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Potassium−Lead-Switched G-Quadruplexes: A New Class of DNA Logic Gates

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DNA computing is thought to be the future of computer technologies,[1] while DNA molecules that perform logic operations are a prerequisite for digital information processing and computing.[2] This has attracted many efforts focused on problems in Boolean logic at the molecular scale to find ideal candidates that satisfy logic operations. Toward this goal, diverse DNA logic gates have been designed and constructed,[3] most of which are based on base pairing or DNAzyme-catalyzed DNA cleavage. Herein, we utilized a cation-driven allosteric G-quadruplex DNAzyme to devise a conceptually new class of DNA logic gate based on cation-tuned ligand binding and release.

G-quadruplexes are four-stranded DNA structures that are generally stabilized by monovalent cations such as K^+ and Na^+.[4] Very importantly, a few K^+-stabilized G-quadruplexes are found to mimic peroxidases (with hemin as a cofactor) and catalyze the H_2O_2-mediated oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS).[5] In comparison with K^+, Pb^{2+} has a higher efficiency for stabilizing G-quadruplexes[6] due to the formation of more compact DNA folds (Figure 1a). For this reason, we hypothesized that Pb^{2+} has the ability to competitively bind to K^+-stabilized G-quadruplex DNAzymes, giving rise to sharp changes in their structures and functions.

Three monomolecular G-quadruplex DNAzymes (i.e., PS2.M, PW17 and T30695)[7] were investigated here. Figure 1b shows their circular dichroism (CD) spectra when stabilized by K^+ or Pb^{2+}. In the presence of K^+, PW17 and T30695 have typical characteristics of parallel G-quadruplexes, whereas PS2.M has those of the antiparallel structure. Upon substitution of K^+ by Pb^{2+}, the CD spectra of PS2.M and PW17 change dramatically. Most notably, a strong positive band appears near 312 nm in their CD spectra, consistent with Pb^{2+}-stabilized antiparallel quadruplex structures.[6a,c,d] In contrast, most of the Pb^{2+}-stabilized T30695 is found to adopt a parallel conformation and coexist with a fraction having the antiparallel structure. These observations strongly suggest structural differences between G-quadruplexes stabilized by K^+ and Pb^{2+}. A previous study[6b] demonstrated that Pb^{2+}-stabilized G-quadruplexes generally have shorter M−O and O−O bonds than those stabilized by K^+ (see Figure 1a). Such compact structures contribute to the unusually high efficiency of Pb^{2+} at stabilizing G-quadruplexes.[6a,b] In general, 1 equiv of Pb^{2+} is enough to hold a G-quadruplex stable, whereas K^+ at the millimolar level is required to do so.

Since PS2.M, PW17, and T30695 all exhibit high hemin-binding affinities and DNAzyme activities in the presence of K^+,[7] we hypothesized that when stabilized by Pb^{2+}, they may have better properties because of higher structural stability. To test this hypothesis, UV−vis absorption spectroscopy was utilized to reveal the binding of three G-quadruplexes to hemin, reflected by the hyperchromicity of the hemin Soret band (Figure 1c). Next, their DNAzyme activities were characterized in the ABTS−H_2O_2 reaction system (Figure 1d). Unexpectedly, Pb^{2+}-stabilized PS2.M and PW17 had almost no ability to bind hemin and thus exhibited no DNAzyme activity. This suggests

