Cooperative binding of oligonucleotides to DNA by triple helix formation: Dimerization via Watson–Crick hydrogen bonds

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · JULY 1991

Impact Factor: 12.11 · DOI: 10.1021/ja00015a076

3 AUTHORS, INCLUDING:

Mark Distefano
University of Minnesota Twin Cities
135 PUBLICATIONS **3,086** CITATIONS

SEE PROFILE

Jumi A Shin
University of Toronto
36 PUBLICATIONS **303** CITATIONS

SEE PROFILE

with the cooperation of two imidazoles in ribonuclease, precisely studied by Breslow using model systems.[4] Contamination of ribonuclease is ruled out by formation of ribonucleoside 3′-phosphate and the 2′-phosphate as the final products in almost a 1:1 ratio.[18] Ribonuclease should produce, if any, the 3′-phosphate in 100% selectivity.[19]

In conclusion, oligoamines N-N, N_4, and N_6 have large catalytic activities for RNA hydrolysis, together with simplicity in structure and stability, being promising as catalytic sites for artificial ribonucleases. Attachment of the oligoamines to sequence-recognizing moieties is now in progress.

Acknowledgment. We thank Dr. Kazunari Taira at Fermentation Research Institute, Agency of Industrial Science and Technology, for valuable comments. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

(17) Furthermore, positive charges of N_4 and N_6 might electrostatically stabilize the transition state of hydrolysis, similarly to the function of lysine 41 of ribonuclease A (Deakyne, C. A.; Allen, L. C. *J. Am. Chem. Soc.* **1979**, *101*, 3951). Participation of the third amino residue (either cationic or neutral) in the catalysis is also plausible.

(18) The ratios of ribonucleoside 3′-phosphate to the 2′-phosphate were determined by HPLC, when the cleavage of RNAs was almost complete. The separation conditions were identical with those described previously (Komiyama, M. *J. Am. Chem. Soc.* **1989**, *111*, 3046).

(19) Dugas, H.; Penney, C. *Bioorganic Chemistry*; Springer-Verlag: New York, 1981.

Cooperative Binding of Oligonucleotides to DNA by Triple Helix Formation: Dimerization via Watson–Crick Hydrogen Bonds

Mark D. Distefano, Jumi A. Shin, and Peter B. Dervan*

Arnold and Mabel Beckman Laboratories of Chemical Synthesis
California Institute of Technology
Pasadena, California 91125

Received March 20, 1991

Cooperative binding by proteins to DNA results in higher sequence specificity as well as greater sensitivity to concentration changes.[1] We recently reported cooperative binding of two oligonucleotides at abutting sites by triple helix formation on double helical DNA.[2] However, the enhanced binding observed was modest (a factor of 3.5) and likely due to favorable base-stacking interactions between adjacent oligonucleotides and/or induced conformational changes propagated to adjacent binding sites.[2] Thus, the issue arises whether cooperativity in oligonucleotide-directed triple helix formation can be enhanced by the addition of *discrete dimerization domains*. We report here the binding properties of oligonucleotides that dimerize by Watson–Crick hydrogen bonds and bind neighboring sites on double helical DNA by triple helix formation.

Our design for effecting cooperativity is a Y-shaped complex formed on double helical DNA by two oligonucleotides, each containing two separate functional domains, binding and dimerization.[3] Each oligonucleotide possesses a pyrimidine segment designed to recognize a specific purine duplex target site through the formation of Hoogsteen hydrogen bonds (TAT and CGC triplets).[5-7] These pyrimidine regions are 9 and 15 nucleotides in length and are designated as recognition domains A and D, respectively (Figure 1).[8] Additionally, each oligonucleotide possesses a segment of mixed sequence composition. These sequences, designated dimerization domains B and C (Figure 1), are complementary to allow formation of eight base pairs of duplex DNA held together by Watson–Crick hydrogen bonds. 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.[9] On the basis of model building studies, the 9 and 15 base pair binding sites were separated by two base pairs in order to accommodate a Y-shaped motif (Figure 2). A series of oligonucleotides, **1–8**, was synthesized to evaluate the role of each domain (Figure 1). The modified base T* was incorporated at the 5′ termini of oligonucleotides **1–3**, each targeted to the nine base pair half site, to allow analysis by the affinity cleaving method.[5,10] Because T* is positioned at the 5′ terminus of domain A, these cleavage experiments monitor the binding of only those oligonucleotides directed to the nine base pair target site. With this experimental design, cooperative binding due to the presence

