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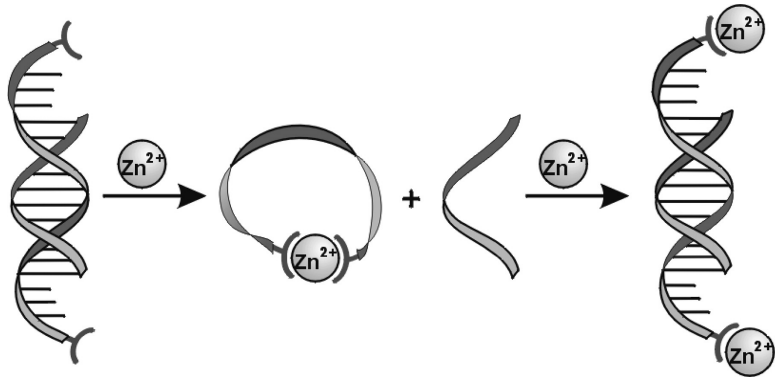

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Allosteric Control of Oligonucleotide Hybridization by Metal-Induced Cyclization

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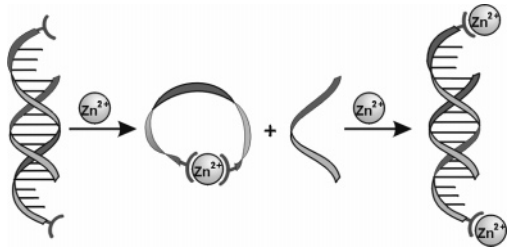
Allosteric[1] control of the biological activity of proteins[2,3] and—to a lesser extent—nucleic acids[4,5] is fundamental for cellular function. Influenced by biomimetic design principles, fully synthetic allosteric receptors and catalysts have been developed.[6] The incorporation of artificial allosteric sites into biomacromolecules would expand the natural repertoire of regulatory mechanisms and allow targeted modulation of biomolecular function by specific small-molecule effectors.

This challenging task has recently been addressed by engineering allosteric receptor proteins,[7] enzymes,[8] and ribozymes,[9] of which the two latter represent an emerging new class of biosensors. Very few examples of hybrid systems containing "chemical" allosteric sites have been reported, such as a fructose-responsive myoglobin−boronic acid conjugate[10] and a DNA-responsive peptidase.[11] Here we describe a chemically modified single-stranded DNA which displays a unique response behavior toward the biometals Fe^{2+} and Zn^{2+} (Scheme 1): in the absence of metal ion, the probe binds a complementary DNA strand; at one equivalent, the metal is a negative allosteric effector and off-regulates binding; with excess metal (in case of Zn), hybridization is reestablished. Two chelating 2,2′:6′,2″-terpyridine (tpy) moieties attached to the 3′ and 5′ end of the oligo consitute the "allosteric site", and the metal ion forces the probe into a cyclic structure such that conformational restrictions destabilize a double helix.

Scheme 1. Cyclization and Control of Hybridization of Bis(tpy) Modified DNA by Zn^{2+}. Complementary DNA (Hairpin Structure when Released) Is Represented as a Linear Strand for Simplicity



Previous examples of metal-ion modulated hybridization of modified nucleic acids include Cu^{2+}- and Gd^{3+}-directed dimerization of short oligonucleotide probes for enhanced DNA binding,[12,13] assembly of metallosalene DNA,[14,15] cross-linking of a peptide nucleic acid/DNA duplex by Pt^{2+},[16] and artificial metallo base-pairs in DNA (Cu^{2+}, Ag^{+})[17,18] and peptide nucleic acid (Ni^{2+}).[19] Bis(tpy)−Fe^{2+} and 2,2′-bipyridine−Ru^{2+} complexes have recently been introduced as stable vertexes into oligonucleotides, to direct the assembly of DNA nanostructures by duplex formation.[20,21] The stability of "Short-stem" 2′-O-Me-RNA hairpins modified with terpyridine at both ends is improved by addition of coordinating metal ions.[22] By incorporating a metal binding site into a peptide nucleic acid, we could recently make the sequence-specific hybridization of short probes conditional upon the presence of Zn^{2+}.[23]

Terpyridine-modified oligo-DNAs **1**−**2** (Scheme 2) were prepared by solid-phase synthesis. A carboxy-functionalized 2,2′:6′,2″-terpyridine was coupled to primary amino functions introduced at the 3′- and/or 5′ terminus of a DNA 20mer (Supporting Information).

