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Energetics of cooperative binding of oligonucleotides with discrete dimerization domains to DNA by triple helix formation

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ABSTRACT **Cooperativity in oligonucleotide-directed sequence-specific recognition of DNA by triple helix formation can be enhanced by the addition of discrete dimerization domains. The equilibrium association constants for cooperative binding of oligonucleotides that dimerize by Watson–Crick hydrogen bonds and occupy adjacent sites on double helical DNA by triple helix formation have been measured by quantitative affinity cleavage titration. For two oligonucleotides that bind unique neighboring 11-bp and 15-bp sites on double helical DNA, and dimerize by formation of an 8-bp Watson–Crick mini-helix, the free energy of binding is −8.0 and −9.7 kcal·mol^{-1}, respectively, and the cooperative energy of interaction is −2.3 kcal·mol^{-1} (1 kcal = 4.18 kJ). The energetics of this artificial nucleic acid cooperative intermolecular assembly can mimic naturally occurring cooperative protein–DNA systems, such as the phage λ repressor.**

Cooperative binding by proteins to DNA results in higher sequence specificity as well as greater sensitivity to concentration changes (1, 2). Oligodeoxyribonucleotide-directed triple helix formation is one of the most versatile methods for the sequence-specific recognition of double helical DNA (3, 4). We recently reported the binding properties of oligonucleotides that dimerize by Watson–Crick hydrogen bonds and bind neighboring sites on double helical DNA by triple helix formation (5). Our design for effecting cooperativity is a Y-shaped complex formed on double helical DNA by two oligonucleotides, each containing separate functional domains for binding and dimerization (5). Each oligonucleotide possesses a pyrimidine recognition domain designed to bind a specific purine duplex target site through the formation of Hoogsteen hydrogen bonds (TAT and CGC triplets) (6–8). Additionally, each oligonucleotide possesses dimerization domains of mixed sequence composition to allow formation of a Watson–Crick mini-helix. The recognition and dimerization domains of each oligonucleotide are connected by a linker base introduced for the purpose of providing conformational flexibility at the junction between triplex and duplex DNA. On the basis of model building studies, the neighboring purine binding sites were separated by 2 bp in order to accommodate a junction in the Y-shaped complex (5).

We report here the energetics of cooperative binding of oligodeoxyribonucleotides that dimerize by formation of an 8-bp mini-helix of Watson–Crick hydrogen bonds and bind to distinct and unique neighboring 11-bp and 15-bp sites on double helical DNA by triple helix formation (Fig. 1). The experimental design involves measuring by affinity cleavage titration at 24°C the equilibrium constants for binding of an oligonucleotide to the 11-bp site in the absence and presence of a second oligonucleotide occupying an adjacent site. We find that, for the system designed here, the presence of a neighboring bound oligonucleotide increases the affinity of an oligonucleotide for the 11-bp site by a factor of 44. The free energy values of this artificial heterodimeric nucleic acid recognition system are comparable to those of naturally occurring cooperative binding repressor proteins from phage λ (2).

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MATERIALS AND METHODS

General. Sonicated, deproteinized calf thymus DNA was purchased from Pharmacia. Nucleotide triphosphates were obtained from Pharmacia. Nucleotide triphosphates labeled with ^{32}P (≥3000 Ci/mmol; 1 Ci = 37 GBq) were purchased from Amersham. Restriction endonucleases were purchased from New England Biolabs and used according to the suggested protocol in the provided buffer. Phosphoramidites were obtained from Applied Biosystems (mC, T). Sephadex resins were obtained from Pharmacia. Sequenase Version 2.0 was obtained from United States Biochemical.

