Interaction between Antibodies to Z-Form Deoxyribonucleic Acid and Double-Stranded Polynucleotides†

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ABSTRACT: Antibodies to Z-DNA have been elicited in rabbits immunized with chloro(diethylenetriamine)platinum(II) chloride (Cl_2dienPt) modified poly(dG-dC)·poly(dG-dC). Under physiological conditions this polynucleotide in which 12% of the bases were substituted is in the Z form. The antibodies are mainly immunoglobulins G as shown by gel filtration of the antiserum and by ultracentrifugation of the antibodies purified on a Sepharose–poly(dG-dC)·poly(dG-dC) column. From the quantitative precipitin curve, it is concluded that each binding site of the antibody covers four nucleotide residues. The Z-form DNA–antibody complex is stabilized by electrostatic interactions. There was no interaction between poly(dI-br^{5}dC)·poly(dI-br^{5}dC) in the Z form and the antibodies, which strongly suggests that the exocyclic amino group of guanine residues in Z-poly(dG-dC)·poly(dG-dC) interacts with the antibody binding site. The modification of poly(dG-dC)·poly(dG-dC) by 2-(acetylamino)fluorene residues decreases but does not prevent the binding to the antibodies. It is also shown that the polynucleotide poly(dG-br^{5}dC)·poly(dG-br^{5}dC) adopts the Z form over a large range of salt concentration [5 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 7.5, to 3 M NaCl].

Almost all, if not all, the assays to elicitate in rabbits antibodies to right-handed double-stranded DNA (B form) by immunization with native DNA complexed or not with proteins have failed [for general reviews, see Lacour et al. (1973) and Stollar (1973, 1975)]. On the other hand, two recent studies (Lafer et al., 1981; Malfoy & Leng, 1981) have shown that the antisera of rabbits immunized with left-handed double-stranded poly(dG-dC)·poly(dG-dC) (Z form) gave a strong positive reaction with Z-DNA. In these studies, the antigens were poly(dG-dC)·poly(dG-dC) modified either by bromine (Lafer et al., 1981) or by chloro(diethylenetriamine)platinum(II) chloride (Cl_2dienPt)[1] (Malfoy & Leng, 1981). One major reason for the choice of these modified poly(dG-dC)·poly(dG-dC) is that they are in the Z form in physiological conditions.

It was first observed by Pohl & Jovin (1972) that poly(dG-dC)·poly(dG-dC) can undergo a salt-induced cooperative transition, the midpoint of the transition being at 2.5 M NaCl. The low-salt form belongs to the B family (Pohl et al., 1974) and the high-salt form to the Z family (Arnott et al., 1980; Thamann et al., 1981; Mitra et al., 1981; Behe et al., 1981; Ramstein & Leng, 1980; Pilet & Leng, 1982). In addition to bromine (Lafer et al., 1981) and monofunctional platinum derivatives (Malfoy et al., 1981; Lippard, 1981), several chemical modifications of base residues can stabilize the Z form as, for example, methylation on the N(7) of guanine residues (Möller et al., 1981) or on the C(5) of cytosine residues (Behe & Felsenfeld, 1981; Behe et al., 1981), arylamidation on the C(8) of guanine residues (Sage & Leng, 1980, 1981; Santella et al., 1981a,b), and modification by mitomycin C (Mercado & Tomasz, 1977).

In this work, we report some results on the antibodies induced in rabbits by immunization with poly(dG-dC)·poly(dG-dC) modified by chloro(diethylenetriamine)platinum(II) chloride (Cl_2dienPt) on the N(7) of guanine residues. This polynucleotide in which 12% of the bases are bound to Cl_2dienPt is in Z form in 0.1 M NaCl–1 mM $MgCl_2$, pH 7.5. We show that the antibodies to Z-DNA are mainly IgG and that they can be purified by affinity chromatography. The number of nucleotide residues covered by each binding site of the antibodies was deduced from the precipitin curve. The affinity of the antibodies toward several polynucleotides was studied by radioimmunoassays. The results are discussed in relation with the conformation of the Z form.

