A monoclonal antibody specific for the duplex DNA poly[d(TC)]·poly[d(GA)]

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Although most duplex DNAs are not immunogenic some synthetic DNAs such as poly[d(Tm^{5}C)]·poly[d(GA)] are weakly immunogenic allowing the production of monoclonal antibodies. The specificity of one of these antibodies, Jel 172, was investigated in detail by a competitive solid-phase radioimmune assay. Jel 172 bound well to poly[d(TC)]·poly[d(GA)] but not to other duplex DNAs such as poly[d(TTC)]·poly[d(GAA)] and poly[d(TCC)]·poly[d(GGA)]. The binding to poly[d(Br^{5}UC)]·poly[d(GA)] was enhanced while that to poly[d(TC)]·poly[d(IA)] was decreased compared to poly[d(TC)]·poly[d(GA)]. Thus, not only is the antibody very specific for a sequence of duplex DNA but it also appears to recognize functional groups in both grooves of the helix.

B-DNA *Major groove* *Minor groove* *Antigenic determinant* *Monoclonal antibody*

1. INTRODUCTION

Antibodies to duplex DNA are notoriously difficult to produce, because, in general, duplex DNA is not immunogenic [1]. Exceptions to this rule include Z-DNA [2,3] which adopts a left rather than right-handed conformation and poly(dG)·poly(dC) [4,5] both of which are highly immunogenic. These antibodies are potentially useful as probes for investigating the occurrence of unusual DNA conformations [6], as well as serving as models for elucidating what features of duplex DNA can be recognized by proteins [7].

In an attempt to increase the range of available duplex specific antibodies we have studied the immunogenicity of other synthetic duplex DNAs. Some of them, such as poly[d(Tm^{5}C)]·poly[d(GA)] described here, are weakly immunogenic, possibly because they adopt conformations which are slightly different from native B-DNA [8,9]. This has allowed the recovery of monoclonal antibodies at low frequency.

2. MATERIALS AND METHODS

2.1. *Nucleic acids*

Synthetic duplex nucleic acids were synthesized with *E. coli* DNA polymerase and characterized as in [8,10,11]. Concentrations were calculated from absorbance measurements at 260 nm assuming an extinction coefficient of 6600 M^{-1} throughout. As judged from agarose gel electrophoresis all of the polymers were greater than 250 base-pairs in length with an average length greater than approx. 500 base-pairs. The single-stranded DNA, poly[d(Tm^{5}C)] was prepared by depurination of the duplex as in [8]. Poly(dT) was purchased from P-L Biochemicals.

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 in phosphate-buffered saline, pH 7.2 (PBS), for several days. The plates were then coated with 1% fetal calf serum in PBS to block non-specific binding of the antibodies. The assay was performed with cell

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culture supernatants as in [12]. For competition experiments with IgM Jel 172, 1 μg of antibody prepared from ascites fluid as in [13] was used routinely. The competitor was added first, followed by Jel 172 and then the assay was continued as for the SPRIA except that all solutions and washes were prepared with a pH 8.0 buffer (10 mM Tris-HCl, 0.1 mM EDTA, 0.1 M NaCl and 0.1% Tween 20) to ensure that (pyrimidine)·(purine) DNAs containing 5-methylcytosine were in the duplex conformation [8].

2.3. *Monoclonal antibodies*

C57/Black mice were injected 3 times at 10 day intervals with 50 μg poly[d(Tm^{5}C)]·poly[d(GA)] complexed to 50 μg methylated bovine serum albumin. Three days after the last injection, analysis of the serum antibodies with the SPRIA showed that a weak but significant response had been produced. The spleen cells of one of these mice were then used to prepare monoclonal antibodies as in [3,12]. Twelve stable hybridoma cell lines which continued to produce antibodies were successfully cloned by limiting dilution.

3. RESULTS

The specificity of the 12 monoclonal antibodies was initially screened by the SPRIA and 3 groups were discernible (table 1). The first of these, exemplified by Jel 156 (an IgG), showed a clear preference for poly[d(Tm^{5}C)]. We have found that immunizing mice with other (pyrimidine)·(purine) DNAs produces a majority of antibodies which show specificity for the pyrimidine single strand (unpublished). The second group (Jel 161, an IgG) showed a preference for the immunogen, but also bound well to poly[d(Tm^{5}C)], poly[d(TC)]·poly[d(GA)] and poly[d(TG)]·poly[d(m^{5}CA)]. This is a very curious pattern of specificity but unfortunately the antibody was not competed by DNAs in the competitive SPRIA so that, as yet, its specificity has not been investigated further.

