Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3546–3550, June 1981
Biochemistry

Antibodies specific for left-handed Z-DNA

[radioimmunoassay/circular dichroism/brominated poly(dG-dC)·poly(dG-dC)/murine lupus]

EILEEN M. LAFER*, ACHIM MÖLLER†, ALFRED NORDHEIM†, B. DAVID STOLLAR*, AND ALEXANDER RICH†

*Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111; and †Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Contributed by Alexander Rich, March 30, 1981

ABSTRACT **We prepared a brominated poly(dG-dC)·poly(dG-dC) which forms a stable Z-DNA helix under physiological salt conditions. Rabbits and mice were immunized with brominated and unbrominated poly(dG-dC)·poly(dG-dC) complexed with methylated bovine serum albumin. Antibodies specific for Z-DNA were produced. These antibodies were found not only in the sera of animals immunized with the low-salt stabilized Z-DNA [Br-poly(dG-dC)·poly(dG-dC)] but also in sera from animals immunized with the unbrominated B-DNA form of the polymer. From this it is inferred that the unbrominated poly(dG-dC)·poly(dG-dC) was partially converted to Z-DNA by its combination with the basic protein methylated bovine serum albumin. In addition to specific anti-Z-DNA antibody populations, two other interesting types of antibody populations were found. One of these reacted with both the Z and B forms of poly(dG-dC)·poly(dG-dC). This antibody may be converting the polymer from the B-DNA to the Z-DNA form. The other type of antibody was specific for a B form of poly(dG-dC)·poly(dG-dC) and did not react at all with the Z form. The antibodies raised to Z-DNA were shown to be highly specific for Z-DNA and did not react with B-DNA, RNA, DNA·RNA hybrids, or a number of other polynucleotides. This specificity for Z-DNA will make possible their use as reagents for determining the presence of Z-DNA in biological systems. Sera of autoimmune lupus mice were also shown to have a considerable amount of naturally occurring anti-Z-DNA antibody activity.**

Atomic resolution x-ray crystallographic analysis has defined a family of left-handed DNA helical structures composed of alternating dG and dC residues (1, 2). This two-stranded helix is built with anti-parallel sugar-phosphate chains, and the bases have Watson–Crick hydrogen bonding; however, the bases have a different orientation relative to the backbone than is found with right-handed B-DNA. The guanine residues are in the *syn* conformation. The helix has 12 base pairs per turn, and there is only one groove in the molecule because the base pairs form the outer convex wall of the helix. The phosphate groups from the chains on either side of the groove are closer together than in B-DNA. The left-handed Z helix which is found in the crystals corresponds to the form of poly(dG-dC)·poly(dG-dC) that was first found to exist in solutions at high ionic strength (3). The reversible interconversion between the Z helix at high ionic strength and a structure resembling right-handed B-DNA at low ionic strength can be measured by a corresponding inversion of the polymer's circular dichroism spectrum (3).

Sequences of alternating dG and dC residues or of alternating purine and pyrimidine occur in natural DNA. Such regions could exist in either a B or Z structure, depending upon the local ionic environment, the presence of binding proteins, or the topological constraints of supercoiling. It thus would be useful to have a specific probe to test for the presence of the Z helix as a small fraction of total nucleic acid structure. Antibodies specific for Z helix may provide such a probe. Antibodies to double-stranded RNA (4), RNA·DNA hybrids (5), native DNA (6), and triple-helical polynucleotides (7) have served as specific reagents that are sensitive to changes in helical shape (8). They probably recognize antigenic sites comprising the pentose-phosphate backbones of adjacent strands over a span of two or three base pairs (6, 9).

Here we describe the production and characterization of antibodies that show specificity for the Z helix of poly(dG-dC)·poly(dG-dC). To study the serological reactions at relatively low ionic strength, the Z form of the helix was stabilized by high-salt bromination of guanine and cytosine residues. Antibodies specific for the Z helix were induced by both the brominated polymer and by unbrominated poly(dG-dC)·poly(dG-dC) complexed to the positively charged carrier methylated bovine serum albumin. The bromine atoms are thus not necessary for antibody production. Antibodies that react with Z-DNA were also found in the sera of mice with an autoimmune disease similar to human systemic lupus erythematosus.

