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Zn^{2+}-Selective Switch of Duplex to Hairpin DNA

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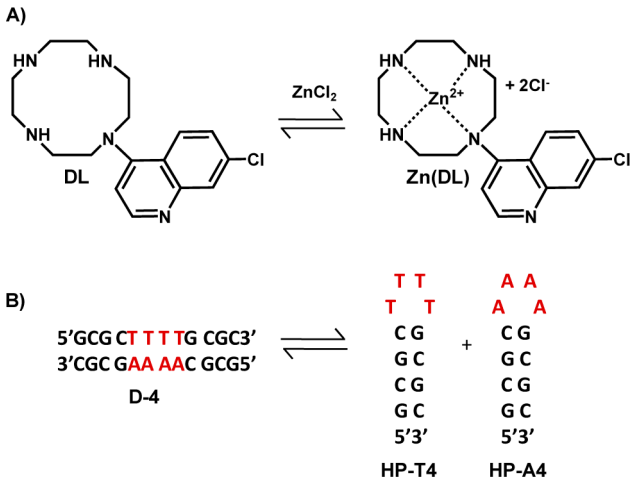
Supporting Information

ABSTRACT: DNA sequences that undergo large changes in secondary structure upon binding of small molecules are the basis for molecular switches. Here we report a Zn^{2+} complex that promotes the conversion of a fully complementary DNA double helix into DNA hairpins. The conformational switch is promoted by an isolated Zn^{2+} complex or by free $ZnCl_2$ and a macrocyclic ligand. The switch is selective for Zn^{2+} over biologically relevant transition-metal ions including Cu^{2+} and Fe^{2+}. The dual ligand/DNA switch is an approach that may improve the selectivity for metal-ion-sensing applications.

Metal-ion-promoted changes in nucleic acid conformation have wide-ranging applications in metal-ion sensing. These applications include the development of DNAzymes that contain conformational switches for selective sensing of metal ions in the environment[1] and mRNA conformational changes that lead to metal-ion-dependent modulation of gene expression.[2,3] For example, Hg^{2+} or Ag^{+} is selectively detected through their binding to thymine−thymine or cytosine−cytosine mismatches to activate DNA-based switches.[4] Examples from biological systems include Mg^{2+} riboswitches[5,6] and Fe^{2+}-promoted conformational changes in ferritin m-RNA that modulate the efficiency of translation.[7] Metal-ion-induced conformational switches are quite remarkable in that coordination to a few groups may significantly alter the nucleic acid structure. However, the limited diversity in ligand donor groups in DNA and RNA makes it challenging to develop switches that are specific for certain metal ions. An alternative approach is the combination of a conventional ligand and DNA to create a dual sensor of enhanced selectivity. In this approach, the conventional ligand binds the metal ion to produce a complex that recognizes the nucleic acid structure.[8−11] Dual sensors may be especially useful for distinguishing between the biologically relevant transition-metal ions and Zn^{2+}.

Here we show that the Zn^{2+} ion in the presence of a macrocyclic ligand [DL = 7-chloro-4-(1,4,7,10-tetraazacyclododecan-1-yl)quinoline][12] converts a DNA duplex containing multiple AT base pairs into two hairpin loops at moderate ratios of Zn^{2+}-to-DNA (Scheme 1). The switch is promoted by Zn^{2+} and not by Cu^{2+} or Fe^{2+}, enabling us to construct a nucleic acid sensor for the Zn^{2+} ion. The DNA sequence was chosen based on the propensity of certain Zn^{2+} macrocyclic complexes to bind to noncanonical DNA containing unpaired thymines such as loop structures or bulges[13] and to denature DNA duplexes containing multiple AT base pairs. Early studies in Kimura's laboratory showed that the selectivity of Zn^{2+} complexes for thymine nucleobases arises from interaction of the metal center with the deprotonated N3 imide of thymine and stacking of the aromatic pendent group on the thymine nucleobase.[11,14,15]

Scheme 1. (A) Equilibrium between DL and Zn(DL) and (B) Equilibrium between Hairpins HP-T4 and HP-A4 with Duplex D-4



Studies in our laboratory have recently focused on the design of Zn^{2+} complexes that selectively bind to unpaired thymine nucleobases in noncanonical DNA.[9,13,15] These complexes have pendent groups with either two or three fused rings as well as different types of linkages to the macrocyclic complex. Zn(DL) (Scheme 1) was shown to interact selectively with thymines in bulges and in G-quadruplex loops.[13] The selective binding of Zn(DL) to unpaired thymines led us to study this complex for the development of a DNA switch.