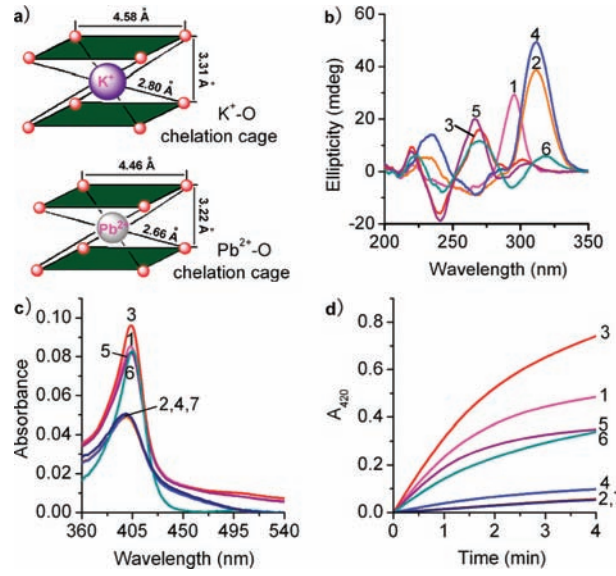

Figure 1. Structures and properties of PS2.M, PW17 and T30695 in 10 mM Tris-Ac buffer (pH 8.0, 21 °C): (1) K^+−PS2.M; (2) Pb^{2+}−PS2.M; (3) K^+−PW17; (4) Pb^{2+}−PW17; (5) K^+−T30695; (6) Pb^{2+}−T30695; (7) hemin. (a) K^+−O and Pb^{2+}−O chelation cages (the structural data are available in ref 6b). (b) CD spectra of PS2.M, PW17, and T30695 in the presence of 10 mM K^+ or 10 μM Pb^{2+}. (c) UV−vis absorption spectra of 1 μM hemin−G-quadruplex complexes. (d) Kinetic curves for the ABTS−H_2O_2 reaction catalyzed by 0.2 μM complexes of hemin and G-quadruplexes. Absorbance at 420 nm (A_{420}) was always monitored.

that both PS2.M and PW17 are K^+-dependent DNAzymes. In contrast, T30695 appeared to be independent of the cation, since it bound hemin and exhibited DNAzyme activity in each case, even without any coordination cation.[8]

Figure 1 shows that substitution of K^+ by Pb^{2+} results in structural and functional changes of three G-quadruplexes. For PW17 and PS2.M, K^+ and Pb^{2+} are responsible for hemin binding and unbinding, respectively. A similar rule was also obtained utilizing another G-quadruplex-interactive ligand, zinc protoporphyrin IX (ZnPPIX), in place of hemin (see Figure S1 in the Supporting Information). These observations suggest that ligand binding to or release from the G-quadruplexes can be tuned by K^+ or Pb^{2+}. This allowed us to utilize PW17 and PS2.M to construct a conceptually new class of DNA logic gate.

In comparison with PS2.M, PW17 has more remarkable changes in the CD spectra, Soret absorption band, and DNAzyme activity upon substitution of K^+ by Pb^{2+}. Hence, this G-quadruplex DNA was here chosen as the logic device, with hemin as the cofactor (Figure 2). As demonstrated above, K^+ favors hemin binding to PW17, thereby promoting DNAzyme activity. In contrast, Pb^{2+} drives hemin to release from PW17, resulting in deactivation of

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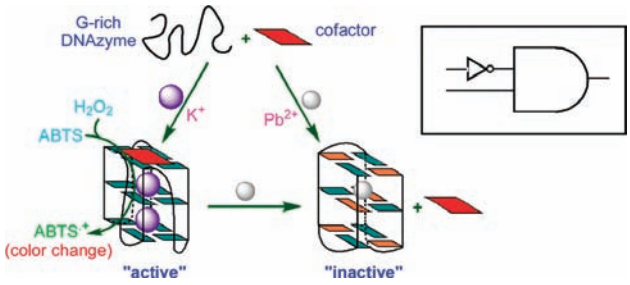
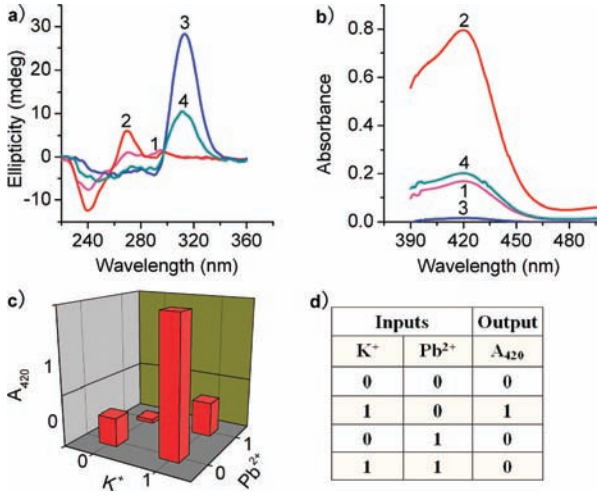


Figure 2. Construction of an INHIBIT logic gate (represented in the box) based on the G-rich DNAzyme PW17, with K^+ and Pb^{2+} as two inputs and absorbance as an output. In the quadruplex structures, anti and syn guanines are colored cyan and orange, respectively.