MATERIALS AND METHODS

Polynucleotides. Poly(dG), poly(dG-dC)·poly(dG-dC), and poly(dG)·poly(dC), were purchased from P-L Biochemicals; poly(A)·poly(dT) was from Collaborative Research (Waltham, MA). Calf thymus DNA was obtained from Worthington. Baby hamster kidney cell RNA was provided by Victor Stollar. Polynucleotides were radioactively labeled by the *in vitro* nick-translation technique described by Rigby *et al.* (10). [^{3}H]Thymidine-labeled *Escherichia coli* DNA was prepared as described (11).

Bromination of Poly(dG-dC)·poly(dG-dC). Poly(dG-dC)·poly(dG-dC) was dissolved in 20 mM sodium citrate, pH 7.2/1 mM EDTA/3.5 M NaCl. The high salt concentration lowered the pH to 6.4. Aqueous bromine reagent was prepared by adding bromine to distilled water and mixing thoroughly until the water became saturated with bromine at room temperature. The bromine-saturated water was added to the polymer in a ratio of 1.3:1 (bromine/nucleotide). The reaction was allowed to proceed at room temperature for 10 min with occasional mixing. Excess bromine was subsequently removed by bubbling air through the reaction mixture (kept in an ice-water bath) for 10 min. The solution was then exhaustively dialyzed against 15 mM Tris·HCl, pH 7.2/150 mM NaCl/1 mM EDTA. A more complete description of brominated poly(dG-dC)·poly(dG-dC) will be published elsewhere.

Antisera. Two New Zealand white rabbits (441 and 442) were immunized first by injection, at multiple intradermal sites, with 50 μg of Br-poly(dG-dC)·poly(dG-dC) complexed with 75 μg of methylated bovine serum albumin (Sigma) and emulsified with complete Freund's adjuvant. Another rabbit (443) was immunized first at multiple intradermal sites with 50 μg of unbrominated poly(dG-dC)·poly(dG-dC) similarly complexed with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

methylated albumin and emulsified. Similar injections, but with incomplete Freund's adjuvant, were given on days 15, 21, 35, and 54 after the first immunization. Sera were obtained on days 28, 41, 47, and 61 and were heated at 56°C for 10 min before use. C57BL/6 mice received 50 μg of brominated or unbrominated polymer with 75 μg of methylated albumin in 0.2 ml intraperitoneally, without adjuvant, on days 1, 15, and 27 and were bled on day 35. MRL/lpr mice were obtained from Edwin Murphy and John Roth (The Jackson Laboratory, Bar Harbor, ME) through the courtesy of Robert Schwartz (Tufts University Cancer Research Center). The sera used in these studies were obtained from 5-month-old mice.

Radioimmunoassays were performed as described (11) except that 0.2 M NaCl was included in the standard buffer.

RESULTS

Right-handed B-DNA differs from left-handed Z-DNA in a number of features, the most significant of which are the conformational differences in the deoxyguanosine residues and the phosphate-sugar folding. In low-salt solutions, poly(dG-dC)·poly(dG-dC) is stable as B-DNA; it converts to Z-DNA only in the presence of increased concentrations of salt (3). This difference in stability is principally associated with the fact that the phosphate groups are closer together in Z-DNA than in B-DNA, and salt provides electrostatic shielding. It is likely that the Z-DNA conformation can be stabilized by other conditions such as topological constraints or the presence of basic proteins.

In order to work with the molecule under physiological salt conditions, we elected to utilize the difference in deoxyguanosine *syn* and *anti* conformations as a way of stabilizing the molecule in the Z conformation. The C8 position in the imidazole ring of guanine is on the outer surface of Z-DNA whereas in B-DNA it is blocked by the sugar-phosphate chain. Addition of bulky groups to the C8 position stabilizes the Z-DNA conformation. This has already been demonstrated with the carcinogen acetoxyaminofluorene (12, 13).

In this investigation, low-salt stabilization of Z-DNA was accomplished by brominating the molecule, which replaces H atoms by larger Br atoms in the C8 position of guanine and, to a lesser extent, in the C5 position of cytosine. Brominating 45% of the guanine and 20% of the cytosine residues stabilized the Z conformation as shown by the complete inversion of the circular dichroism spectrum. Spectra of the brominated and unbrominated forms of poly(dG-dC)·poly(dG-dC) are shown in Fig. 1. The brominated polymer's circular dichroism in 0.15 M NaCl is similar to that displayed by the unbrominated polymer under high-salt conditions (3). Furthermore, this spectrum was maintained in the solutions used for subsequent serological assays.