DNA conformational changes in the presence of Zn^{2+} and DL added separately to a buffered DNA solution or with the isolated Zn(DL) complex were studied initially by using a molecular beacon approach.[16] The thymine-containing strand of a duplex was modified with fluorophores on the 5′ and 3′ ends (HP-T4F) with the expectation that denaturation of the duplex (D-4F) would produce hairpins. Hairpin formation would bring the two fluorophores closer together and lead to fluorescence quenching through fluorescence resonance energy transfer (FRET) between the dyes [Scheme S1 in the Supporting Information (SI) and Figure 1]. Notably, the change in the fluorescence intensity between the duplex and hairpin is relatively small (Figure S1 in the SI), suggesting that there is energy transfer between fluorophores even in the duplex form.[17] The combination of Zn(DL) or $ZnCl_2$ and DL with the D-4F duplex

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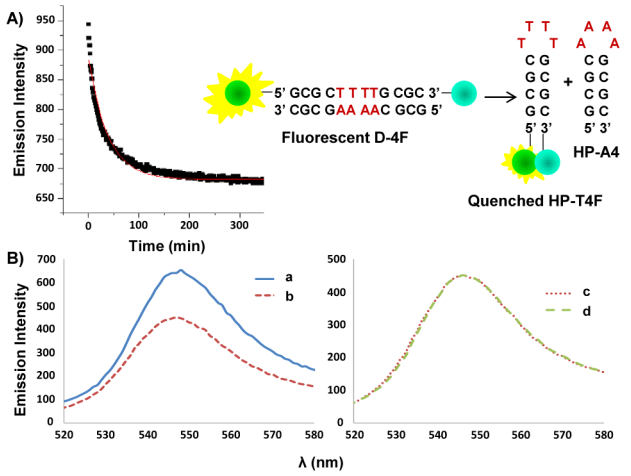

Figure 1. (A) Fluorescence emission decay curve of D-4F with Zn(DL) at a 1:8 ratio. Excitation was at 495 nm, and emission was followed at 545 nm. The curve was fit to a first-order decay with $k = 3.5 \times 10^{-4}$ s^{-1} or $t_{1/2}$ = 33 min. (B) Emission spectra of D-4F and HP-T4F collected under the following conditions: (a) D-4F, $ZnCl_2$, DL, immediately after mixing; (b) D-4F, $ZnCl_2$, DL at 2 h; (c) D-4F and Zn(DL) at 2 h; (d) HP-T4F and Zn(DL) at 2 h. All solutions contained 25 μM DNA, 0.080 M NaCl, 0.020 M HEPES, pH 7.5. Fluorophores were 3′-6-FAM and 5′-TET (Scheme S1 in the SI).

led to a decrease in the fluorescence emission intensity (Figure 1B). A 1:8 DNA-to-Zn(DL) ratio [0.67 Zn(DL)-to-nucleotide] gave complete conversion of the duplex to the hairpin as judged by the decrease in the fluorescence emission at 545 nm. Under these conditions, the final emission intensity was identical within experimental error with that of the thymine-containing hairpin (HP-T4F) in the presence of Zn(DL) (Figure 1B, c and d). Notably, the addition of Zn^{2+} ion and DL separately produced identical fluorescence changes when added to the duplex DNA (Figure 1B, b and c) compared to that of Zn(DL), consistent with the formation of Zn(DL) under the conditions of the experiment. The change in the fluorescence emission intensity with time was fit to a first-order decay curve. In the presence of a 1:8 duplex-to-Zn(DL) ratio, the half-life for hairpin formation was 33 min (Figure 1A), a relatively short half-life compared to most computational DNA logic gates.[18−20] The relatively fast conformational change and stable final equilibrium state highlights the potential of this DNA switch.