Jel 172, on the other hand, bound well to many different nucleic acids. A plausible explanation for this behaviour is that the antibodies are, in part, recognizing the phosphate backbone so that a weak non-specific interaction is sufficient to attach a deca-functional IgM to the DNA on the SPRIA plate. However, the specificity of Jel 172 could be studied in more detail by competition experiments. Although the plates were coated with poly[d(Tm^{5}C)] only the duplex competes effectively. Fig.1A shows competition binding experiments for DNAs having an altered sequence compared to poly[d(Tm^{5}C)]·poly[d(GA)] whereas fig.1B and C investigates the binding of analogues containing modified purines and pyrimidines, respectively. The amount of DNA required to achieve 50% competition is proportional to the binding constant and these are detailed in table 2 relative to poly[d(Tm^{5}C)]·poly[d(GA)] as the standard.

Table 1

Solid-phase radioimmune assay results for monoclonal antibodies binding to various DNAs

	Jel 156	Jel 161	Jel 172
Poly[d(Tm^{5}C)]·poly[d(GA)]	<5	100	80
Poly[d(Tm^{5}C)]	100	51	100
Poly[d(TC)]·poly[d(GA)]	<5	65	71
Poly[d(TG)]·poly[d(m^{5}CA)]	12	76	57
Native calf thymus DNA	17	<5	67
Heat-denatured calf thymus	27	22	70
Poly(dG)·poly(dm^{5}C)	13	<5	71
Poly(dA)·poly(dT)	<5	<5	71
Poly[d(Tm^{5}Cm^{5}C)]·poly[d(GGA)]	10	<5	82
Poly[d(TTm^{5}C)]·poly[d(GAA)]	19	<5	89
Poly(dT)	18	9	79
Blank well	<5	<5	<5

Results are expressed as percentage of maximum binding after subtraction of the background. The maximum cpm was approx. 2000 with a background of 300 in all cases

The specificity of the antibody is remarkable. Firstly, there is no significant binding to the sequence isomer poly[d(TG)]·poly[d(m^{5}CA)] and competition with calf thymus DNA is only observed at very high concentrations. Although at pH 8.0, poly[d(Tm^{5}C)]·poly[d(GA)] is a duplex, at lower pH values it dismutates to a triplex [8]; therefore the possibility was considered that the antibody was recognizing a minor component of the DNA. However, a sample of the duplex which had been treated at pH 5 before returning to pH 8.0 for the competition experiment behaved in a similar manner (not shown). Thus it would appear that the antibody is indeed duplex specific.