Oligonucleotide Preparation. Oligonucleotides were synthesized by standard automated solid-support chemistry on an Applied Biosystems model 380B DNA synthesizer using *O*-cyanoethyl *N,N*-diisopropylphosphoramidites (6). The phosphoramidite of thymidine-EDTA (T*) was prepared according to published procedures (7). Controlled pore glass derivatized with T* was prepared as described by Strobel (8). Oligonucleotides **1** and **3** containing T* were deprotected with 0.1 M NaOH at 55°C for 24 hr, neutralized with glacial acetic acid, desalted on Pharmacia Sephadex (G-10-120) spin columns, and dried *in vacuo*. Oligonucleotide **2** was deprotected with concentrated ammonium hydroxide at 55°C for 24 hr and dried *in vacuo*. The crude 5′-terminal-dimethoxytrityl protected oligonucleotides were purified by reverse-phase FPLC on a ProRPC 16/10 (C_2/C_8) column (Pharmacia LKB) and a gradient of 0–40% CH_3CN in 0.1 M triethylammonium acetate (pH 7.0), detritylated in 80% AcOH, and chromatographed a second time. Triethylammonium acetate was removed via repeated resuspensions of the oligonucleotides in water followed by lyophilization. Oligonucleotide concentrations were determined by UV absorbance at 260 nm using extinction coefficients ($M^{-1}\cdot cm^{-1}$) of 8800 (T and T*) and 5700 (mC).

DNA Preparation. The 3′-^{32}P-labeled duplex was prepared by digestion of the plasmid pMD5556 with *Eco*RI, followed by treatment with [α-^{32}P]dATP and [α-^{32}P]TTP in the presence of Sequenase. To remove nonincorporated radioactivity, the fragment was passed through a Pharmacia Nick column. The DNA was then digested with *Xmn* I, followed by separation of the resulting products on a 5% nondenaturing polyacrylamide gel (19:1, monomer/bis). The gel band corresponding to the desired 852-bp fragment was visualized by autoradiography, excised, crushed, and soaked in 10 mM Tris, pH 8.0/0.2 M EDTA, at 37°C for 12 hr. The resulting

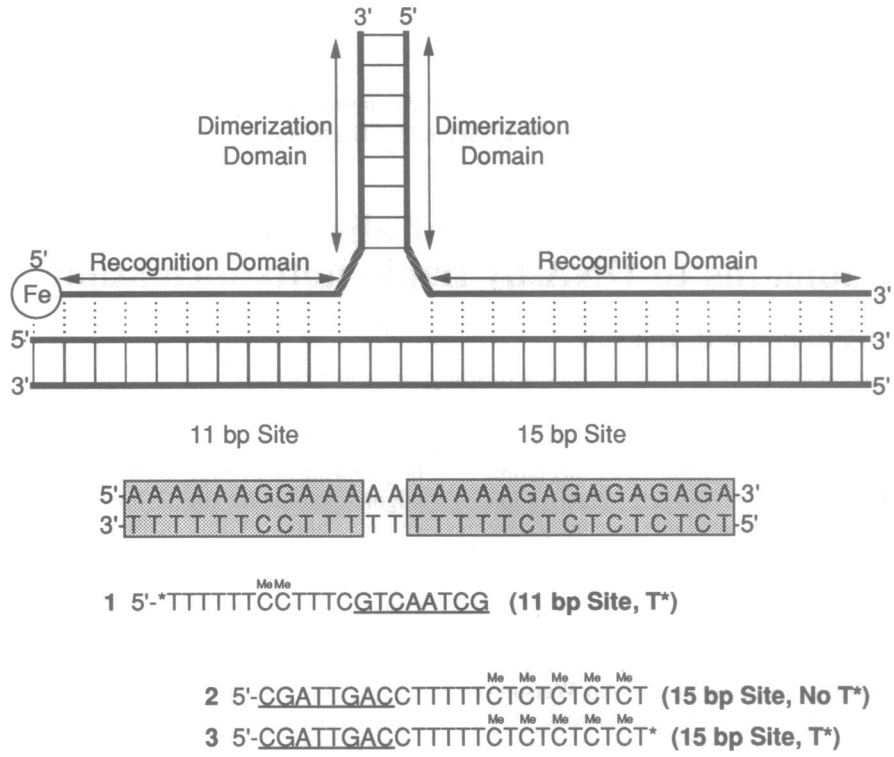

FIG. 1. Schematic representation of a complex composed of two oligonucleotides with recognition domains for triple helix formation and dimerization domains to permit cooperative binding. Thick solid lines represent the DNA backbone of the target site and associated oligonucleotides. Thin solid lines represent Watson–Crick hydrogen bonds; dashed lines indicate Hoogsteen hydrogen bonds. The dimerization domain of each oligonucleotide is underlined.

suspension was filtered through a 0.45-μm filter, and the eluted DNA present in the supernatant was precipitated with NaOAc/EtOH. The DNA pellet was washed once with 70% EtOH, dried *in vacuo*, resuspended in TE buffer (10 mM Tris, pH 8.0/1.0 mM EDTA), extracted five times with phenol, extracted twice with 24:1 $CHCl_3$/isoamyl alcohol, and reprecipitated with NaOAc/EtOH. The DNA pellet was washed with 70% EtOH, dried *in vacuo*, resuspended in 25 mM Tris-acetate/100 mM NaCl, pH 7.0, at a final activity of 100,000 cpm/μl, and stored at −20°C.

