Antibodies to Z DNA stabilized with polyarginine

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The left-handed form of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) induced by heating the copolymer in the presence of magnesium and stabilized with polyarginine can be used to raise antibodies in rabbits. These antibodies are able to recognize the Z conformation of both methylated and nonmethylated forms of the copolymers. In the same experimental conditions, hypermethylated B DNA is not recognized by these antibodies.

Polyarginine *Z DNA* *Antibody*

1. INTRODUCTION

Stabilization of the left-handed form of poly(dG-dC)·poly(dG-dC) by polyarginine suggested that arginine-rich proteins could stabilize Z DNA in vivo [1]. This observation prompted us to test the immunogenicity of such protein-Z DNA complexes prepared with the methylated form of the copolymer. Poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was induced to adopt the left-handed configuration, stabilized with polyarginine and injected into rabbits. The reactivity of antibodies obtained after immunization was tested against the methylated and nonmethylated forms of the copolymer, in the absence of polyarginine, and compared with the reactivity of antibodies raised through the use of poly(dG-m^{5}dC)·poly(dG-m^{5}dC)dien Pt (0.12) [2] or of poly(dG-Br^{5}dC)·poly(dG-Br^{5}dC) [3].

2. MATERIALS AND METHODS

The procedure to convert poly(dG-m^{5}dC)·poly(dG-m^{5}dC) to the Z configuration and to stabilize the left-handed form with polyarginine was essentially the same as that described by Klevan and Schumaker [1] for the nonmethylated copolymer.

Poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) were from PL Biochemicals.

Rabbits were immunized with the polyarginine-Z DNA complexes according to the procedure used for raising antibodies to m^{7}guanosine and m^{6}adenosine [4,5]. Immunoglobulins were extracted from antisera as described [4]. DNA affinity columns were prepared with sheared calf thymus DNA according to Allfrey and Inoue [6]. The sheared DNA was linked to carboxylated agarose (Affigel 202, Biorad, Richmond, CA) with carbodiimide. In these conditions, 1.1 mg DNA were bound to 10 ml wet resin. 400 μg immunoglobulins in 10 mM Tris, pH 7.5, 0.14 M NaCl were loaded on the column (0.5 × 5 cm). The column was washed with the same buffer at a flow rate of 1 ml/h. Non-adsorbed proteins were used for filter binding assays.

Antibodies to poly(dG-Br^{5}dC)·poly(dG-Br^{5}dC) were obtained through the procedure of Zarling et al. [3].

Antibodies to poly(dG-m^{5}dC)·poly(dG-m^{5}dC)-dien Pt (0.12) were a gift from Dr M. Leng.

Poly(dG-dC)·poly(dG-dC) was labelled by nick translation [7] with tritiated dGTP and dCTP (spec. act. 12.3 and 19 Ci/mmol, respectively). The specific activity of the radiolabelled copolymer was 3.6 × 10^6 dpm/μg.

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Filter binding assays were performed as described [4].

3. RESULTS

3.1. *Optical measurements*

Differential ultraviolet spectra obtained with poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under various conditions gave the results summarized in table 1. It can be seen that the copolymer was in the B configuration in 0.14 M NaCl in both the absence and presence of magnesium (table 1, expts 1 and 2). After heating at 60°C, the copolymer displayed the shifted spectrum characteristic of the Z conformer (expt 3). Cooling the solution to 0°C for 30 min somewhat decreased the amplitude of the change (table 1, expt 4). When cooled samples were reincubated at 37°C for 10 min the shifts increased (table 1, expt 5). Heating at 60°C for 5 min and addition of polyarginine further increased the observed shift, whereas cooling to 0°C did not promote the same decrease as in expt 4. No change in the ultraviolet spectrum was observed after storage of the solution at 4°C for 3 weeks.

Table 1

Ultraviolet absorbance shifts observed with poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in various experimental conditions

Expt	Buffer	Temperature	A_{295}/A_{260}
1	0.14 M NaCl, 10 mM Tris-HCl, pH 7.4	20°C	0.25
2	0.14 M NaCl, 10 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$	20°C	0.25
3	as in expt 2	60°C for 5 min	0.38
4	as in expt 2	as in expt 3 then cooled in ice for 30 min	0.30
5	as in expt 2	as in expt 4 then 37°C for 5 min	0.35
6	as in expt 2	as in expt 5 then 60°C for 5 min	0.45
7	as in expt 2 + polyarginine	as in expt 6 then cooled in ice for 30 min	0.46

Differential ultraviolet spectra were obtained with a Beckman DB spectrophotometer. Polymer concentration: 50 μg/ml

Circular dichroism spectra were obtained in similar experimental conditions except the polymer concentration was increased to 125 μg/ml. The results are shown in fig.1. No significant changes were observed at room temperature in either the presence or absence of magnesium. After heating at 60°C for 5 min the spectrum obtained was characteristic of the Z form. When the solution was cooled in ice for 5 min the spectrum observed was intermediate between those obtained in previous experiments.