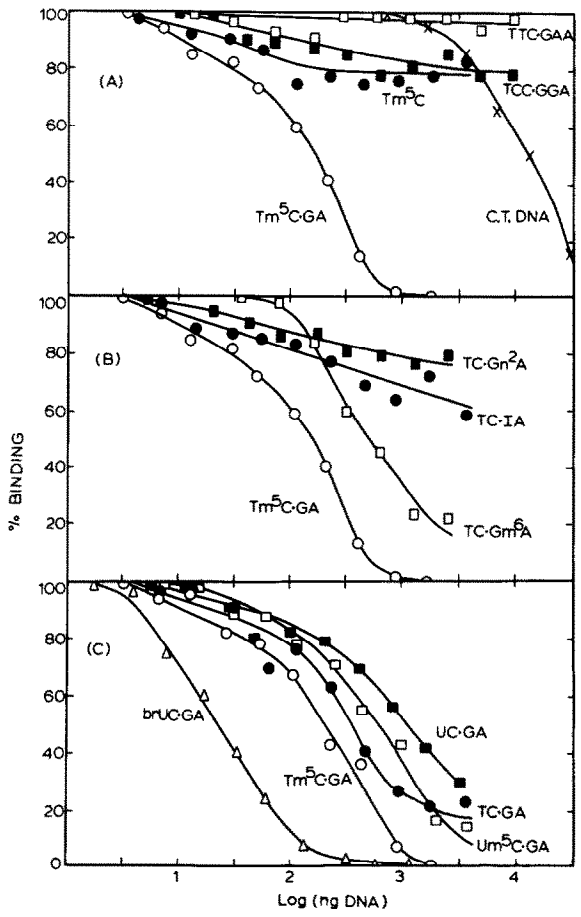

Fig.1. Competitive solid-phase radioimmunoassays. The percent binding of Jel 172 to poly[d(Tm^{5}C)] is shown as a function of the amount of DNA added as competitor. Each data point shown represents the average of 2 independent experiments. (A) Sequence variants: poly-[d(Tm^{5}C)]·poly[d(GA)] (○), poly[d(Tm^{5}C)] (●), poly-[d(TTC)]·poly[d(GAA)] (□), poly[d(TCC)]·poly-[d(GGA)] and poly[d(TG)]·poly[d(m^{5}CA)] (■) (the data points for the latter DNA have been omitted for clarity); calf thymus DNA (×). (B) Modifications of the purines: poly[d(Tm^{5}C)]·poly[d(GA)] (○), poly[d(TC)]·poly-[d(Gn^{2}A)] (■), poly[d(TC)]·poly[d(IA)] (●), poly-[d(TC)]·poly[d(Gm^{6}A)] (□). (C) Modifications of the pyrimidines: poly[d(Tm^{5}C)]·poly[d(GA)] (○), poly-[d(Br^{5}UC])·poly[d(GA)] (△), poly[d(TC)]·poly[d(GA)] (●), poly[d(Um^{5}C)]·poly[d(GA)] (□), poly[d(UC)](□) poly[d(GA)] (■).

Secondly, whereas the unmethylated duplex poly[d(TC)]·poly[d(GA)] competes well, poly-[d(TTC)]·poly[d(GAA)] and poly[d(TCC)]·poly-[d(GGA)] do not. It seems likely that these

Table 2

Relative binding constants for the interaction of Jel 172 with various DNAs and analogues of poly[d(TC)]·poly[d(GA)]

DNA	Relative binding constant
Poly[d(Tm^{5}C)]·poly[d(GA)]	100
Poly[d(TG)]·poly[d(m^{5}CA)]	<4
Poly[d(TC)]·poly[d(GA)]	51
Poly[d(TTC)]·poly[d(GAA)]	<2
Poly[d(TCC)]·poly[d(GGA)]	<2
Calf thymus DNA	1
Poly[d(Tm^{5}C)]	<5
Poly[d(UC)]·poly[d(GA)]	16
Poly[d(Um^{5}C)]·poly[d(GA)]	31
Poly[d(Br^{5}UC)]·poly[d(GA)]	730
Poly[d(TC)]·poly[d(Gm^{6}A)]	31
Poly[d(TC)]·poly[d(IA)]	<4
Poly[d(TC)]·poly[d(Gn^{2}A)]	<6

The relative binding constants were calculated from the ratios of the amount of DNA required to achieve 50% inhibition (fig.1) relative to poly[d(Tm^{5}C)]·poly[d(GA)] as a standard. For those DNAs where 50% inhibition was not reached (< *X*) the value given was deduced from the highest concentration of DNA tested

(pyrimidine)·(purine) DNAs share the same conformation even though it may be slightly different from the classical B-type structure [9]. Since a sequence of 3 base-pairs present in poly[d(TC)]·poly[d(GA)] can be found in the repeating trinucleotide synthetic DNAs, then one must conclude that the preferred binding site contains at least 4 base-pairs, i.e. either $\frac{\text{-TCTC-}}{\text{-AGAG-}}$ or $\frac{\text{-CTCT-}}{\text{-GAGA-}}$. The points of contact between the antibody and duplex were investigated further by preparing analogues of poly[d(TC)]·poly[d(GA)] containing modified bases.

The lack of binding to poly[d(TC)]·poly-[d(Gn^{2}A)] suggests that the antibody makes a close contact to adenine which is prevented by the presence of an amino group in the 2-position of the adenine ring. Also, the binding to poly[d(TC)]·poly[d(IA)] is severely reduced implying that the antibody recognizes the 2-amino group of guanine. It is important to note that both these modifications lie in the minor groove of a B-type helix. There is also a small reduction in binding when a

