K_d(A-A_0)/(A_\infty-A) + [P]_0\,(A-A_0)/(A_\infty-A_0)$$

where $[DNA]_0$ is the initial concentrations of G-quadruplex; $[P]_0$ is the initial concentration of hemin; A_∞ and A_0 are the absorbance of hemin in the presence of a saturated concentration of DNA and in the absence of DNA, respectively; and A is the absorbance of hemin measured in the presence of varying concentrations of G-quadruplex.

7. References

1. X. Cheng, X. Liu, T. Bing, Z. Cao and D. Shangguan, *Biochemistry,* **2009**, 48, 7817.
2. P. L. T. Tran, J. L. Mergny, P. Alberti, *Nucleic Acids Res.* **2011**, 39, 3282.
3. J. L. Mergny, A. T. Phan, L. Lacroix, *FEBS Lett* **1998,** 435, 74.
4. P. Travascio, Y. Li and D. Sen, *Chem. Biol.*, **1998**, 5, 505.
5. Y. Wang, K. Hamasaki and R. R. Rando, *Biochemistry,* **1997,** 36, 768.

FIGURES AND TABLES

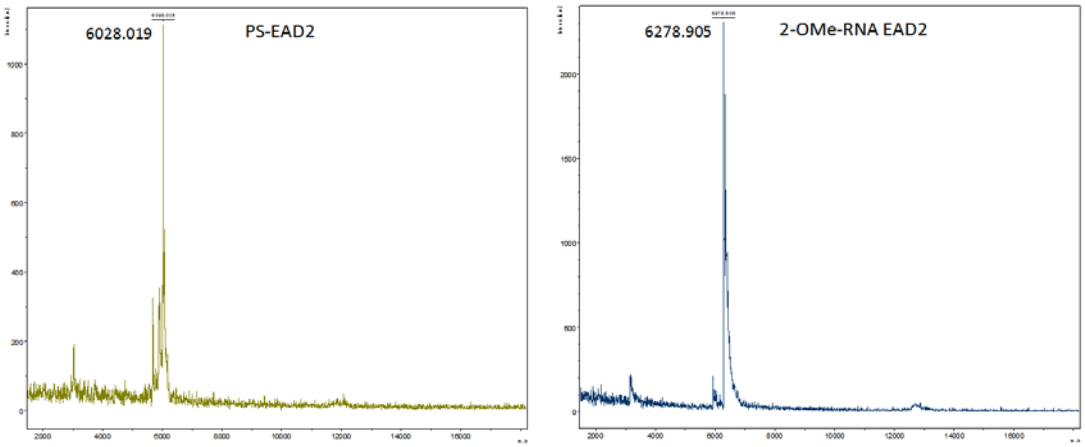

Fig. S1 MALDI-TOF mass spectrum of PS-EAD2 and 2-OMe-EAD2. The calculated molecule weight is 6022.7 of PS-DNA EAD2 and 6274.7 of 2-OMe-RNA EAD2.

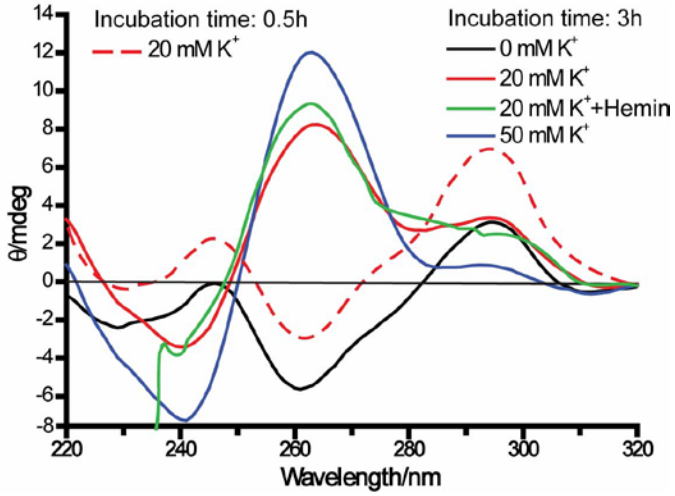

Fig. S2 CD spectra of PS2.M with increase in time and addition of increasing K^+..