3.2. *Filter binding assays*

Antiserum or immunoglobulins were tested for their ability to recognize the Z form of poly(dG-dC)·poly(dG-dC) in the absence of polyarginine.

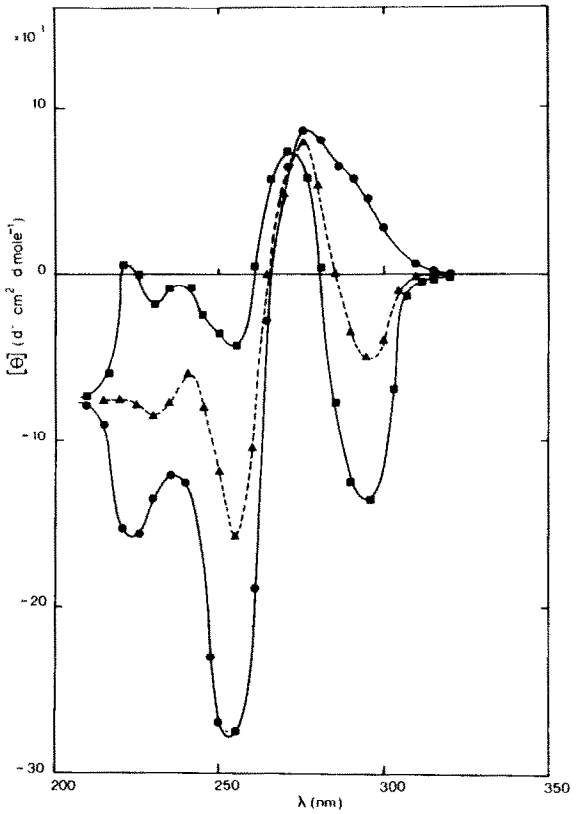

Fig.1. Circular dichroism spectra of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under various experimental conditions. (●—●) 0.14 M NaCl, 5 mM $MgCl_2$ at 20°C; (■—■) 0.14 M NaCl, 5 mM $MgCl_2$ after 5 min at 60°C; (▲---▲) as above after cooling in ice. Polymer concentration, 125 μg/ml.

The radiolabelled tracer was retained on the filter by antiserum at high ionic strength and, to a lesser extent, in 0.2 M NaCl. No binding was observed with the preimmune sera. Tests performed with total immunoglobulins and immunoglobulins not retained by the affinity column are summarized in table 2. The results obtained show that both configurations of the copolymer were recognized by total immunoglobulins whereas antibodies not retained on the DNA column bound the tracer in high salt only. Control experiments carried out with DNA from *Xanthomonas oryzae* XP12 bacteriophage, in which all cytosines are replaced by 5-methylcytosine [8], indicated that these antibodies did not react with a hypermethylated DNA in the B form. Therefore it can be assumed that a fraction of antibodies raised with the polyarginine-methylated copolymer complex recognize the Z configuration of poly(dG-dC)·poly(dG-dC). To ascertain further the specificity of these antibodies, competition experiments were done with nonlabelled methylated and nonmethylated forms of the copolymer, in salt conditions giving rise to the Z form. Antibodies were preincubated with increasing amounts of competitors for 15 min at 37°C. Then the radiolabelled tracer was added and incubation pursued for 30 min at 37°C and 30 min in ice. The results obtained are summarized in table 3. Preincubation with the nonlabelled competitor gave a high percentage of inhibition of the retention of the labelled tracer by antibodies. When an equimolar amount of the methylated form of the copolymer was used under the same conditions, the retention of the tracer was inhibited by 57%. From these results, it can be assumed that the Z configuration of the methylated copolymer is able to compete efficiently with the non-methylated tracer for antibody binding.