Bromine-stabilized Z-DNA solutions were then prepared for both immunization and radioimmunoassays. New Zealand White rabbits were immunized with either brominated or unbrominated poly(dG-dC)·poly(dG-dC) complexed with methylated bovine serum albumin. Sera were collected at various times and tested for their ability to bind ^{3}H-labeled brominated Z form or ^{3}H-labeled unbrominated B form. Fig. 2*A* shows a direct binding curve for serum collected from rabbit 441 on day 28. This rabbit was immunized with Br-poly(dG-dC)·poly(dG-dC), and the serum bound strongly to ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC) (Z form) with a titer of 1200 (the reciprocal of the serum dilution at the midpoint of the titration curve); in contrast, even undiluted serum did not bind to ^{3}H-labeled poly(dG-dC)·poly(dG-dC) (B form). Subsequent serum samples from this animal showed the gradual appearance of an activity capable of binding to ^{3}H-labeled poly(dG-dC)·poly(dG-dC) (B form) (Fig. 2*B*).

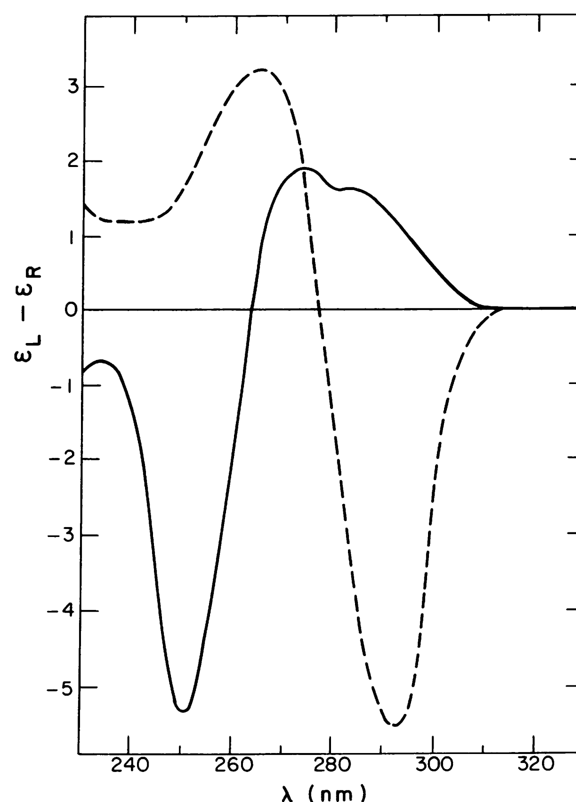

FIG. 1. Circular dichroism spectra of poly(dG-dC)·poly(dG-dC) (B form) (——) and Br-poly(dG-dC)·poly(dG-dC) (Z form) (----) in 15 mM Tris·HCl, pH 7.2/150 mM NaCl/1 mM EDTA.

The reaction with ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC) (Z form) was slightly stronger than that of the 28-day serum, with a titer of 2100; the titer for binding to ^{3}H-labeled poly(dG-dC)·poly(dG-dC) (B form) was 50. Sera collected from rabbit 442, which was immunized in the same way as rabbit 441, displayed similar strong binding of ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC) but no binding of ^{3}H-labeled poly(dG-dC)·poly(dG-dC) even after 2 months of immunization.

Rabbit 443 was immunized with unbrominated poly(dG-dC)·poly(dG-dC) complexed with methylated bovine serum albumin. Even though the injected polymer was in the B conformation, as judged by circular dichroism, the serum collected on day 28 bound primarily to the brominated Z form of the polymer, with a titer of 20. This serum did display a small amount of binding to unbrominated poly(dG-dC)·poly(dG-dC), but this was seen clearly only with undiluted serum.

C57BL/6 mice were also immunized with both brominated and unbrominated poly(dG-dC)·poly(dG-dC) complexed with methylated bovine serum albumin. The results were similar to those seen with the rabbits. Mice injected with Br-poly(dG-dC)·poly(dG-dC) (Z form) complexed with the albumin produced a vigorous anti-Z response (titers >1000), whereas mice injected with unbrominated poly(dG-dC)·poly(dG-dC) (B form) complexed with the albumin produced a weaker yet significant anti-Z response. At a 1:10 serum dilution, sera from the latter animals bound 10–20% of the Z form of the polymer and showed no binding of the B form.