Materials and Methods

Poly(dG-dC)·poly(dG-dC) and poly(dI-br^{5}dC)·poly(dI-br^{5}dC) were bought from P-L Biochemicals. They were treated with phenol and then exhaustively dialyzed as previously described (Sage & Leng, 1981). Poly(dG-m^{5}dC)·poly(dG-m^{5}dC) and brominated poly(dG-dC)·poly(dG-dC) were prepared according to the procedures of Behe & Felsenfeld (1981) and Lafer et al. (1981), respectively. *Escherichia coli* DNA polymerase large fragment was purchased from Boehringer Mannheim, the restriction endonuclease *Hha*I from P-L Biochemicals, and micrococcal nuclease from Worthington.

Poly(dG-br^{5}dC)·poly(dG-br^{5}dC) was synthesized in a manner similar to that used for preparation of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) (Behe & Felsenfeld, 1981). The poly(dI-dC)·poly(dI-dC) template was digested by *Hha*I, poly(dG-br^{5}dC)·poly(dG-br^{5}dC) being resistant to this enzyme. The yield of the reaction was about 1 mg for 1 mg of template. The extinction coefficient was determined after treatment of the polynucleotide by micrococcal nuclease. The reaction mixture contained 50 mM Tris-HCl, pH 8, 0.25 mM $CaCl_2$, the polynucleotide (0.15 mM), and 300 units of enzyme/mL. At 260 nm, the extinction coefficient of poly(dG-br^{5}dC)·poly(dG-br^{5}dC) was 6000 M^{-1} cm^{-1}. Sedimentation velocity was performed in a Beckman Model E analytical centrifuge. The sedimentation coefficient was 8 S in 0.1 M NaCl–5 mM Tris-HCl, pH 7.5.

The preparation of ^{3}H-labeled poly(dG-dC)·poly(dG-dC) and poly(dG-dC)·poly(dG-dC) chemically modified by *N*-acetoxy-2-(acetylamino)fluorene and by chloro(diethylenetriamine)platinum(II) chloride was previously described

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[1] Abbreviations: Cl_2dienPt, chloro(diethylenetriamine)platinum(II) chloride; IgG, immunoglobulin G; RIA, radioimmunoassay; AAF, 2-(acetylamino)fluorene; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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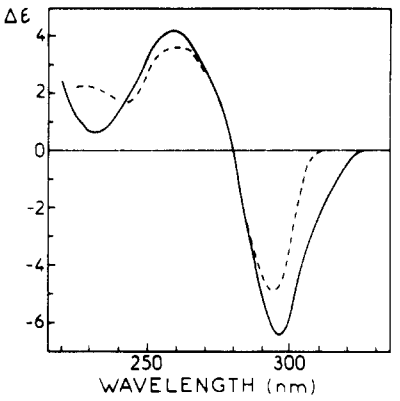

FIGURE 1: Circular dichroism spectra of poly(dG-br^{5}dC)·poly(dG-br^{5}dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC): (—) poly(dG-br^{5}dC)·poly(dG-br^{5}dC) in 5 mM Tris-HCl, pH 7.5, or in 3 M NaCl–5 mM Tris-HCl, pH 7.5, plus 0.1 mM EDTA; (---) poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 3 M NaCl–5 mM Tris-HCl, pH 7.5.

(Malfoy et al., 1981; Sage & Leng, 1981). We will write poly(dG-dC)–AAF (0.12) or poly(dG-dC)–Cl_2dienPt (0.12) for a poly(dG-dC)·poly(dG-dC) in which 12% of the base residues were substituted by 2-(acetylamino)fluorene residues or Cl_2dienPt residues, respectively.

The antibodies were purified by affinity chromatography on a Sepharose–poly(dG-dC)·poly(dG-dC) column. Poly(dG-dC)·poly(dG-dC) was linked to a Sepharose 4B through a spacer, 6-aminohexanoic acid. The activation of the Sepharose 4B with BrCN, the reaction with 6-aminohexanoic acid, and then the covalent binding of poly(dG-dC)·poly(dG-dC) to the spacer in presence of carbodiimide were performed as described for the preparation of a Sepharose–poly(A)·poly(U) column (Guigues & Leng, 1976). About 0.1 mg of poly(dG-dC)·poly(dG-dC) was linked per mL of wet Sepharose. Radioimmunoassays, precipitin assays, and absorption and circular dichroism spectroscopies were performed as previously described (Sage et al., 1979).

