Functional groups on 'Z' DNA recognized by monoclonal antibodies

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Both brominated poly[d(GC)] and poly[d(Gm^{5}C)] form stable left-handed Z-DNA structures at physiological ionic strengths. These two antigens were used to prepare monoclonal antibodies from immunized mice. The specificity of the antibodies was studied in detail with a solid-phase radioimmune assay as well as by means of competition experiments. Both immunogens produced several relatively non-specific antibodies but two types of very specific antibody were also distinguished. The first binds poly[d(Gm^{5}C)] but not brominated poly[d(GC)] while the other has the opposite specificity and will only bind the brominated polymer.

Z-DNA *8-Bromoguanine* *5-Methylcytosine* *Antigenic determinant* *Monoclonal antibody*

1. INTRODUCTION

The availability of specific antibodies has facilitated the exploration of the physiological role of Z-DNA. Antibodies have demonstrated the presence of Z-DNA in isolated chromosomes of many species and in various supercoiled plasmids under physiological conditions [1–4]. Proteins have also been discovered which bind to Z-DNA suggesting that this unusual DNA plays some part in controlling gene activity [5]. Therefore, it is of considerable interest to elucidate what features of the Z-DNA helix can be recognized by proteins. Monoclonal antibodies specific for Z-DNA can act as model proteins to investigate this problem.

We have used brominated poly[d(GC)] and poly[d(Gm^{5}C)] as immunogens in mice to produce monoclonal antibodies. The first of these polymers is brominated primarily at the 8-position of guanine with some modification of the 5-position of cytosine [6], whereas the latter polymer contains only 5-methylcytosine. Thus monoclonal antibodies raised against these two polymers are expected to show different patterns of specificity.

2. MATERIALS AND METHODS

2.1. *Nucleic acids*

Brominated poly[d(GC)] and poly[d(GBr^{5}C)], prepared as in [6,7], were generously donated by Dr J.H. van de Sande (University of Calgary). Poly[d(GC)] was purchased from P-L Biochemicals. Poly[d(Gm^{5}C)] and the other synthetic polymers were synthesized with *E. coli* DNA polymerase I as in [8]. Concentrations were calculated from absorbance measurements assuming an extinction coefficient of 6600 M^{-1} throughout.

2.2. *Solid-phase radioimmune assay (SPRIA)*

For screening the fusions and testing antibody specificities, PVC plates were coated with 2 μg/ml of the appropriate nucleic acid. The assay was performed as in [9] except that all operations were performed in the presence of 5 mM $MgCl_2$ to ensure that poly[d(Gm^{5}C)] was in the Z conformation [11]. A suitable dilution of the cell culture supernatant or of ascites fluid was used routinely. For competition experiments, the competitor was added first, followed by the DNA-binding antibody and then the assay was continued as usual.

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2.3. *Monoclonal antibodies*

Female C57 black mice were given 3 injections at 10-day intervals of 25 μg of either brominated poly[d(GC)] or poly[d(Gm^{5}C)] coupled to 25 μg of methylated bovine serum albumin. The first two injections were given intraperitoneally after emulsification with Freund's adjuvant while the final injection was intravenous. Three days later the spleens were removed and the splenocytes were cultured for 24 h in 20 μg/ml of lipopolysaccharide (Sigma). The fusion protocol with myeloma MOPC 315.43 and culture conditions were carried out as in [9] except that all media contained 0.1 μM sodium selenite. Ten days later testing was initiated using the SPRIA and positive cell lines were cloned by limiting dilution.

2.4. *Determination of isotype*

Ascites fluid was prepared from some of the hybridoma clones as in [10]. Gel filtration on Sephacryl S-200 was performed to separate IgM from IgG fractions. The isotype was then determined by measuring antibody titres in these fractions [9].

3. RESULTS AND DISCUSSION

From the two fusions a total of 11 hybridomas were cloned and, as judged by the SPRIA, they continued to secrete antibodies which bound to Z-DNA. Results for 4 of the monoclonal antibodies tested against various DNAs are shown in table 1. Since these experiments were performed in 5 mM $MgCl_2$ only poly[d(Gm^{5}C)], poly[d(GBr^{5}C)] and brominated poly[d(GC)] are in the 'Z' conformation. Much higher ionic strengths are required to flip poly[d(GC)] and poly[d(TG)] poly[d(m^{5}CA)] from the B form [11,12]. Jel 129 and Jel 131 were derived from the mouse immunized with poly[d(Gm^{5}C)] while Jel 149 and Jel 150 were produced from the brominated polymer. Both Jel 129 and Jel 149 show only a slight preference for Z-DNA and bind to some extent to all the DNAs. Indeed, 9 of the 11 monoclonal antibodies produced were of this type. A plausible explanation for this behavior is that the antibodies are binding to the phosphate backbone and that even a weak nonspecific interaction is sufficient to attach a deca-functional ligand such as an IgM to the DNA on the SPRIA plate. Because of an apparent lack of specificity these antibodies were not investigated further.