The specificities of the various antibody populations were studied by competitive radioimmunoassay. Antibody dilutions corresponding to the linear portion of the binding curves were chosen. The ability of various unlabeled nucleic acids and polynucleotides to inhibit the reaction between antibody and ^{3}H-labeled antigen was determined. With serum collected on day

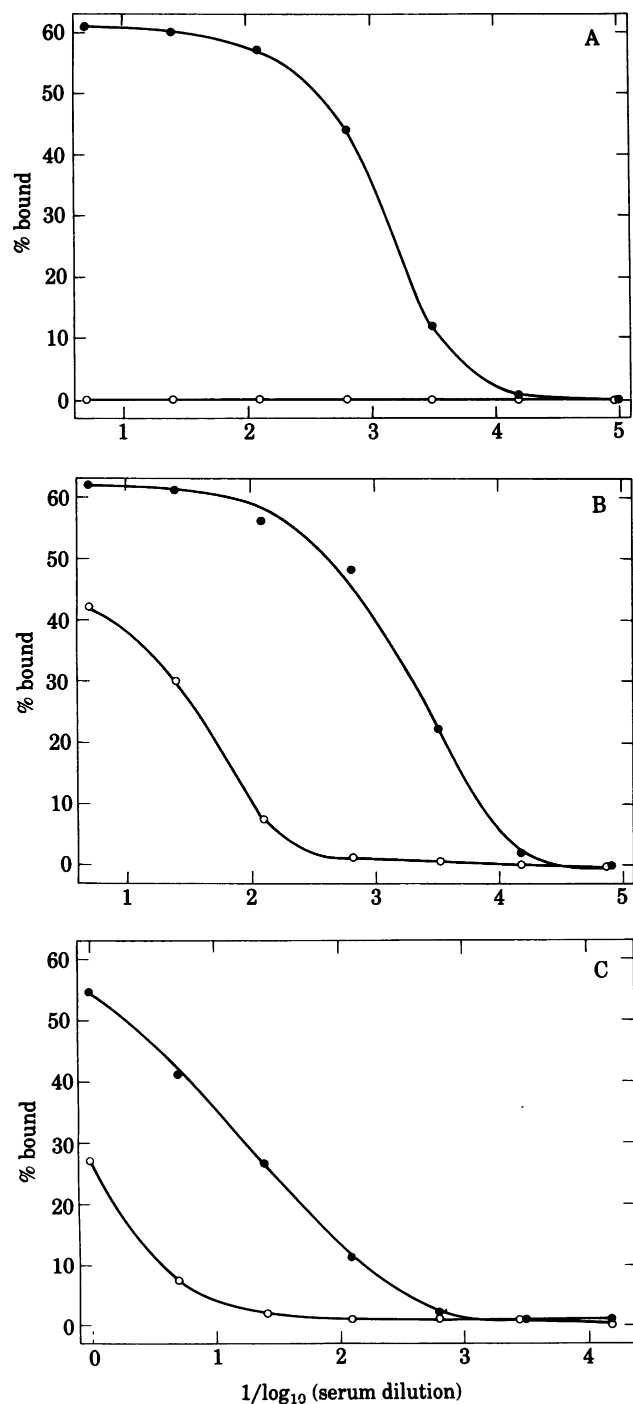

FIG. 2. Binding of rabbit antisera to ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC) (Z form) (●——●) and ^{3}H-labeled poly(dG-dC)·poly(dG-dC) (B form) (○——○). (*A*) Serum Ra 441a drawn on day 28 from rabbit 441; (*B*) serum Ra 441h drawn on day 61 from rabbit 441; (*C*) serum Ra 443a drawn on day 28 from rabbit 443. Undiluted normal rabbit serum bound 0.4% of the ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC) and 0.2% of the ^{3}H-labeled poly(dG-dC)·poly(dG-dC).

