An Immunological Examination of Acetylaminofluorene-modified Poly(dG-dC) · Poly(dG-dC) in the Z-Conformation*

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Immunization of rabbits with a complex of methylated bovine serum albumin and N-2-acetylaminofluorene (AAF)-modified poly(dG-dC) · poly(dG-dC), a polynucleotide that can assume the Z-DNA conformation, yielded several populations of antibodies specific for Z-DNA determinants. The Z-DNA determinants were analyzed by examination of the antisera and of antibody preparations purified on immunoadsorbsents. The following was found: AAF-poly(dG-dC) · poly(dG-dC) shared Z-DNA determinants in common with poly(dG-dC) · poly(dG-dC) in 3.0 M NaCl, poly(dG-m6dC) · poly(dG-m6dC) in 1.5 M NaCl, and brominated poly(dG-dC) · poly(dG-dC) in 0.2, 1.5, and 3.0 M NaCl. Included among the antibodies induced by these determinants was a subpopulation whose reaction with brominated poly(dG-dC) · poly(dG-dC) was sensitive to increased ionic strength. Another distinct population of antibodies recognized determinants present on AAF-poly(dG-dC) · poly(dG-dC) but not on the other Z-DNAs. Only a small portion of this population was specific for the AAF moiety; the greater part appeared to recognize Z-DNA-associated conformational characteristics that were unique to AAF-poly(dG-dC) · poly(dG-dC). These findings are consistent with the existence of a continuum of Z-DNA determinants, which might be capable of functioning as recognition signals for regulatory DNA-binding proteins.

While studying the circular dichroism spectrum of poly(dG-dC) · poly(dG-dC), Pohl and Jovin (1) noticed an inversion under conditions of high salt concentration and suggested the possibility that the CD inversion signaled a change in the structure of the double-stranded polymer from a right-handed to a left-handed helix. Subsequent proof of the existence of the left-handed helical structure came from the crystallographic studies of Wang et al. (2, 3) and Crawford et al. (4) and Drew et al. (5) and Drew and Dickerson (6) and from fiber diffraction data (7). NMR (8–10) and laser Raman (11) studies confirmed that poly(dG-dC) · poly(dG-dC) could assume a left-handed structure (Z) in aqueous solution.

Modification of the guanine or cytosine residues by substitution with bulky groups can lead to stabilization of the left-handed Z-conformation, in some cases even under physiological conditions. Some of the modified guanine and cytosine residues include 5-methylcytosine (12), 7-methylguanine (13), 7-(dien)PtCl₂-guanine (14, 15), 8-Br'guanine-5-Br'-cytosine (16), and 8-AAF-guanine (17–21).

A major advance in the effort to determine the biological relevance of the left-handed Z-DNA helix came about with the finding that Z-DNA, unlike B-DNA, is immunogenic (16). This work was funded by National Institutes of Health Grants AI-06860, CA-21111, CA-13696, and 5T-32-GM-07367. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. Section 1734 solely to indicate this fact.

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§ Will submit this work as part of a dissertation in partial fulfillment of the requirements for the Ph.D. degree in Microbiology.
A similar protocol in which the methylated BSA was omitted failed to elicit an anti-Z-DNA response in the one rabbit immunized.

Preparation of [3H]Poly(dG-dC)-Poly(dG-dC) by Nick Transliteration—This was carried out by a modification of the method of Schleif and Wensink (30) using [3H]dCTP (Amersham Corp., 16.5 Ci/mmol) and unlabeled dGTP (Sigma). Incorporation of the label was determined at the end of the reactions. DNaase was assayed by the method of Maniatis et al. (31). The major modification was in the isolation of the product.7 After a 1.5-h incubation, the tubes were put on ice.

After removing an aliquot for assay of label incorporation, enough 250 mM EDTA was added to the reaction mixture to bring the final concentration to 20 mM. At least 1 volume of 0.1% sodium dodecyl sulfate, 10 mM Tris-HCI, pH 7.4, was added, and the solution was chromatographed on a Sephadex G-50 (Pharmacia Fine Chemicals) column using 0.1% sodium dodecyl sulfate, 10 mM Tris-HCI, pH 7.4, as the elution buffer; 0.7–0.8-ml fractions were collected. The labeled product was located by counting 5 pl of each fraction. The appropriate fractions were pooled and ethanol-precipitated using cold poly(dG-dC)-poly(dG-dC) as carrier.