Scheme 2. Tpy-Modified 20mer DNAs **1**,**2**, Unmodified DNA **3**, Complementary DNA **4**, Complementary "Molecular Beacon" DNA **5** (Self-Complementary Part of Sequence Underlined)

At first, our investigations of the reaction of **1** and **2** with transition metals were focused on iron(II), which forms thermodynamically and kinetically rather stable, highly colored, octahedral bis(tpy) complexes.[24] Complexation by two tpy units is highly cooperative, i.e., mono(tpy) complexes are not formed even in the presence of excess Fe^{2+}.[25] Spontaneous complexation of Fe(II) by the tpy groups of **1** was followed by the increase of both optical absorbance at $\lambda_{max} = 558$ nm (characteristic of the $(tpy)_2Fe$ moiety) and UV absorbance at 320 nm (Supporting Information). Formation of a 1:1 complex between **1** and Fe(II) is confirmed by electrospray MS (negative mode, m/z 1796 [M + Fe(II)],$^{4-}$ m/z 1437 [M + Fe(II)]$^{5-}$ and by MALDI-TOF MS (m/z = 7189); no signals of unmetalated **1** or of oligomeric products were found. In particular, gel shift analysis by denaturing PAGE (Figure 1, lanes 2 and 6)

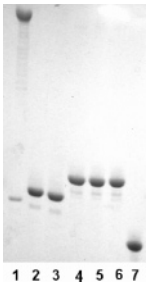


Figure 1. Denaturing 20% PAGE analysis. (Lane 1): **2** + Fe(II). (Lane 2): **1** + Fe(II). (Lane 3): **1** + Ni(II). (Lane 4): **1** + Zn(II). (Lane 5): **1** + Cu(II). (Lane 6): **1**. (Lane 7): **3**. Spots are visualized by UV shadowing.

indicates smooth formation of a product (**1**)Fe (confirmed by elution and MALDI-TOF MS of the spot), which has a somewhat higher mobility than the linear probe **1**, as expected for a relatively small circular DNA.[26] A band of similar retention factor is observed in the gel for a (**1**)Ni(II) 1:1 mixture (Figure 1, lane 3). In contrast, the intermolecular complex $(\mathbf{2})_2Fe$ formed with the mono-tpy compound **2** has a very low mobility (lane 1) due to its double DNA chain length.

We have next investigated the effect of Fe(II) ions on the hybridization of **1** with complementary oligo-DNA. To detect duplex

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formation at room temperature, the target DNA was designed as a fluorogenic molecular beacon **5** with a 16-base complementary loop and a 6-base-pair self-complementary stem (Scheme 2). Free **5** adopts a hairpin conformation, so that the fluorescence of the 5′-appended tetramethyl rhodamine is intrinsically quenched by promixity to 3′-attached Dabcyl. On addition of **1** to a solution of **5**, the fluorophor−quencher contact is disrupted by formation of a rigid 16mer double helix, and the red fluorescence of tetramethyl rhodamine strongly increases by a factor of 35. On addition of (**1**)-Fe to **5**, only a 4-fold increase of fluorescence is observed; obviously the cycle does not efficiently hybridize with **5**. This observation is consistent with the strong destabilization of a 25mer DNA duplex when one of the strands is circularized by a covalent linker.[26] Addition of the strong chelator EDTA does not increase the fluorescence of **5**.

