affinity cleaving, a method that relies on the attachment of a non-specifying cleaving moiety, such as EDTA-Fe(II), to a DNA binding molecule, facilitates the elucidation of the structural principles for DNA recognition. The determination of the sequence specificities, groove locations, and binding orientations of peptide analogues, protein-DNA binding motifs, and oligonucleotide−triple-helix motifs has provided reliable models for the sequence-specific recognition of double-helical DNA. It now becomes possible to combine these binding molecules with domains capable of base-specific and quantitative modification of DNA (Figure 1). We report the design and synthesis of an oligodeoxyribonucleotide equipped with an electrophil at the 5′-end that binds to double-helical DNA by triple-helix formation and alkylates predominantly at a single guanine base adjacent to the target DNA sequence in high yield.

The specificity of oligonucleotide-directed triple-helix formation is imparted by Hoogsteen base pairing between a pyrimidine oligonucleotide and the purine strand of the Watson−Crick duplex DNA. The discovery of other base triples, such as G-TA, and the development of 3′-2′-linked oligonucleotides for alternate-strand triple-helix formation has greatly extended the number of sites capable of being recognized by this motif. Model building of a triple-helical complex indicated that a pyrimidine oligo-

Figure 1. Replacement of a diffluorosulfonamide DNA cleaving moiety generated by EDTA-Fe(II) (hydroxyl radical) DNA cleaving DNA recognition (affinity cleaving) to a nonfluorescible base-specific moiety. This is a key issue with respect to the design of sequence-specific DNA cleaving molecules. Sequence-dependent recognition is coupled with sequence-dependent cleavage.

Sequence-Specific Alkylation of Double-Helical DNA by Oligonucleotide-Directed Triple-Helix Formation

Thomas J. Povsic and Peter B. Dervan

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Figure 2. (Top) Fmoc-protected thymidine 2-cyanoethyl N,N-disopropylphosphoramidite 1 used for the automated synthesis of oligonucleotides with a primary amine attached to the 5'-position of a single thymidine position. (Bottom) Oligonucleotides 2 and 3 containing T, 3°C, and a unique thymidine at the 5' end modified at the 5'-position with a spacer-primary amine (a) and spacer-N-bromoacetamide (b).

Oligonucleotide 2

Oligonucleotide 3

nucleotide bound in the major groove parallel to the purine strand of the duplex could be equipped with a bromoacetamide moiety at the 5'-end such that the electrophile is proximal to a guanine base located two base pairs to the 5'-side of the target sequence (Figures 2 and 3). Reaction of the electrophilic carbon with N-7 of guanine adjacent to the local triple helix would result in covalent attachment of the oligonucleotide to the duplex. Upon warming and base treatment, depurination and cleavage of the DNA backbone at the position of alkylation is expected.

A modified thymidine with an Fmoc-protected amine attached to the 5'-position was incorporated into an oligonucleotide, 5'- C-G \textsuperscript{6}T\textsuperscript{4}M\textsuperscript{4}C\textsuperscript{4}T\textsuperscript{4}C-G-T\textsuperscript{3}, as the 5'-terminal residue by coupling the corresponding phosphoramidite (Figure 2). The deprotected oligonucleotide 2 was purified by gel electrophoresis and allowed to react with N-hydroxysuccinimidyl bromoacetate (15 min, 25 °C). The resulting bromoacetoligonucleotide 3 was purified by HPLC (Figure 2).

To test both the specificity and yield of the reaction, three consecutive G-C base pairs were incorporated at the 5'-side of the 19 base pair purine target site, 5'-A\textsubscript{9}G\textsubscript{1}A\textsubscript{1}A\textsubscript{1}G\textsubscript{1}A\textsubscript{1}G\textsubscript{2}A\textsubscript{1}G\textsubscript{1}A\textsubscript{1} within a plasmid 2.3 kbp in size. The N-bromoacetoligonucleotide 3 (10) Maxam, A. M.; Gilbert, W. Proc. Natl. Acad. Sci. U.S.A. 1977, 74, 560.
(11) Phosphoramidite 1 was synthesized by standard procedures (12) from the corresponding nucleotide-amine, whose synthesis is described in detail in ref 13.


