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Immunology

A recognition site on synthetic helical oligonucleotides for monoclonal anti-native DNA autoantibody

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ABSTRACT **The binding site in native DNA for a murine monoclonal anti-DNA autoantibody was investigated by measurements of competitive binding of a series of synthetic helical oligonucleotides. The antibody bound to a (dG-dC)$_3$ or (dG-dC)$_4$ core in the center of a base-paired octadecanucleotide. Reactions of analogues containing modifications or substitutions at specific sites indicated that the antibody bound to portions of cytosine and guanine in the major groove, a limited region of the backbone, and the 2-amino group of one guanine in the minor groove. For these interactions to occur, the antibody combining site would straddle the backbone of one of the helical strands of DNA.**

Recognition of specific nucleic acid regions by proteins is an essential feature of many steps in gene packaging, regulation of transcription and translation, and selective degradation of DNA (reviewed in refs. 1 and 2). In several cases this recognition depends to a large extent on interactions of helical protein segments with bases in the major groove of the DNA and contacts of other portions of the protein with the phosphate backbone and the minor groove (1, 2). Experimentally induced antibodies also recognize specific features of nucleic acid structure (reviewed in refs. 3–7). Some are directed against nucleosides or nucleotides, interacting primarily with the purine or pyrimidine bases (8). They recognize the bases in denatured DNA but do not bind to fully helical DNA. Other experimentally induced antibodies react selectively with the following helical forms: double-stranded RNA; RNA·DNA hybrid; Z-DNA; triple-helical RNA; or triple-helical DNA·RNA (reviewed in refs. 3–7).

Still other anti-nucleic acid antibodies are found in sera of patients with the autoimmune disease systemic lupus erythematosus (3, 6, 7, 9) and in related diseases in animals (10). Some lupus autoantibodies react with denatured but not native DNA, whereas others react with both forms—usually better with the denatured DNA. Less common are antibodies with a true selectivity for the native form (9, 11). As native DNA has not been an effective immunogen in experimental animals, the autoimmune subjects are the primary sources of antibodies that react with it.

Monoclonal representatives of anti-DNA autoantibodies have been studied intensively (reviewed in refs. 7 and 12). Most react much more strongly with denatured than with native DNA, as do most serum autoantibodies. Some, however, clearly bind to native DNA and even discriminate among helical DNAs of differing base sequence, certain ones showing selectivity for poly(dA-dT) (13) and others for poly(dG-dC) (14). We have studied reactions of one of the latter with both synthetic polynucleotides and DNA fragments (15, 16). This example (antibody H241) was produced by a hybridoma derived from the MRL-lpr/lpr lupus mouse strain. In the present study, we examined its recognition of a series of helical oligonucleotides containing a defined target that was modified at specific sites of the bases or backbone. The results indicate that this antibody interacts with adjacent guanine and cytosine bases in the major groove and with more limited regions of the backbone and minor groove. The binding site of this antibody thus straddles a single backbone of the polynucleotide.

MATERIALS AND METHODS

Reagents. [^{3}H]Thymidine-labeled *Escherichia coli* DNA was prepared as described (11). dGMP and dCMP were purchased from Sigma. Poly(dG-dC), poly(dG-dm^{5}C), and (dG-dC)$_5$ were purchased from P-L Laboratories (Pharmacia Molecular Biologicals).

5′-DMT (dimethoxytrityl), 3′-methoxy(*N*,*N*-diisopropylamino)phosphine deoxynucleosides, which have either *N*-benzoyl (for dA, dC, and dm^{5}C) or *N*-isobutyryl (for dG and dI) blocking groups, and corresponding β-cyanoethyl phosphoramidite reagents, were purchased from Applied Biosystems (Foster City, CA) or American Bio Nuclear (Emeryville, CA). They were used as 0.1 M solutions (except for dI, 0.2 M) in acetonitrile, which was obtained from Burdick and Jackson (0.01% water; Muskegon, MI) and dried over type 4A molecular sieves. 1*H*-Tetrazole (>99% pure) was purchased from Aldrich and was resublimed before use as a 0.5 M solution in acetonitrile. Long-chain alkylamine-type controlled-pore glass having 3′-linked 5′-DMT-deoxynucleoside at 20–40 μmol/g was purchased from Applied Biosystems and American Bio Nuclear.

Monoclonal Antibody H241. The IgG2b,κ immunoglobulin H241 was prepared from a cloned hybridoma derived from spleen cells of an unimmunized autoimmune MRL-lpr/lpr mouse (17). The monoclonal antibody was purified from culture fluid by affinity chromatography with a column of purified goat anti-mouse IgG-conjugated Sepharose 4B.