Native polyacrylamide gel electrophoresis (PAGE) experiments were used to study the denaturation of a native DNA duplex containing four AT base pairs. Similar to the fluorescent D-4F duplex, the D-4 duplex denatures to produce two DNA strands that form hairpin structures (Scheme 1). Studies using native PAGE showed that, in the absence of the Zn^{2+} complex, the equilibrium between two complementary DNA single strands is toward hybridization into a duplex (Figure 2). In the presence of Zn^{2+} and DL, the D-4 duplex converts to products that are assigned as the T- and A-loop hairpins. Notably, similar thymine-containing sequences have been shown to fold into hairpins under conditions comparable to those here.[21] In addition, both DNA single strands (HP-T4 and HP-A4) comigrate on the gels with the DNA switch products and stain with ethidium bromide, supporting a hairpin stem that binds the fluorescent dye.

Conversion of the D-4 duplex into hairpins is nearly complete at a 6:1 ratio of Zn^{2+} and DL to DNA (Figure 2). Similar results were obtained with the isolated Zn(DL) complex (Figure S2 in

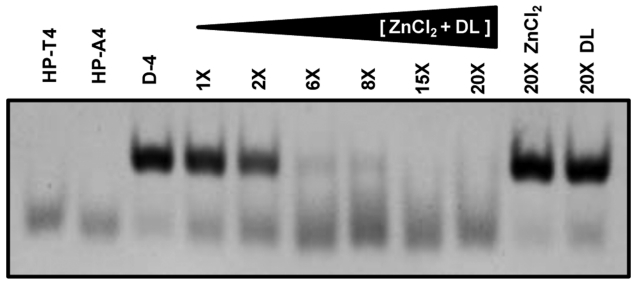

Figure 2. Native PAGE of the D-4 duplex (25 μM DNA, 0.080 M NaCl, 0.020 M HEPES, pH 7.5) with increasing ratios of Zn^{2+} and DL (equimolar) to DNA. The right lanes show $ZnCl_2$ or DL controls.

the SI). Studies of D-4 incubated with Zn(DL) for 1 h, 24 h, or 1 week at 22 °C gave similar results (data not shown). This finding suggests that denaturation of the duplex is complete in 1 h or less, consistent with the studies using D-4F discussed above. Thermal melting experiments confirmed that the addition of Zn(DL) to D-4 destabilized the duplex (Figure S3 in the SI). Notably, the D-4* duplex, which also contains four AT pairs but has a higher melting temperature (D-4, 41.8 ± 0.2 °C; D-4*, 54.7 ± 0.7 °C), was not denatured by Zn(DL) (Figure S4 in the SI).

The DNA conformational change is dependent both on the Zn^{2+} center and on the pendent group. The dependence on Zn^{2+} is shown by the lack of DNA structural changes for D-4 in the presence of the free (DL) ligand alone (Figures 2 and S5 in the SI). Furthermore, sequestration of Zn^{2+} reverses the DNA structural changes. Solutions containing the Zn(DL)-denatured D-4 duplex were treated with ethylenediaminetetraacetic acid (EDTA) to remove Zn^{2+} from the macrocycle. This led to rehybridization of the HP-T4 and HP-A4 strands (Scheme 1B) to give the duplex as shown by gel electrophoresis (Figure S6A in the SI). Removal of Zn^{2+} from Zn(DL) by EDTA is also confirmed by fluorescence studies (Figure S6B in the SI). Finally, the Zn^{2+} complex of the parent cyclen macrocycle (1,4,7,10-tetraazacyclododecane) failed to denature the duplex (Figure S7 in the SI). These results confirm the importance of the pendent group and the bifunctional nature of the Zn^{2+} DNA switching agent.