28 from rabbit 441, 45 ng of unlabeled Br-poly(dG-dC)·poly(dG-dC) (Z form) was required to inhibit binding of the ^{3}H-labeled brominated Z polymer by 50% (Fig. 3*A*). No inhibition was seen with up to 10 μg of poly(dG-dC)·poly(dG-dC) (B form), Br-poly(dG), poly(dG), Br-poly(dG)·poly(dC), or poly(dG)·poly(dC) or with the same amount of native or denatured calf thymus DNA, baby hamster kidney RNA, or poly(A)·poly(dT) (data not shown). The same result was observed with the 60-day serum

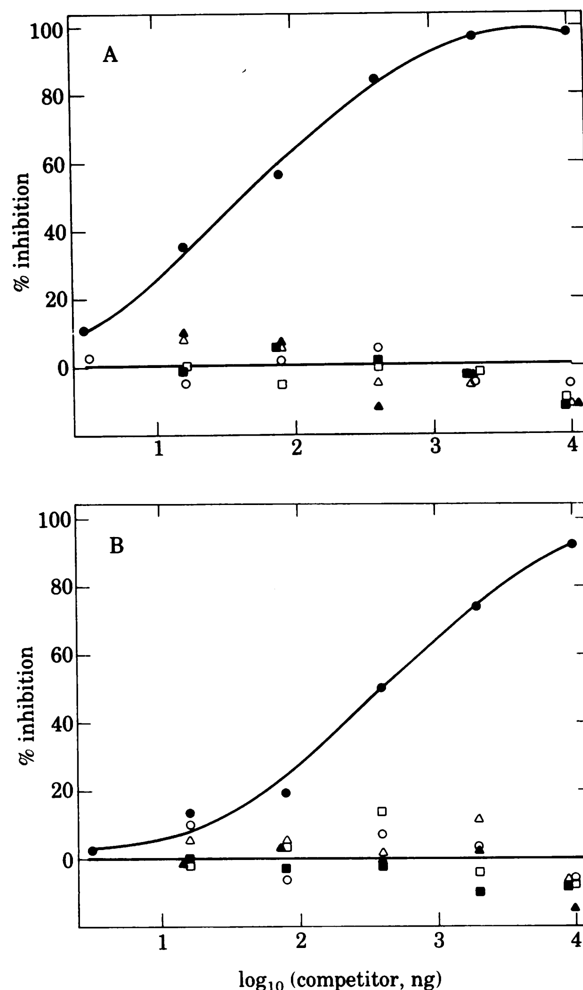

FIG. 3. Competitive radioimmunoassays with ^{3}H-labeled Z-DNA. Rabbit serum binding to ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC) was subjected to competition by Br-poly(dG-dC)·poly(dG-dC) (●), poly(dG-dC)·poly(dG-dC) (○), Br-poly(dG) (▲), poly(dG) (△), Br-poly(dG)·poly(dC) (■), and poly(dG)·poly(dC) (□). (*A*) Serum Ra 441a was used at a 1:625 dilution; it bound 28% of the ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC) in the absence of competitors. (*B*) Serum Ra 443a was used at a 1:25 dilution; it bound 26% of the ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC) in the absence of competitors. A 1:25 dilution of normal rabbit serum bound 0.9% of the ^{3}H-labeled Br-poly(dG-dC)·poly(dG-dC).

of this rabbit (tested in competition for binding labeled Z polymer) and with the serum from rabbit 442.

Serum Ra 443a bound the labeled Z polymer even though the immunogen was in the B form. This binding was specific for the Z form in competitive assays also (Fig. 3*B*); 400 ng of unlabeled Br-poly(dG-dC)·poly(dG-dC) (Z form) caused 50% reduction in binding. Again, no competition was seen with up to 10 μg of any of the above nucleic acids, including the unbrominated immunizing polymer. It is apparent that even though sera Ra 441h and Ra 443a could bind both the Z and B forms of poly(dG-dC)·poly(dG-dC), their antibody populations that bound the Z form were in fact specific only for that form and did not show cross competition with the B polymer.

The specificities of the antibody populations that bound labeled B polymer were then tested by competitive radioimmunoassays (Fig. 4). The serum from rabbit 441h (immunized with Z form) showed competition by both Z- and B-DNA but not by native or denatured calf thymus DNA, poly(dG), or poly(dG)·poly(dC). Similar amounts (30–40 ng) of the Z and B forms were required for 50% competition. Because this assay