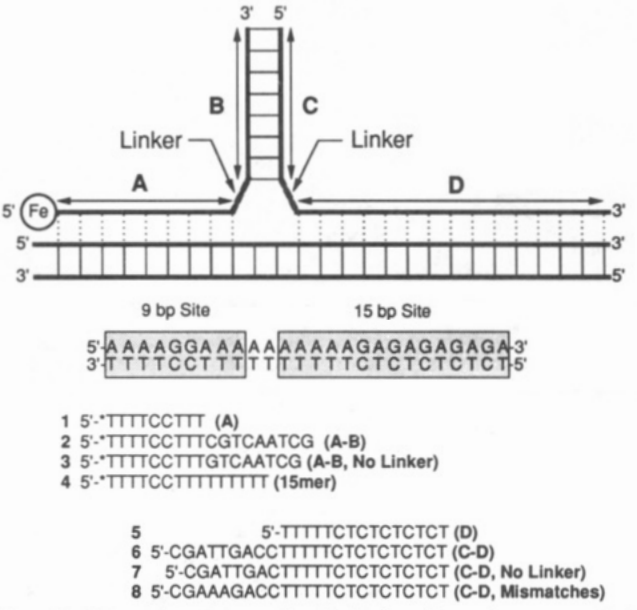

Figure 1. Schematic representation of a Y-shaped complex composed of two triplex forming oligonucleotides which cooperate through formation of a small segment of Watson–Crick double helical DNA. Two oligonucleotides possessing duplex recognition domains (A and D) and dimerization domains (B and C) connected by a linker base are designed to bind cooperatively to a double helical DNA target site. Thick solid lines represent the DNA backbone of the target site and associated oligonucleotides. Thin solid lines represent Watson–Crick hydrogen bonds, while dashed lines indicate Hoogsteen hydrogen bonds. The domains contained within each oligonucleotide are given in parentheses.

(1) (a) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry Part III: The Behavior of Biological Macromolecules*; W. H. Freeman and Co.: San Francisco, CA, 1980. (b) Hill, T. L. *Cooperativity Theory in Biochemistry: Steady State and Equilibrium Systems*; Springer-Verlag: New York, 1985. (c) Ptashne, M. *A Genetic Switch*; Blackwell Scientific Publications and Cell Press: Palo Alto, CA, 1986. (d) Johnson, A. D.; Poteete, A. R.; Lauer, G.; Sauer, R. T.; Ackers, G. K.; Ptashne, M. *Nature* **1981**, *294*, 217. (e) Adhya, S. *Ann. Rev. Genet.* **1989**, *23*, 227.

(2) Strobel, S. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 7286.

(3) In a formal sense, this Y-shaped structure is a nucleic acid version of the scissor grip-leucine zipper protein motif, proposed for certain sequence specific DNA binding proteins.[4]

(4) (a) Vinson, C. R.; Sigler, P. B.; McKnight, S. L. *Science* **1989**, *246*, 911. (b) Oakley, M.; Dervan, P. B. *Science* **1990**, *248*, 847 and references cited therein.

(5) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645, and references cited.

(6) Praseuth, D.; Perroault, L.; Doan, T. L.; Chassignol, M.; Thuong, N.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *58*, 1349.

(7) For recent structural studies, see: (a) Rajagopal, P.; Feigon, J. *Biochemistry* **1989**, *28*, 7859. (b) de los Santos, C.; Rosen, M.; Patel, D. *Biochemistry* **1989**, *28*, 7282. (c) Sklenar, V.; Feigon, J. *Nature* **1990**, *345*, 836.

(8) Binding sites 9 and 15 base pairs in size were chosen because at μM oligonucleotide concentrations (25 °C, pH 6.6) the former would be largely unoccupied while the latter nearly saturated. Since our experiments monitor the binding of oligonucleotides targeted to the 9 bp site, this arrangement maximizes any possible binding enhancement resulting from oligonucleotides present at the 15 bp site.

(9) Due to steric considerations pyrimidines are favored over purines for use as a linker base. Cytidine was chosen over thymidine to avoid possible Hoogsteen hydrogen bonding with the adenine bases separating the 9 and 15 bp half sites.

(10) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A* **1985**, *82*, 968.