Brominated Poly(dG-dC)-Poly(dG-dC)—This was prepared by the method of Lafer (32). In addition to UV spectra, CD spectra were sometimes taken to ensure that a left-handed polymer had been obtained.

Immunoadsorption—The method used was a modification of Papallian et al. (33). The buffer used in the experiments was 0.06 M phosphate, 0.06 M EDTA, pH 8.0, with 0.1% BSA. BSA was used to minimize nonspecific binding of nucleotide to the plastic microfuge tubes in which the assay was performed. For assays involving poly(dG-dC)-poly(dG-dC) in the Z-conformation, 4 m NaCl was added to the elution buffer. One-hundred-thousand units of either heat-inactivated serum or purified antibody in the above buffer were mixed with 100 pl of inhibitor in buffer or other solutions specified below. The tubes were incubated 0.5 h at room temperature, and 90–100 ng of labeled antigen, either [3H]AAF-poly(dG-dC)-poly(dG-dC) or [3H]poly(dG-dC)-poly(dG-dC), were added in the above buffer containing the appropriate amount of NaCl. In order to ensure that poly(dG-dC)-poly(dG-dC) was in the Z-conformation when it was added, the stock solution was incubated at 37 °C in 3.5 M NaCl for at least 15 min. The tubes were incubated for 1 h, followed by the addition of 100 ml of goat anti-rabbit IgG (containing 3.5 M NaCl, if necessary) and another 1-h incubation at room temperature. The tubes were centrifuged in a microfuge (Eppendorf) for 15 min at 4°C, and the supernatants were counted. Two controls were used: 1) diluted antibody or serum without inhibitor but with components of the inhibitor solution, and 2) the described buffer mixed with 100 ml of the solvent in which the inhibitor was dissolved.

AAF-Poly(dG-dC)-Poly(dG-dC)—precipitated from solution in 3.5 M NaCl, we were unable to determine inhibition of binding to [3H]AAF-poly(dG-dC)-poly(dG-dC) by poly(dG-dC)-poly(dG-dC) in its Z-conformation.

Preparation of Affinity Immunoabsorbents—Based on the method of Smith and Stollar, 2 ml of Affi-Gel 102 (Bio-Rad) were washed with 50 ml of 120 mM NaCl for the AAF derivative or 120 mM NaCl for the brominated derivative, and about 325 pg of Z-DNA in 3–4 ml of the appropriate solution were added. To this, 3–4 mg of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added. The reaction mixture, which was at a pH of 6.5–7.0, was incubated overnight at 4°C on an Omnishaker (Buchler Instruments) and then centrifuged. The supernatant, when assayed by measuring absorbance at 260 nm, showed very little remaining polynucleotide. The substituted resin was washed with 200–1200 ml of NaCl, followed by PBS (0.01 M KH2PO4-Na2HPO4, 0.15 M NaCl, pH 7.4) and PBS, 0.02% NaN3, in which it was stored.

Purification of Antibodies on Immunoabsorbents—This was accomplished by a combination of published procedures (22, 32). Five milliliters of serum containing 20 mM EDTA were added to 2 ml of immunosorbent. The mixture was incubated overnight at 4°C on an Omnishaker (Buchler Instruments) and then centrifuged. The supernatant was then centrifuged immediately against 5 ml Tris-HCl, 50 mM NaCl, pH 7.4. Dialysis was repeated against several changes of PBS. After dialysis, the purified fraction was concentrated by vacuum dialysis to about 100 pg/ml of protein. The immunoabsorbent was washed with PBS, 0.02% azide, in which it was stored.

Quantitative Precipitin Analysis—This was carried out essentially as described by Lafer (32). Briefly, 100–250 ml of antiserum, clarified by centrifugation three times at 12,000 × g, were added to an antigen solution in a 1.5-ml Eppendorf microfuge tube. The addition of antiserum always brought the final volume to 500 ml. The resulting solution contained 0.06 M Na2HPO4, 0.03 M EDTA, pH 8.0, and the appropriate amount of NaCl. After being thoroughly mixed, the tubes were incubated for 2 h at 37°C and overnight at 4°C. The resulting precipitates were washed three times with the appropriate salt solution and dissolved in 0.1 M NaOH, and the antibody was measured by the method of Lowry et al. (34).