The reactivity of these antibodies was compared with the ability to bind Z DNA of the antibodies raised against poly(dG-m^{5}dC)·poly(dG-m^{5}dC)-dien Pt (0.12) or poly(dG-Br^{5}dC)·poly(dG-Br^{5}dC). Inhibition tests were performed with ^{3}H-labelled poly(dG-dC)·poly(dG-dC) as a tracer and increasing amounts of the nonmethylated or methylated form of the polymer, in 3 M NaCl. The results obtained are summarized in table 4 and fig.2. A 50% inhibition of antibody binding was obtained with poly(dG-dC)·poly(dG-dC) for approximately the same range of magnitude for the 3 types of antibodies (table 3). Inhibition tests performed with poly(dG-m^{5}dC)·poly(dG-m^{5}dC) revealed that antibodies to the brominated polymer were slightly less affected by competition between the methylated and nonmethylated forms (fig.2).

Table 2

Tracer-antibody binding observed with various fractions of immunoglobulins

Antibodies (μg)		% tracer retained	
		[^{3}H]Poly(dG-dC)·poly(dG-dC) input: 10 ng (3.6 × 10^7 dpm/μg)	
		3 M NaCl	0.2 M NaCl
Total IgGs	16	24.7	31.1
	32	60.5	74.4
IgGs not retained on the DNA column	12	15.8	0.43
	24	30	0.56
		[^{35}S]XP12 phage DNA input: 10 ng (1.82 × 10^9 dpm/μg)	
IgGs not retained on the DNA column	12	0	0.09
	24	0.28	0.09

Nitrocellulose filter binding assays were performed in a 200 μl volume as described in section 2

Table 3

Inhibition of tracer-antibody binding by preincubation with nonlabelled competitors

Competitor (ng)	% inhibition
Poly(dG-dC)·poly(dG-dC)	
0.5	95
1.0	84
2.5	83
Poly(dG-m^{5}dC)·poly(dG-m^{5}dC)	
0.5	12
1.0	36
2.5	57

Volume of the assay, 200 μl; [^{3}H]poly(dG-dC)·poly(dG-dC) input, 2.5 ng (spec. act. 3.6 × 10^7 dpm/μg); antibodies, 120 μg/ml

Table 4

Tracer-antibody binding inhibition by poly(dG-dC)·poly(dG-dC) observed with antibodies from various origins

Antibodies	Number of residues for 50% inhibition in 3 M NaCl
Antipolyarginine-poly(dG-m^{5}dC)·poly(dG-m^{5}dC), 70 μg	4.5×10^{13}
Antipoly(dG-m^{5}dC)·poly(dG-m^{5}dC)dien Pt (0.12), 40 μg	1.7×10^{14}
Antipoly(dG-Br^{5}dC)·poly(dG-Br^{5}dC), 50 μg	7.2×10^{13}

Hapten concentration, 1.5×10^{-8} M; buffer, 3 M NaCl, 10 mM Tris, pH 7.5; volume of the assay, 200 μl

4. DISCUSSION

Polyarginine was shown to stabilize the Z form of poly(dG-dC)·poly(dG-dC) [1]. On the other hand, reversible interconversion of B and Z forms was obtained when the methylated form of the copolymer was heated [9,11]. These properties were used to test the possibility to raise antibodies directed against Z DNA. Poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was induced to adopt the Z configuration by heat, at moderate ionic strength, in the presence of magnesium and was stabilized with polyarginine. The protein-Z DNA complex was used as an antigen and rabbit antisera were obtained which contained immunoglobulins able to retain both the methylated and nonmethylated copolymers on nitrocellulose filters in high salt. The analysis of circular dichroism spectra indicated that poly(dG-m^{5}dC)·poly(dGm^{5}dC) was induced to adopt the Z conformation by heating at 60°C for 5 min, at moderate ionic strength in the presence of magnesium. Cooling the solution gave a spectrum intermediate between the B and Z forms. A linear regression analysis performed on 18 points indicated that 46% (± 3%) of the polymer was in the B form and 49% (± 7%) in the Z form according to the following equation:

$$[\theta]_{\lambda BZ} = [\theta]_{\lambda B} f(B) + [\theta]_{\lambda Z} f(Z)$$

These data are in accordance with the results reported by others [9]. Ultraviolet spectra confirmed the appearance of the Z form on heating and its stabilization by polyarginine, as described