0002-7863/91/1513-5901$02.50/0

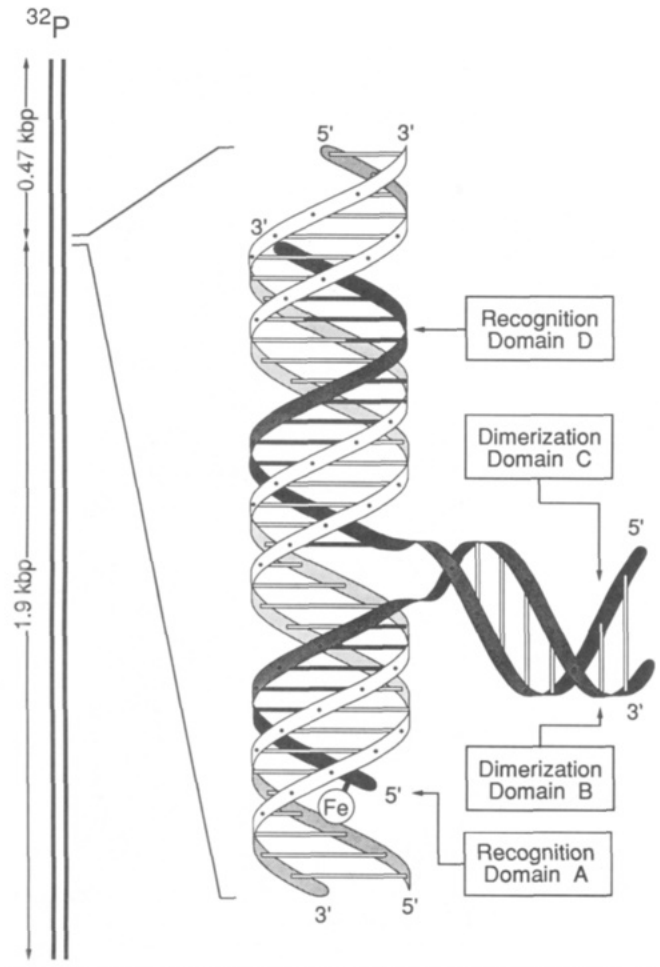

Figure 2. Location of the target site within a 2.3 kbp fragment of DNA and a ribbon model of two oligonucleotides bound at that target site. Single site cleavage of the DNA labeled on one end with ^{32}P yields one fragment, 0.47 kbp in size.

of domains B, C, and D should result in an enhancement of cleavage at this nine base pair site.

Affinity cleavage experiments were performed on a 2.3 kbp ^{32}P end-labeled restriction fragment[11] containing a 26 base pair target site (Figure 2). Reaction of the 9mer oligonucleotide-EDTA·Fe **1** with the target site results in little cleavage (lane 1, Figure 3). This indicates that the 9mer (domain A) possesses low affinity for its target site at 25 °C (pH 6.5), as expected.[5] A reaction containing oligonucleotides **1** and **5**, binding domains A and D only, also results in little cleavage (lane 2, Figure 3). This demonstrates that there is no binding enhancement of the 9mer when the nearby 15 bp target site is occupied. The reactions containing oligonucleotides **1** and **6** (lane 3) and **2** and **5** (lane 5) both show no cleavage, thus providing evidence that a dimerization domain present on only one oligonucleotide provides no cooperativity (Figure 3). However, the affinity cleavage reaction containing oligonucleotides **2** and **6**, which possess complementary dimerization domains, results in *enhanced* cleavage (lane 6, Figure 3). The weak cleavage observed in the reaction containing oligonucleotides **3** and **7** underscores the requirement for the linker base which is absent from these two oligonucleotides (lane 7, Figure 3). The absence of cleavage in the reaction containing oligonucleotides **2** and **8** illustrates that a two base pair mismatch in the dimerization domain is sufficient to abolish the cooperative binding (lane 8, Figure 3). The intensity of cleavage by oligonucleotides-EDTA·Fe **2** and **6** cooperativity bound in the Y-shaped

