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Variations in duplex DNA conformation detected by the binding of monoclonal autoimmune antibodies

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ABSTRACT

Four monoclonal antibodies (Jel 229, 239, 241, 242) which bound to duplex DNA were prepared from two autoimmune female NZB/NZW mice. Their binding to various nucleic acids was investigated by a competitive solid phase radioimmune assay which allows the estimation of relative binding constants. None of the antibodies showed any consistent variation of binding constant with base composition and thus they must recognize features of the DNA backbone. Jel 241 binds across the major groove but the interaction with poly(pyrimidine).poly(purine) DNAs was barely detectable. This antibody appears to recognize the "alternating-B" conformation which is promoted by methylation of pyrimidines in alternating sequences. The other three antibodies bind in the minor groove. In particular, for Jel 229 the preferred antigen was poly(dG).poly(dC) with only weak binding to poly(dA).poly(dT). This suggests a requirement for a wide minor groove. Thus autoimmune antibodies provide examples of "analogue" recognition and can be used to detect structural variations in the grooves of duplex DNA.

INTRODUCTION

X-ray fibre diffraction studies have revealed that the conformation of duplex DNA falls into various structural families named A, B, C etc (1). Naturally-occurring DNA is predominantly B-form. Therefore it was felt that sequence-specific DNA-binding proteins would have to recognize features of the bases rather than the structure of duplex DNA. However this view of protein-DNA interactions now appears to be too simplistic and three experimental approaches have suggested that structural features may also be important determinants of protein recognition.

Firstly, X-ray crystallographic studies on short duplex DNA fragments have demonstrated that the sequence has a marked effect on the structure of the sugar-phosphate backbone. In particular, base-stacking interactions alter the pitch of the helix between successive base-pairs and AT rich and GC rich regions adopt conformations characterized by very narrow and very wide minor grooves, respectively (2,3). Secondly, spectroscopic studies of repeating-sequence synthetic DNAs show structural variations. For example,

the circular dichroism spectra of poly[d(TG)].poly[d(CA)] is rather different from the sequence isomer poly[d(TC)].poly[d(GA)] (4). More recently, by phosphorous NMR it has been shown that poly[d(AT)].poly[d(AT)] (and other polymers such as poly[d(Gm^{5}C)].poly[d(Gm^{5}C)]) have a repeating dinucleotide motif, i.e. 5'TpA3' and 5'ApT3' are not equivalent (5,6). Finally, nucleases cleave different sequences at different rates. Several restriction enzymes which recognize d(GGCC) will cleave this sequence more quickly if it is surrounded by AT base-pairs compared to GC base-pairs (7). On the other hand, pancreatic DNase I is believed to show little sequence specificity but binds best to sequences which have a minor groove of intermediate width (8,9).

Thus sequence does have a profound effect on structure. In view of this, Drew and Travers (9) suggest that the terms "sequence" and "structure" specificity be replaced by "digital" and "analogue" specificity. This emphasizes the fact that the first recognition process is discrete, while the other is continuous. In other words, a protein binding to DNA must first make sure that the conformation is correct before recognizing functional groups on the base-pairs.

In this paper, we explore the possibility of using the binding of monoclonal autoimmune antibodies to synthetic duplexes in order to understand DNA structural motifs as well as protein-DNA interactions. Synthetic DNA polymers may be particularly useful for structural studies since any variations in structure may be amplified and stabilized by the regular repetition (10,11). Thus structural differences may be revealed which would not be apparent in short sequences. Previous studies have shown that duplex-binding antibodies from autoimmune mice show very little specificity and generally prefer a B-type conformation (12,13,14,15). However, the detailed studies reported here, using many synthetic DNAs, reveal that considerable variation in binding constants can be detected. This phenomenon is due to analogue recognition.

MATERIALS AND METHODS

Monoclonal Antibodies. Hybridomas were prepared from several female NZB/NZW mice from 4 to 9 months of age as described previously (16). The mice which were purchased from Jackson Laboratories develop a severe autoimmune disease which resembles systemic lupus erythematosus (SLE) (17,18,19). The hybridoma supernatants were screened initially for binding to duplex calf thymus DNA with the aid of a solid phase radioimmune assay (SPRIA) (16,20). However, notwithstanding this initial selection, more detailed studies showed that the

majority of these autoimmune antibodies had a preference for single-stranded DNA (21,22,23). Duplex specific monoclonal antibodies were only successfully prepared from 8 or 9 month old mice, at which age they develop severe SLE and die. Thus the production of useful antibodies is largely a matter of chance.

