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Probing the Surface of Z-DNA with Anti-Nucleoside Antibodies†

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ABSTRACT: Antibodies specific for cytidine (C) and guanosine (G) were used to probe the surface of two Z-DNA conformers. When tested by ELISA, anti-G reacted with poly(dG-dC)·poly(dG-dC) treated with bromine water [Br-poly(dG-dC)·poly(dG-dC)] but anti-C did not. A weak reaction with anti-C was detected by dot immunobinding. In contrast, anti-C reacted strongly with poly(dG-dC)·poly(dG-dC) treated with *N*-acetoxy-2-(acetylamino)fluorene [AAF-poly(dG-dC)·poly(dG-dC)]; anti-G reacted weakly, despite the fact that most G residues had not been substituted with AAF. Neither antinucleoside bound to the B conformation of poly(dG-dC)·poly(dG-dC). In competition experiments, GMP was the most efficient competitor of the reaction of anti-G with Br-poly(dG-dC)·poly(dG-dC); AMP and TMP were 100-fold less efficient, and CMP did not compete to a significant extent. In contrast, the reaction of anti-Z with Br-poly(dG-dC)·poly(dG-dC) was not inhibited by nucleotides. Of five possible sites recognized on guanosine by anti-G antibodies (N1, C6, O6, N7, and C8), AMP and TMP share three or their equivalent and CMP only one. The binding of anti-C to AAF-poly(dG-dC)·poly(dG-dC) was inhibited best by CMP; AMP was 8 times less efficient; GMP and TMP were about 35-fold less efficient than CMP. Thus, although the amino group on the C4 position of CMP appears to be immunodominant, the capacity of GMP and TMP to inhibit the reaction indicates that other sites are also recognized in AAF-poly(dG-dC)·poly(dG-dC), e.g., the exposed C5 position. Recognition of cytidine in denatured DNA by anti-C is more specific: CMP is the best competitor; AMP did not compete, and GMP and TMP were 53-fold and 115-fold, respectively, less efficient. Therefore, different antigenic determinants are recognized in the two systems although parts may overlap. The data show that purine and pyrimidine residues in a Z-type double helix are accessible to nucleotide-specific proteins, in this case, specific antibodies. In addition, the accessibility of nucleotides depends upon the chemical nature of the Z duplex, illustrating that Z-DNA is polymorphic. Our findings also show that proteins with purine or pyrimidine specificity, but not necessarily specific for the Z conformation, can also bind to Z-DNA.

The possible existence of a novel form of double-stranded helical DNA was first suggested (Pohl & Jovin, 1972) when it was observed that the CD spectrum of poly(dG-dC)·poly(dG-dC) underwent an inversion when the concentration of salt in the solution was increased to 4 M NaCl. Subsequent proof that it was a left-handed or Z-DNA came from crystallographic studies, from fiber diffraction data, and from NMR and laser Raman studies [reviewed in Rich et al. (1984)].

The structure of left-handed Z-DNA is unlike that of right-handed DNA, e.g., B-DNA, in many respects (Rich et al., 1984). Z-DNA does not have a major and minor groove but, instead, has one deep helical groove analogous to the minor groove of B-DNA. In place of the major groove is a convex surface in which regions of the paired purines and pyrimidines in poly(dG-dC)·poly(dG-dC) bases are displayed. This is in marked contrast to B-DNA, in which the paired bases are internally located, i.e., "buried", and not accessible at the surface of the molecule.

The presence of purine and pyrimidine residues on the surface of Z-DNA has made possible the development of specific chemical probes that can detect Z regions in DNA that is predominantly right-handed (Herr, 1985; Johnson & Rich, 1985). These probes, e.g., diethyl pyrophosphate, are of low molecular weight. In the context of a possible role of Z-DNA in gene regulation (Rich et al., 1984), it would be of interest to determine whether the purine and pyrimidine bases are also accessible for binding to base-specific proteins.

Antibodies can be made that are specific for nucleosides and nucleotides sequences [Erlanger & Beiser, 1964; Wallace et al., 1971; reviewed in Stollar (1980)]. These antibodies have been shown to be highly specific for the base (or bases) in the immunogen and capable of reacting with the specific base in DNA only in regions where there is no base pairing, i.e., in denatured regions of DNA. In this regard, antinucleoside antibodies were recently used to detect local denaturation in

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DNA modified by platinum derivatives (Sundquist et al., 1986). Thus, native B-DNA is unreactive. As noted above, however, portions of the G and C residues are on the outer surface of Z-DNA. In this paper, we report on studies in which anti-C[1] and anti-G antibodies were used to probe the surface characteristics of two forms of Z-DNA, looking for, among other things, elements of polymorphism.