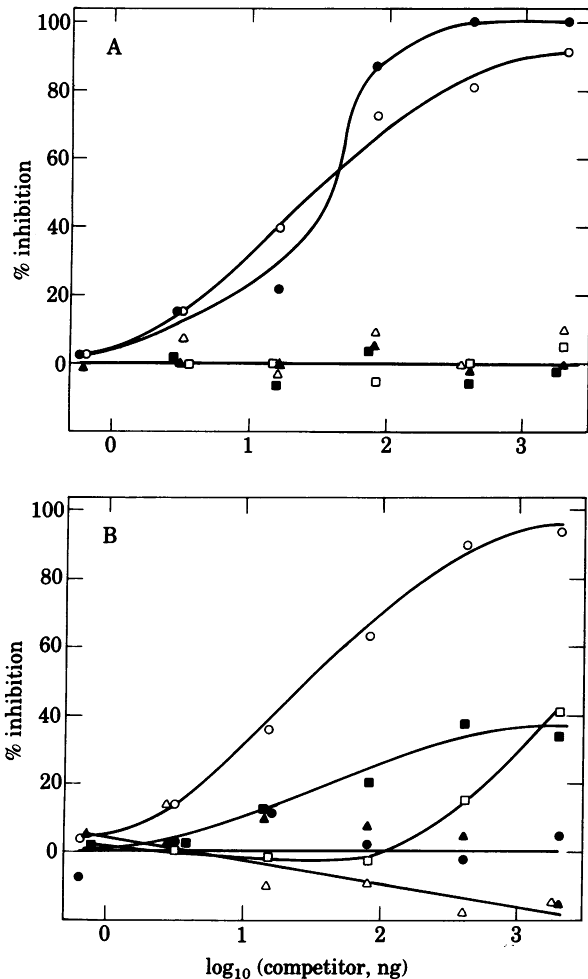

FIG. 4. Competitive radioimmunoassay with ^{3}H-labeled B form of poly(dG-dC)·poly(dG-dC). Binding to ^{3}H-labeled poly(dG-dC)·poly(dG-dC) was subjected to competition by Br-poly(dG-dC)·poly(dG-dC) (●); poly(dG-dC)·poly(dG-dC) (○); poly(dG) (△); poly(dG)·poly(dC) (□); native calf thymus DNA (▲), and denatured calf thymus DNA (■). (A) Serum Ra 441h was used at 1:10 dilution which bound 42% of the ^{3}H-labeled poly(dG-dC)·poly(dG-dC) in the absence of competitors. (B) Serum Ra 443a was used undiluted and bound 25% of the ^{3}H-labeled poly(dG-dC)·poly(dG-dC) in the absence of competitors. Undiluted normal rabbit serum bound 1.5% of the ^{3}H-labeled poly(dG-dC)·poly(dG-dC).

was performed with a 1:10 dilution of serum, much of the Z polymer may have been bound by coexisting Z-specific antibody, so that the amount of free polymer available for competition would be correspondingly reduced. If so, the Z polymer was in fact a more potent competitor than the B polymer for this antibody population.

A different result was observed with the B-binding population from rabbit 443a (immunized with B form). In this case, the unbrominated poly(dG-dC)·poly(dG-dC) (B form) was an effective competitor, whereas the brominated Z polymer was not (Fig. 4B). Some competition was observed with denatured DNA and poly(dG-dC)·poly(dG-dC) at higher concentrations; native calf thymus DNA and poly(dG) again were unreactive.

With antigenic structures that depend on high salt concentration, such as core histone octamers, antibody reactivity can be maintained in the presence of concentrated salt (14). Additional confirmation of the presence of Z-specific antibodies was obtained by carrying out radioimmunoassays in 4 M NaCl. Poly(dG-dC)·poly(dG-dC) was bound to antibodies in serum Ra 441a in 4 M NaCl, in which the polymer is known to have the

Table 1. Binding of MRL/lpr sera to polynucleotides

	Binding				
Mouse	Br-poly(dG-dC)·poly(dG-dC) (Z form)	Poly(dG-dC)·poly(dG-dC) (B form)*†	Denatured DNA*†	Native DNA*	Native DNA‡
MRL 39	28.6	1.5	32.7	1.9	33.6
MRL 40	41.6	1.0	55.5	3.6	36.0
MRL 45	38.3	1.8	71.8	4.8	28.1
MRL 46	43.3	1.0	23.4	1.7	27.2
MRL 47	41.7	0.8	24.8	4.3	28.0
MRL 51	13.5	2.1	51.4	1.3	28.2
MRL 53	19.9	1.6	40.4	4.8	34.6
MRL 54	48.9	1.3	55.4	6.5	35.6
MRL 57	8.3	0.9	10.4	2.5	14.6
MRL 58	45.8	0.6	37.3	2.0	26.5
MRL 59	8.9	0.9	79.2	5.4	31.0
MRL 60	12.4	1.1	29.7	1.3	28.5
Normal C57BL/6	2.0	0.5	0.4	0.1	1.6

* Binding of a 1:20 serum dilution in 0.06 M sodium phosphate, pH 8.0/0.03 M sodium EDTA/0.2 M NaCl.