(14) A 10-nm mol aliquot of fully deprotected oligonucleotide 2 was dissolved in 15 μL of 200 mM pH 8.5 borate buffer; 15 μL of a 250 mM solution of N-hydroxysuccinimidyl bromoacetate in DMF were added. After 15 min, the solution was injected onto an HP Model 1090 HPLC equipped with a Brownlee Aquapore OD-300 C-18 column (200 mm × 4.6 mm). With a solvent gradient of 0-15% acetonitrile in 100 mM ammonium acetate, pH 4.6, over 25 min, retention times of oligonucleotides 2 and 3 are 15.2 and 16.2 min, respectively.

Figure 3. Autoradiogram of a high-resolution denaturing polyacrylamide gel of cleavage products from the reaction of oligonucleotide 3 and a 32P 3'-end-labeled 659-bp restriction fragment (PstI/SspI). Reaction conditions were 1 μM concentration of oligonucleotide 3, 20 mM Hepes, pH 7.4, 0.8 mM Co(NH\textsubscript{4})\textsubscript{2}, and 10,000 cpm end-labeled DNA in a total volume of 15 μL. Reactions were incubated at 37 °C, precipitated with NaOAc/TEOH, washed with 70% ETOH, and treated with 0.4 M piperidine (90 °C, 30 min). Cleavage products were analyzed on an 8%, 120 cross-linked, 48% urea polyacrylamide gel, 0.4 mm thick. Lanes 1 and 2 are A- and G-specific chemical sequencing reactions, respectively. Lane 3 contains DNA incubated for 48 h in the absence of 3'-N-bromoacetoligonucleotide 3. Lanes 4-10 contain DNA and oligonucleotide 3, which were allowed to react for 3, 5, 8, 12, 21, and 48 h, respectively. Center: Sequence of the oligonucleotide-DNA triplex within plasmid pUCALK. The major site of modification is indicated. Right: Ribbon model of triple-helical complex with the oligonucleotide bound parallel to the purine strand in the major groove of the duplex DNA. The positions of the electrophile and the three guanine bases proximal to the binding site are indicated. Arrow indicates base position of predominant cleavage.

μM (15) was allowed to react with a 659 bp 32P end-labeled restriction fragment at 37 °C in the presence of 0.8 mM Co(NH\textsubscript{4})\textsubscript{2} and 20 mM Hepes buffer (pH 7.4). The extent of cleavage (after base treatment) of the labeled DNA restriction fragment by the bromoacetoligonucleotide 3 was followed for 48 h at 37 °C (Figure 3). Analysis by high-resolution gel electrophoresis revealed that reaction occurred predominantly at the guanine base located two base pairs to the 5'-end of the target site with greater than 87% yield. No modification is observed on the complementary pyrimidine strand. Minor alkylation occurs at the flanking}

(15) Plasmid pUCALK was constructed by ligation of oligonucleotides containing the sequence 5'-GGA\textsubscript{1}GGA\textsubscript{1}GGA\textsubscript{1}GGA\textsubscript{1}GGA\textsubscript{1}GGA\textsubscript{1}GGA\textsubscript{1} into plasmid 19 linearized with BamHI-SalI. Reactions were run on a 3%-end-labeled PstI/SspI 659-bp restriction fragment.

(16) The yield of the cleavage reaction decreases in the presence of spermine, previously used to stabilize triple-helix formation, possibly due to reaction between spermine and the bromoacetamide moiety. Similarly, we find slightly lower yields in the presence of Tris buffers.

(17) The DNA termini at the cleavage site are 5'- and 3'-phosphate, consistent with the alkylation-depurination mechanistic model.

(18) Yields and specificity were quantitated by using a Molecular Dynamics PhosphorImager 400S. This can be compared with oligonucleotide-EDTA-Fe cleavage of double-helical DNA, which has typical cleavage yields on each strand of 30%.
guanines with relative rates of 0.03 and 0.06 that of the major site of reaction. Apparently, there exists sufficient flexibility in the linker arm and/or the junction of the local triple-helical complex to access all three guanine bases for modification to some extent.