Materials and Methods

Materials

Antisera. Rabbits were immunized with bovine serum albumin conjugates of cytidine or guanosine, or with a 5-methyluridine–ovalbumin conjugate (Erlanger & Beiser, 1964). Globulin preparations were made by precipitating twice with ammonium sulfate (Strauss et al., 1960). Normal immunoglobulins were obtained in the same way from pooled rabbit sera.

Anti-Z-DNA antiserum was prepared by immunization of a rabbit (533) with AAF-poly(dG-dC)·poly(dG-dC) (Hanau et al., 1984).

A human autoimmune serum was from a patient with juvenile rheumatoid arthritis, adult-type, and was the gift of D. Estes, Department of Medicine, Columbia University. This serum reacted with *Escherichia coli* DNA in a Farr assay and with native DNA from salmon sperm, calf thymus, and pUC9, as well as with Br-poly(dG-dC)·poly(dG-dC) in solid-phase (ELISA and dot immunobinding) assays.

The anti-immunoglobulin antibodies, peroxidase or alkaline phosphatase tagged, were from Sigma (St. Louis, MO).

Antigens. Poly(dG-dC)·poly(dG-dC) (Pharmacia Molecular Biologicals, Piscataway, NJ) was treated with *N*-acetoxy-2-(acetylamino)fluorene as described in Santella et al. (1981). The extent of modification determined from the ratio A_{305}/A_{260} was 28%.

Brominated poly(dG-dC)·poly(dG-dC) was prepared by the method of Lafer (1982). The A_{295}/A_{260} absorbance ratio was 0.38, corresponding to bromination of 41% of the guanine residues and of 19% of the cytosine residues (Moller et al., 1984).

Salmon sperm DNA was obtained from Sigma (St. Louis, MO), as were AMP, CMP, TMP, and GMP.

Methods

ELISA. (A) Direct Binding Assay. The method described by Zouali and Stollar (1986) was used, with modifications.

Polystyrene 96-well plates (Corning 25855, Corning Glass Works, NY) were UV irradiated (Zouali & Stollar, 1986) for 18 h. They were then coated with 100 μL of antigen solution in PBSE. All nucleic acids were at 5 μg/mL, except for AAF-poly(dG-dC)·poly(dG-dC) which was at a concentration of 0.5 μg/mL. After 2.5 h at room temperature, the plates were washed 3 times with PBSET, blocked with PBSET containing 1% ovalbumin for 1 h at room temperature, and washed 3 times with PBSET. The antibody (100 μL/well) was diluted in PBSET containing normal rabbit serum at a final dilution 1:50 and 1% ovalbumin (w/v). The plates were incubated for 1 h at room temperature and washed 4 times with PBSET; bound antibodies were detected with 100 μL of peroxidase-tagged second antibody diluted in PBSET–ovalbumin (1%), using hydrogen peroxide as substrate and *o*-phenylenediamine as indicator. The absorbance at 490 nm was determined. Controls without antigen were assayed simultaneously, and their values were subtracted from the experimental values.

For assay of AAF-poly(dG-dC)·poly(dG-dC), addition of normal serum to the antibody diluent was necessary to decrease nonspecific binding. We chose to test Br-poly(dG-dC)·poly(dG-dC) under the same conditions, even though the results were identical with or without addition of normal serum.

For testing naturally occurring DNA, ovalbumin and normal rabbit serum were omitted.

(B) Inhibition Assay. Serial dilutions of inhibitors were incubated for 1 h at room temperature in glass tubes with the chosen dilution of antibody, in PBSET containing normal rabbit serum (1:50) and 1% ovalbumin. Aliquots (100 μL) of each mixture were added to the plates after the blocking step, and the procedure was continued as in the direct binding assay.

The percentage of inhibition was calculated by the formula (OD without inhibitor − OD with inhibitor)/(OD without inhibitor − OD background) × 100.