Immunization. Two random-bred rabbits were injected with a mixture of equal weights of poly(dG-dC)–Cl_2dienPt (0.12) and of methylated bovine serum albumin in 0.1 M NaCl, 1 mM $MgCl_2$, and 5 mM Tris-HCl, pH 7.5 (Nahon-Merlin et al., 1973). The mixture (100 μg/mL), emulsified in complete Freund's adjuvant, was injected at weeks 0 and 2. At week 3, the same mixture without adjuvant was injected intravenously. An intravenous booster was done at weeks 8 and 13. The bleedings were done at weeks 4, 9, and 14.

Results

Poly(dG-br^{5}dC)·Poly(dC-br^{5}dC). Most conformations of the polynucleotides used in this work have been already described (see references given in the introduction). In our experimental conditions, they are in either B or Z conformations. A new polynucleotide, poly(dG-br^{5}dC)·poly(dG-br^{5}dC), has been synthesized. In Figure 1, we present the circular dichroism spectrum of this polymer. This spectrum is characteristic of the Z-form DNA with a first negative band and a positive band centered respectively at 295 and 260 nm. This spectrum was found salt-concentration independent over a range of 5 mM Tris-HCl, pH 7.5, to 3 M NaCl. As a comparative purpose, we also show in Figure 1 the circular dichroism spectrum of poly(dG-m^{5}dC)·poly(dG-m^{5}dC), which has already been reported by Behe & Felsenfeld (1981). Except for minor differences, both spectra are similar.

Reactivity of Antiserum. Most of the results reported in this paper were obtained with the antiserum corresponding to the bleeding done after the first booster. The reactivity of the antiserum was studied by radioimmunoassays and by quantitative precipitation.

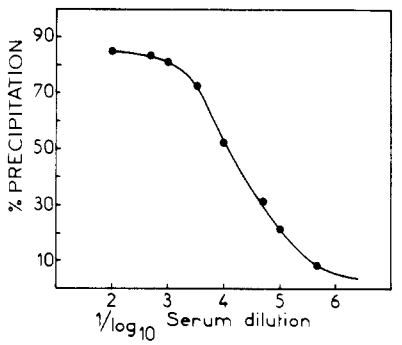

FIGURE 2: Binding of antiserum to [^{3}H]poly(dG-dC)–Cl_2dienPt (0.12). The antiserum to poly(dG-dC)–Cl_2dienPt (0.12) was exhaustively dialyzed against 0.1 M NaCl, 1 mM $MgCl_2$, and 5 mM Tris-HCl, pH 7.5. For each dilution, the total amount of proteins was kept constant by addition of a nonspecific rabbit antiserum dialyzed against the same buffer. After incubation for 30 min at 4 °C, goat anti-rabbit IgG was added in order to precipitate all the antibodies. The concentration of the antigen was 0.2 μM. The volume of the assay was 50 μL. The total radioactivity of the assay was of the order of 800 dpm.

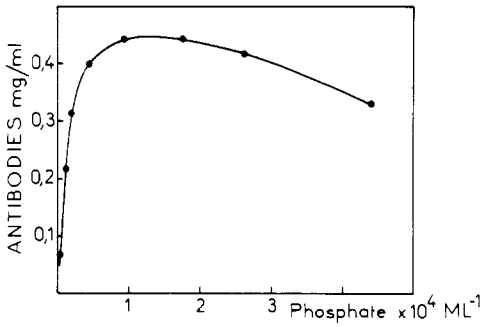

FIGURE 3: Precipitin curve. Amount of antibodies per milliliter of antiserum precipitated by poly(dG-dC)·poly(dG-dC). Solvent 3 M NaCl–5 mM Tris-HCl, pH 7.5; temperature 4 °C. The concentration of the polynucleotide is expressed in moles of nucleotide residues.