Quantitative Affinity Cleavage Titrations. In a typical experiment, a 5 nmol aliquot of the desired oligonucleotide-EDTA was dissolved in 50 μl of aqueous 200 μM $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ to produce a solution that was 100 μM in oligonucleotide. This solution was then diluted serially to yield 100 μM, 10 μM, 1 μM, 100 nM, 10 nM, and 1 nM solutions, and the appropriate volume of these solutions was then distributed among eighteen 1.5-ml microcentrifuge tubes to afford the appropriate concentration range. A stock solution, containing labeled target DNA, Tris-acetate buffer, NaCl, calf thymus DNA, the second oligonucleotide where needed, and water, was distributed to each reaction tube in 12-μl aliquots, and the appropriate volume of water and 4 μl of 10 mM spermine were added to each tube to bring the total volume to 36 μl. The oligonucleotide-EDTA·Fe(II) and the DNA were allowed to equilibrate for 24 hr at 24°C. The cleavage reactions were initiated by the addition of 4 μl of a 40 μM aqueous dithiothreitol solution to each tube. Final reaction conditions in 40 μl of association buffer were 25 mM TrisOAc at pH 7.0, 10 mM NaCl, 1 mM spermine, 100 μM bp calf thymus DNA, 4 mM dithiothreitol, and ≈10,000 cpm of labeled DNA. The reaction mixtures were incubated for 6 hr at 24°C. Cleavage reactions were terminated by NaOAc/EtOH precipitation and the reaction tubes were then stored at −20°C for 30 min before centrifugation. The DNA cleavage products were washed with 70% aqueous EtOH, dissolved in 50 μl of water, and dried *in vacuo*. The DNA was then resuspended in 10 μl of formamide/TBE loading buffer, heated at 55°C for 10 min to effect dissolution, and transferred to a new tube. The Cerenkov radioactivity of the solutions was then measured with a scintillation counter, followed by dilution to the same cpm/μl with more formamide/TBE buffer. The DNA was denatured at 95°C for 3 min, and 5 μl from each sample was loaded onto an 8% denaturing polyacrylamide gel.

Quantitation. Gels were exposed to photostimulable storage phosphor imaging plates (Kodak storage phosphor screen S0230 obtained from Molecular Dynamics, Sunnyvale, CA) in the dark at 24°C for 5–12 hr. A Molecular Dynamics 400S PhosphorImager was used to obtain data from the phosphor-imaging screens. Rectangles of the same dimensions were drawn around the cleavage bands at the target and at the reference sites. The ImageQuant v. 3.0 program running on an AST Premium 386/33 computer was used to integrate the volume of each rectangle.

Affinity Cleavage Titration Fitting Procedure. A detailed description of the affinity cleavage titration procedure used has been published (9, 10). The relative cleavage efficiencies at the target site for each oligonucleotide concentration were determined by using the following equation:

$$I_{site} = I_{tot} - \lambda I_{ref}, \quad [1]$$

where I_{tot} and I_{ref} are the intensities of the cleavage bands at the target site and at the reference site, respectively, and where λ is a scaling parameter defined as the ratio I_{tot}/I_{ref} at the lowest oligonucleotide concentration. A theoretical binding curve, represented by Eq. 2, where I_{sat} is the apparent maximum cleavage, K_i is the equilibrium association constant for the oligonucleotide i, and $[O]_{tot}$ is the oligonucleotide-EDTA concentration, was used to fit the experimental data using I_{sat} and K_i as adjustable parameters:

$$I_{fit} = I_{sat} \frac{K_i[O]_{tot}}{1 + K_i[O]_{tot}}. \quad [2]$$

KaleidaGraph software (version 2.1, Abelweck Software) running on a Macintosh IIfx computer was used to minimize the difference between I_{fit} and I_{site} for all data points. All values reported in the text are the means of three to five experimental observations ± SEM. For graphical representation and comparison, I_{site} values were converted to fractional occupancies (θ_{app}) by dividing I_{site} by I_{sat}.