Nucleic acids. The repeating sequence synthetic nucleic acids were prepared as described previously (20,24) or purchased from P-L Biochemicals. Duplex DNAs were characterized by the fluorescence upon binding of ethidium and thermal denaturation measurements (20,24). Particular care was taken with (pyrimdine).(purine) DNAs to ensure that they adopted a duplex conformation (20).

Poly[d(GGCC)].poly[d(GGCC)] arose serendipitously from a synthetic reaction containing dGTP, dCTP and E. coli polymerase I (24). The Tm of the polymer was 76°C, compared to 85°C for poly[d(GC)].poly[d(GC)] and 71°C for poly(dG).poly(dC) under identical conditions (20). Because the DNA "snapped back" (i.e. there was a 100% return of the fluorescence of bound ethidium after heating and cooling (25)) it was tentatively identified as poly[d(GGCC)].poly[d(GGCC)] rather than poly[d(GGC)].poly[d(GCC)]. This conclusion was confirmed initially by nearest neighbour analysis (R.P. Braun, unpublished results). A polymer having a Tm identical to that of the original poly[d(GGCC)].poly[d(GGCC)] was also prepared by priming a synthetic reaction with the self-complementary dodecamer $d(GGCC)_3$.

The following nucleic acids were generously donated: poly[d(n^2AT)].poly[d(n^2AT)] (Dr. J.H. van de Sande), poly(ADP-ribose) (Dr. J.T. Sibley), phage XP12 DNA (Dr. M. Ehrlich) and phage ØW14 DNA (Dr. T. Warren). Concentrations were calculated from absorbance measurements at 260 nm with extinction coefficients listed previously (4) or assumed to be $6{,}600M^{-1}$. The molecular weights of the duplex DNAs were estimated by electrophoresis on 1.5% Agarose gels with the Hinf III fragments of phage DNA as markers (22,000 to 140 base-pairs).

Relative binding constants. Polyvinyl chloride 96-well plates were coated with 2 μg/ml of poly[d(AT)].poly[d(AT)] for at least 24 hrs. After washing, 80 μl of the most concentrated solution of the competitor was added to the first well and 22 serial, two-fold dilutions were made by transferring 40 μl, with mixing, to 40 μl of buffer in each subsequent well. 40 μl of a suitable dilution of the hybridoma supernatant was then added immediately before continuing the SPRIA as described previously (16,20). The dilution of supernatant (in buffer with 1% fetal calf serum) was chosen to be the greatest dilution which still gave maximum counts (in the range of 1 in 8 to 1 in 64,

depending on the antibody). Under these conditions there is a maximum loss of binding to the plate as a function of added competitor. The assays were performed in duplicate and a competition with duplex calf thymus DNA was always included as an internal standard to provide a reference. Under these conditions the ratio of competing nucleic acids required to reach 50% inhibition is directly proportional to the binding constants which for calf thymus DNA was assigned a value of unity. Depending on the antibody, maximum CPM varied between 2,000 and 8,000 with a background of less than 200 CPM. Although the error in these experiments is difficult to estimate, repeated measurements suggest that the relative binding constants are accurate to within $\pm$ 20%. As will be discussed below, the molecular weights of the various nucleic acids are a far more important consideration.

Antibody isotypes. Ascites fluid was prepared for each of the four duplex specific antibodies described herein. Chromatography on Sephacryl S-200 followed by analysis of the concentration in each peak showed that the antibodies were all IgG rather than IgM (21).