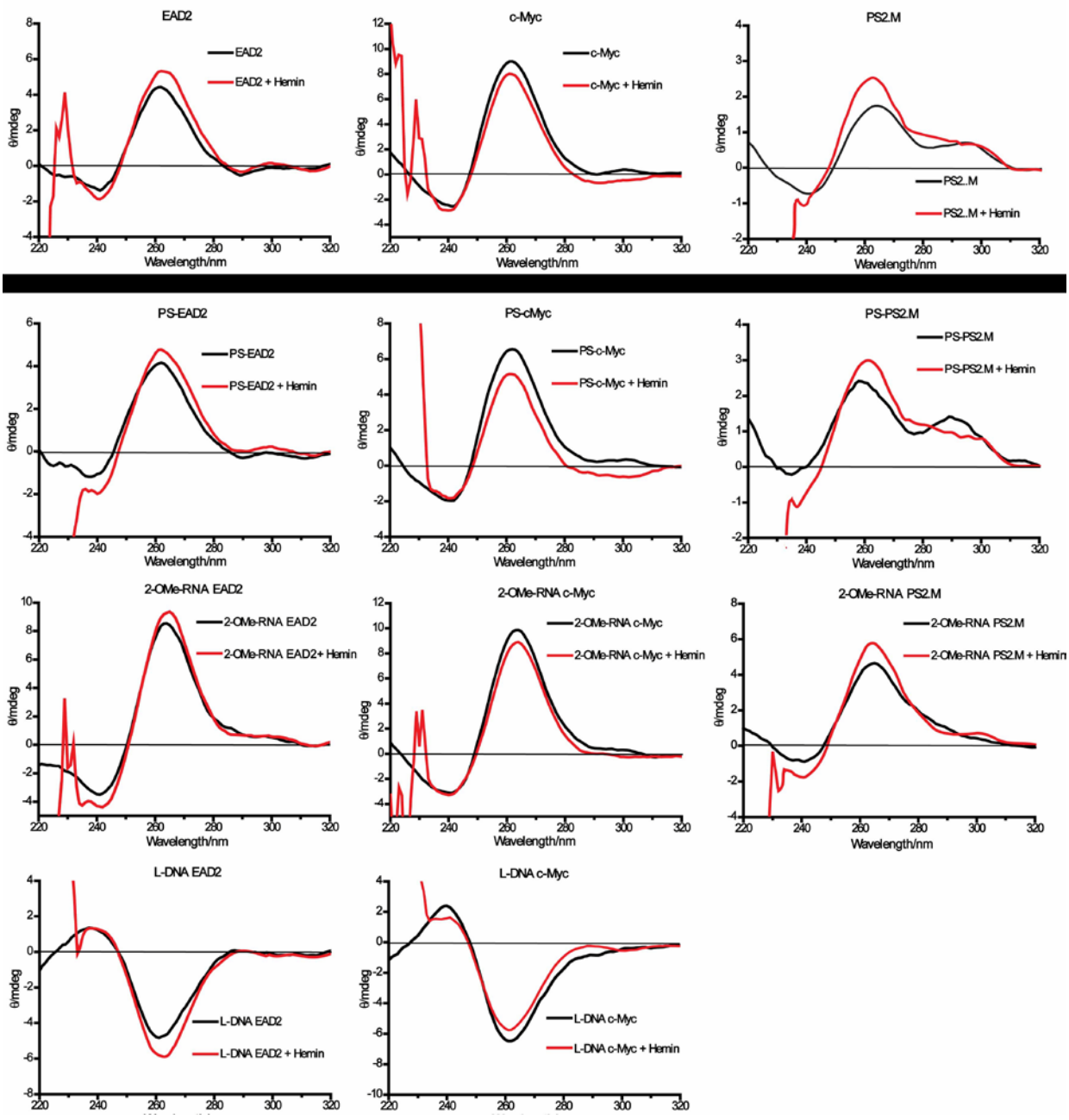

Fig. S3 CD spectra of quadruplex-hemin complexes with 1:1 concentration ratio.

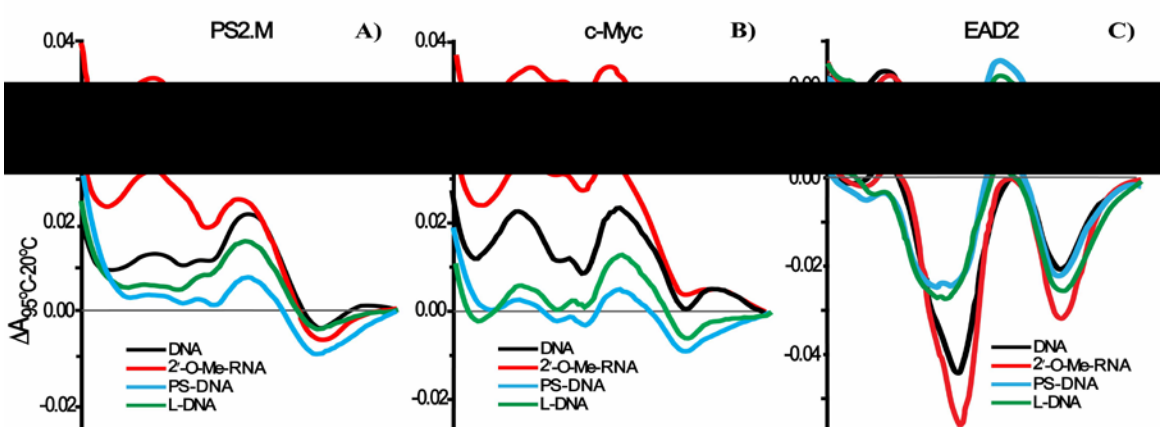

Fig. S4 Thermal difference spectra in the 220-320 nm regions for quadruplexes at 2 μM concentration in 0.1 M sodium phosphate buffer with 20 mM K^+.