RESULTS AND DISCUSSION

Affinity Cleavage Reactions. Oligonucleotides **1** and **2** were allowed to equilibrate with an 852-bp 3′-^{32}P-end-labeled duplex DNA fragment at 24°C, pH 7.0 for 24 hr, followed by addition of dithiothreitol to initiate cleavage. After 6 hr the reactions were stopped by ethanol precipitation, and the products were separated by denaturing polyacrylamide gel electrophoresis (Fig. 2). The small amount of cleavage in a reaction containing **1** at 100 nM concentration indicates that **1** binds to the 11-bp target site weakly under these conditions (Fig. 2, lane 2). In the presence of 1.0 μM concentration of oligonucleotide **2**, a dramatic increase in cleavage is observed for the reaction of **1** at the same concentration (100 nM) (Fig. 2, lane 3). The addition of oligonucleotide **2** at near-saturating concentration allows complete formation of a Y-shaped cooperative complex which results in a large increase in the binding affinity of **1**.

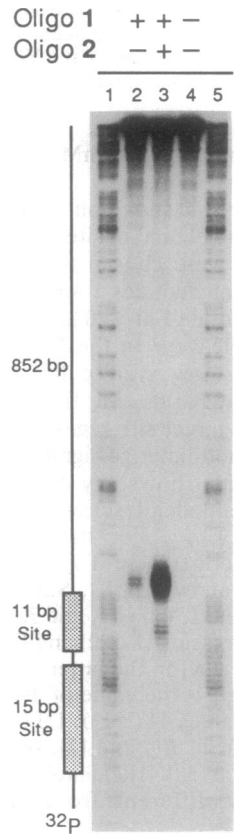

FIG. 2. Autoradiogram of an 8% denaturing polyacrylamide gel of reaction products from affinity cleavage reactions with oligonucleotides **1** and **2** and a ^{32}P-labeled restriction fragment containing the target site (Fig. 1). Oligonucleotides **1** and **2** are present at 100 nM and 1 μM concentrations, respectively. Lane 1, "A" specific sequencing reaction (11); lane 2, oligonucleotide **1**; lane 3, oligonucleotides **1** and **2**; lane 4, no oligonucleotide; lane 5, same as lane 1.

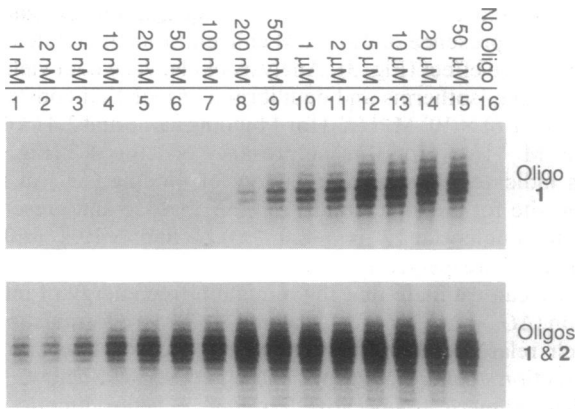

FIG. 3. Expanded views of autoradiograms of 8% denaturing polyacrylamide gels of reaction products from quantitative affinity cleavage titration with oligonucleotides **1** and **2**. Only the regions containing the cleavage products are shown here. The panel labeled "Oligo 1" shows the cleavage products from reactions containing **1** at various concentrations. The panel labeled "Oligos 1 & 2" shows the cleavage products from similar reactions containing **1**, but supplemented with **2** at 1 μM. The concentration of **1** in each reaction is indicated.

Equilibrium Association Constants. To measure the magnitude of cooperativity, quantitative affinity cleavage titrations were performed over an extensive concentration range (9, 10) (Figs. 3 and 4). The extent of cleavage for each reaction is determined by phosphorimaging analysis and an equilibrium association constant, K, is obtained by fitting a Langmuir binding isotherm to the experimental data. Quantitative affinity cleavage titrations were carried out with **1** alone and with **1** in the presence of oligonucleotide **2** at a concentration sufficient to saturate the nearby 15-bp site. In an independent quantitative affinity cleavage titration experiment, the equilibrium binding constant of **3**, an oligonucleotide with the same sequence as **2** but containing T*, to the 15-bp site was shown to be 1.3 (±0.6) × 10^7 M^{-1}. Since we have demonstrated previously that the substitution of T* for T has negligible influence on the binding affinity, we estimate that at 1 μM concentration, the 15-bp site would be ≥93% occupied by oligonucleotide **2** (9).