RESULTS

Duplex specificity. Because of the complex pattern of specificities exhibited by autoimmune antibodies (15,16,22), initial experiments were performed to ensure that the preferred antigen was duplex DNA (Figure 1). For comparison with Jel 229, 239, 241 and 242, we have included results for Hed 10 and Jel 205. Hed 10 has been characterized extensively by fluorescence quenching techniques (21) and was shown to be specific for T-rich sequences in single-stranded DNA. Competition experiments confirm this conclusion (Figure 1(a). The amount of competing poly(dT) required to remove 50% of the antibody from binding to the DNA on the solid phase is about 1 pmole. In the case of heat-denatured calf thymus DNA about 100 pmoles are required to reach 50% binding and thus the binding constant of Hed 10 to poly(dT) is about 100 fold greater than to heat-denatured calf thymus DNA. Competition with native calf thymus DNA is only observed at very high concentrations and 50% binding could not be achieved but, by extrapolation, the binding constant is probably about 100 fold less than heat-denatured calf thymus DNA. For Jel 205 (produced from a four-month old autoimmune mouse), as with Hed 10, heat-denatured calf thymus is a much better competitor than the duplex (Figure 1(b)) but we have been unable to identify the preferred single-stranded antigen.

On the other hand, Jel 229, 239 and 242 clearly have a preference for duplex calf thymus compared to heat-denatured calf thymus DNA (Figure

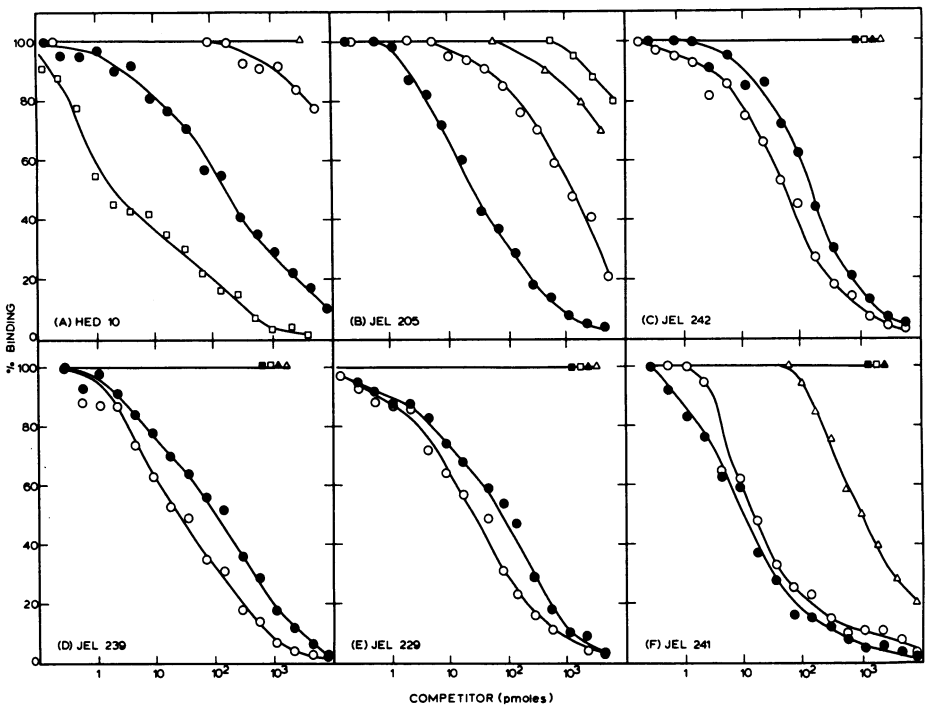

Figure 1. Competition binding experiments with diluted hybridoma supernatants from (a) Hed 10, (b) Jel 205, (c) Jel 242, (d) Jel 239, (e) Jel 229, and (f) Jel 241. In (a) and (b) the plates were coated with heat-denatured calf thymus DNA while for the others poly[d(AT)].poly[d(AT)] was used. The percentage inhibition of binding is shown as a function of added competitor in the Solid Phase Radioimmune Assay (SPRIA): □ = poly(dT); O = calf thymus; ● = heat-denatured calf thymus DNA; △ = poly(dI); ■ = poly(dA) and ▲ = ribosomal RNA from E. coli.