Synthesis of Oligonucleotides. Oligodeoxyribonucleotides were synthesized on a 1-μmol scale using an automated version (18) of the solid-phase phosphoramidite coupling method of Caruthers *et al.* (19) with Applied Biosystems DNA synthesizers (Models 380A and 380B). Thiono phosphotriester linkages were incorporated by the use of a solution of elemental sulfur (0.4 M) in 2,6-dimethylpyridine (20). The 5′-DMT group was retained on the final nucleotide unit that was coupled at the end of each synthesis to allow the isolation of DMT-bearing oligonucleotides by reverse-phase (RP)-HPLC (21–23). Coupling yields were derived from successive relative absorbance values measured at 530 nm for the collected DMT cation after dilution to 5 ml with 0.1 M *p*-toluenesulfonic acid in acetonitrile.

Purification and Analysis of the Oligonucleotides. HPLC was carried out with a Waters system with a Waters μBondapak C$_{18}$ column (7.8 mm × 30 cm), a Waters Z-

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Abbreviations: DMT, dimethyltrityl; RP, reverse phase; bp, base pair(s); Et$_3$NHOAc, triethylamine adjusted to pH 7.0 with acetic acid.

Module with Radial Pak SAX cartridge (8 mm × 10 cm), a Whatman Partisil SAX column (4.6 mm × 25 cm) or a DuPont Zorbax ODS column (21.2 mm × 25 cm). Data were recorded and integrated with a Waters Model 730 or a Hewlett Packard Model 3390A signal recorder–integrator. The μBondapak column, SAX cartridge, Partisil column, and Zorbax column were used with flow rates of 4, 2, 1, and 11.25 ml/min, respectively. Standard RP-HPLC employed a 1% per min linear gradient (I) of 20–30% acetonitrile against Et_3NHOAc (0.1 M triethylamine adjusted to pH 7.0 with acetic acid) buffer. RP-HPLC under denaturing conditions employed a 1% per min linear gradient (II) of 0–40% acetonitrile/formamide (1:1; vol/vol; Kodak spectro-grade formamide) against Et_3NHOAc buffer/formamide (1:1; vol/vol) at a column temperature of 60°C. HPLC using the Radial Pak SAX cartridge under denaturing conditions at a column temperature of either 25 or 60°C employed a 2% per min linear gradient (III) of 10–80% 1 M potassium phosphate buffer (pH 6.8) against 1 mM potassium phosphate buffer (pH 6.8), each of which contained 5 M urea (Bethesda Research Laboratories, enzyme grade) and water/ethanol (70:30; vol/vol).

Oligonucleotides were labeled at their 5′ end with T4 polynucleotide kinase (P-L Biochemicals) and [γ-^{32}P]ATP (24). Absorbance measurements were made with a Hewlett Packard Model 8451A diode array UV-visible spectrophotometer.

Radioimmunoassay. Competitive radioimmunoassays were performed as described (11), with 5 μg of purified antibody H241 (100 μl), [^{3}H]thymidine-labeled native DNA (125 ng in 50 μl), and various amounts of unlabeled competing oligonucleotides. Immune complexes were precipitated with 50 μl of the gamma globulin fraction of goat anti-mouse IgG serum, washed with phosphate/EDTA buffer, and dissolved in 200 μl of 0.1 M NaOH. The radioactivity was then quantified.

Computer Graphics. Solids modeling of the modified DNA sequences was carried out on an Apollo DN-600 computer using the National Institutes of Health molecular graphics system (25). An ideal B-DNA helix (26) was generated, and the positions of the chemical modifications were denoted by a change of coloring. Distortions in the helix due to the substitutions or deletions are expected to be small and were not included in the modeling.

RESULTS

Oligonucleotide Synthesis and Purification. Oligonucleotides were synthesized with coupling yields of 96–99.7% and overall yields of 5–50%. Most of the preparations showed one major DMT-bearing component on analytical RP-HPLC with gradient I at 25°C. The crude "parent" octadecamer (polymer B, Table 1), however, yielded two major DMT-bearing components (11.2 and 14.6 min), tentatively assigned to double- and single-stranded material, respectively. When the sample was detritylated with acetic acid at pH 2.5–2.7 (5 min, 25°C), extracted with 2 ml of ethyl acetate, and twice precipitated by ethanol from 0.2 M NaCl, 45 A_{260} units of the final polymer B product were obtained, a 24% overall yield. Under denaturing conditions, this product showed one major component that eluted at 23 min from the Radial Pak SAX (25°C) and 16 min with Partisil SAX (60°C) columns.