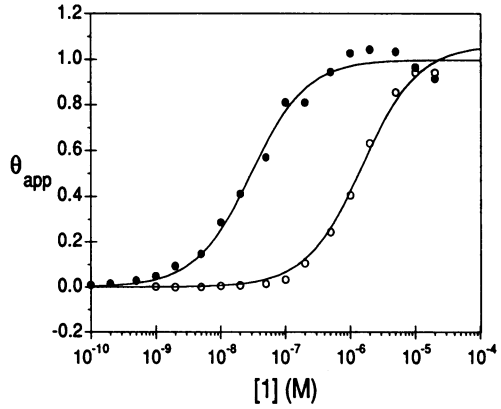

FIG. 4. Binding isotherms obtained for oligonucleotide **1** alone (○) and in the presence of 1.0 μM oligonucleotide **2** (●), using quantitative affinity cleavage titration. The data points represent the average site-specific cleavage signal intensities from five (**1** alone) and four (**1** in the presence of **2**) experiments. The sigmoidal curves represent the titration binding isotherms calculated from Eq. **2** using the mean of association constants obtained from individual experiments. For each data set, the individual I_{site} points were divided by I_{sat} to obtain θ_{app}.

The increase in cleavage at low concentrations of **1** alone and in the presence of **2** at 1 μM concentration illustrates the cooperative effect (Fig. 3). Analysis of cleavage data yielded the binding isotherms and equilibrium association constants of $7.7 (\pm 1.6) \times 10^5\ M^{-1}$ (K_1) for **1** binding alone and $3.4 (\pm 0.7) \times 10^7\ M^{-1}$ ($K_{1,2}$) for **1** in the presence of 1.0 μM **2** (Fig. 4). This indicates that the free energy of binding (ΔG) at the 11-bp site for oligonucleotide **1** in the absence and presence of oligonucleotide **2** is -8.0 (± 0.1) and -10.2 (± 0.2) kcal·mol^{-1}, respectively (1 kcal = 4.18 kJ) (Table 1). From this we can estimate that the minimum free energy of interaction ($\Delta G_{1,2}$–ΔG_1) is -2.2 kcal·mol^{-1}. A more formal model which relates these experimental data to the cooperative interaction energy between **1** and **2** is described below.

Heterodimeric Cooperative Systems. A heterodimeric cooperative system is one in which two different ligands, L_A and L_B, bind uniquely to two distinct sites and each ligand perturbs the binding equilibrium at the noncognate site. Hill (1) has derived a grand partition function for such a system:

$$\xi = 1 + K_A[L_A] + K_B[L_B] + yK_A[L_A]K_B[L_B], \quad [3]$$

where K_A and K_B are equilibrium association constants for L_A and L_B binding in the absence of each other, and y is related to the interaction energy, E_{coop}, between L_A and L_B by

$$y = \exp(-E_{coop}/RT). \quad [4]$$

The fractional occupancy of site A by L_A is given by

$$\theta_A = [L_A]\frac{\partial \ln \xi}{\partial [L_A]} = \frac{\phi K_A[L_A]}{1 + \phi K_A[L_A]}, \quad [5]$$

where

$$\phi = \frac{1 + yK_B[L_B]}{1 + K_B[L_B]}. \quad [6]$$

Eqs. **4** and **6** can be combined and rearranged to give the following expression for the interaction energy:

$$E_{coop} = -RT\ln\left[\frac{\phi(1 + K_B[L_B]) - 1}{K_B[L_B]}\right]. \quad [7]$$

