Communications to the Editor

Cooperative Triple-Helix Formation via a Minor Groove Dimerization Domain

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Cooperative interactions between DNA-binding proteins are critical to the regulation of gene expression.[1] When cooperative interactions occur between two oligonucleotides bound at neighboring sites on double-helical DNA, the specific binding of each oligonucleotide is enhanced.[2] Pyrimidine oligodeoxyribonucleotides 11 bases in length bind cooperatively to abutting sites on double-helical DNA by triple-helix formation.[2,3] Cooperative interactions up to 2.3 kcal mol^{-1} were obtained by addition of dimerization domains to oligonucleotides such as those capable of forming a short Watson−Crick helix.[4] The designed polyamide ImPyPy binds sequence specifically to the minor groove of DNA at 5′-(T,A)G(T,A)C(T,A)-3′ sites as a cooperative side-by-side antiparallel homodimer.[5] We report here that a pyrrole−imidazole polyamide oligonucleotide can bind cooperatively to a double-stranded DNA template via a sequence specific polyamide dimerization domain.

A pyrimidine oligonucleotide 11 bases in length covalently tethered to the polyamide H_2N-γ-ImPyPy (Im = *N*-methylimidazole, Py = *N*-methylpyrrole, γ = γ-aminobutyric acid) cooperatively binds as a dimer to 27 noncontiguous base pairs of double-helical DNA by simultaneous formation of a 2:1 polyamide−DNA complex in the minor groove and triple-helix formation in the major groove (Figure 1). A polyamide oligonucleotide containing a mismatched polyamide H_2N-γ-PyPyIm shows no cooperative binding (Figure 2).

Polyamide oligonucleotides **1** and **2** were prepared by automated synthesis of the amino-modified oligonucleotide, followed by manual stepwise Boc-chemistry solid phase synthesis of the polyamide.[6,7] The molecular composition of purified polyamide oligonucleotides was verified by MALDI-TOF mass spectroscopy and HPLC analysis of enzymatic phosphodiester hydrolysis products. A DNA fragment was constructed containing the symmetrical 31 base pair target site 5′-$T_3C_2T_6A_2$TGTCA$T_2A_6G_2A_3$-3′, composed of two 11 base pair triple-helix sites separated by a 9 base pair region containing the 5 base pair polyamide binding site.[8] Quantitative DNase I footprint titration experiments on the 268 base pair restriction fragment afford the binding affinities of the designed polyamide oligonucleotides **1** and **2**.[9]

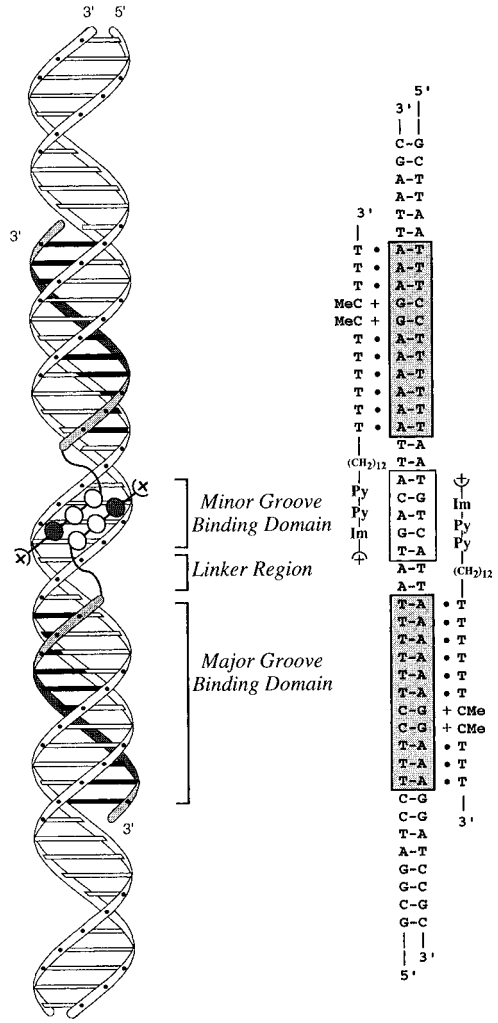

Figure 1. Ribbon model depicting two pyrrole−imidazole polyamide oligonucleotides binding as a dimer to double-helical DNA.