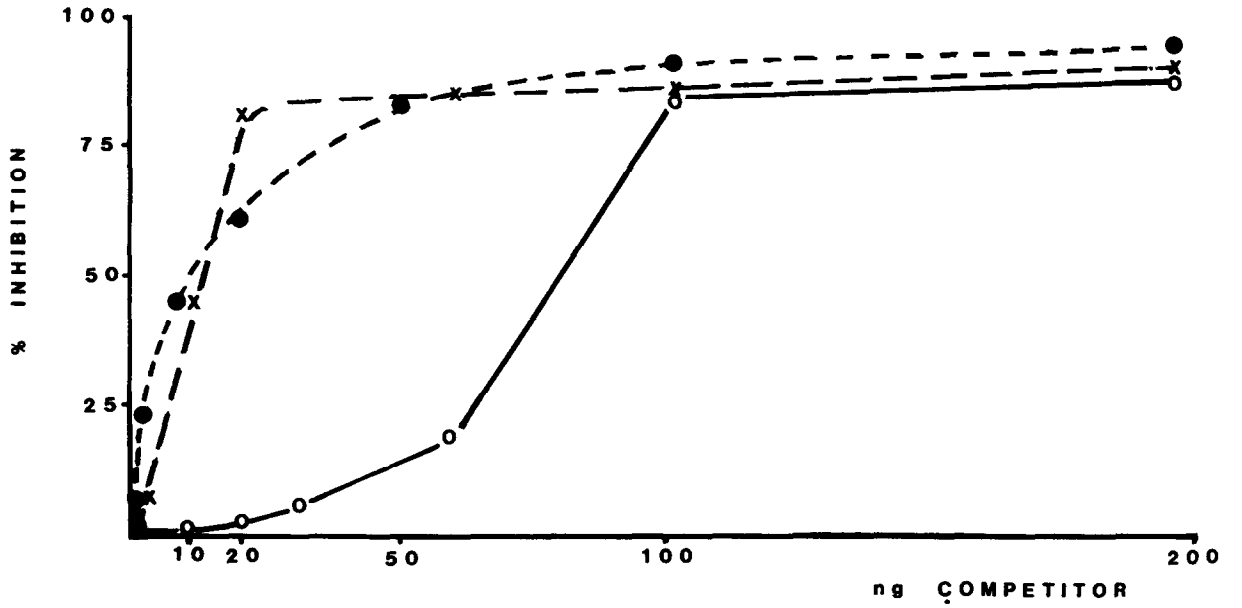

Fig.2. Antibodies from various origins were incubated with 10 ng [^{3}H]poly(dG-dC)·poly(dG-dC) in the presence of increasing amounts of poly(dG-m^{5}dC)·poly(dG-m^{5}dC), in high salt. (●---●) Antipolyarginine-poly(dG-m^{5}dC)·poly(dG-m^{5}dC), 70 μg/assay; (×—×) antipoly(dG-m^{5}dC)·poly(dG-m^{5}dC)dien Pt (0.12), 40 μg/assay; (○—○) antipoly(dG-Br^{5}dC)·poly(dG-Br^{5}dC), 50 μg/assay.

by Klevan and Schumaker [1] for poly(dG-dC)·poly(dG-dC). No aggregation of the polymer was detected as judged from spectroscopic data.

Antibodies obtained after immunization of rabbits with a protein-Z DNA complex were able to recognize the left-handed conformation of both methylated and nonmethylated copolymer in the absence of polyarginine. Therefore it can be assumed that the Z conformation of DNA was at least partially conserved and recognized in vivo. The fact that antibodies recognize both the methylated and nonmethylated Z conformation of the hapten indicated that the methyl group in position 5 of the pyrimidine ring was not immunodominant in the hapten response. This was confirmed by the absence of reaction of antibodies with hypermethylated B DNA extracted from XP12 phage. Antibodies to Z DNA have generally been raised through the use of chemically modified synthetic polynucleotides [10–14]. Here we demonstrate that poly(dG-m^5dC)·poly(dG-m^5dC) stabilized in the Z conformation by polyarginine at moderate ionic strength can be used to obtain antibodies which recognize a left-handed DNA structure.

Monoclonal antibodies raised through the use of brominated poly(dG-dC)·poly(dG-dC) were shown to recognize different parts of Z DNA [3,15]. In antisera obtained after immunization of rabbits with acetylaminofluorene-modified Z DNA, various populations of antibodies were elicited, which were specific for Z DNA determinants [14,16]. These results suggest that subtle differences in DNA conformation could play a key role in the recognition of specific sites by regulatory proteins. Then the interconversion between B and Z DNA configurations could affect the mechanisms involved in the control of differentiation, growth and malignancy. Proteins which bind selectively to Z DNA [17] or to methylated B DNA [18] have been detected in tissues from various origins. Investigations dealing with the nature of these proteins should reveal whether arginine residues are important in stabilizing various DNA configurations.

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