With the mono- and polyphosphorothioate analogues (polymer H and F, Table 1), major fast- and slow-eluting DMT-bearing components were obtained at 10.5 and 12.0 min on RP-HPLC. When these polymers were separated on a preparative scale, ^{32}P-labeled, and tested by PAGE, the fast eluting peak was found to be nearly pure (>95%) octadecamer (ca. 10 A_{260} units), with minor contaminants that were 1–3 nucleotides shorter.

Analytical RP-HPLC of the crude analogue containing 5-methylcytosine (polymer I, Table 1) showed a major and several minor components with gradient I at 25°C (Fig. 1*a*) or with gradient II at 60°C (Fig. 1*b*). Collected fractions a–g and 1–4 were further processed as described above for polymer B, and PAGE analysis of the resulting ^{32}P-labeled oligonucleotides indicated that peak 1 was nearly pure (>95%) octadecamer (Fig. 2). A yield of 20 A_{260} units of this peak was obtained from preparative HPLC.

Table 1. Inhibition of the binding of labeled native DNA

Competitor	Structure	Competitor required for 50% inhibition of [^{3}H]native-DNA binding, M
A	gatgaGCGCGCGCtcatc	2.3×10^{-7}
B	atataGCGCGCGCtatat	5.4×10^{-7}
C	atataCGCGCGCGtatat	6.8×10^{-7}
D	auauaCGCGCGCGuauau	8.4×10^{-7}
E	auauaGCGCGCGCuauau	1.3×10^{-6}
F	atataGsCsGsCsGsCsGsCstatat	1.5×10^{-6}
G	GCGCGCGCGC	2.4×10^{-6}
H	atataGCGCsGCGCtatat	2.9×10^{-6}
I	atataGCGmCGCGCtatat	3.0×10^{-6}
J	atataGCGCHCGCtatat	3.1×10^{-6}
K	atataHCHCHCHCtatat	3.9×10^{-6}
L	mCGmCGmCGmCGmCGmCG	n
M	atatamCGmCGmCGmCGtatat	n
N	atataGmCGmCGmCGmCtatat	n
O	atataGCGATCGCtatat	n
P	atataGCGTACGCtatat	n
Q	atataGCGCGTGCtatat	n

In the listing of structures, bases in the target region for antibody binding are in upper case letters, and bases that flank the target region are in lowercase letters. The phosphorothioate internucleotide link is designated by s, 5-methylcytosine by mC, and hypoxanthine by H. n, less than 10% inhibition at 10^{-5} M competitor.

Reactions of Monoclonal Antibody H241. H241 binds native DNA slightly better than denatured DNA and shows a marked preference for poly(dG-dC) (B-form) over native DNA or poly(dG)·poly(dC) (16). It reacts only very weakly with poly(dG-dA)·poly(dT-dC) and does not react detectably with poly(dA-dT) or poly(dA)·poly(dT) (16). Although it has a higher affinity for DNA of high molecular weight than for DNA of small size, it does bind to helical fragments in the range of 15–100 base pairs (bp) (15).

Purified H241 (5 μg) bound approximately 50% of a 125-ng sample of labeled native DNA in a double-antibody radioimmunoassay. A 2.4×10^{-6} M concentration of the decanucleotide $(dG\text{-}dC)_5$ caused 50% inhibition of native DNA binding by H241 (Table 1). As described above, we then prepared the following more stable, helical oligonucleotide with a central target of 8 G·C bp and unreactive flanking sequences of 5 A·T bp on either side:

ATATAGCGCGCGCTATAT
. . . . . .
TATATCGCGCGCGATATA

The self-complementary octadecanucleotide had a base-paired helical structure, as it melted sharply with a melting temperature of 60°C in PBS (10 mM sodium phosphate, pH 7.4/150 mM NaCl). A 5.4×10^{-7} M concentration of this oligonucleotide (Table 1, polymer B) caused 50% inhibition of the DNA binding. (dC-dG) or (dG-dC) sequences in the central core were equally potent (Table 1, compare polymers B and C). Modification of the flanks had little effect on binding. Addition of 2 G· C bp (Table 1, polymer A) to this region raised the melting temperature slightly with little effect on the competitive potency of the oligonucleotide. The presence of A·U bp in the flanks in place of A·T bp also had