Dot Immunobinding Assay. (A) Direct Assay. A 0.5-μL portion of the antigen solution in PBSE (50–200 μg/mL) was deposited on nitrocellulose strips (BA 85; Schleicher & Schuell, Keene, NH) and allowed to dry. The strips were shaken in PBSET for 1 h at room temperature. The primary antibody was diluted in PBSET, and the incubation was performed under continuous agitation for 1 h and 30 min at room temperature. After three washes of 10 min each in PBSE containing 0.05% Tween 20 (v/v), the strips were incubated for 1 h at room temperature with the appropriate alkaline phosphatase linked anti-Ig, diluted in PBSET. The washing was repeated, and the bound antibody was revealed by using 3-indoxyl phosphate as substrate and nitro blue tetrazolium as indicator.

(B) Inhibition. Serial dilutions of inhibitors were incubated in glass tubes with the appropriate dilution of primary antibody, in PBSET, for 2 h at room temperature and 17 h at 4 °C. After having applied the antigen onto nitrocellulose as described in the direct assay, the membrane was cut into small squares, each bearing an individual dot, which were distributed in a 96-well microplate containing the mixtures of inhibitor and primary antibody, and the procedure was continued as indicated for the direct assay.

Results

Reactions with Br-Poly(dG-dC)·Poly(dG-dC). Figure 1 shows that, in an ELISA, anti-G reacted strongly with Br-poly(dG-dC)·poly(dG-dC) whereas no reaction was seen with anti-C. Anti-Z-DNA, a positive control, reacted strongly. These results were confirmed by dot immunobinding. Anti-G was positive at a 1:100 dilution, at which concentration anti-C was negative. Anti-C gave a weak positive reaction at a 1:20 dilution (results not shown).

Reactions with AAF-Poly(dG-dC)·Poly(dG-dC). In contrast, anti-G reacted very weakly with AAF-poly(dG-dC)·poly(dG-dC) (Figure 2). Anti-C, in contrast to its negligible reaction with the brominated polymer, reacted strongly with AAF-poly(dG-dC)·poly(dG-dC) (Figure 2). A positive reaction was also seen with anti-Z-DNA. These observations were confirmed by dot immunobinding (not shown).

Dot immunobinding carried out with antibodies to thymidine (anti-T) showed no reaction with Br-poly(dG-dC)·poly(dG-

[1] Abbreviations: AAF-poly(dG-dC)·poly(dG-dC), poly(dG-dC)·poly(dG-dC) treated with *N*-acetoxy-2-(acetylamino)fluorene; Br-poly(dG-dC)·poly(dG-dC), poly(dG-dC)·poly(dG-dC) treated with bromine water; anti-G, anti-C, anti-A, and anti-T, antibodies to guanosine, cytidine, adenosine, and 5-methyluridine, respectively, raised according to Erlanger and Beiser (1964); PBSE, 0.01 M KH_2PO_4, 0.15 M NaCl, and 0.001 M Na_2EDTA, pH 7.4; PBSET, PBSE + 0.1% (v/v) Tween 20; EDTA ethylenediaminetetraacetic acid.

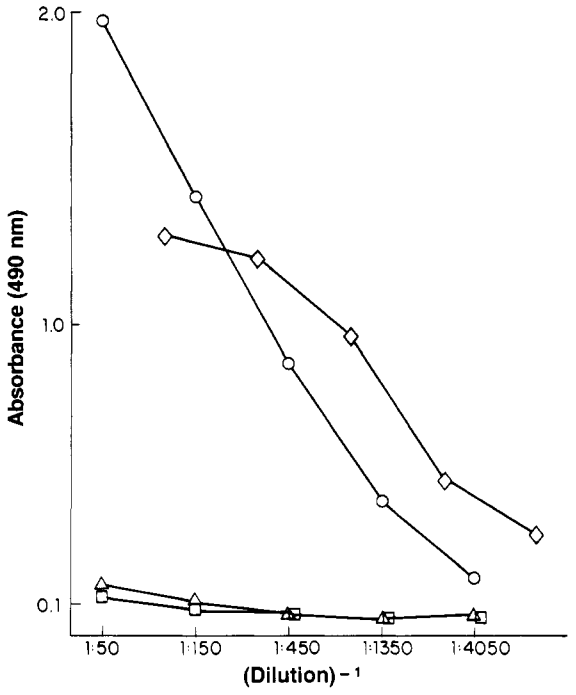

FIGURE 1: Binding of anti-G (○), anti-C (△), anti-AAF-poly(dG-dC)·poly(dG-dC) (◇), and normal rabbit immunoglobulins (□) to Br-poly(dG-dC)·poly(dG-dC). Antibody binding was measured by ELISA.