1(c),(d),(e)). Although the difference in relative binding constants is only of the order of 2-4 fold, this is readily measured by competition binding experiments. Also, it must be remembered that heat-denatured calf thymus adopts a conformation containing 50% duplex regions (25,26), and therefore some binding to heat-denatured DNA is to be expected even for a duplex specific antibody. As confirmation of this specificity, no competition was observed with other single-stranded DNAs (poly(dI), poly(dT) and poly(dA)) (Figure 1(c),(d),(c)) nor with poly(ADP-ribose), rRNA and left-handed `Z'-DNA (Brominated poly[d(GC)].poly[d(GC)]) (See Table I). In this regard, Jel 241 is somewhat of a mystery since the binding to heat-denatured calf thymus is marginally (1.5 fold) better than to native duplex DNA (Figure 1(f)).

Table I: Relative Binding Constants to Various Nucleic Acids[a]

Nucleic Acid	Antibody			
	JEL 242	JEL 239	JEL 229	JEL 241
Calf thymus DNA (42% (G+C))	1.0	1.0	1.0	1.0
Heat-Denatured calf thymus DNA	0.39	0.25	0.28	1.5
rRNA	N.C.	N.C.	N.C.	N.C.
poly(ADP-ribose)	<0.01	<0.01	<0.01	<0.01
poly(dI)	<0.01	N.C.	N.C.	0.02
poly(dT)	<0.01	N.C.	N.C.	N.C.
poly(dA)	N.C.	N.C.	N.C.	N.C.
Brominated poly[d(GC)].poly[d(GC)]	<0.01	N.C.	N.C.	N.C.
phage XP12 DNA	1.9	3.0	2.5	1.0
phage ØW14 DNA	0.50	0.68	0.31	0.02
phage T4 DNA	1.0	0.75	0.55	0.07
M. luteus DNA (70% (G+C))	0.81	2.0	0.93	0.86
E. coli DNA (50% (G+C))	1.1	1.6	1.2	1.8
C. perfringens DNA (30% (G+C))	0.95	1.0	0.70	0.86
Sonicated calf thymus DNA	0.22	0.22	0.55	0.77
low M.W. poly[d(AT)].poly[d(AT)]	0.29	0.55	0.68	0.44
normal M.W. poly[d(AT)].poly[d(AT)]	0.95	1.3	1.5	0.92

[a]Binding constants were determined from competition experiments and were measured relative to calf thymus DNA. N.C. = No competition. < 0.01 means that 50% inhibition of binding was not reached at the highest concentration of competitor tested.

Repeated measurements show that this small difference is reproducible (data not shown). Jel 241, is also competed to a much lesser extent by poly(dI) but is clearly different from the single-strand specific antibodies, Hed 10, and Jel 205. For this, and other reasons, to be explained below, we believe that Jel 241 is duplex specific.

Effect of molecular weight. Since an IgG can span up to 50 base-pairs on a duplex DNA, it is not surprising that molecular weight effects are important (27,28). The synthetic DNAs used in this study all have average lengths greater than 450 base-pairs and therefore, one would expect that changes in

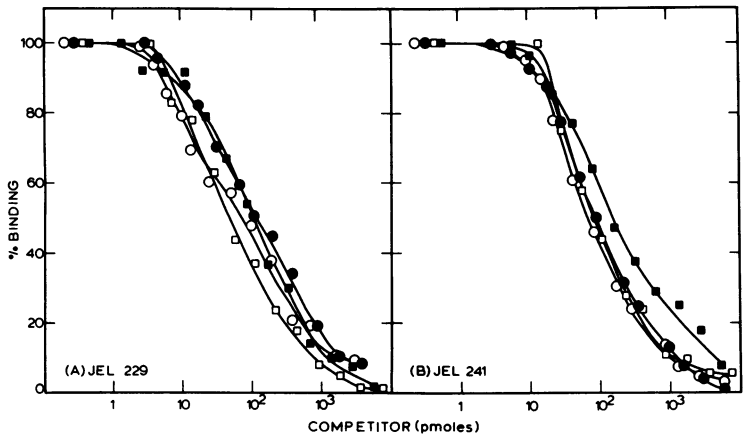

Figure 2. The effects of DNA length on the binding of (a) Jel 229 and (b) Jel 241 to plates coated with poly[d(AT)].poly[d(AT)] investigated by the competitive SPRIA. O = long calf thymus DNA; ● = sonicated calf thymus DNA; □= long poly[d(AT)].poly[d(AT)] and ■ = short poly[d(AT)].poly[d(AT)]. See text for details.

relative binding constants due to end effects would be small (29). On the other hand, Papalian et al. (27) showed that there was a difference in the binding constant of autoimmune sera for a duplex DNA of 400 base-pairs compared to high molecular weight DNA. For this reason, the effects of molecular weight on relative binding constants were investigated.