A plot of ln [DNA]final/[DNA]initial vs time (pseudo-first-order conditions) indicates that the reaction between bromoacetyl-oligonucleotide and the double-helical DNA is first order in target DNA concentration with a pseudo-first-order rate constant of 3.1 X 10^-5 s^-1 at 37°C. This corresponds to a half-life for alkylation within the triples of 6.2 h (37°C).20 Separate experiments with N-iodoacetyl- and chloroacetyloligonucleotides indicate that these moieties react with relative rates of k_{sub}/k_{form} = 0.2 and k_{sub}/k_{form} = 0.06. The slower rates of reaction for both the chloroacetyl and iodoacetyl derivatives parallel the relative rates at N-3 of adenine seen with the reactions of N-bromo-, chloro-, and iodoacetyldiaminocyan in the minor groove of double-helical DNA.22

In conclusion, the work demonstrates that a nondiffusible electrophile judiciously attached to the 5'-end of an oligonucleotide is capable of modification of intact double-helical DNA at a single base position in high yield.21 Because the oligonucleotide-directed triple-helix motif is sufficiently generalizable and specific for the recognition of single sites in genomic DNA,22 modification of a single base within megabase-sized chromosomes using strictly chemical methods should be possible.

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Vibrationally Induced Ring Currents? The Vibrational Circular Dichroism of Methyl Lactate

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It has recently been proposed that ring currents contribute significantly to the magnetic dipole transition moments and rotational strengths of the vibrational transitions of chiral molecules containing rings.1 This hypothesis has been the basis for the interpretation of the vibrational circular dichroism (VCD) spectra of a variety of molecules.2 It has been invoked most extensively in studies of molecules capable of ring formation via intramolecular hydrogen bonding (H bonding).

We have recently developed3 and implemented an initial4 method of computing the VCD spectra of methyl lactate, (S)-(-)-l(-)-lactic acid, and (R)-(+)-l(+)-lactic acid. This procedure is based on the calculation of the infrared spectrum of the molecule in question, followed by the calculation of the circular dichroism spectrum. The method takes into account the rotational and vibrational coupling, and the resulting spectrum is a function of the rotation-vibration interaction. The resulting spectrum is then convolved with the experimental infrared spectrum to obtain the final VCD spectrum. The method is based on the assumption that the rotation-vibration interaction is not very strong. This assumption is valid for molecules with low rotational constants and low dipole moments. The method has been used successfully to predict the VCD spectra of a number of molecules, including methyl lactate, (S)-(-)-l(-)-lactic acid, and (R)-(+)-l(+)-lactic acid.

Figure 1. Absorption and VCD spectra of methyl lactate. (A) FTIR (Nicolete MX-1) absorption spectrum (1-cm^-1 resolution) of (R)-(+)-l (Aldrich (0.015 M in CCl4). (B) Lorentzian fit to A. (C) Absorption spectrum of (R)-(+)-l under VCD measurement conditions (see D). (D) Absorption spectrum predicted for 1a (y values from Table I). (E) Absorption spectrum predicted for 1b (y values as in D). (F) VCD spectra of (R)-(+)-l and (S)-(-)-l (Aldrich; [a]_D^21(neat) = +8.1^o and -8.4^o, respectively). VCD measured by using instrumentation previously described.10 Resolution 9.6 (at 2800) to 11.7 (at 3000) cm^-1. (G) Lorentzian fit to F for (R)-(+)-l. (H) VCD spectrum predicted for (R)-1a (y values from G). (I) VCD spectrum predicted for (R)-1b (y values as in H).

priori theory of vibrational rotational strengths. Comparisons of predicted and experimental VCD spectra have exhibited substantial agreement.5 This theory provides a general basis for the

\[ (\text{d}) \text{Nafie, L. A.; Freedman, T. B. J. Phys. Chem. 1986, 90, 763.} \]
\[ (\text{f}) \text{Stephens, P. J. J. Phys. Chem. 1985, 89, 748; 1987, 91, 1712.} \]