We find that unmodified oligonucleotide, 5′-d(TTTTTT^{Me}C-^{Me}CTTT)-3′, binds each triple-helix site with an equilibrium association constant of K_a = 1.7 (±0.1) × 10^6 M^{-1}. The untethered polyamide ImPyPy was found to bind its 5′-TGTCA-3′ target site with an equilibrium association constant K_a = 1.5 (±0.3) × 10^5 M^{-1}. Polyamide oligonucleotide **1** binds the designated 31 base pair target site with an equilibrium association constant K_a = 1.7 × 10^8 M^{-1}, representing a 100-fold increase in apparent affinity over the unmodified oligonucleotide (Table 1). Polyamide oligonucleotide **2** contains a mismatch in the polyamide moiety and is found to bind without positive cooperativity, demonstrating that the observed cooperative interaction is mediated by sequence specific recognition in the minor groove. The 2.7 kcal/mol binding enhancement reported here is achieved with an unconstrained 12 carbon linker domain for crossing from the major to the minor groove. An incremental two atom increase or decrease in the linker domain

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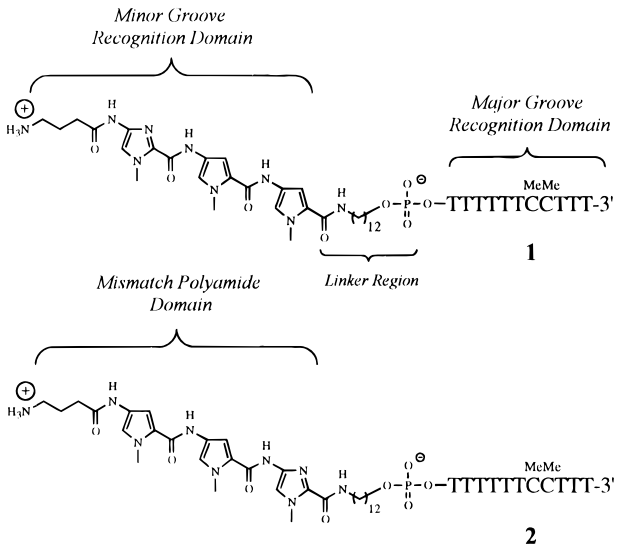

Figure 2. The cognate pyrrole−imidazole polyamide oligonucleotide **1** and pyrrole−imidazole polyamide oligonucleotide **2** containing a mismatch polyamide.

results in a 2- or 10-fold reduction in affinity, respectively. The enhanced affinity is consistent with formation of the dimeric complex.

It is interesting to compare the energetics of this artificial cooperative ligand−DNA complex with certain DNA-binding proteins which have discrete dimerization domains.[10] For example, the λ phage repressor can associate as a dimer at adjacent DNA binding sites with a ~2 kcal/mol cooperative interaction energy.[1d,10] By mimicking the complex behavior of such DNA-binding proteins, cooperative polyamide oligonucleotides provide a new model for the design of synthetic molecules for control of gene expression.

(8) The plasmid pJWS8 was constructed by ligation of an insert, 5′-d(GATCCTTTCCTTTTTTAATGACATTAAAAAAGGAAATTA)-3′ and 5′-d(AGCTTAATTTCCTTTTTTAATGTCATTAAAAAAGGAAAG)-3′, into pUC19 previously cleaved with Bam HI and Hind III. The plasmid was digested with Eco RI, labeled at the 3′ end, and digested with Pvu II. The 268 base pair restriction fragment was isolated by nondenaturing gel electrophoresis and used in all experiments described here.