† Binding of these polynucleotides was similar with or without the addition of 0.2 M NaCl to the standard radioimmunoassay buffer.

‡ Binding of a 1:10 serum dilution in 0.06 M sodium phosphate, pH 8.0/0.03 M sodium EDTA without added NaCl.

Z conformation. However, the polymer is not bound in 0.2 M NaCl. In 4 M NaCl, the serum showed a titer of 3200 with brominated Z-DNA and 1500 with unbrominated Z-DNA. Native *E. coli* DNA was not bound under these conditions. The binding of labeled poly(dG-dC)·poly(dG-dC) in 4 M NaCl was inhibited by 0.65 μg of unbrominated Z-DNA and 0.19 μg of the brominated polymer. Thus, the antibody population recognizes the two forms similarly.

Mice of the MRL/lpr strain have a severe form of lupus, and they produce anti-nucleic acid antibodies similar to those found in human systemic lupus erythematosus. Sera from 5-month-old MRL/lpr mice bound significant amounts of Z-DNA as well as native or denatured DNA (Table 1). There was no correlation between the amount of Z-DNA and the amount of native or denatured DNA bound. The reaction of these sera with native DNA was quenched by addition of 0.2 M NaCl to the standard buffer, whereas Z-DNA reacted well under this condition. Unbrominated poly(dG-dC)·poly(dG-dC) did not react in the presence or absence of 0.2 M NaCl.

DISCUSSION

The major finding in this work is that antibodies specific for Z-DNA were induced in both rabbits and mice by immunization with either brominated or unbrominated poly(dG-dC)·poly(dG-dC) complexed with methylated bovine serum albumin. The brominated Z-DNA was a particularly potent immunizing stimulus. These results are consistent with the facts that antibodies to helical nucleic acids are generally sensitive to changes in the conformation of the sugar-phosphate backbone and most forms of B-DNA complexed to methylated albumin are not immunogenic (6). The backbone of Z-DNA differs considerably from that found in B-DNA and probably differs from that found in most other polynucleotides.

The interesting finding that unbrominated poly(dG-dC)·poly(dG-dC) induced a significant amount of anti-Z antibody may reflect the potential of this polymer for forming Z-DNA. The latter conformation is stabilized by a high concentration of cations, as in concentrated salt solutions. The complex injected into rabbit 443 contained methylated bovine serum

albumin in which surface negative charges are eliminated by methylation of exposed carboxyl groups so that a high concentration of unopposed positively charged groups remains. The positively charged groups of the protein combine with the negative charges of poly(dG-dC)·poly(dG-dC), possibly creating microenvironments in which the Z-DNA conformation is stabilized. No inversion of the circular dichroism spectrum was apparent when the complex was measured, however, indicating that if Z-DNA were present in the complex it comprised less than 5% of the structure. A low percentage of Z-DNA content would also be consistent with the finding that this complex induced a much lower titer of antibody than did the DNA fully in Z form.

An important implication of the fact that rabbit 443 developed Z-DNA-specific antibody even though the umbrominated polymer was used for immunization is that these anti-Z-DNA antibodies were not directed against the Br atoms used for stabilizing the Z-DNA. This interpretation was reinforced by the competition experiments in which other brominated polymers, including the homopolymer Br-poly(dG) and the double-stranded Br-poly(dG)·poly(dC), were not reactive with the antibodies. This was true even when the antibody was induced by Br-poly(dG-dC)·poly(dG-dC). Collectively, the evidence points to the presence of antibodies specific for the Z-DNA conformation *per se* in all three rabbits rather than to antibodies reactive with Br atoms or brominated bases.

The antibody reactions in 4 M NaCl also reinforces this conclusion. The Z form of poly(dG-dC)·poly(dG-dC) is stable in the high-salt solution, and the antibody reacts with it, even in the absence of bromine atoms.