RESULTS

Double Diffusion in Two Dimensions (Ouchterlony) (35)

Double diffusion patterns of two sera, tested against AAF-poly(dG-dC)-poly(dG-dC) and Br-poly(dG-dC)-poly(dG-dC), are shown in Fig. 1. The pattern for serum R532 (Fig. 1A) indicated partial identity with additional determinants being present on AAF-poly(dG-dC)-poly(dG-dC). Serum R533 (Fig. 1B) showed precipitation with both Z-DNA polymers, but the patterns were not sharp enough for a determination of partial or nonidentity.

Quantitative Precipitation Analysis of Various Z-DNAs

Quantitative precipitation curves of serum R533 (14 days after the fifth booster) with AAF-poly(dG-dC)-poly(dG-dC), Br-poly(dG-dC)-poly(dG-dC), and poly(dG-dC)-poly(dG-dC) in 3 M NaCl as antigens are shown in Fig. 2. Sixty to seventy per cent of the antibodies, maximally precipitated by AAF-poly(dG-dC)-poly(dG-dC), were precipitated by the brominated derivative at its equivalence point; poly(dG-dC)-poly(dG-dC) in 3 M NaCl precipitated only 45% of these antibodies. In 1.5 and 3 M NaCl, the amount of antibody precipitated by the brominated polymer fell to a value close to that of poly(dG-dC)-poly(dG-dC) (not shown). A similar precipitation titer was found for poly(dG-dC)-poly(dG-dC) in 1.5 M NaCl (not shown). The effect of NaCl concentration on the precipitation of AAF-poly(dG-dC)-poly(dG-dC) could not be determined because of its insolubility in high salt solutions. Less than 10% of the antibody was precipitated by AAF-calf thymus DNA (not shown). Thus, only a small portion of the antibody not precipitated by Br-poly(dG-dC)-poly(dG-dC) was specific for non-Z-DNA determinants.

Fig. 1. Ouchterlony patterns of antisera R532, day 7, after third booster (A) and R533, day 8, after fifth booster (B). Lines of R533 were stained with Amido black. Wells 1 and 4, Br-poly(dG-dC)-poly(dG-dC); wells 2 and 5, AAF-poly(dG-dC)-poly(dG-dC); wells 3 and 6, poly(dG-dC)-poly(dG-dC) in 0.15 M NaCl (A), 0.15 M NaCl (B).

7 C. Milcarek, personal communication.
8 K. Smith and B. D. Stollar, personal communication.
binding by radioimmunoassay is less than by precipitation.

poly(dG-dC) .poly(dG-dC); AAF-poly(dG) .poly(dC), which does not assume the Z-configuration and is susceptible to S1 nuclease digestion (17), at the antibodies binding AAF-poly(dG-dC) .poly(dG-dC) also that react either with the AAF moiety or with single-stranded poly(dG-dC) in 3.5 M NaCl. The ordinate refers to the amount of antibody precipitated per ml of serum tested; the abscissa refers to the amount of antigen added per ml of serum tested.

FIG. 2. Quantitative precipitation curve (R533, day 14, after fifth booster). Q, AAF-poly(dG-dC)-poly(dG-dC); □, Br-poly(dG-dC)-poly(dG-dC); ◆, poly(dG-dC)-poly(dG-dC) in 3.0 M NaCl. The ordinate refers to the amount of antibody precipitated per ml of serum tested; the abscissa refers to the amount of antigen added per ml of serum tested.

Table I

Maximal inhibition of binding of [3H]AAF-poly(dG-dC)-poly(dG-dC) by antisera R533

| Inhibitor                  | Maximal inhibition | Ratio to antigen
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>AAF-poly(dG-dC)-poly(dG-dC)</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>Br-poly(dG-dC)-poly(dG-dC)</td>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td>AAF-poly(dG)-poly(dC)</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>AAF-calf thymus DNA</td>
<td>20</td>
<td>70</td>
</tr>
</tbody>
</table>

At dilution of R533 that gave 30-50% binding of [3H]AAF-poly(dG-dC)-poly(dG-dC).

Represents results of a series of experiments in which increasing amounts of inhibitor were used. Although maximal inhibition occurred at lower concentrations (see footnotes below), the numbers in this column are an indication of the fraction of the population of antibodies specific for the inhibitor. Ratio is calculated on a weight basis (but, see Footnote d).

a Maximum was reached at ratio of 0.9.
b Based on AAF content.
c Maximum was reached at ratio of 5.5.
d Maximum was reached at ratio of 6.