Inputs		Output
K^+	Pb^{2+}	A_{420}
0	0	0
1	0	1
0	1	0
1	1	0

Figure 3. Operation of K^+−Pb^{2+}-switched PW17 as a two-input INHIBIT logic gate. (a) CD spectra monitoring the conformational transition of 10 μM PW17−hemin complex (pH 8.0) for four input modes: (1) no input; (2) 2 mM K^+; (3) 100 μM Pb^{2+}; (4) 2 mM K^+ + 100 μM Pb^{2+}. (b) UV−vis absorption spectra (4 min) for analyzing the DNAzyme activity of 0.2 μM PW17−hemin complex in the ABTS−H_2O_2 reaction system for the four input modes. (c) Absorbance changes at 420 nm in the form of a bar representation, with a threshold of $A_{420} = 0.3$ for output 1 or 0. (d) Truth table for the two-input INHIBIT logic gate.

the DNAzyme. That is, K^+ and Pb^{2+} can be utilized to switch PW17 between two states, "active" and "inactive". This is in accordance with a two-input INHIBIT logic gate behavior.[9]

To demonstrate how this logic gate operates, CD measurements and UV−vis absorption spectroscopy were utilized to monitor the conformation and activity changes of PW17 for four input modes (Figure 3). With no input, PW17 was in the random coil state, whereas it folded into a parallel or antiparallel quadruplex structure upon input of K^+ or Pb^{2+} (Figure 3a). It was found that Pb^{2+} can induce K^+-stabilized PW17 to undergo a parallel-to-antiparallel conformation transition (curve 2 → 4) as a result of its unusually high efficiency at stabilizing G-quadruplexes. The conformational transition is accompanied with a remarkable change in the DNAzyme activity (Figure 3b). Since the enzyme activity can be straightforwardly represented in the form of absorbance at 420 nm (i.e., the characteristic absorption of the product $ABTS^{\bullet+}$), A_{420} here serves as the output (1 or 0) with a threshold that is three times above the background imparted by hemin catalysis (Figure 3c). It was observed that when K^+ was input alone, the output was 1; otherwise it was 0. These observations demonstrate that K^+−Pb^{2+}-switched PW17 really functions as a two-input INHIBIT logic gate, for which the truth table is shown in Figure 3d. Some common cations (e.g., Mg^{2+} and Ca^{2+}) have no obvious influence on the DNAzyme activity and the output, and thus, this logic gate can operate in the presence of these cations without any interference (see Figure S2 in the Supporting Information).

Likewise, another DNA logic gate can be constructed via introduction of a Pb^{2+} chelator, i.e., EDTA (see Figure S3 in the Supporting Information). We found that EDTA is able to capture Pb^{2+} from the Pb^{2+}−PW17 complex, thereby allowing K^+ to bind to PW17 again. This reactivates the DNAzyme, i.e., the logic system is reset to the initial state. Such a process is in accordance with reversible two-input IMPLICATION logic gate behavior,[10] but the inputs here are Pb^{2+} and EDTA. Furthermore, we envisage the construction of additional DNA logic gates via replacement of the cofactor hemin by other G-quadruplex-interactive ligands (e.g., ZnPPIX), where the output is a fluorescence signal.

In conclusion, we have constructed a conceptually new class of DNA logic gate utilizing a K^+−Pb^{2+}-switched G-quadruplex DNAzyme. Unlike most of previous counterparts based on base pairing or DNA cleavage, which cannot be reversibly operated, this logic gate employs different cations to control ligand binding to or release from the G-quadruplex, thereby modulating the output reversibly. Such a logic process includes two unique features different from the conventional: (1) it provides the first demonstration of the transformation from the parallel to the antiparallel G-quadruplex induced by cations, in contrast to the usually reported antiparallel-to-parallel conformation transition, and (2) it indicates that a stable G-quadruplex does not always favor ligand binding, in contrast to the conventional opinion. Our study not only introduces a new concept for devising DNA logic gates but also provides new insight into the structures and functions of DNA G-quadruplexes.

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Supporting Information Available: Experimental details and supporting figures and discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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