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Affinity-Labeling Study for Determining Interacting Sites of an Anti-DNA Antibody on DNA Double Helix

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Previously, a purified monoclonal mouse (MRL-lpr/lpr) IgG anti-DNA autoantibody, H241, was reported to interact with major groove and possibly with phosphate backbone of synthetic helical oligonucleotide of 18 base pairs in affinity-labeling experiment. Experiments were performed to determine further the interacting sites of the autoantibody, H241, on the DNA double helix. Two oligonucleotides which were specifically modified in the minor groove of double-stranded DNA were cross-linked covalently to H241. The ratio of L chain to H chain cross-linked was decreased with the modified oligonucleotide, compared to that with an unmodified helical oligonucleotide. With the more extensively modified oligonucleotide in the minor groove, the ratio was further decreased. This indicates that the anti-DNA autoantibody, H241, possibly interacts possibly with the minor groove as well.

Key Words: Anti-DNA antibody, Affinity-labeling, Interacting sites

INTRODUCTION

A central feature of systemic lupus erythematosus (SLE) is the production of anti-DNA autoantibodies[1~4]. Among the anti-DNA antibodies, much attention has been paid to the IgG antibodies that react with native DNA, because their presence is correlated with disease activity in many patients[5,6] and these antibodies interact with DNA self-antigens to form circulating immune complexes which deposit in the tissues and lead to the activation of complement and a general inflammatory response[7]. These facts raise the possibility that these antibodies are particularly pathogenic. The potential pathogenicity of anti-DNA antibodies raises an interest in the characterization of how these antibodies bind to DNA. However, the binding sites of native DNA are not clearly defined yet. Deoxyribose and phosphate groups may contribute to the specificity for DNA over RNA[8]. The backbones of native DNA may contribute to the determinant site, and the orientation of the two strands relative to each other may be also important. Some antibodies which react with dsDNA as well as ssDNA may recognize features that occur in a single backbone chain of either native or denatured DNA. Alternatively, these antibodies may recognize a precise geometry that is characteristic of dsDNA, but that also occurs locally in base-paired regions of ssDNA[9~11]. Interacting sites on native DNA have been analyzed with helical polynucleotides[12~14], and with oligonucleotides that have defined sequence variations and modified bases[15,16]. Certain anti-DNA autoantibodies discriminate among helical DNAs of differing base sequence, some showing selectivity for poly(dA-dT)[17] and others for poly (dG-dC)[18]. Studies with base-paired synthetic oligonucleotides with or without modified bases indicate the combining site of one anti-dsDNA antibody binds to the major groove[15,16] and to more limited regions of the backbone and minor groove[15]. A detailed crystal structure gives a picture of one monoclonal antibody with and without antigen[19].

Previously, the modifications in the major groove and

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phosphate backbone have been reported to decrease the affinity of H241 to DNA[15,16] and alter the relative cross-linking to H and L chains[16]. It was indicated that the antibody, H241, interact with the major groove and possibly with the phosphate backbone. In this report, we tested whether H241 contacts the minor groove as well by zero-length affinity-labeling technique, UV cross-linking experiment, with two oligonucleotides modified in the minor groove.

MATERIALS AND METHODS

Oligonucleotides

Oligonucleotides were synthesized and purified by the solid-phase phosphoramidite method with an automated synthesizer (Applied Biosystems, Foster, CA). Oligonucleotides 1138, d(ATATAGCGCGCGCTATAT); 1284, d(ATATAGCGCICGCTATAT) and 1285, d(ATATAICICICICTATAT) are self-complementary, in which the letter I represents hypoxanthine, were anealed to form a duplex.

End-labeling of oligonucleotides

Oligonucleotides were radiolabeled at their 5'ends (to sp. act. of 3×10^7 to 5×10^7 cpm/μg) with T4 polynucleotide kinase and γ[^{32}P]ATP. The reaction was carried out at 37℃ for 1 h with a buffer containing 70 mM Tris(pH 7.6), 10 mM $MgCl_2$, and 5 mM dithiothreitol and was stopped by the addition of EDTA (to 20 mM) in ice. The labeled oligonucleotides were purified from free [^{32}P]ATP with a Sep-Pak C18 cartridge (Millipore). Purified samples were boiled for 5 min and allowed to cool down slowly to room temperature.

mAb

MRL-lpr/lpr murine hybridoma H241 was grown in Dulbecco's modified Eagle's medium supplemented with L-glutamine, penicillin, streptomycin, gentamycin, nonessential amino acids, 10mM HEPES buffer, and 10% FCS. The cells were grown in 750-ml microcarrier stirrer bottles and maintained at 37℃ in humidified 5% CO_2 incubators.The cell line was tested for both antobody production and antibody binding to DNA by an ELISA. The H241 antibody was affinity purified from tissue culture fluid with a single-stranded DNA column (GIBCO/BRL).