Energetics of Cooperatively Binding Oligonucleotides. This simple model requires two important assumptions. First, the ligands do not interact in solution. An 8-bp mini-helix was chosen to minimize the amount of oligonucleotides **1** and **2** that dimerize in the absence of the neighboring binding site (Fig. 5). To calculate the concentration of **1**–**2** dimer in the absence of the binding sites, the method of Breslauer and co-workers (14) was used to estimate ΔG for association of the 8-base dimerization domains of **1** (5′-CGATTGAC) and **2** (5′-GTCAATCG). This value of ΔG was corrected for the difference in ionic strength between the Breslauer conditions and those employed here using the thermodynamic relationship $1/t_m = [\Delta S + R\ln(C_T/4)]/\Delta H$ (15) and the empirical function $t_{m2} - t_{m1} = 18.5 \log(\mu_2/\mu_1)$ (16) assuming that ΔH does not vary with ionic strength. This analysis yields a ΔG value for the 8-bp duplex of 5.8 kcal·mol^{-1} and suggests that in the concentration range of 1 nM to 1 μM, <2% of **1** is present as **1**–**2** dimer.

Table 1. Equilibrium association constants and free energies for triple helix formation

Oligonucleotide	K, M^{-1}	ΔG, kcal·mol^{-1}
1	$7.7 (\pm 1.6) \times 10^5$	-8.0 (± 0.1)
1 + **2** (1.0 μM)	$3.4 (\pm 0.7) \times 10^7$	-10.2 (± 0.2)

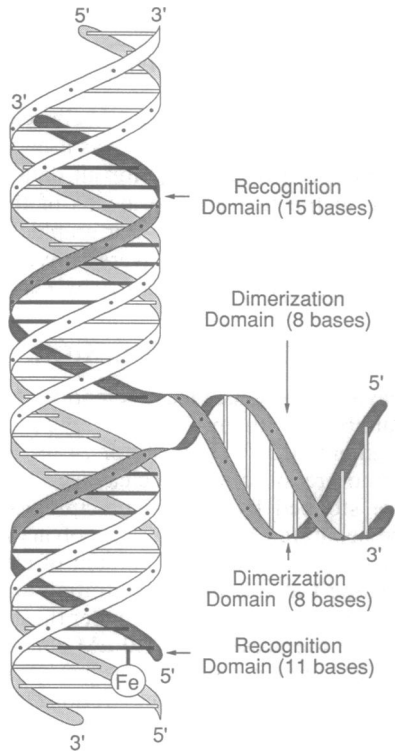

FIG. 5. Ribbon model of oligonucleotides **1** and **2** bound at adjacent sites on neighboring DNA sites by triple helix formation. The DNA binding sites for triple helix formation are neighboring heterodimeric sites separated by 2 bp. The dimerization domain is an 8-bp mini-helix of Watson–Crick base pairs. This putative Y-shaped nucleic acid structure is reminiscent of the Y-shaped leucine zipper-basic region dimeric, sequence-specific proteins important in transcription (12, 13).

The second required assumption is that L_A does not bind to site B and L_B does not bind to site A. The sequences of the 11- and 15-bp sites were chosen to maximize the binding specificities of oligonucleotides **1** and **2** for their designated sites. For oligonucleotide **1** at ≤ 20 μM concentrations, there was no detectable cleavage in the 15-bp target site region, evidence that **1** has low affinity for the 15-bp site. For oligonucleotide **2**, at ≤ 1.0 μM, there was no detectable binding in the 11-bp target site region.

Since **1** and **2** do not dimerize significantly in solution and neither oligonucleotide shows any detectable affinity for its noncognate site, the model described above can be reliably used to determine E_{coop}. For the cooperatively binding oligonucleotides described here, L_A is oligonucleotide **1**, site A is the 11-bp site, and K_A is K_1. Similarly, L_B is oligonucleotide **2**, site B is the 15-bp site, and K_B is K_2. Comparison of the Langmuir expression (Eq. **2**) and Eq. **5** shows that $K_{1,2}$ in the former equals ΦK_1 in the latter. Thus, $\Phi_{1.0} = K_{1,2}/K_1 = 44 \pm 13$, where $\Phi_{1.0}$ is the value of Φ when [**2**] = 1.0 μM. Substitution of the values 44 (± 13), 1.3 (± 0.6) $\times 10^7$, and 1.0 (± 0.1) $\times 10^{-6}$ M for Φ, K_2, and [**2**], respectively, in Eq. **7** affords a value of -2.3 (± 0.4) kcal·mol^{-1} for E_{coop}. This value is not significantly different from $\Delta G_{1,2}$–$\Delta G_1 = -2.2$ kcal·mol^{-1}.