Two distinct types of anti-B-DNA antibodies were also found. One occurred in rabbit 441 after prolonged immunization with brominated Z-DNA (Fig. 2*B*). With this antibody, competitive binding occurred with both B and Z polymers. One interpretation of this result is that the antibody recognized a structure common to both B and Z forms; this is unlikely, however, because the geometries of both the pentose-phosphate backbones and the base stacking are quite distinct in the two conformations. A more probable explanation is that the antibody can recognize a small amount of Z-DNA in an equilibrium mixture and it appears to convert the polymer from the B to the Z form.

The B-reactive antibody formed by rabbit 443 was different. When it bound labeled B-form polynucleotide, the Z-DNA did not compete. This antibody was specific for the B form. It also recognized some features of denatured DNA and poly(dG)·poly(dC). It is interesting that native calf thymus DNA did not compete, suggesting that the B form of poly(dG-dC)·poly(dG-dC) has a conformation that differs slightly from that found in the bulk of natural B-DNA. In both poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC), which have strict alternation of purine and pyrimidine sequences, the conformation of the purine and pyrimidine residues are likely to differ slightly from each other, forming an alternating B conformation (15). This could explain the finding that native DNA did not compete for the antibody that bound poly(dG-dC)·poly(dG-dC). This was also supported by the finding that sera of MRL/lpr mice that bound native DNA did not bind poly(dG-dC)·poly(dG-dC).

It is of great interest that antibodies reactive with Z-DNA were found in sera of autoimmune lupus mice. These are spontaneously occurring antibodies for which the immunogen is unknown. It is possible that they are unique populations. This is suggested by the lack of correlation between the anti-Z-DNA activity and the anti-denatured DNA and anti-native DNA activities. Furthermore, these antibodies reacted with Z-DNA under ionic conditions in which native DNA did not react. The presence of these naturally occurring autoantibodies in mice provides a stimulus for a similar search for their presence in sera from humans with systemic lupus erythematosus.

An important feature of the Z-DNA structure is that it represents a double-helical DNA that still maintains antiparallel sugar-phosphate chains and Watson–Crick base pairs but nonetheless has a conformation that is radically different from that of B-DNA. The natural question that arose after the discovery of Z-DNA is whether this structure has any biological relevance (1). The significance of the present work is that it provides us with highly specific tools in the form of antibodies that combine with Z-DNA but not with B-DNA. These tools should make it possible to test, both by radioimmunoassay and by immunohistochemical analysis, for the presence of Z-DNA in various biological materials.

This work was supported by grants from the National Institutes of Health (AM 27232 and CA 04186), the National Science Foundation (PCM 79-04057), and the American Cancer Society. A.M. and A.N. are supported by grants from the Deutsche Forschungsgemeinschaft.

1. Wang, A. H. J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. & Rich, A. (1979) *Nature (London)* **282**, 680–686.
2. Wang, A. H. J., Quigley, G. J., Kolpak, F. J., van der Marel, G., van Boom, J. H. & Rich, A. (1981) *Science* **211**, 171–176.
3. Pohl, F. M. & Jovin, T. M. (1972) *J. Mol. Biol.* **67**, 375–396.
4. Stollar, B. D., Koo, R. & Stollar, V. (1978) *Science* **200**, 1381–1383.
5. Rudkin, G. T. & Stollar, B. D. (1977) *Nature (London)* **265**, 472–473.
6. Stollar, B. D. (1975) *Crit. Rev. Biochem.* **3**, 45–69.
7. Stollar, B. D. & Raso, V. (1974) *Nature (London)* **250**, 231–234.
8. Lacour, F., Nahon-Merlin, E. & Michelson, M. (1973) *Curr. Top. Microbiol. Immunol.* **62**, 1–39.
9. Leng, M., Guiges, M. & Genest, D. (1978) *Biochemistry* **17**, 3215–3220.
10. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
11. Papalian, M., Lafer, E., Wong, R. & Stollar, B. D. (1980) *J. Clin. Invest.* **65**, 469–477.
12. Sage, E. & Leng, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4597–4601.
13. Santella, R. M., Grunberger, D., Weinstein, I. B. & Rich, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1451–1455.
14. Rekvig, O. & Haunestad, K. (1980) *J. Exp. Med.* **152**, 1720–1733.
15. Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z. & Steitz, T. A. (1979) *J. Mol. Biol.* **131**, 669–680.