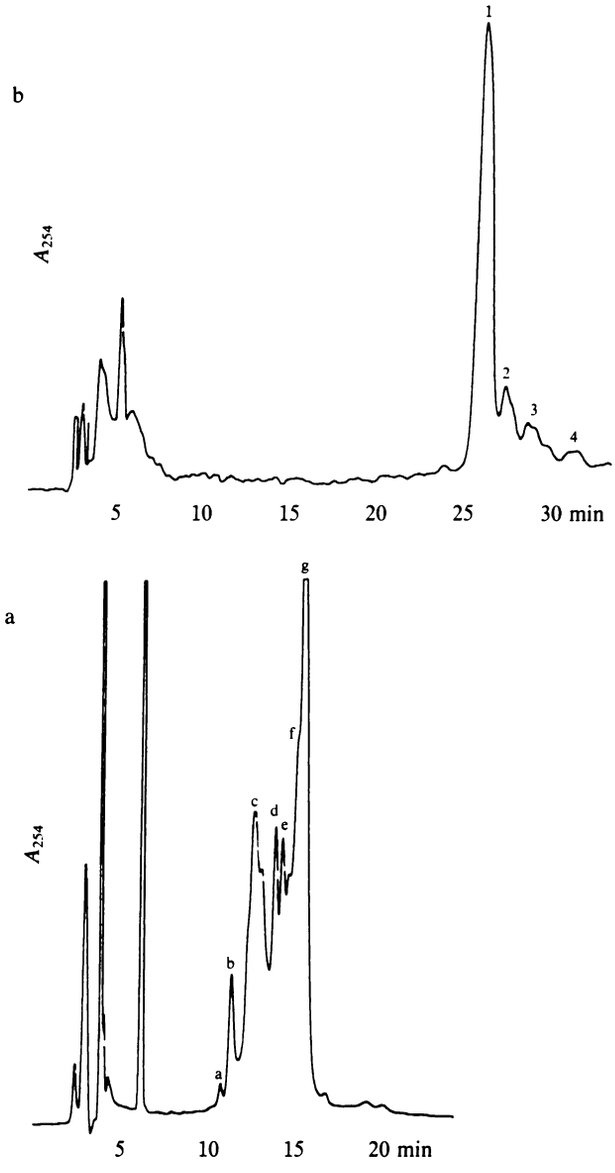

FIG. 1. RP-HPLC of the crude synthetic mixture for ATAT-AGCGmCGCGCTATAT (polymer I, Table 1). (*a*) Separation obtained with a 1% per min linear gradient of 20–30% acetonitrile against 0.1 M Et_3NHOAc buffer (pH 7.0) at 4 ml/min on a μBondapak C_{18} column (7.8 mm × 30 cm) at 25°C. The fast-eluted peaks at 2–4 min contain 5′-hydroxyl truncated "failure" sequences. The peak at 6 min is benzamide, a product of base deprotection. The peaks at 10–16 min (peaks a–g) are DMT-bearing oligonucleotides, which were collected, detritylated, 5′-end labeled with ^{32}P, and analyzed by PAGE (Fig. 2, lanes a–g, respectively). (*b*) Separation obtained with a 1% per min linear gradient of 0–40% acetonitrile/formamide (1:1; vol/vol) against 0.1 M Et_3NHOAc buffer (pH 7.0)/formamide (1:1; vol/vol) at 4 ml/min, 60°C on the same column described in *a*. Peaks 1–4 are DMT-bearing oligonucleotides, which were collected and treated as in *a* (Fig. 2, lanes 1–4, respectively).

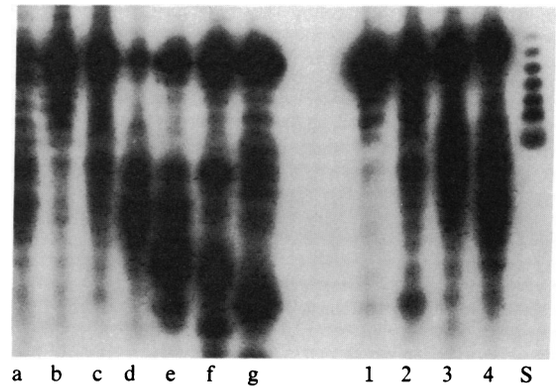

FIG. 2. Print of an overexposed autoradiogram obtained after PAGE of ^{32}P-labeled oligonucleotide fractions a–g and 1–4 derived by RP-HPLC, as described in Fig. 1. Lanes a–g and 1–4 represent the corresponding fractions, and lane S contains [^{32}P]p(dT)12–18 as size standards. The octadecanucleotide makes up more than 95% of the sample in lane 1.

little effect (Table 1, compare polymer C with D and polymer B with E).