Radioimmunoassay

Direct Binding—The reactivity of the serum R533 with [3H]AAF-poly(dG-dC)-poly(dG-dC) and [3H]poly(dG-dC)poly(dG-dC) in 3.5 M NaCl, both of which assume the Z-conformation, was assayed by direct binding. Fifty per cent binding to [3H]AAF-poly(dG-dC)-poly(dG-dC) occurred at a 1/250 dilution and to [3H]poly(dG-dC)-poly(dG-dC) in 3.5 M NaCl at a 1/125 dilution.

Inhibition Studies—Inhibition assays were used to assess the antisera’s reactivity with other polymers, some of which can assume the Z-form. Inhibition of binding to [3H]AAF-poly(dG-dC)-poly(dG-dC) by unlabeled polymer, which was used as a positive control, was as high as 90% (Table I). Inhibition by Br-poly(dG-dC)-poly(dG-dC) reached a maximum of 16%. Thus, by radioimmunoassay, a subpopulation at the antibodies binding AAF-poly(dG-dC)-poly(dG-dC) also recognized determinants on the brominated polymer. A population with this specificity was also seen by quantitative precipitation analysis (Fig. 2), although its proportion of binding by radioimmunoassay is less than by precipitation. AAF-poly(dG)-poly(dC), which does not assume the Z-conformation and is susceptible to S1 nuclease digestion (17), served as an indicator for the presence of antibody populations that react either with the AAF moiety or with single-stranded polymers. Inhibition by this polymer reached a maximum of 22%. Similar results were obtained with AAF-calf thymus DNA.

Other non-Z-DNA polymers, such as poly(dG-dC)-poly(dG-dC) at low salt concentration and poly(dG)-poly(dC), were not inhibitors. Nor were AAF-Guo-BSA or AAF-dG.

Poly(dG-dC)-poly(dG-dC) in its Z-conformation could not be tested as an inhibitor of [3H]AAF-poly(dG-dC)-poly(dG-dC) binding because the latter precipitates in 3.5 M NaCl. On the other hand, binding to [3H]poly(dG-dC)-poly(dG-dC) in 3.5 M NaCl was completely inhibited by Br-poly(dG-dC)-poly(dG-dC) (not shown).

Fractionation on a Br-Poly(dG-dC)-poly(dG-dC) Immunoadsorbent (Scheme I)

Exposure of the antisera to a Br-poly(dG-dC)-poly(dG-dC) immunoadsorbent yielded two fractions: a supernatant containing antibodies that did not bind to the immunoadsorbent and a fraction that bound and was subsequently eluted with 1.8 M KSCN. The latter bound both [3H]poly(dG-dC)-poly(dG-dC) and [3H]AAF-poly(dG-dC)-poly(dG-dC) (Fig. 3). The supernatant, on the other hand, bound only AAF-poly(dG-dC)-poly(dG-dC) (not shown).

In radioimmunoassay inhibition studies (Table II), binding by the specifically purified fraction to [3H]AAF-poly(dG-dC)-poly(dG-dC) was inhibited about equally (approximately 75%) by AAF-poly(dG-dC)-poly(dG-dC) and by Br-poly(dG-dC)-poly(dG-dC); AAF-poly(dG)-poly(dC) did not inhibit. Binding to [3H]poly(dG-dC)-poly(dG-dC) in high salt was completely inhibited by the brominated polymer at 5-fold excess (not shown). Thus, this purified preparation includes a population of antibodies that recognizes, to an equal extent, determinants that are shared by AAF-poly(dG-dC)-poly(dG-dC), Br-poly(dG-dC)-poly(dG-dC), and poly(dG-dC)-poly(dG-dC) in high salt. The supernatant, on the other hand, recognized determinants on AAF-poly(dG-dC)-poly(dG-dC) but not on poly(dG-dC)-poly(dG-dC) in high salt as determined by direct binding studies (not shown).

Inhibition studies (Table III) showed that the supernatant (i.e. the fraction that did not bind to the immunoadsorbent) did not recognize Br-poly(dG-dC)-poly(dG-dC). Only a small percentage of the binding of the supernatant could be identified as being specific for a non-Z-DNA epitope, as shown by the fact that a 10-fold excess of AAF-poly(dG-dC)-poly(dG-dC) inhibited only about 20% of the binding; AAF-poly(dG-dC)-poly(dG-dC) inhibited completely. We conclude (see "Discussion") that unique Z-conformational determinants on AAF-poly(dG-dC)-poly(dG-dC) are being detected.