Comparison of Artificial Nucleic Acid and Natural Repressor Protein Assemblies. The value of -2.3 kcal·mol^{-1}, obtained for E_{coop}, represents the cooperativity energy for the heterodimeric oligonucleotide system described here. This value and the individual site binding free energies can be compared to similar data obtained for the naturally occurring, homotrimeric phage λ repressor system (17, 18). The indi-

vidual site binding free energies for the phage λ system range from −10.1 to −11.7 kcal·mol^{-1} and are similar to those from the heterodimeric triple helix system (−8.0 to −9.7 kcal·mol^{-1}). The cooperative interaction energies for the oligonucleotides (−2.3 kcal·mol^{-1}) and λ repressor (−1.9 to −2.0 kcal·mol^{-1}) are also comparable. Thus, we have designed an artificial, nucleic acid-based cooperative system that mimics the energetics of protein–DNA interactions.

Implications. An important feature of the artificial system described here is that the functional domain structures are *modular* (Fig. 5). The individual site binding and cooperative interaction energies can be *varied independently* by changing the length of the recognition and/or dimerization domains. This is in contrast to natural protein complexes where recognition and cooperativity elements are less easily separated. Moreover, because the dimerization domain in the artificial system is a Watson–Crick double helix, the stability of the nucleic acid dimerization domain can be enhanced by known sequence-specific DNA binding ligands (19). This could form the basis for small molecule-promoted control of oligonucleotides binding cooperatively to DNA analogous to naturally occurring inducible protein systems.

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1. Hill, T. L. (1985) *Cooperativity Theory in Biochemistry: Steady State and Equilibrium Systems* (Springer, New York).
2. Ptashne, M. (1986) *A Genetic Switch* (Blackwell Sci. Pub. and Cell Press), Palo Alto, CA.
3. Moser, H. E. & Dervan, P. B. (1987) *Science* **238**, 645–650.
4. LeDoan, T., Perrouault, L., Praseuth, D., Habhoub, N., Decout, J.-L., Thuong, N. T., Lhomme, J. & Helene, C. (1987) *Nucleic Acids Res.* **15**, 7749–7760.
5. Distefano, M. D., Shin, J. A. & Dervan, P. B. (1991) *J. Am. Chem. Soc.* **113**, 5901–5902.
6. Felsenfeld, G., Davies, D. R. & Rich, A. (1957) *J. Am. Chem. Soc.* **79**, 2023–2024.
7. Arnott, S. & Selsing, E. (1974) *J. Mol. Biol.* **88**, 509–521.
8. Hoogsteen, K. (1959) *Acta Crystallogr.* **12**, 822–823.
6. Gait, M. J., ed. (1984) *Oligonucleotide Synthesis: A Practical Approach* (IRL, Oxford).
7. Dreyer, G. B. & Dervan, P. B. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 968–972.
8. Strobel, S. A. (1992) Dissertation (California Institute of Technology, Pasadena, CA).
9. Singleton, S. F. & Dervan, P. B. (1992) *J. Am. Chem. Soc.* **114**, 6956–6965.
10. Singleton, S. F. & Dervan, P. B. (1992) *Biochemistry* **31**, 10995–11003.
11. Iverson, B. L. & Dervan, P. B. (1987) *Nucleic Acids Res.* **15**, 7823–7830.
12. Vinson, C. R., Sigler, P. B. & McKnight, S. L. (1989) *Science* **246**, 911–916.
13. Oakley, M. & Dervan, P. B. (1990) *Science* **248**, 847–850.
14. Breslauer, K. J., Frank, R., Blöcker, H. & Marky, L. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3746–3750.
15. Marky, L. A., Blumenfeld, K. S., Kozlowski, S. & Breslauer, K. J. (1983) *Biopolymers* **22**, 1247–1257.
16. Dove, W. F. & Davidson, N. (1962) *J. Mol. Biol.* **5**, 467–478.
17. Johnson, A. D., Poteete, A. R., Lauer, G., Sauer, R. T., Ackers, G. K. & Ptashne, M. (1981) *Nature (London)* **294**, 217–223.
18. Ackers, G. K., Johnson, A. D. & Shea, M. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1129–1133.
19. Distefano, M. D. & Dervan, P. B. (1993) *J. Am. Chem. Soc.*, in press.