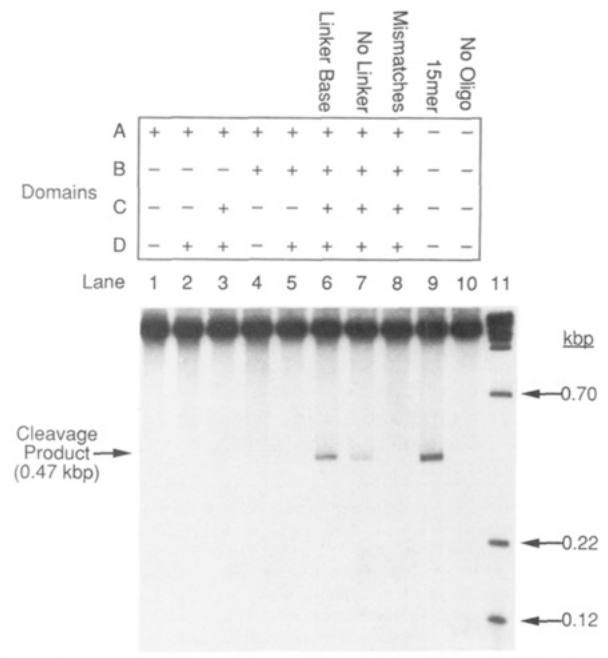

Figure 3. Autoradiogram of a nondenaturing polyacrylamide electrophoresis gel of reaction products from affinity cleavage reactions with the oligonucleotides described below and a ^{32}P end-labeled DNA fragment containing the target site shown in Figures 1 and 2. The radiolabeled restriction fragment was prepared by digestion of pMD5556 with EcoO 109 followed by treatment with ^{32}P dGTP and the Klenow fragment of DNA polymerase I. Digestion with Xmn I yielded the 2.3 kbp DNA fragment used in the experiments below. Affinity cleavage reactions were performed by preincubating the desired oligonucleotides (1 μM) together with Tris-Acetate, pH 6.6 (25 mM), NaCl (10 mM), spermine (1 mM), $Fe(NH_4)_2SO_4$ (10 μM), sonicated calf thymus DNA (100 μM in bp), and 10 000 cpm ^{32}P labeled DNA in a volume of 40 μL for 1 h. The cleavage reactions were initiated by addition of sodium ascorbate (2 mM, final concentration) and allowed to react for 10 h at 25 °C at which time they were terminated by NaOAc/EtOH precipitation. The samples were washed with 70% EtOH, dried in vacuo, dissolved in TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA) and subjected to electrophoretic separation on a 5% nondenaturing polyacrylamide gel (19:1, monomer/bis): lane 1, oligonucleotide **1**; lane 2, **1** and **5**; lane 3, **1** and **6**; lane 4, **2**; lane 5, **2** and **5**; lane 6, **2** and **6**; lane 7, **3** and **7**; lane 8, **2** and **8**; lane 9, **4**; lane 10, no oligonucleotide; and lane 11, ^{32}P labeled BstE II digest of λ DNA. Sizes of the λ fragments are indicated on the right side of the autoradiogram. The expected size of the cleavage product is given on the left side of the autoradiogram. The domains A, B, C, and D are defined in Figure 1.

dimeric motif is within a factor of 2 of that observed by the single 15mer-EDTA·Fe **4** targeted to that site (lane 9, Figure 3).

Quantitation of the cleavage yields by phosphorimaging analyses of the gel shown in Figure 3 allows an estimate of the magnitude of binding enhancement.[12,13] Our results suggest a 10–15-fold increase in the binding of oligonucleotide-EDTA·Fe **2** in the presence of oligonucleotide **6**. This is comparable in magnitude to the cooperativity observed in biological systems such as the phage λ repressor.[14] We conclude that hydrogen bonding between nucleic acid bases can be used to generate dimerization domains with strong, specific interactions.

Acknowledgment. This work was supported by the National Institutes of Health (GM 35724), Fellowship DRG-1003 of the Damon Runyon–Walter Winchell Cancer Research Fund to M.D.D., and an N.I.H. predoctoral fellowship for J.A.S.

(11) The 26 base pair target shown in Figure 1 was incorporated into a plasmid, pMD5556, by ligation of an insert, 5′AGCTTCCTAAAAAAGGAAAAAAAAAAGAGAGAGAGATCTG-3′ and 5′GATCCAGATCTCTCTCTTTTTTTTTTCCTTTTTTAGGA-3′, with pUC18 previously cleaved with Hind III and BamH I.

(12) The percentages of specific cleavage were quantitated with a Molecular Dynamics 400S PhosphorImager and yielded the following results (%): lane 1, 0.30; lane 2, 0.30; lane 3, 0.18; lane 4, 0.31; lane 5, 0.13; lane 6, 3.0; lane 7, 1.8 lane 8, 0.25; lane 9, 6.0; lane 10 (background), 0.12.

(13) The cooperative binding enhancement is the ratio of cleavage obtained in reactions containing oligonucleotide **2** in the presence and absence of oligonucleotide **6**.

(14) (a) Johnson, A. D.; Meyer, B. J.; Ptashne, M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5061. (b) Ackers, G. K.; Johnson, A. D.; Shea, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 1129.