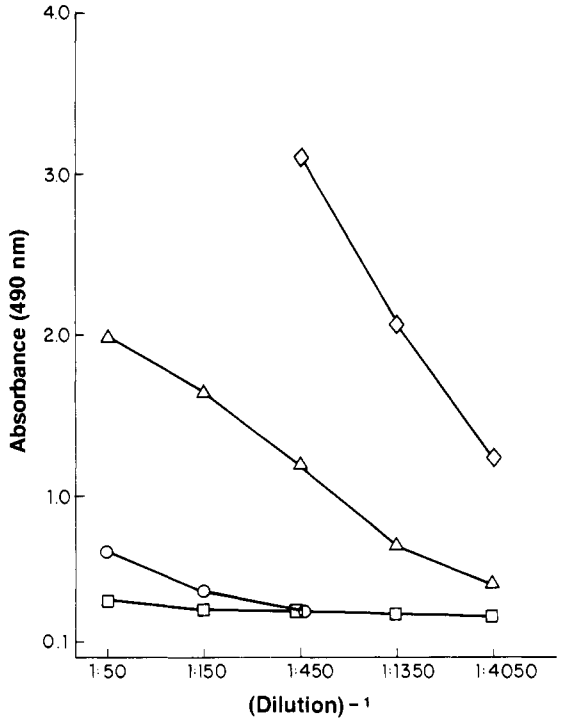

FIGURE 2: Binding of anti-G (○), anti-C (△), anti-AAF-poly(dG-dC)·poly(dG-dC) (◇), and normal rabbit immunoglobulins (□) to AAF-poly(dG-dC)·poly(dG-dC).

dC). As expected, anti-T did bind to denatured DNA from salmon sperm.

Reactions with B-DNA. As opposed to their reactions with base residues in Z-DNA conformers, neither anti-C nor anti-G reacted significantly by ELISA with poly(dG-dC)·poly(dG-dC) in its B conformation, i.e., in low salt (Rich et al., 1984). Control reactions with anti-Z were also negative, but a reaction was seen with a human serum containing autoantibodies to DNA. Anti-Z exhibited a very slight reaction by the dot immunobinding technique when it was carried out in PBSE.

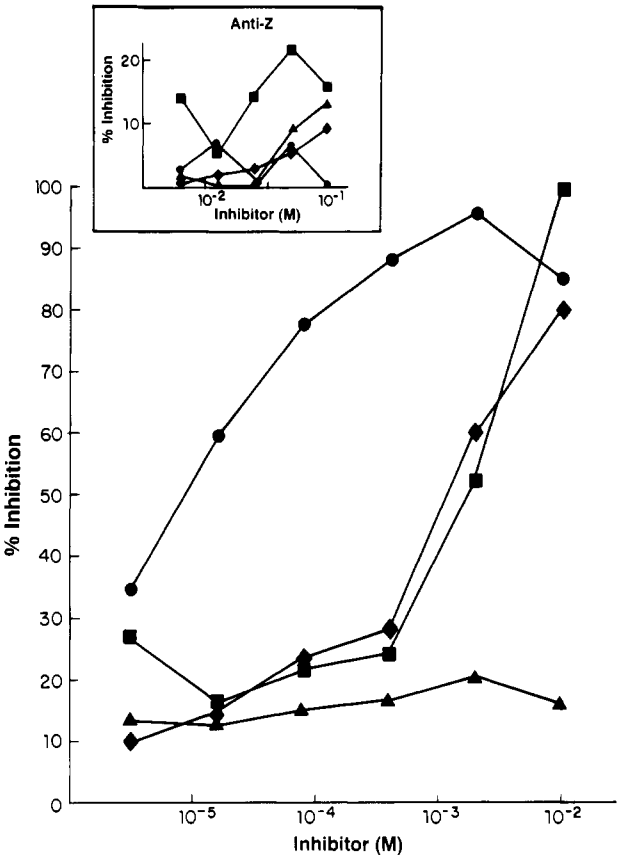

FIGURE 3: Inhibition of binding of anti-G to brominated poly(dG-dC)·poly(dG-dC) by GMP (●), AMP (■), TMP (♦), and CMP (▲). Inset: Same experiment with anti-AAF-poly(dG-dC)·poly(dG-dC). The reactions were examined by ELISA after preincubation of the antibodies with increasing amounts of competitors and expressed as a percentage of binding in the absence or inhibitor.