(9) The quantitative footprint titration experiments were executed in a total volume of 40 μL with a final concentration of each species as indicated. The ligands were added to solutions of radiolabeled restriction fragment (15 000 cpm), NaCl (10 mM), Bis Tris·HCl (10 mM, pH 7.0), and spermine (250 μM), incubated for 24 h at 22 °C. Footprinting reactions were initiated by addition of 4 μL of stock solution of DNase I (0.8 units/mL) containing $MgCl_2$ (50 mM), $CaCl_2$ (50 mM), Bis Tris·HCl (10 mM), and glycerol (5%) and allowed to proceed for 6 min at 22 °C. The reactions were quenched by addition of 12.8 μL of a solution made up of 205 μL of NaOAc (3 M), 160 μL of glycogen, and 160 μL of NaEDTA (50 mM) and ethanol precipitated. The reactions were resuspended in 80% formamide loading buffer and electrophoresed on an 8% polyacrylamide denaturing gels at 2000 V for 1 h. Data analysis were performed as previously described.[11]

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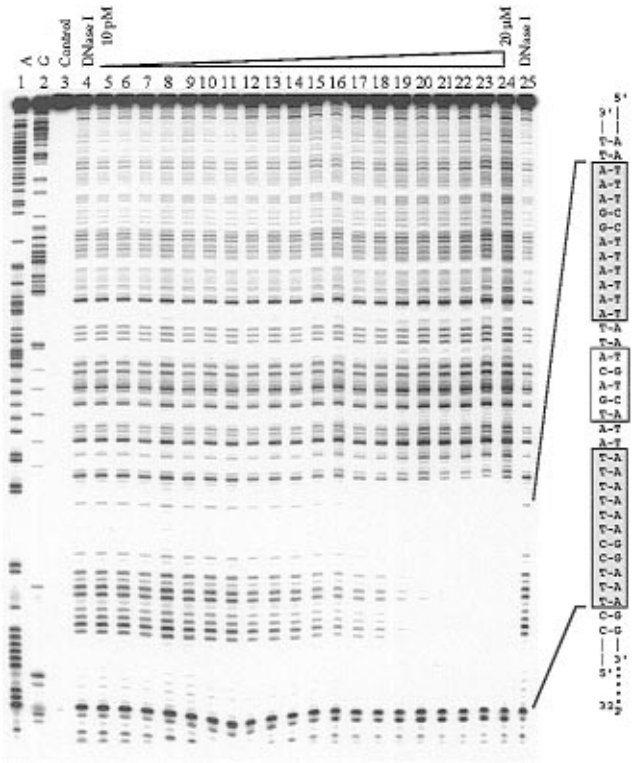

Figure 3. Quantitative DNase I footprint titration of the match pyrrole−imidazole polyamide oligonucleotide **1** with a ^{32}P end-labeled 268 bp restriction fragment. Gray scale representation of a storage phosphor autoradiogram of a 8% denaturing polyacrylamide gel. The binding site is shown to the right side of the autoradiogram. Lane 1, A reaction; lane 2, G reaction; lane 3, intact DNA; lane 4 and 25, DNase I standard; lanes 5−24 contain 10 pM, 20 pM, 50 pM, 100 pM, 200 pM, 500 pM, 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM, 1 μM, 2 μM, 5 μM, 10 μM, and 20 μM polyamide oligonucleotide **1**, respectively.

Table 1. Equilibrium Association Constants (M^{-1})[a,b]

ligand	K_a (M^{-1})
1	1.7 (±0.9) × 10^8
2	2.5 (±0.3) × 10^6
5′-d(TTTTTT^{Me}C^{Me}CTTT)-3′	1.7 (±0.1) × 10^6

[a] Experiments performed at 22 °C in the presence of 10 mM NaCl, 10 mM Bis Tris·HCl (pH 7.0), and 250 μM spermine. [b] Values reported are the mean values measured from three or more footprint titration experiments.

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