The size of the recognition site was investigated by preparation of oligonucleotides with the same dA-dT flanks but with central regions of (dG-dC), $(dG\text{-}dC)_2$, $(dG\text{-}dC)_3$, $(dG\text{-}dC)_4$, or $(dG\text{-}dC)_5$. With the single dG-dC sequence or the $(dG\text{-}dC)_2$ sequence, no competition for native DNA binding was detected (Fig. 3). This latter combination of 10 A·T bp and the central 4 G·C bp formed a stable tetradecanucleotide, with a melting temperature of 50°C, but did not present a complete recognition site. By contrast, an oligonucleotide with $(dG\text{-}dC)_3$ between the dA-dT flanks was an effective competitor (Fig. 3). The $(dG\text{-}dC)_4$ sequence was approximately five times more potent and appeared to be optimal. Addition of a fifth (dG-dC) dinucleotide pair did not increase the competitive activity (Fig. 3). Assuming a separation of 3.6 Å between bp and a diameter of 20 Å for the helix, the area of a surface presented on the $(dG\text{-}dC)_3$ segment may be estimated to be approximately 18 × 20 Å^2.

The importance of the (dG-dC) sequence of appropriate size was confirmed further with preparations in which (A-T) pairs in the central target eliminated binding (Table 1, polymers O and P), as did a pair of (G-T) mismatches (Table 1, polymer Q). More subtle modifications were then introduced into the central target region to determine which portions of the bases or backbone were being recognized. Replacement of one central phosphodiester by a phosphorothioate internucleotide linkage (without separation of *R* and *S* isomers) caused a reduction in binding effectiveness by a

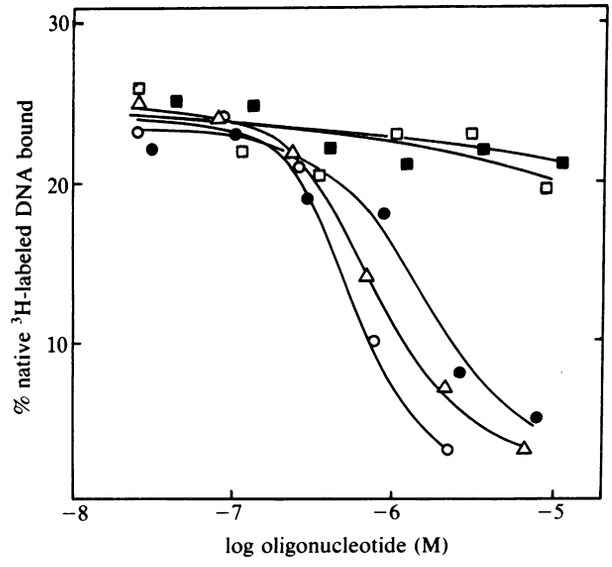

FIG. 3. Competitive binding of purified H241 monoclonal murine autoantibody with ^3H-labeled *E. coli* native DNA (125 ng) and various amounts of oligonucleotides with (A-T) flanks as in polymer B (Table 1) and a central region of (dG-dC) (■); $(dG\text{-}dC)_2$ (□); $(dG\text{-}dC)_3$ (●); $(dG\text{-}dC)_4$ (○); or $(dG\text{-}dC)_5$ (△).

factor of 5 (Table 1, polymer H). Additional modification of all the (G-C) internucleotide linkages in this way, however, did not cause a further loss of binding (Table 1, polymer F). These results suggested that some interaction occurs with a limited region of the backbone. Interaction with the backbone was also indicated by the ability of cardiolipin to inhibit the DNA binding (not shown); as relatively high concentrations of this phospholipid were required ($1–10 \times 10^{-5}$ M phosphate), only part of the binding affinity could be accounted for by interaction with the phosphodiesters.

When the central cytosine of the parent oligonucleotide was replaced by 5-methylcytosine, binding efficiency was reduced, again by a factor of 5 (Table 1, polymer I). Replacement of the other three cytosines with 5-methylcytosines led to a dramatic further loss of binding (Table 1, polymers M and N). This is consistent with the lack of reaction with thymine-containing segments, which also have the 5-methyl substituent. Although the 5-methylcytosine substitution had a marked effect, it did not completely eliminate the binding site. A high molecular weight polymer form of poly(dG-dm^{5}C) did inhibit binding to DNA. It was less effective by a factor of 20–50 than the unmodified poly(dG-dC) (not shown).

Computer graphics modeling of the helical oligonucleotides predicted that the 7-N of the central guanosine should be in the center of the binding site. An oligonucleotide with 7-deazaguanosine in this position was synthesized. In comparison with the parent polymer, its ability to bind to H241 was reduced by a factor of 5 (not shown).