Quantitative precipitation (not shown) of the supernatant (i.e. Fraction A of Table III) confirmed the radioimmunoassays. No precipitation was seen with the brominated derivative, with poly(dG-dC)-poly(dG-dC) in 3 M NaCl or with poly(dG-m4dC)-poly(dG-m4dC) in 1.5 M NaCl. Precipitation with AAF-poly(dG-dC)-poly(dG-dC) was decreased by about 70% compared to the unfractionated serum. This is in agreement with earlier findings on the fraction of the antisera that precipitates the brominated polymer (Fig. 2) and agrees qualitatively but not quantitatively with the radioimmunoassay binding studies (Table I).

Purification of Fraction A (Table III) on an AAF-Poly(dG-dC)-Poly(dG-dC) Immunoadsorbent (Scheme I)

An AAF-poly(dG-dC)-poly(dG-dC) immunoadsorbent was used to purify the populations of antibody that did not bind to the Br-poly(dG-dC)-poly(dG-dC) immunoadsorbent. Bind-
**Scheme 1**

**Table II**

Maximal inhibition of binding of $^3$H]AAF-poly(dG-dC)-poly(dG-dC) using the Br-poly(dG-dC)-poly(dG-dC)-purified fraction

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Maximal inhibition</th>
<th>Ratio to antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAF-poly(dG-dC)-poly(dG-dC)</td>
<td>78</td>
<td>46:1</td>
</tr>
<tr>
<td>Br-poly(dG-dC)-poly(dG-dC)</td>
<td>73</td>
<td>36:1</td>
</tr>
<tr>
<td>AAF-poly(dG)-poly(dC)</td>
<td>0</td>
<td>0:1</td>
</tr>
</tbody>
</table>

*At a dilution of the purified antibody that gave between 30 and 50% binding of $^3$H]AAF-poly(dG-dC)-poly(dG-dC).

The highest ratio of inhibitor tested.

*Ratios are based on weight for Br- and AAF-poly(dG-dC)-poly(dG-dC). Ratios for AAF-poly(dG)-poly(dC) were calculated on the basis of AAF content.

*73% inhibition was detected with a 10.5-fold excess.

*70% inhibition was detected with a ratio of 0.53.

**Table III**

Inhibition studies on a fraction not bound by Br-poly(dG-dC)-poly(dG-dC) (Fraction A) and on a subfraction subsequently purified on an AAF-poly(dG-dC)-poly(dG-dC) immunoadsorbent (Fraction B)

<table>
<thead>
<tr>
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<th>Fraction B</th>
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<tbody>
<tr>
<td>AAF-poly(dG-dC)-poly(dG-dC)</td>
<td>89.5</td>
<td>14:1</td>
<td>13:1</td>
</tr>
<tr>
<td>Br-poly(dG-dC)-poly(dG-dC)</td>
<td>0</td>
<td>5:1</td>
<td>9:1</td>
</tr>
<tr>
<td>AAF-poly(dG)-poly(dC)</td>
<td>20</td>
<td>10:1</td>
<td>11:1</td>
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*At a dilution that gave between 30 and 50% binding of $^3$H]AAF-poly(dG-dC)-poly(dG-dC).

Numbers in parentheses are the ratios of inhibitor to antigen which are calculated as described in the preceding tables. The ratio given is one at which maximal inhibition was observed.

Based on results of two different preparations, one of which was subsequently used to make Fraction B.

Based on results of three different preparations, one of which was subsequently used to make Fraction B.

**Fig. 3. Binding of Br-poly(dG-dC)-poly(dG-dC)-purified fraction to [3H]AAF-poly(dG-dC)-poly(dG-dC) (C) and to [3H]poly(dG-dC)-poly(dG-dC) in 3.5 M NaCl (C).**

Maximal inhibition of binding of [3H]AAF-poly(dG-dC)-poly(dG-dC) using the Br-poly(dG-dC)-poly(dG-dC)-purified fraction

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Numbers in parentheses are the ratios of inhibitor to antigen which are calculated as described in the preceding tables. The ratio given is one at which maximal inhibition was observed.