As shown in Figure 2, the antiserum binds strongly to the tracer [^{3}H]poly(dG-dC)–Cl_2dienPt (0.12) with a titer of about 1/10000. Almost the same curves were obtained with the antisera corresponding to the bleedings done at weeks 4 and 14.

The nature of the immunoglobulin (IgG, IgM) was determined by gel filtration on a Ultrogel ACA 34 column. The antibody activity [against poly(dG-dC)–Cl_2dienPt (0.12)] was mainly found in the IgG fraction.

Quantitative precipitation was performed in 3 M NaCl by adding increasing amounts of poly(dG-dC)·poly(dG-dC) to a constant volume of antiserum. In this salt concentration, poly(dG-dC)·poly(dG-dC) was in the Z form. The results are shown in Figure 3. At the equivalence point, about 0.45 mg of antibodies/mL of antiserum is precipitated by the polynucleotide. The molar ratio of nucleotide residues over IgG in the precipitate, corresponding to the antibody excess region, is about 8. Thus, each Fab fragment binding site covers about four nucleotide residues.

Interaction with Poly(dG-dC)·Poly(dG-dC). The relative affinity of the antiserum toward poly(dG-dC)·poly(dG-dC) and poly(dG-dC)–Cl_2dienPt (0.12) has been compared. As shown in Figure 4A, there is no displacement of the tracer binding to the antiserum by poly(dG-dC)·poly(dG-dC) in 0.1 M NaCl, 1 mM $MgCl_2$, and 5 mM Tris-HCl, pH 7.5, conditions in which poly(dG-dC)·poly(dG-dC) is in the B form. On the other hand, in 3 M NaCl the inhibition of the tracer

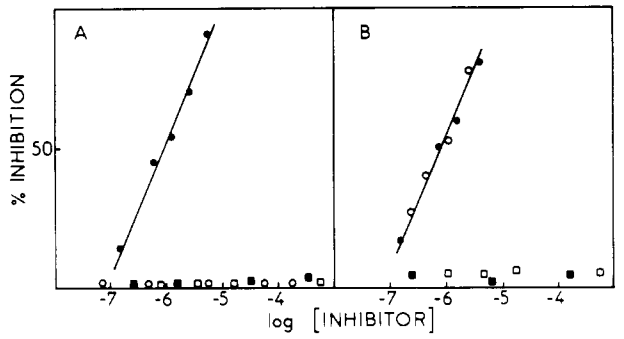

FIGURE 4: Inhibition of tracer–antibody binding of various DNAs in competitive RIA: Tracer [^{3}H]poly(dG-dC)–Cl_2dienPt (0.12), 0.2 μM; antiserum dilution $1/10^4$; competitors (in M) (●) poly(dG-dC)–Cl_2dienPt (0.12), (○) poly(dG-dC)·poly(dG-dC), and (□) native or (■) denatured *M. lysodeikticus* DNA. (A) Solvent 0.1 M NaCl, 1 mM $MgCl_2$, and 5 mM Tris-HCl, pH 7.5; (B) solvent 3 M NaCl–5 mM Tris-HCl, pH 7.5.

binding is the same by poly(dG-dC)·poly(dG-dC) and poly(dG-dC)–Cl_2dienPt (0.12) (Figure 4B). Thus the antibodies recognize the Z form and are not directed against platinum residues. There was no reaction with native or denatured G-C-rich DNA (*Micrococcus lysodeikticus*).

Effect of Ionic Strength. The interaction between the antiserum and poly(dG-dC)–Cl_2dienPt (0.12) or brominated poly(dG-dC) has been studied as a function of salt concentration in the range 0.1–3 M NaCl by RIA. In these salt conditions, the two polymers were in the Z form. The reference conditions were such that 50% of the tracer was precipitated by the antiserum in 0.1 M NaCl–1 mM $MgCl_2$. The percentage of precipitated tracer was the same in the range 0.1–3 M NaCl. Similar results were obtained with brominated poly(dG-dC) (results not shown). Experiments at salt concentrations lower than 0.1 M NaCl are difficult because nonspecific interactions occur between the antiserum and double-stranded polynucleotides.

