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. 23 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,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 H₂N-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 major groove and triple-helix formation in the major groove (Figure 1). A polyamide oligonucleotide containing a mismatched polyamide H₂N-γ-ImPyPyIm 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 hydrolysate products. A DNA fragment was constructed containing the symmetrical 31 base pair target site 5′-T₃C₅T₃A₅T₃GTCAT₂₃A₅G₃A₅₃′, 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′-dT(TT)₃CT₃MeC-T₃TTT₃), binds each triple-helix site with an equilibrium association constant of Kₐ = 1.7 (±0.1) × 10⁶ M⁻¹. The untethered polyamide ImPyPy was found to bind its 5′-TGTCAT₃ sites with an equilibrium association constant Kₐ = 1.5 (±0.3) × 10⁶ M⁻¹. Polyamide oligonucleotide 1 binds the designated 31 base pair target site with an equilibrium association constant Kₐ = 1.7 × 10⁸ 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...
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. For example, the λ phage repressor can associate as a dimer at adjacent DNA binding sites with a ~2 kcal/mol cooperative interaction energy. 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.

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(8) The plasmid pJWS8 was constructed by ligation of an insert, 5′-d(GATCCTTTCCTTTTTTAATGACATTAAAAAGGAAATTA)-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 MgCl2 (50 mM), CaCl2 (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 glycerol, 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


Table 1. Equilibrium Association Constants (M⁻¹)ᵃᵇ

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<tr>
<th>ligand</th>
<th>Kₐ (M⁻¹)</th>
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<td>1</td>
<td>1.7 ± (0.9) × 10⁸</td>
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<td>2</td>
<td>2.5 ± (0.3) × 10⁶</td>
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<tr>
<td>5′-d(TTTTTCMcCTT)-3′</td>
<td>1.7 ± (0.1) × 10⁶</td>
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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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Figure 2. The cognate pyrrole–imidazole polyamide oligonucleotide 1 and pyrrole–imidazole polyamide oligonucleotide 2 containing a mismatch polyamide.

Figure 3. Quantitative DNase I footprint titration of the match pyrrole–imidazole polyamide oligonucleotide 1 with a ³²P 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.

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