The reaction was greatly enhanced at 3.5 M NaCl (data not shown).

Inhibition Studies. The specificity of the reaction of anti-G with Br-poly(dG-dC)·poly(dG-dC) was examined further by inhibition studies. Dot immunobinding was inhibited completely by GMP at 0.1 mM; no inhibition by CMP was seen even at 10 mM (not shown). The analysis was carried out further by ELISA, which confirmed that GMP was the most efficient competitor (Figure 3). A 100-fold higher concentration of AMP and TMP were required for 50% inhibition; inhibition by CMP was less than 20% even at 10 mM.

The reaction of anti-Z-DNA with Br-poly(dG-dC)·poly(dG-dC) could not be inhibited by as much as 100 mM GMP, CMP, or TMP using either dot immunobinding or ELISA (Figure 3, inset). AMP appeared to inhibit slightly (Figure 3, inset).

The reaction of anti-C with AAF-poly(dG-dC)·poly(dG-dC) was inhibited to some extent by all four nucleotides studied, with CMP being the most potent inhibitor (Figure 4). Fifty percent inhibition required 8 times more AMP than CMP. At the maximal concentration of inhibitor used, 10 mM, GMP inhibited 40% of the reaction and TMP 35%. Extrapolation of the curves to 50% inhibition gave an inhibitory capacity of about 30, relative to CMP, for both nucleotides. These were unforeseen observations since the antibodies directed toward nucleosides conjugated to a protein carrier by the periodate procedure are usually more selective (Erlanger & Beiser, 1964; Garro et al., 1971). In order to check the specificity of the antibodies used in this study, we analyzed the capacity of nucleotides to inhibit the reaction of anti-C with denatured

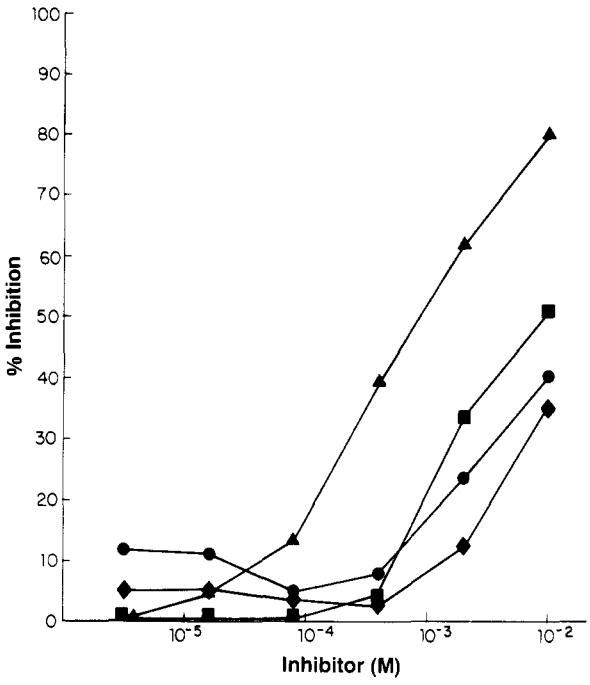

FIGURE 4: Inhibition of binding of anti-C to AAF-poly(dG-dC)·poly(dG-dC) by GMP (●), AMP (■), TMP (♦), and CMP (▲), as determined by ELISA (see legend to Figure 3).