Effect of Temperature. The experimental conditions were identical with those used in the study of the ionic strength effect; i.e., 50% of [^{3}H]poly(dG-dC)–Cl_2dienPt (0.12) was precipitated by the antiserum at 4 °C. The amount of precipitated tracer was the same at 4 and 40 °C.

Interactions with Modified Double-Stranded Polynucleotides. The affinity of the antiserum toward several modified polynucleotides has been studied by RIA in 0.1 M NaCl–1 mM $MgCl_2$ and in 3 M NaCl.

In 3 M NaCl, all the polynucleotides are in the Z form. The concentrations of inhibitors necessary to get 50% inhibition of tracer binding [[^{3}H]poly(dG-dC)–Cl_2dienPt (0.12)] are given in Table I. Poly(dG-br^{5}dC)·poly(dC-br^{5}dC) and brominated poly(dG-dC) behave almost as poly(dG-dC)·poly(dG-dC) while poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is much less effective. Poly(dG-dC)–AAF (0.025) binds to the antiserum slightly less than the tracer. Higher percentages of AAF residues result in a decrease of the affinity, but poly(dG-dC)–AAF (0.07) and poly(dG-dC)–AAF (0.13) have the same affinity.

In 0.1 M NaCl–1 mM $MgCl_2$, poly(dG-dC)–AAF (0.025) is partly in the Z form and interacts weakly with the antiserum. Poly(dG-dC)–AAF (0.07) and poly(dG-dC)–AAF (0.13) are in the Z form; they bind to the antiserum but less than poly(dG-dC)–Cl_2dienPt (0.12). It is interesting to note that the differences in the affinities are smaller in 0.1 M NaCl than in 3 M NaCl. Also, in this low salt concentration poly(dG-br^{5}dC)·poly(dG-br^{5}dC) is a better inhibitor than poly(dG-dC)–Cl_2dienPt (0.12). The behavior of poly(dG-br^{5}dC)·poly(dG-br^{5}dC) as a function of salt concentration differs from that of brominated poly(dG-dC).

Table I: Inhibition of Tracer–Antibody Binding in Competitive RIA[a]

competitors	concn for 50% inhibition in 0.1 M NaCl	concn for 50% inhibition in 3 M NaCl
poly(dG-dC)·poly(dG-dC)		10^{-6}
poly(dG-dC)–Cl_2dienPt (0.12)	10^{-6}	10^{-6}
poly(dG-m^{5}dC)·poly(dG-m^{5}dC)		2×10^{-4}
poly(dG-br^{5}dC)·poly(dG-br^{5}dC)	2×10^{-7}	3×10^{-6}
brominated poly(dG-dC)	2×10^{-6}	2×10^{-6}
poly(dG-dC)–AAF (0.025)	4×10^{-4}	4×10^{-6}
poly(dG-dC)–AAF (0.07)	10^{-5}	10^{-4}
poly(dG-dC)–AAF (0.13)	10^{-5}	10^{-4}

[a] The values given in the columns are the concentrations (in nucleotide residues) of competitors to get 50% inhibition of the tracer [[^{3}H]poly(dG-dC)–Cl_2dienPt (0.12)]-antibody binding. Tracer concentration 0.2 μM; solvent 5 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, and 0.1 M NaCl or 3 M NaCl; temperature 4 °C.

Poly(dI-br^{5}dC)·poly(dI-br^{5}dC) is in the Z form in 4 M NaCl at room temperature (Hartmann et al., 1982). RIAs were performed in 4 M NaCl and at 25 °C. No significant inhibition of tracer binding to the antiserum was observed up to 1 mM poly(dI-br^{5}dC)·poly(dI-br^{5}dC). Some other compounds have been tested such as poly(dG)·poly(dC), poly(dG), or double-stranded RNA. None of these products inhibited the tracer binding to the antiserum (results not shown).