UV cross-linking

Antibody(10 μg) was mixed with ^{32}P-labeled oligonucleotide (0.25 μg) in TBE buffer in a total volume of 50 μl in a well of a polystyrene microtiter plate. The plate, resting in an ice water bath, was irradiated with short-wavelength (peak, 254 nm)UV light with a Mineralight lamp (model UVGL-25; UVP Inc.) at a distance of 4 cm for 10 min. The irradiated samples were boiled in SDS dissociation buffer (0.0625M Tris-HCl, pH6.8, 5% SDS, 5% 2-ME, 20% glycerol, 0.015% bromophenol blue) for 3 min and analyzed by discontinuous SDS-PAGE. After electrophoresis, the gel was fixed in 5% methanol-7.5% acetic acid and stained with freshly prepared 0.1% Coomassie brilliant blue solution in 50% TCA for 1 h. The gel was destained with 5% methanol-7.5% acetic acid, dried, and exposed to X-OMAT AR film for autoradiography at −70℃. Intensities of the H and L chain bands cross-linked were scanned following detailed procedures described in the GelScan XL software package analysis made by the manufacturer of the GelScan XL Laser Densitometer.

RESULTS

H241, an IgG2a mAb from an MRL-lpr/lpr mouse, binds native DNA in preference to denatured DNA[15]. It binds to poly(dG-dC) but not to poly(dA-dT)[20]. Its DNA recognition site consists of several base pairs of alternating dG-dC sequence within the helical self-complementary oligonucleotide 1138[15]. For minor groove modification of oligonucleotide 1138, guanosine(s) was(were) replaced by a hypoxanthine (oligonucleotide 1284) or four hypoxanthines (oligonucleotide 1285). UV cross-linking of H241 and oligonucleotides 1284 (figure not shown), or 1285 (Fig. 1) mixture yielded oligonucleotide labeling of the Ig protein. As with oligonucleotide 1138[16], three bands of L chain were visible on the autoradiogram (Fig.1). Cross-linking was quantified by densitometric scanning of lightly exposed autoradiograms in a linear density range. It has been known that the total radioactivity of oligonucleotide 1138 associated with the L chain was approximately 1.43 times that associated with the H chain[16]. The L/H labeling ratio was altered with oligonucleotides 1284 and 1285 (Table 1). With the modification of a guanosine to a hypoxanthine in the minor groove

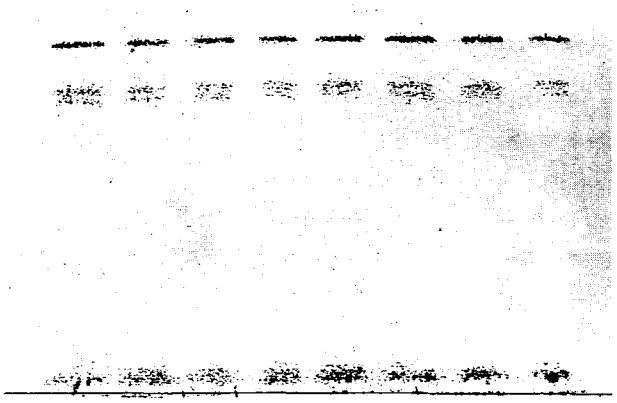

Fig. 1. Analysis of cross-linked oligonucleotide 1285-antibody H241 complexes. Mixtures of end-labeled 1285 and antibody H241 placed in the several wells of polystyrene plate were UV-irradiated at the same time. Each samples were electrophoresed in 10% polyacrylamide gels under denaturing and reducing conditions.

Table 1. Relative cross-linking of modified oligonucleotides to H and L chains of H241

Oligonucleotide (Modification)	L/H Ratio (Mean)[a]	n[b]	SD	Student's *t*-test Result[c]
1284	1.1	3	0.27	0.17
1285	0.9	8	0.18	0.004

[a]Replicate UV-exposed mixtures of labeled oligonucleotide and antibody H241 were electrophoresed in polyacrylamide gels under denaturing and reducing conditions. The amount of cross-linking was determined by two-dimensional laser densitometric scanning of lightly exposed autoradiograms. Scans of all the L chain and H chain derivatives were integrated for determination of the L/H ratio.

[b]Number of replicate samples electrophoresed and scanned.

[c]The t-test was applied to a comparison of the L/H ratios of the modified oligonucleotides to that(1.43) of the standard, 1138, which result was published in reference 16.

(oligonucleotide 1284), the ratio decreased. The presence of four hypoxanthines in place of all guanosines (oligonucleotide 1285) led to a more significant decrease.

CONCLUSION AND DISCUSSION

UV irradiation, a "zero-length" cross-linker of residues, provides a direct measurement of close interactions of H and/or L chains with DNA[16]. It was shown that modification of base at minor groove sites altered the relative cross-linking of L and H chains from 1.43[16] to 1.1 or 0.9 (Table 1). The antibody H241 appears to interact with the minor groove as well as with the major groove and the phosphate backbone. This results are consistent with previous indications, based on the results from solid-phase assays or competitive RIA in solution, that H241 can possibly interact with sites in the minor groove[15].

Photochemical cross-linking, like other types of affinity labeling, measures only a fraction of the DNA-antibody interactions. High resolution crystal structures of protein-DNA complexes have been reported in detail for EcoRI[21], 434 cro repressor fragment[22,23], Klenow fragment of *E.coli* DNA polymerase I[24,25], DNAse I[26], λcI repressor fragment[27]. In the absence of crystallographic data, affinity labeling can identify some of the close antibody-hapten contacts but provides only a partial picture[28,29].

Crystallographic analysis will be needed to define the precise three-dimensional structure of DNA-anti-DNA antibody complexes. For the present, affinity-labeling is helpful in identifying the antigenic determinants.

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