To test for the effect of changes in the minor groove, oligonucleotides were prepared with hypoxanthine in place of guanine. With one hypoxanthine in the center of the $(dG\text{-}dC)_4$ core, the oligomer was about one-eighth as potent a competitor as the purely (dG-dC)-containing molecule (Table 1, polymer J). The oligomer that contained hypoxanthines in place of all four guanines was no less effective than the singly substituted one (Table 1, polymer K). A single replacement by hypoxanthine of the first guanine of a $(dG\text{-}dC)_3$ central core had no effect on binding; replacement of only the second guanine decreased binding by a factor of 5–10, and replacement of only the third guanine by hypoxanthine (comparable to the replacement in polymer J) decreased binding by a factor of 100 (data not shown). These results indicated a primary role for the 2-amino group of one guanine in the minor groove in forming the binding site.

DISCUSSION

The target for immunological recognition of DNA is of interest in relation to the nature of autoantibodies in systemic lupus erythematosus and to how proteins interact selectively with certain regions of nucleic acids. Early studies with lupus sera indicated that oligonucleotides could inhibit the reactions of some antibodies with denatured DNA, in a way that suggested a major role of the purine and pyrimidine bases in the antigenic sites (3, 6). Results of affinity chromatography with nucleoside–protein conjugates supported this interpretation (27). Some sera, however, reacted with both native and denatured DNA in a way that suggested the involvement of