Purification of Antibodies. The purification has been performed by affinity chromatography on a Sepharose–poly(dG-dC)·poly(dG-dC) column. Wet Sepharose–poly(dG-dC)·poly(dG-dC) (1 mL) (prepared as described under Materials and Methods) was washed 3 times with 3 M NaCl (10 mL) at room temperature. NaCl was added slowly to the antiserum (0.18 g/mL of antiserum), and then the gel was added to the antiserum (1 mL of wet Sepharose/3 mL of antiserum). After incubation under gentle stirring at 37 °C for 30 min and then at 4 °C for 30 min, the gel was poured into a column and washed with 3 M NaCl solution until the absorbance at 280 nm of the effluent was less than 0.03. The column was then washed with 1 mM NaCl (5 mL). The antibodies were eluted with a 1.8 M NaSCN solution in the cold and immediately dialyzed against 50 mM NaCl–5 mM Tris-HCl, pH 7.5, in the cold. The antibodies were concentrated by precipitation with ammonium sulfate and then exhaustively dialyzed against 0.1 M NaCl–5 mM Tris-HCl, pH 7.5. About 90% of the anti-Z-DNA antibodies were recovered, as estimated from the absorption of the solution at 280 nm.

The same procedure of purification was done in 0.3 M NaCl instead of in 3 M NaCl. Poly(dG-dC)·poly(dG-dC) linked to the Sepharose was in the B form. The antibodies reacting with Z-DNA were not retained on the column.

The purity of the antibodies was studied by ultracentrifugation and double diffusion. By ultracentrifugation, only one component was detected and its sedimentation coefficient was 7 S in 0.15 M NaCl–5 mM Tris-HCl, pH 7.5. By double diffusion in agar, precipitation lines were observed with goat antiserum anti-rabbit IgG but not with goat antiserum anti-rabbit IgM (results not shown).

The binding capacity of the purified antibodies was slightly less than that of the antiserum. The concentration of the purified antibody solution was adjusted to 0.45 mg/mL (same concentration as that of the antibodies to Z-DNA in the antiserum). The titer of this solution deduced from the precipitation of [^{3}H]poly(dG-dC)–Cl_2dienPt (0.12) as a function of the dilution of the antibody solution (same conditions as

in Figure 2) was about 1/2600. Almost all the experiments reported on the antiserum were also done with the purified antibodies, and similar results were obtained.

Discussion

Two rabbits were immunized with poly(dG-dC)·poly(dG-dC) modified by chloro(diethylenetriamine)platinum(II) chloride. This polynucleotide in which 12% of the total bases are modified on the N(7) of guanine residues has been chosen because it has the Z form under physiological conditions. The two antisera gave a strong precipitation with poly(dG-dC)–Cl_2dienPt (0.12) (Malfoy & Leng, 1981). In this work, we report some properties of the antisera and of the purified antibodies.

The antiserum precipitates poly(dG-dC)–Cl_2dienPt (0.12) and poly(dG-dC)·poly(dG-dC) in the Z form. This has been shown by RIA and precipitin assays. By RIA in 3 M NaCl, the affinity of the antiserum toward these two polynucleotides is the same, and poly(dG-dC)·poly(dG-dC) inhibits up to 100% the binding of poly(dG-dC)–Cl_2dienPt (0.12) to the antiserum. Thus, the antiserum recognizes poly(dG-dC)·poly(dG-dC) in the Z form, and the platinum residues are not involved in the antigenic determinant. The platinum residues are bulky, and consequently, the N(7) of guanine residues are not in close contact with the amino acids residues of the antibody binding site. In 0.1 M NaCl, poly(dG-dC)·poly(dG-dC) is in the B form and does not inhibit the binding of poly(dG-dC)–Cl_2dienPt (0.12) to the antiserum.

The amount of antibodies to Z-poly(dG-dC)·poly(dG-dC) was determined by quantitative precipitation. This experiment was performed in 3 M NaCl with poly(dG-dC)·poly(dG-dC) as the antigen. At the equivalence point, the concentration of antibodies is about 0.45 mg/mL of antiserum. In the region of antibody excess (at the left of the equivalence point), all the poly(dG-dC)·poly(dG-dC) is precipitated. The molar ratio of nucleotide residues over antibodies in the precipitate is about equal to 8. Thus each antibody molecule covers about eight nucleotide residues.