Jel 131, on the other hand, is also an IgM but shows considerable specificity and will bind to poly[d(Gm^{5}C)] and poly[d(GBr^{5}C)] but not to brominated poly[d(GC)] or DNAs in the B conformation. Jel 150, an IgG, has the opposite specificity and will only bind to the brominated polymer and not to Z-DNAs modified at the 5-position of cytosine. As noted in [10,13], because the SPRIA tends to be a rather poor discriminator of specificity, the binding of Jel 131 and Jel 150 was studied in more detail by competition experiments (fig.1).

For Jel 150 the only successful competitor is brominated poly[d(GC)] confirming the results of the SPRIA. For Jel 131 poly[d(GBr^{5}C)] is approx.

Table 1

Solid-phase radioimmune assay results for monoclonal antibodies binding to B and Z DNAs

	Jel 129 IgM	Jel 131 IgM	Jel 149 IgM	Jel 150 IgG
Poly[d(Gm^{5}C)]	36	75	61	<5
Poly[d(GBr^{5}C)]	50	100	70	<5
Brominated poly[d(GC)]	100	<5	100	100
Poly[d(GC)]	18	<5	67	<5
Poly[d(TG)] poly[d(m^{5}CA)]	21	<5	57	<5
Native calf thymus DNA	35	<5	69	<5
Blank well	<5	<5	<5	<5

Results (average of at least two measurements) are expressed as percentage of maximum binding after subtracting the background. The maximum cpm was approx. 2000 with a background of 200 in all cases

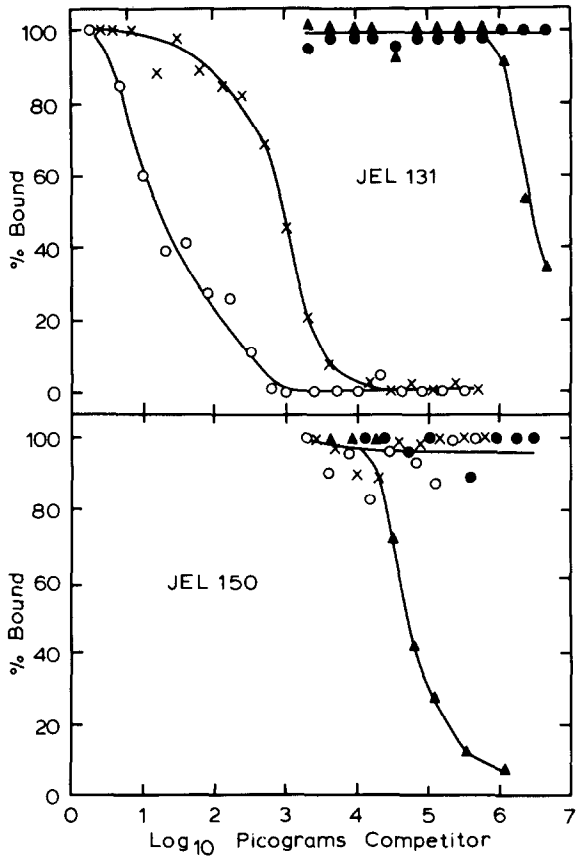

Fig.1. Competition binding experiments for Jel 131 and Jel 150. The % bound to the SPRIA plate is shown as a function of the amount of DNA added as competitor. (×) Poly[d(Gm^{5}C)], (▲) brominated poly[d(GC)], (○) poly[d(GBr^{5}C)], (●) poly[d(GC)]. The plates were coated with poly[d(Gm^{5}C)] and brominated poly[d(GC)] for Jel 131 and Jel 150, respectively.

100-times more effective than poly[d(Gm^{5}C)] suggesting that a modification at the 5-position of cytosine forms a positive recognition site for the antibody. Other proteins such as the lac repressor or antibody Hed 10 which also recognize the 5-position of pyrimidines also show increased binding to bromine compared to methyl moieties [10,14]. Competition by brominated poly[d(GC)] is extremely weak confirming that a bromine at the 8-position of guanine interferes with the interaction. The fact that some competition is detectable is presumably due to the presence of short random stretches of the polymer which have not been brominated. It is worth noting that with this competition assay sub-nanogram quantities of Z-DNA can be detected. For both Jel 131 and Jel 150 no interaction could be detected with poly[d(GC)] which only adopts the Z conformation at much higher ionic strengths [11].

Thus two distinct types of antibody interaction with functional groups on the Z-DNA helix are discernible. The first type of antibody is prevented from binding by modifications at the 5-position of cytosine; monoclonal antibodies corresponding to this class were reported in [13]. The second type of monoclonal antibody is novel and is characterized by a positive interaction at the 5-position of cytosine and a negative interaction at the 8-position of guanine. Both of these groups are found in close proximity on the convex surface of a Z-DNA helix. With these antibodies it may be possible to investigate the role of methylation in the formation of Z-DNA in vivo.

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