methyl group is attached to the 6-amino group of adenine (poly[d(TC)] · poly[d(Gm^{6}A)]). This modification lies in the major groove of a DNA helix. Further evidence for an interaction with functional groups in the major groove was obtained by studying pyrimidine analogues. Thus poly[d(TC)] · poly[d(GA)] binds half as well as poly[d(Tm^{5}C)] · poly[d(GA)] and also the binding constant of poly[d(UC)] · poly[d(GA)] is half that of poly[d(Um^{5}C)] · poly[d(GA)]. The similar effect of a methyl group on the two series of polymers suggests, not only that the 5-methyl of cytosine is being recognized, but also that the binding of the antibody is not determined primarily by small conformational differences which may be exhibited by the various polymers. Finally, comparison of poly[d(Tm^{5}C)] · poly[d(GA)] with poly[d(Um^{5}C)] · poly[d(GA)] and of poly[d(TC)] · poly[d(GA)] with poly[d(UC)] · poly[d(GA)] shows that removal of the 5-methyl of thymine leads to a 3-fold reduction in the binding constant in both cases. It is also noteworthy that poly[d(Br^{5}UC)] · poly[d(GA)] has the highest binding constant. Similar changes in binding constants upon modification of the 5-position of pyrimidines have been observed for other proteins interacting with DNA [13,14].

4. DISCUSSION

Although the SPRIA results suggest that Jel 172 is non-specific, the competition experiments demonstrate a high degree of discrimination between different duplex sequences. The fact that the SPRIA is a rather poor discriminator of specificity for some antibodies has been noted previously in [13,15] and is probably due to antibody recognition of the phosphate backbone. Evidence has also been presented for an interaction of Jel 172 in both grooves of the helix, although the relative binding constants need to be interpreted with caution (table 2). At present, it is not possible to evaluate the effects of small changes in polymer length or flexibility on the binding affinity. It has been shown in [16] that the maximum binding constant for an IgG is only achieved when both arms of the antibody are bound simultaneously to the DNA. This occurs when the DNA is longer than about 50 base-pairs. The polymers used here were over 250 base-pairs in length but Jel 172 is an IgM and therefore polymer length may play an even more important role in determining the binding affinity. Even so some of the differences are quite large. For example the binding to poly[d(Br^{5}UC)] · poly[d(GA)] is 15-times greater than to poly[d(TC)] · poly[d(GA)] while the binding to poly[d(TC)] · poly[d(IA)] is at least 12-fold lower.

Based upon these observations a possible binding site for the antibody is shown in fig.2. Recognition of the pyrimidines occurs in the major groove and close contact has been demonstrated with the purines in the minor groove. Other interactions which could not be evaluated with the use of modified bases may well occur but the most interesting feature of this model is the straddling of the sugar-phosphate backbone. Presumably the antibody must contain a deep groove into which the backbone fits, allowing, at the same time, sequence-specific recognition of a right-handed duplex DNA. In this regard the weak but detectable interaction of Jel 172 with calf thymus DNA

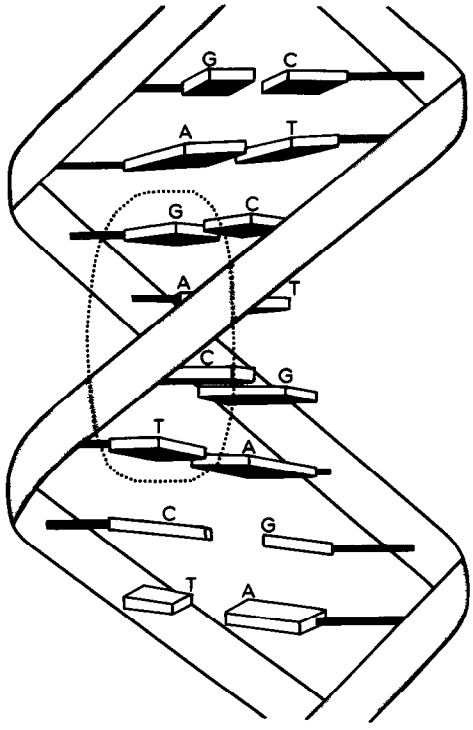

Fig.2. Proposed binding site of Jel 172 on poly[d(TC)] · poly[d(GA)]. The area of contact between the antibody and DNA is shown enclosed by the dotted ellipse. Thus the antibody straddles the sugar-phosphate backbone with interactions in both grooves of the helix.

is interesting since (pyrimidine)·(purine) tracts are found frequently in the 5'-flanking regions of eucaryotic genes [17,18]. Future work will be directed to developing this and other antibodies as probes for these unusual sequences.

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