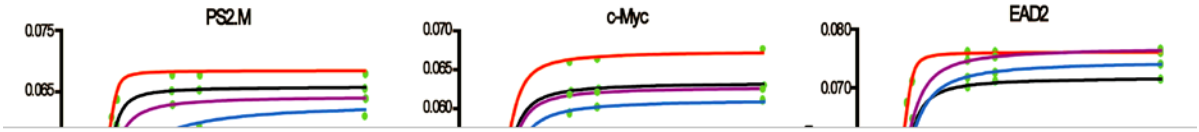

Fig. S5 Absorbance at 404 nm versus varying concentrations of G-quadruplexes.

Table S1. Kinetic parameters for peroxidation of ABTS catalyzed by modified G-quadruplex - Hemin complexes

Name		V_{obs} (nM s^{-1})		K_{cat}(s^{-1})	K_{cat}/K_{uncat}
		Without K^+	With 20 mM K^+		
Hemin		3.87 ± 0.19	3.27 ± 0.39	0.00437 ± 0.00035	1.0 ± 0.1
Random		3.33 ± 0.76	3.15 ± 0.44	0.00504 ± 0.00103	1.2 ± 0.3
PS2.M	DNA	16.4 ± 0.2	261 ± 6	0.439 ± 0.063	100 ± 17
	PS-DNA	57.7 ± 2.2	175 ± 14	0.303 ± 0.028	69 ± 9
	2-OMe -RNA	186 ± 15	370 ± 16	0.977 ± 0.075	224 ± 27
	L-DNA	11.0 ± 0.8	114± 6	0.170 ± 0.018	39 ± 5
	LNA	2.60 ± 0.36	3.75 ± 0.11	0.00614 ± 0.00036	1.4 ± 0.2
c-Myc	DNA	167 ± 2	330 ± 4	0.830 ± 0.043	190 ± 20
	PS-DNA	153 ± 7	234 ± 3	0.565 ± 0.048	129 ± 16
	2-OMe-RNA	308± 11	505 ± 13	1.93 ± 0.15	442 ± 54
	L-DNA	123 ± 2	381 ± 9	0.950 ± 0.062	217 ± 25
	LNA	3.83	5.63	0.021	4.8
EAD2	DNA	108 ± 8	438 ± 5	1.05 ± 0.08	241 ± 29
	S-DNA	183 ± 10	367 ± 16	0.883 ± 0.060	202 ± 23
	2-OMe-RNA	572 ± 34	814 ± 23	3.10 ± 0.25	708 ± 87
	L-DNA	120 ± 9	417 ± 18	0.967 ± 0.085	221 ± 28
	LNA	3.87 ± 0.56	14.6 ± 1.3	0.020 ± 0.002	4.5 ± 0.6

V_{obs} were calculated from the slope of the initial linear portion of the increase in absorbance. K_{cat} were obtained from the equation $K_{cat} = V_m / [E]$, and K_{uncat} was the "blank" (hemin) catalytic constant.

Table S2. T_ms of DNA, PS-DNA, 2-OMe-RNA and L-DNA analogs in 20 mM K^+ at different concentrations

Name		4 uM	10 uM	20 uM
PS2.M	DNA	52.3±0.6	53.0±0.0	52.5±0.7
	PS-DNA	46.3±0.6	47.7±0.6	48.7±1.5
	2-OMe-RNA	53.3±0.6	55.3±0.6	57.3±0.6
	L-DNA	49.0±1.0	51.0±0.0	51.5±0.7
c-Myc	DNA	74.7±1.5	74.3±0.6	74.3±0.6
	PS-DNA	71.7±0.1	72.7±1.0	72.7±0.6
	2-OMe-RNA	86.0±1.0	86.7±0.6	85.3±0.6
	L-DNA	71.0±1.7	72.7±0.6	72.0±1.0
EAD2	DNA	65.0±1.5	64.7±0.5	65.4±0.6
	PS-DNA	64.0±1.7	63.7±0.5	63.7±0.6
	2-OMe-RNA	86.5±0.5	84.7±0.5	85.7±0.6
	L-DNA	63.7±0.6	62.7±0.5	64.7±0.6