Conversion of the duplex to hairpin DNA by the Zn^{2+} ion and DL macrocycle added independently to DNA prompted us to determine whether other biologically relevant metal ions would promote the conformational switch. Similar studies carried out with Cu^{2+} or Fe^{2+} in the presence of the DL macrocycle did not give rise to denaturation of the duplex (Figure S8 in the SI) even in the presence of a 15-fold excess of the metal ion and ligand. This result is consistent with the weak complexation of Fe^{2+} ions to cyclen derivatives under the conditions of the experiment and on the relatively poor Lewis acidity of the Cu^{2+} complex of the cyclen macrocycle.[22]

Interaction of the Zn(DL) complex with DNA was studied by using isothermal calorimetry (ITC) and surface plasmon resonance (SPR) in order to gain insight into the duplex-to-hairpin conversion. Previous studies showed that Zn(DL) binds to a G-quadruplex with thymine-containing loops and to thymine bulges but does not bind appreciably to DNA hairpins lacking thymine.[13] The instability of the DNA duplex in the presence of Zn(DL) made it difficult to quantify the interaction of the complex with D-4F. Instead of a duplex, the oligonucleotide SS-T4 (Figure S4A in the SI) was studied to determine the magnitude and stoichiometry of the interaction of Zn(DL) with consecutive thymines in a structurally flexible context. Data were

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fit to a binding equation for 2:1 stoichiometry to give a dissociation constant (K_d) of 28.1 ± 0.2 μM for two identical sites in solutions containing 0.080 M NaCl and 0.020 M HEPES, with pH 7.5 at 25 °C (Equation S1 in the SI). Alternatively, data could be fit to a two-site binding model for two inequivalent sites (Equation S2 in the SI) to give similar K_d values of 22.9 ± 0.3 and 56.0 ± 2.8 μM (Figure S9A in the SI). Fitting of the data to a 1:1 binding isotherm gave a K_d value of 55.6 ± 3.4 μM but with a slightly poorer fit (Figure S9B in the SI).

Binding of the Zn(DL) complex to hairpin DNA, HP-T4, was studied using SPR. SPR enabled us to monitor binding at micromolar concentrations of Zn(DL), whereas ITC binding studies required much higher concentrations of Zn(DL) for sufficient heat evolution to be observed. Data were fit to SPR binding isotherms to give K_d values of 6.3 ± 0.2 and 76.0 ± 5.0 μM (Figure S10A in the SI). Data could also be fit to a 1:1 binding isotherm, but with a poorer fit than to the 2:1 binding isotherm (Figure S10B in the SI). The Zn(DL) complex thus binds strongly to the hairpin loop despite crowding of the thymines in the constrained 4-nucleotide loop.[22]

These data suggest that the Zn(DL) complex binds with micromolar affinity to unpaired thymines, most likely in a 2:1 ratio to DNA sequences that contain four sequential unpaired thymines. One interpretation of the switching event is that two Zn(DL) complexes interact with the Watson−Crick face of the thymines as they are exposed in transient breathing of the duplex[23] to give duplex denaturation. Conversion would then be favored by the inhibition of DNA duplex formation by loop-bound Zn(DL) complexes. Thus, the switch is driven by the binding of Zn(DL) to unpaired thymines. Along these lines, studies in Kimura's group showed that Zn^{2+} complexes promoted the denaturation of DNA duplexes containing long thymine (T) tracts at high ratios of the Zn^{2+} complex to DNA.[11] Nuclease cleavage only occurred on the adenine (A) tract, confirming the selective binding and protection of the thymine-containing strand by the Zn^{2+} complex.[23]

This work constitutes the first example of a DNA switch from duplex to hairpin promoted by a Zn^{2+} macrocyclic complex, to the best of our knowledge. The dual nature of the sensor for Zn^{2+} has the advantage of being tunable through modification of the conventional ligand as well as through optimization of the nucleic acid sequence.

■ ASSOCIATED CONTENT

Supporting Information

Experimental and synthetic methods, fluorescence emission, ITC, and gel electrophoresis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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