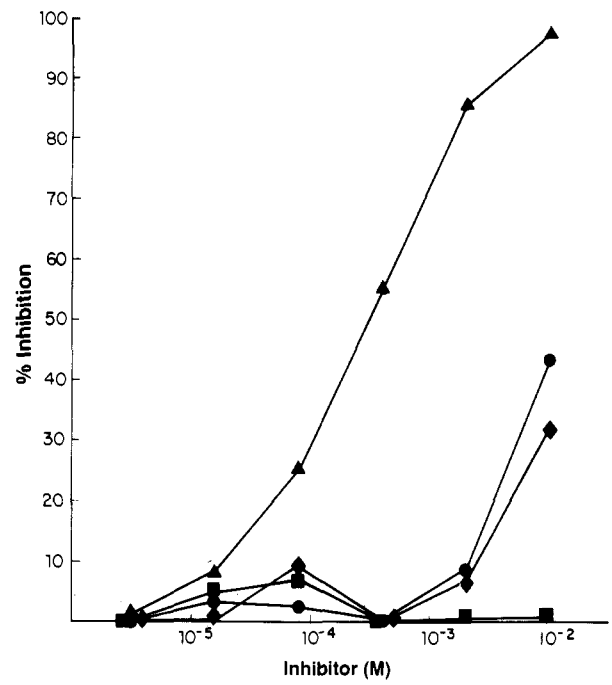

FIGURE 5: Inhibition of binding of anti-C to denatured DNA from salmon sperm by GMP (●), AMP (■), TMP (♦), and CMP (▲).

DNA from salmon sperm by the ELISA procedure. As seen in Figure 5, no inhibition by AMP was observed, and the inhibitory capacities of GMP and TMP were about 1/53 and 1/115, respectively, that of CMP.

Discussion

We have demonstrated that anti-guanosine and anti-cytidine antibodies react with base residues in left-handed Z-DNA. This is in agreement with the model proposed by Wang et al. (1979), in which the G·C base pairs are at the periphery of the double-stranded helix, with the C5 of cytosine and the N7 and C8 of guanine in the most exposed portions. However, in two Z-conformers obtained by different modifications of poly(dG-dC)·poly(dG-dC), the bases were recognized to a different extent: anti-G reacted strongly with Br-poly(dG-dC)·poly(dG-dC) but weakly with AAF-poly(dG-dC)·poly(dG-dC). The reverse was observed with anti-C, which reacted only with the AAF derivative.

The Br-poly(dG-dC)·poly(dG-dC) studied had 41% of its guanine residues modified (Moller et al., 1984), leaving many unsubstituted guanines available for reaction with antibody. Since our antibody was polyclonal and capable of reacting with a variety of epitopes on guanine, it is possible that reaction also occurred with the brominated purines in spite of their modification. Whatever the case, the specificity of the reaction was shown by the fact that GMP was at least 100-fold more efficient an inhibitor than the other nucleotides (Figure 4).

The weak reaction of anti-C with Br-poly(dG-dC)·poly(dG-dC) (Figure 1) was unexpected as only 19% of the C residues were brominated. Reaction was observed only by dot immunobinding, at a dilution of 1:20. None was seen by ELISA. In Z-DNA, cytosine is in a somewhat recessed position on the convex outer surface of the molecule with the guanine imidazole rings projecting further away from the axis than the cytosine rings (Fujii et al., 1982). Indeed, Moller et al. (1984) ascribed the finding that the number of guanine residues brominated was twice that of cytosine residues to a difference in reactivity associated with the ready access of bromine to the guanine imidazole ring. As only 19% of the cytidines were brominated in the Z polymer used in the present work, steric hindrance by the bromine atoms could account only in part for the low reactivity with anti-C. We therefore conclude that, unlike the guanosine residues, the cytosine residues in Br-poly(dG-dC)·poly(dG-dC) are recessed to a degree that they are not accessible to reaction with specific antibody.

The situation is reversed with AAF-poly(dG-dC)·poly(dG-dC): anti-C reacted well; the reaction with anti-G was very weak (Figure 2). AAF binds to the C8 position of guanine and stabilizes the Z conformation (Rich et al., 1984). Two possible models of Z-DNA with *syn*-guanosine have been proposed: In the first one, the carcinogen bound to the G residue is situated outside the double helix and does not induce conformational distortion but stabilizes the Z form (Sage & Leng, 1980; Santella et al., 1981). In this case, the very bulk of AAF could block accessibility to the substituted guanines. Alternatively, a base displacement (insertion–denaturation) model (Grunberger et al., 1970; Fuchs & Daune, 1972) would predict that the fluorene residue is stacked with the adjacent cytosine on the same strand and the modified guanosine is located at the exterior of the helix (Hingerty & Broyde, 1982; Grunberger et al., 1985). In both conformations, the unsubstituted guanines, i.e., 72% of the purines, should be free to react with anti-G. Yet only a slight reaction is seen (Figure 2). An explanation may lie in the possibility [cf. Sage and Leng (1980)] that the distribution of the AAF residues is random throughout the molecule with no strong clustering. If so, reaction of unsubstituted G residues with antibody might be hindered by the large AAF groups on neighboring guanines.