Most of the antibodies to poly(dG-dC)–Cl_2dienPt (0.12) are IgG. This was shown by gel filtration on the antiserum, by ultracentrifugation, and by double diffusion in agar on the purified antibodies. By gel filtration, the antibodies that can precipitate poly(dG-dC)–Cl_2dienPt (0.12) migrate as the marker (nonspecific rabbit IgG). The sedimentation coefficient of the purified antibodies is equal to 7 S. By double diffusion, precipitation lines were only observed with goat antiserum against rabbit IgG. These experiments demonstrate that we are dealing with IgG. Thus, each Fab fragment binding site covers four nucleotides. This is slightly smaller than the values reported for the antibodies to double-stranded polynucleotides (six to eight nucleotide residues) by each Fab fragment binding site (Guigues & Leng, 1976; Leng et al., 1978; Johnson & Stollar, 1978). By taking into account the differences in the pitches of A′ and Z double helices (36 and 45 Å, respectively; Drew et al., 1980), one gets about the same size for the binding site of these antibodies to double-stranded polynucleotides.

Several experiments were performed in order to characterize some properties of the antibody–polynucleotide complexes and the nature of the antigenic determinant. We first studied the effect of temperature and ionic strength on the binding affinities. These were characterized qualitatively by measuring the variations of the amounts of polynucleotide precipitated by the antiserum (in the reference conditions, the antiserum was diluted until 50% of the polynucleotide was precipitated, see Figure 2).

The same amount of [^{3}H]poly(dG-dC)–Cl_2dienPt (0.12) was precipitated at 0 and 40 °C. The binding constant is temperature independent, indicating that the enthalpy of the reaction is very small and thus the reaction is entropy driven. This seems to be a general result concerning nucleic acid–protein interactions (Hélène & Lancelot, 1982).

The analysis of the results as a function of the ionic strength is not straightforward. The quantity of poly(dG-br^{5}dC)·poly(dG-br^{5}dC) precipitated by the antibodies varies as a function of the salt concentration in contrast to that of poly(dG-dC)–Cl_2dienPt (0.12) or brominated poly(dG-dC) [we recall that poly(dG-br^{5}dC)·poly(dG-br^{5}dC) was enzymatically synthesized and that brominated poly(dG-dC) was prepared by the reaction between bromine and poly(dG-dC)·poly(dG-dC) as described by Lafer et al. (1981)].

According to Record et al. (1976, 1981), the binding of a protein with n positive charges to a nucleic acid neutralizes n phosphate groups and releases $n\Psi$ counterions from the nucleic acid (Ψ is the fraction of counterions thermodynamically bound per phosphate). The consequence is a decrease of the association constant as the ionic strength is increased.

From the competition experiments, by knowing the concentrations of the inhibitor to get 50% inhibition (Table I), one can estimate the relative association constants for the antibody–poly(dG-br^{5}dC)·poly(dG-br^{5}dC) complexes in 0.1 and 3 M NaCl (Cebra, 1971). We deduce that the association constant decreases by a factor of 10 from 0.1 to 1 M NaCl and thus electrostatic interactions are involved. According to Record et al. (1976, 1981), about one phosphate group is involved in an electrostatic interaction with the antibody binding site (we emphasize that this is a rough approximation because the theory probably has to be reconsidered for Z-DNA).

No effect of ionic strength has been found with poly(dG-dC)–Cl_2dienPt (0.12). This can be understood because this polynucleotide is less negatively charged than poly(dG-br^{5}dC)·poly(dG-br^{5}dC) [in poly(dG-dC)–Cl_2dienPt, each modified base bears two positive charges].

With concern to antibody binding in 3 M NaCl, poly(dG-br^{5}dC)·poly(dG-br^{5}dC), poly(dG-dC)·poly(dG-dC), and brominated poly(dG-dC) behave similarly (Table I). The surprising result is that the inhibition of brominated poly(dG-dC) in 0.1 and 3 M NaCl is the same. Since we have concluded that a phosphate group interacts with the antibody binding site, we have to assume that in brominated poly(dG-dC), the bromine atom on the C(8) of guanine residue hinders or at least reduces these electrostatic interactions either by direct competition or by inducing a slight conformational change.