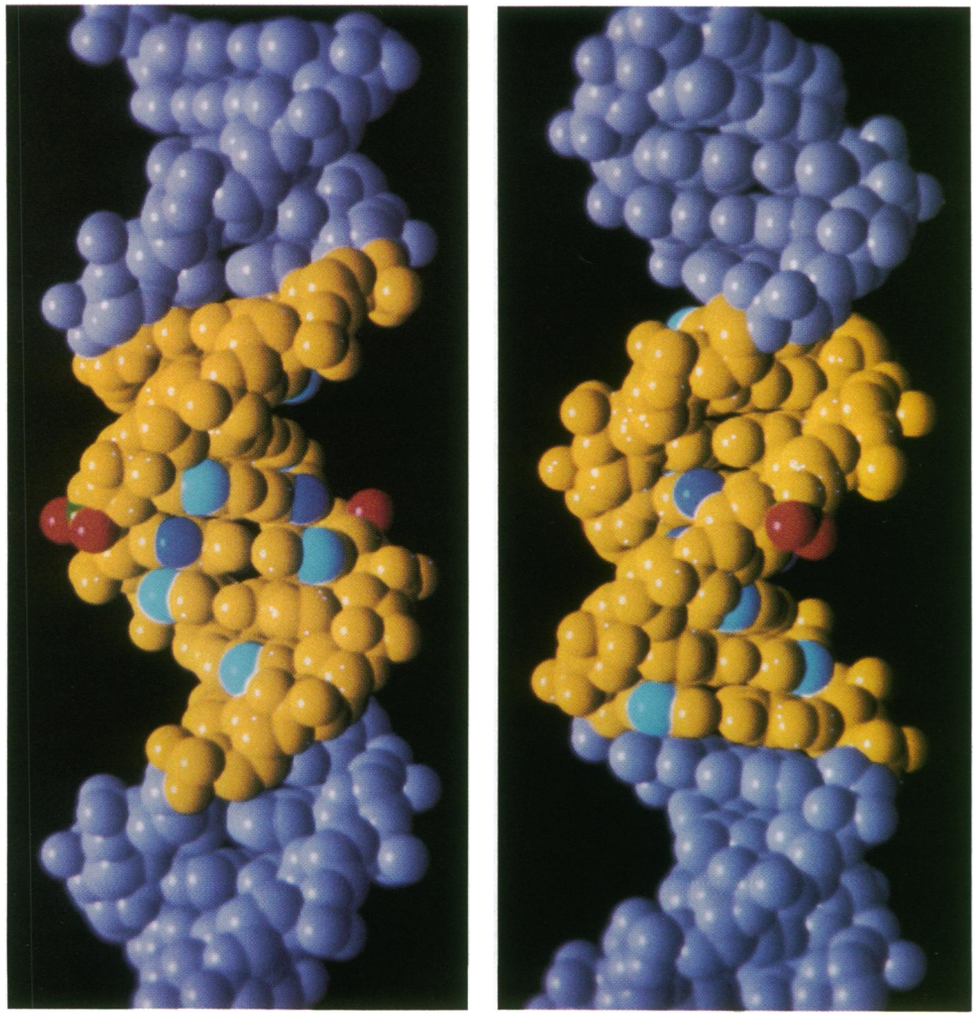

FIG. 4. Computer graphic models of helical octadecanucleotide A-TATAGCGCGCGCTATAT (polymer B). The A-T regions are designated in blue, and the G-C region in yellow. The highlighted atoms are those on which modification (*cf* deletion of which) caused decreased binding by antibody. The two views indicate that the antibody binding site would have to straddle the backbone to contact all the atoms involved: the C-5 of at least two cytosines (turquoise) and N-7 of guanine (darker blue) in the major groove, a portion of the backbone, and the N-2 of guanine (darker blue) in the minor groove. The phosphate oxygens of the central C-G sequence are red and the phosphorus is green.

the sugar-phosphate backbone as well (6, 9, 11). This interpretation was supported by the finding that certain monoclonal anti-DNA antibodies could bind to the phospholipid cardiolipin (28), which presents phosphodiesters separated by three carbon atoms, as does the backbone of DNA.

We have studied this interaction with the monoclonal autoantibody H241, which binds both native and denatured DNA, slightly favoring the native form (16). This antibody showed a remarkable ability to distinguish among synthetic helical polynucleotides of various base sequence, favoring poly(dG-dC) above all others tested. The affinity of H241 for the optimal target is high enough so that its binding can be measured with a small helical oligonucleotide such as $(dG\text{-}dC)_5$, which probably allows only monovalent binding for a given antibody molecule. Because the antibody does not recognize A-T sequences, a series of stable helix-forming self-complementary oligonucleotides with a central G-C core and unreactive A-T flanks was constructed to test specific atoms or groups of atoms as components of the central target site. Our findings indicate that a single antibody can make contact with both the backbone and portions of bases in the grooves of DNA.

The major contacts appear to be in the major groove, within a sequence of bases that, in this case, must include a C-G-C-G-C-G core. Part of the binding, however, also appears to involve the backbone, as indicated by (*i*) the ability of cardiolipin to compete with DNA and (*ii*) the decrease in binding caused by a phosphorothioate modification in the center of the target. The backbone interaction appears to involve a limited region at the center of the C-G core, as further phosphorothioate modifications did not further decrease the binding. Finally, there appears to be involvement of a limited portion of the minor groove near the center of the target, as the substitution of one hypoxanthine for guanine in the center, removing the 2-amino group of guanine in the minor groove, also decreased binding.

A computer graphics analysis highlighted the atoms where modifications decreased the binding of the oligonucleotide (Fig. 4). This analysis indicates that the cavity or groove of the antibody combining site may straddle the backbone of a single DNA chain, with one side of the binding site projecting into the major groove of DNA and a smaller part of the other side projecting into the minor groove. This type of binding could account for the ability of the antibody to react with both native and denatured DNA. It has not been established, however, whether H241 binds to purely single-stranded or base-paired regions in denatured DNA.

These interpretations are based on the assumption that the substitutions we used did not cause changes in the overall conformation of the oligonucleotides. If conformational changes did occur but were very localized, the substitutions would still define the regions of interaction. That any such changes were localized is supported by the finding that only one of the hypoxanthine substitutions for guanine decreased the binding; the same base substitution at the other three guanines of the core did not affect it. Similarly, only one phosphorothioate substitution altered binding.

As with other DNA-binding proteins, hydrogen bond formation between amino acid side chains and the bases may account for much of the interaction. Unlike several other DNA-binding proteins (1, 2), however, the antibody molecule does not contain an α-helix (29). Contacts must depend on the specifically folded conformation of the hypervariable regions built onto the end of a framework that consists of β-pleated sheet structure (30).

In addition to defining the recognition site for a particular DNA-binding antibody, these experiments confirm that synthesis of self-complementary oligonucleotides with defined modifications is of general use in studies of protein–helical DNA interactions (20, 31, 32). The choice of useful oligonucleotides is facilitated by computer graphics analysis that highlights the positions of particular atoms in the helix.