There is considerable reaction between anti-C and AAF-poly(dG-dC)·poly(dG-dC) (Figure 2). Immunological recognition of a purine or pyrimidine base cannot occur if it is involved in a B- or A-type helix [Erlanger & Beiser, 1964; Nahon et al., 1967; reviews by Lacour et al. (1973) and Stollar (1975, 1986)] although an exception, which will be discussed later, has been described. Therefore, the antibody is able to bind cytosine either because the base is accessible in a Z conformation or because AAF-poly(dG-dC)·poly(dG-dC) is denatured. The latter possibility has already been raised

(Grunberger et al., 1985) in the context of the base displacement model, but denaturation should also allow reaction with anti-G, which is not seen. That AAF-poly(dG-dC)·poly(dG-dC) is in the Z form has been amply documented (Sage & Leng, 1980; Santella et al., 1981; Grunberger et al., 1985) and is illustrated by its reaction with a serum specific for Z-DNA (Figure 2). Thus, we can conclude that the cytosines in the Z conformation of AAF-poly(dG-dC)·poly(dG-dC) are accessible to antibody, in contrast to their inaccessibility in the brominated polymer (Figure 1). This is consistent with Z-DNA being polymorphic.

The reaction of anti-G with Br-poly(dG-dC)·poly(dG-dC) was inhibited best by GMP; AMP and TMP were less efficient by 2 orders of magnitudes, and CMP did not compete at all (Figure 3). In a study of antigenic domains within nucleosides, Munns et al. (1984) concluded that the major epitope of guanine encompassed the N1, C6, O6, and N7 atoms. Analyzing the reaction of a monoclonal antibody to GMP with its specific hapten or with denatured DNA, Stollar et al. (1986a) concluded that major interactions involved the purine base in the region of N1, C6, and N7. They also suggested that the C8 proton was involved with interaction with antibody, although to a limited extent. Taken together, epitopes recognized by anti-G include N1, C6, O6, N7, and C8 of guanosine. Of these, AMP shares C6, N7, and C8; the C4, O4, and N3 groups of TMP are equivalent to C6, O6, and N1 of guanosine. Both AMP and TMP inhibit the reaction of anti-G with Br-poly(dG-dC)·poly(dG-dC), although not as well as GMP (Figure 3). CMP which does not inhibit (Figure 3), shares only one group, C4, which might be equivalent to C6 in G. Moreover, O6, N7, and C8, which are on the surface of Z-DNA (Wang et al., 1979), are missing in CMP.

Less is known about the reaction of anti-C with its homologous hapten. Munns et al. (1984), in studies on the specificity of polyclonal anti-C antibodies, concluded that the major epitope in C is dominated by the C4 and N4 atoms and that the NH_2 group attached to C4 was of particular importance. This is in accord with our inhibition data (Figure 4). The best heterologous inhibitor of the reaction of anti-C with AAF-poly(dG-dC)·poly(dG-dC) was AMP which has an amino group attached to C6, equivalent to C4 in cytosine; however, GMP and TMP, in which the NH_2 group is replaced by a keto oxygen, still possess significant inhibitory capacity, an indication that anti-C recognizes additional regions besides N4 on the cytidines.

Although AMP competed very efficiently in the reaction of anti-C with AAF-poly(dG-dC)·poly(dG-dC) (Figure 4), it was devoid of any inhibitory capacity when anti-C was tested with denatured DNA (Figure 5). Thus, different antigenic determinants are recognized in the two systems although parts may overlap. GMP and TMP competed but were less efficient inhibitors of the single-stranded form than of the Z form. Recognition of cytidine in denatured DNA by anti-C is, therefore, more specific than its recognition in AAF-poly(dG-dC)·poly(dG-dC). In the latter case, the cytosines are involved both in base pairing and in base stacking, whereas in the former, they are free to react with the antibody which, in all probability, recognizes more epitopes in the single-stranded form. Indeed, assuming that 50% inhibition is related to K, there is a difference of about 0.75 kcal between the reaction of anti-C with the single-stranded DNA or with the Z conformer.