Disruption of a preformed duplex **1**:**5** on addition of Fe is concluded from a strong and spontaneous decrease of fluorescence, indicating the release of the closed, nonfluorescent form of **5** (Figure 2, right). In contrast, fluorescence of a **2**:**5** duplex decreases only slightly on addition of Fe(II); apparently the duplex remains intact on intermolecular linking of two **2**:**5** hybrids by Fe(II). With Ni(II), UV and fluorescence titrations compare well to those with Fe(II) (Supporting Information).

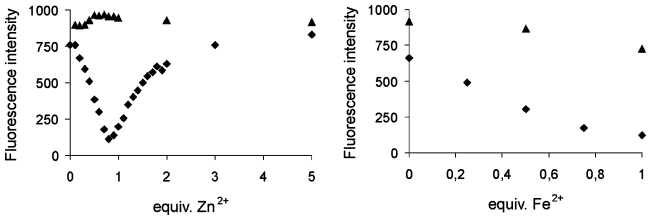

Figure 2. Titration of preformed duplexes **1**:**5** (◆) and **2**:**5** (▲) with zinc(II) sulfate (left) and iron(II) sulfate (right). Conditions: 10 mM HEPES, 150 mM NaCl, 10 mM $MgCl_2$, pH 7, $T = 25$ °C; **1**, **2**, **5** 1 μM each.

Unmodified, fully complementary 20mer ss-DNA **4** does not appear to hybridize with the preformed cycle (**1**)Fe since an ethidium bromide fluorescence assay for duplex detection is negative (Supporting Information). Attempts to characterize the influence of Fe (and Zn) on the **1**:**4** hybridization by temperature-dependent hyperchroism (T_m measurements) were complicated by the decreased thermodynamic and kinetic stability of the metal complexes at elevated temperatures (Supporting Information).

While the bis(tpy) complexes of Fe(II) display high kinetic stability at room temperature, the ligand exchange rates for tpy−Zn complexes are much higher[24] and both 1:1 and 2:1 tpy−Zn complexes are formed, depending on ligand/metal ratio. The Zn−tpy interaction in (**1**)Zn is disrupted in the denaturing gel, and a band identical to that of unmetalated **1** is observed (just as for a 1:1 mixture of **1** and exchange-labile Cu(II), Figure 1). However, (**1**)Zn is detectable by MALDI-TOF mass spectrometry, and spontaneous complexation in solution is confirmed by formation of a UV band at 320 nm (Supporting Information). When Zn(II) is added to a preformed duplex **1**:**5**, fluorescence of the solution strongly decreases for 0−1 equiv of Zn(II), as observed on addition of Fe(II) (Figure 2, left). Fluorescence is restored by addition of one equivalent of EDTA, indicating that Zn(II) binding by **1** is fully reversible. Remarkably, the fluorescence also increases to nearly its initial value when a second equivalent of Zn(II) is added. A straightforward explanation is the re-opening of the ring (**1**)Zn by formation of linear (**1**)Zn_2 (Scheme 1, right), supported by rehybridization with **5** and hydration of tpy-bound Zn. While the decrease of fluorescence is spontaneous at room temperature, the increase at 1−2 equiv of Zn is incomplete and not well reproducible. For smooth conversion, the sample was heated briefly to 50 °C. The first Zn(II) ion is a negative, and the second, a positive allosteric effector of target DNA binding; as a result, binding is "switched off" only in a narrow concentration range ([Zn ≈ [**1**]) of the metal ion.

In conclusion, a stable, single-stranded DNA circle is smoothly formed from a linear precursor by Fe^{2+}-assisted ring closure. With Zn^{2+}, cyclization is reversible, and a novel, complex type of allosteric regulation of hybridization is observed.

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Supporting Information Available: Syntheses of **1** and **2**, mass spectra, ethidiumbromide fluorescence assay, UV−vis and fluorescence titrations, melting profiles of **1**:**4** with Fe and Zn. This material is available free of charge via the Internet at http://pubs.acs.org.

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