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<td>20</td>
<td>10:1</td>
<td>11:1</td>
</tr>
</tbody>
</table>

*At a dilution that gave between 30 and 50% binding of [3H]AAF-poly(dG-dC)-poly(dG-dC).

Numbers in parentheses are the ratios of inhibitor to antigen which are calculated as described in the preceding tables. The ratio given is one at which maximal inhibition was observed.

Based on results of two different preparations, one of which was subsequently used to make Fraction B.

Based on results of three different preparations, one of which was subsequently used to make Fraction B.
Immunization with AAF-poly(dG-dC)-poly(dG-dC), as a methylated BSA complex, yielded several populations of Z-specific antibodies which were characterized by precipitation and competitive binding assays, as well as by purification on immunoabsorbents. Populations were found that were stimulated by conformational characteristics of Z-DNA shared in common by poly(dG-dC)-poly(dG-dC) in 3.5 M NaCl, poly(dG-m5dC)-poly(dG-m5dC) in 1.5 M NaCl, and Br-poly(dG-dC)-poly(dG-dC) in 0.2, 1.5, and 3.0 M NaCl. Also present was a subpopulation that precipitated the brominated polymer only at low ionic strength. Electrostatic forces apparently make up the major binding component of the interaction of this antibody population with Z-DNA, since precipitation could be suppressed by raising the NaCl concentration from 0.2 to 1.5 M with no further decrease occurring in 3.0 M NaCl. It would appear, therefore, that the major determinant recognized by this subpopulation of antibody includes the deoxyribose phosphate backbone. The remaining Z-DNA-specific populations recognized conformational characteristics defined by other surface components of Z-DNA, with the phosphate groups making either a minor or no contribution to the overall binding free energies.

Purification of the antiserum by binding and subsequent elution from an immunoabsorbent containing Br-poly(dG-dC)-poly(dG-dC) yielded a fraction of the antibody population that reacted equally as well with AAF-poly(dG-dC)-poly(dG-dC) and Br-poly(dG-dC)-poly(dG-dC) (Table II). More than 70% inhibition of binding to [3H]AAFPoly(dG-dC)-poly(dG-dC) was attainable with both polynucleotides (Table II). Moreover, this fraction bound to [3H]poly(dG-dC)-poly(dG-dC) in 3.5 M NaCl (Fig. 3), leaving no doubt that the specifically purified preparation contained populations of antibody specific for Z-DNA.

Also elicited was another population of antibodies that was stimulated by structural characteristics unique to AAF-poly(dG-dC)-poly(dG-dC). This fraction, which passed through the Br-poly(dG-dC)-poly(dG-dC) immunoabsorbent unbound, did not react with Br-poly(dG-dC)-poly(dG-dC), poly(dG-dC)-poly(dG-dC) in 3.5 M NaCl, or with polynucleotides in 1.5 M NaCl. After purification by binding to and elution from an AAF-poly(dG-dC)-poly(dG-dC) immunoabsorbent, it displayed similar binding properties (Table III). Only a small fraction recognized a non-Z, AAF-bearing polynucleotide, AAF-poly(dG-poly(dG-dC) (Table III). The rest, therefore, must have been produced in response to immunogenic conformational characteristics unique to AAF-poly(dG-dC)-poly(dG-dC). Since B-DNA is not immunogenic and, indeed, we found no antisera components that could bind poly(dG-dC)-poly(dG-dC) in its B-conformation (i.e. at low ionic strength), the major portion of the population reacting with AAF-poly(dG-dC)-poly(dG-dC) appears to be directed toward unique Z-DNA determinants of this polynucleotide. Our findings, therefore, add to the reports of others (22, 37) concerning Z-DNA polymorphism as detected immunochemically. Electron microscopic studies in our laboratory also support Z-DNA polymorphism (38).

Thus, there appears to be a continuum of Z-DNA conformations (cf. Ref. 6) having differences sufficient to be recognized by specific antibodies. There is no reason to believe that antibodies, as proteins, recognize specific DNA structures by processes that differ fundamentally from those used by DNA-binding regulatory proteins. Therefore, the various left-handed forms, which differ in fine structure, might yield an array of recognition signals for specific proteins involved in the highly sophisticated regulation of DNA expression.
AAF-Poly(dG-dC)·Poly(dG-dC) in Z-Conformation

An immunochemical examination of acetylaminofluorene-modified poly(dG-dC) X poly(dG-dC) in the Z-conformation.
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