Let us now discuss the behavior of poly(dG-dC)–AAF. The covalent binding of AAF residues to the C(8) of guanine residues induces the transition of poly(dG-dC)·poly(dG-dC) from the B form to the Z form (Sage & Leng, 1980, 1981; Santella et al., 1981a,b). In 0.1 M NaCl–1 mM $MgCl_2$, poly(dG-dC)–AAF (0.025) is partly in the Z form and poly(dG-dC)–AAF (0.07) and poly(dG-dC)–AAF (0.13) are in the Z form as judged by circular dichroism (results not shown). Poly(dG-dC)–AAF (0.025) inhibits the tracer binding to the antibodies less than poly(dG-dC)–AAF (0.07) and poly(dG-dC)–AAF (0.13), these two last polymers giving the same inhibition. In 3 M NaCl, poly(dG-dC)–AAF (0.07) and poly(dG-dC)–AAF (0.13) behave similarly but are less efficient inhibitors than in 0.1 M NaCl. This is due to a decrease of the electrostatic interactions. On the other hand, the smaller affinity of AAF-modified poly(dG-dC)·poly(dG-dC) as compared to that of poly(dG-dC)·poly(dG-dC) could reflect some

conformational changes of the polynucleotide.

A steric hindrance due to the bulky AAF residues cannot explain this effect since two AAF-modified poly(dG-dC)·poly(dG-dC)'s (0.07 and 0.13) inhibit similarly. If AAF residues bound to some sequences of natural DNA induce the Z form, these antibodies can be useful to detect in situ these Z sequences.

To better determine the antigenic determinant, we have studied two samples modified on the C(5) of cytosine residues, poly(dG-m^{5}dC)·poly(dG-m^{5}dC) and poly(dG-br^{5}dC)·poly(dG-br^{5}dC). In 3 M NaCl, the binding of the antibodies to poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is smaller by 2 orders of magnitude than the binding to poly(dG-br^{5}dC)·poly(dG-br^{5}dC). This effect cannot be only due to a steric hindrance since the size of methyl groups and bromine atoms is about the same. We assume that this is due to slight differences in the conformation of these two polynucleotides. This could be an evidence for the solution conformational continuum of the Z form described by Drew & Dickerson (1981). It is interesting to note that bromine is more efficient than methyl group for stabilizing the Z form. The midpoint of the B–Z transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is found at about 0.7 M NaCl (Behe & Felsenfeld, 1981), while poly(dG-br^{5}dC)·poly(dG-br^{5}dC) is in the Z form in the range of 5 mM Tris-HCl, pH 7.5, to 3 M NaCl.

In a recent work (Hartmann et al., 1982), we have demonstrated that poly(dI-br^{5}dC)·poly(dI-br^{5}dC) has the Z conformation in 4 M NaCl. By competition experiments, in this work, we show that poly(dI-br^{5}dC)·poly(dI-br^{5}dC) does not inhibit the binding of poly(dG-dC)·poly(dG-dC) to the antibodies. Even if the conformation of these two polymers is slightly different, this result strongly suggests an interaction between the exocyclic amino group of guanine residues and the amino acid residues of the antibody binding site. This behavior is quite different from that of the antibodies to poly(I)·poly(C), which does not interact with the base residues (Leng et al., 1978).

All these results were obtained with the antiserum although similar results were founds with the purified antibodies. The antibodies can be purified by affinity chromatography on a Sepharose–poly(dG-dC)·poly(dG-dC) column. The antibodies were eluted with 1.8 M NaSCN solution, and almost all the anti-Z antibodies were recovered. The column was not destroyed by the NaSCN solution and can be used several times.

Antibodies to Z-poly(dG-dC)·poly(dG-dC) have been also elicited in rabbits immunized with chemically brominated poly(dG-dC)·poly(dG-dC) (Lafer et al., 1981). There are some minor differences in the reactivity of these antibodies and those described in this paper [for example, in the binding to poly(dG-m^{5}dC)·poly(dG-m^{5}dC)]. A major point is that both antibodies can detect Z-form natural DNA as shown by the binding to polytene chromosomes (Nordheim et al., 1981; Rio et al., 1982).

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