The binding of anti-Z to Br-poly(dG-dC)·poly(dG-dC), in contrast to that of anti-G, was not inhibited by any of the nucleotides tested (Figure 3, inset). In this respect, it resembles a number of antibodies that recognize nucleic acid conformations (Z and others) (Stollar, 1975, 1986; Malfoy et al., 1982; Pohl et al., 1982; Zarling et al., 1984; Lacour et al., 1973; Lee et al., 1984). Whether directed toward double-stranded RNA, RNA·DNA hybrids, or right- or left-handed DNA, they have in common their inability to be completely inhibited by nucleotides or by one of the constituent polymers. These antibodies recognize areas on the surface of duplexes that encompass accessible portions of the bases only, or one or two phosphodiester chains in addition (Stollar, 1986; Stollar et al., 1986b; Zarling et al., 1984; Delage et al., 1984). Our results are therefore consistent with what has been found with other conformation-specific antibodies, namely, that epitopes on individual constituent bases make only small contributions to the overall energies of binding.

The fact that anti-G and anti-C recognize Z-DNA as opposed to the B form is reinforced by our finding that poly(dG-dC)·poly(dG-dC), under conditions in which it is in the right-handed B-DNA conformation, does not react with either anti-Z or the antinucleosides. It could, however, be detected by a serum containing autoantibodies to DNA. Adouard et al. (1985) reported a reaction between antinucleoside, anti-5-methylcytidine, and native DNA from *Xanthomonas oryzae* XP12 bacteriophage and suggested the possibility that it could be due to a special conformation of the DNA (different from the Z form) or to adjacent clusters of methylated cytosines. Fujii et al. (1982) pointed out, however, that the methyl groups on the C5 of cytosine protrude from the major groove of the B-DNA helix. This also could explain recognition by an antinucleoside antibody of a B-DNA in which 34% of the bases are 5-methylcytosines (Kuo et al., 1980).

In summary, we have used antibodies directed against guanosine and cytidine to probe the surface of two Z-DNA conformers and have found that guanine and cytosine are not equally accessible to protein binding in the two conformers. We have thus confirmed by immunochemical means the observation (Wang et al., 1981; Drew et al., 1980; Leslie et al., 1980) that Z-DNA is polymorphic. This polymorphism should also serve to regulate the recognition of Z-DNA by other specific proteins, in particular those that might be involved in gene regulation [cf. Lafer et al. (1985)]. Our findings also show that proteins specific for purine or pyrimidines, but not *necessarily* specific for the Z conformation, can also bind to Z-DNA. This must be taken into consideration in procedures designed to isolate Z-DNA binding proteins that might have regulatory function [reviewed in Rich et al. (1984)].

Registry No. Poly(dG-dC)·poly(dG-dC), 36786-90-0.

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Primary Structure of the Mouse Laminin B2 Chain and Comparison with Laminin B1[†,‡]

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ABSTRACT: One of the major components of basement membranes is the glycoprotein laminin, made up of three disulfide-bonded subunits, the A, B1, and B2 chains. We have isolated and sequenced overlapping mouse laminin B2 chain cDNA clones covering 7562 base pairs. The deduced amino acid sequence predicts that the mature B2 chain consists of 1572 residues, has an unglycosylated molecular weight of 173 541, and possesses 14 potential N-linked glycosylation sites. Analysis of the predicted secondary structure shows the presence of six domains, two rich in α-helical structure, two composed of homologous cysteine-rich repeat units, and two globular regions. The organization of the molecule is very similar to that of the mouse laminin B1 chain, and significant sequence homology between the B1 and B2 chains was found in their two cysteine-rich domains and in their amino-terminal globular domains.

Laminin is a major and widely distributed component of basement membranes (Chung et al., 1979; Timpl et al., 1979). The molecule consists of three nonidentical glycoprotein subunits linked by disulfide bonds, the A (M_r 400 000), B1 (M_r 230 000), and B2 (M_r 220 000) chains (Cooper et al., 1981; Howe & Dietzschold, 1983). Laminin is involved in a variety of biological processes including cell adhesion, migration, morphogenesis, metastasis, differentiation, and neurite outgrowth [reviewed by Kleinman et al. (1985)]. Binding of laminin to other basement membrane constituents including type IV collagen, heparan sulfate proteoglycan, and entactin

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