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1. Pabo, C. O. & Sauer, R. T. (1984) *Annu. Rev. Biochem.* **53**, 293–321.
2. Takeda, Y., Ohlendorf, D. H., Anderson, W. F. & Matthews, B. W. (1983) *Science* **221**, 1020–1026.
3. Stollar, B. D. (1973) in *The Antigens*, ed. Sela, M. (Academic, New York), pp. 1–85.
4. Lacour, F., Nahon-Merlin, E. & Michelson, M. (1973) *Curr. Top. Microbiol. Immunol.* **62**, 1–39.
5. Stollar, B. D. (1975) *CRC Crit. Rev. Biochem.* **3**, 45–69.
6. Munns, T. W. & Liszewski, M. K. (1980) *Prog. Nucleic Acid Res. Mol. Biol.* **24**, 109–165.
7. Stollar, B. D. (1986) *CRC Crit. Rev. Biochem.* **20**, 1–36.
8. Erlanger, B. F. & Beiser, S. M. (1964) *Proc. Natl. Acad. Sci. USA* **52**, 68–74.
9. Casperson, G. F. & Voss, E. W., Jr. (1983) *Mol. Immunol.* **6**, 581–588.
10. Andrews, B. S., Eisenberg, R. S., Theofilopoulos, A. N., Izui, S., Wilson, C. B., McConahey, P. J., Murphy, E. D. & Roths, J. B. (1978) *J. Exp. Med.* **148**, 1198–1215.
11. Stollar, B. D. & Papalian, M. (1980) *J. Clin. Invest.* **66**, 210–219.
12. Eilat, D. (1982) *Mol. Immunol.* **19**, 943–955.
13. Tron, F., Charron, D., Bach, J.-F. & Talal, N. (1980) *J. Immunol.* **125**, 2805–2809.
14. Hahn, B. H. & Ebling, F. M. (1984) *J. Immunol.* **132**, 187–190.
15. Ali, R., Dersimonian, H. & Stollar, B. D. (1985) *Mol. Immunol.* **22**, 1415–1422.
16. Stollar, B. D., Huang, B.-S. & Blumenstein, M. (1986) in *Fourth Conversation in Biomolecular Stereodynamics*, ed. Sarma, R. H (Adenine, Guilderland, NY), in press.
17. Andrzejewski, C., Rauch, J., Lafer, E., Stollar, B. D. & Schwartz, R. S. (1981) *J. Immunol.* **126**, 226–231.
18. Stec, W. J., Zon, G., Egan, W., Byrd, R. A., Phillips, L. R. & Gallo, K. A. (1985) *J. Org. Chem.* **50**, 3908–3913.
19. Caruthers, M. H., Beaucage, S. L., Becker, C., Efcavitch, W., Fisher, E. F., Galluppi, G., Goldman, R., deHaseth, P., Martin, F., Matteucci, M. & Stabinsky, Y. (1982) in *Genetic Engineering Principles and Methods*, eds. Setlow, J. K. & Hollaender, A. (Plenum, New York), Vol. 4, pp. 1–17.
20. Stec, W. J., Zon, G., Egan, W. & Stec, B. (1984) *J. Am. Chem. Soc.* **106**, 6077–6079.
21. Ikuta, S., Chattopadhyaya, R. & Dickerson, R. E. (1984) *Anal. Chem.* **56**, 2253–2256.
22. Stec, W. J., Zon, G. & Uznanski, B. (1985) *J. Chromatogr.* **326**, 263–280.
23. Becker, C. R., Efcavitch, J. W., Heiner, C. R. & Kaiser, N. F. (1985) *J. Chromatogr.* **326**, 293–299.
24. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 449–560.
25. Feldmann, R. J., Bing, D. J., Furie, B. C. & Furie, B. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5409–5412.
26. Arnott, S., Campbell, P. J. & Chandrasekaran, R. (1976) in *CRC Handbook of Biochemistry and Molecular Biology: Nucleic Acids*, ed. Fasman, G. D. (CRC, Cleveland, Ohio), Vol. 2, p. 411.
27. Munns, T. W., Liszewski, M. K. & Hahn, B. H. (1984) *Biochemistry* **23**, 2958–2964.
28. Lafer, E. M., Rauch, J., Andrzejewski, C., Jr., Mudd, D., Furie, B., Furie, B., Schwartz, R. S. & Stollar, B. D. (1981) *J. Exp. Med.* **153**, 897–909.
29. Edmundson, A. B., Ely, K. R., Abola, E. F., Schiffer, M. & Panagiotopoulos, N. (1975) *Biochemistry* **14**, 3953–3961.
30. Davies, D. R. & Metzger, H. M. (1983) *Annu. Rev. Immunol.* **1**, 87–117.
31. Potter, B. V. L. & Eckstein, F. (1984) *J. Biol. Chem.* **259**, 14243–14248.
32. Noble, S. A., Fisher, E. F. & Caruthers, M. H. (1984) *Nucleic Acids Res.* **12**, 3387–3404.