Representative results are shown in Figure 2 for Jel 229 and 241. The sonicated calf thymus DNA had an average molecular weight of 2,000 base-pairs compared to greater than 20,000 base-pairs for the untreated DNA. The long and short poly[d(AT)].poly[d(AT)] samples had average lengths of 10,000 base-pairs and 350 base-pairs respectively; the former is rather longer than the other synthetic DNAs while the latter is slightly shorter. It can be seen from the amount of competitor required to reach 50% binding that large changes in molecular weight alter relative binding constants by about a factor of two fold for both calf thymus and poly[d(AT)].poly[d(AT)]. These differences were reproducible and significant. For Jel 242 and Jel 239 the effect of molecular weight is slightly larger (Table I). For all four antibodies, since the natural DNAs are all of high molecular weight, then differences of binding constant within this group of more than two fold are considered to be significant. Similarly, because the synthetic DNAs are all relatively short, binding constant changes of more than two-fold between these DNAs are likely

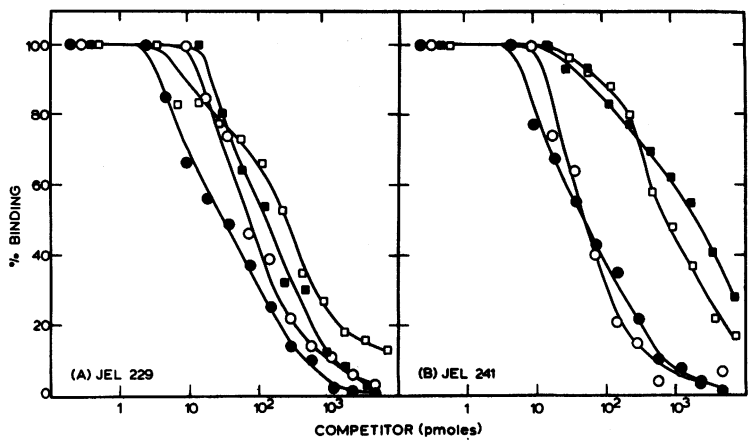

Figure 3. The binding of (a) Jel 229 and (b) Jel 241 to phage DNAs investigated by the competitive SPRIA. O = calf thymus DNA (included as a standard); ● = XP12 DNA; □ = ØW14 DNA, and ■ = T4 DNA.

to represent a real difference. A possible explanation for these effects of molecular weight is presented in the discussion.

Naturally-occurring DNAs. Relative binding constants to bacterial DNAs are compared in Table I. None of the antibodies show much specificity based upon differences in overall base-composition. On the other hand, the binding to phage DNAs is more variable; phage XP12 DNA contains all m^5C residues (30) and all the antibodies except Jel 241 show a small preference for this DNA compared to calf thymus DNA (Table I and Figure 3). Phage ØW14 contains putrescinyl thymine residues which help to reduce the negative charge density of the phosphate backbone (31). This unusual base substantially occludes the major groove which is also the case for phage T4 DNA containing glycosylated hydroxymethyl cytosine residues (32). Jel 241 binds poorly to these DNAs (Figure 3) suggesting that it interacts in or across the major groove. The other antibodies (e.g. Jel 229, Figure 3) show only small or negligible reductions in binding constants suggesting that they interact primarily in the minor groove.

Synthetic nucleic acids - Jel 241. The binding of Jel 241 in the major groove was studied with various alternating (pyrimidine-purine) DNAs containing methylated pyrimidines (Figure 4 and Table II). Comparison of poly[d(AU)].poly[d(AU)] (Figure 4(b)) with poly[d(AT)].poly[d(AT)] (Figure 4(d)) shows that the methylated DNA has a 30 fold higher binding constant. Similarly, Jel 241 has a large preference for the polymers containing m^5C

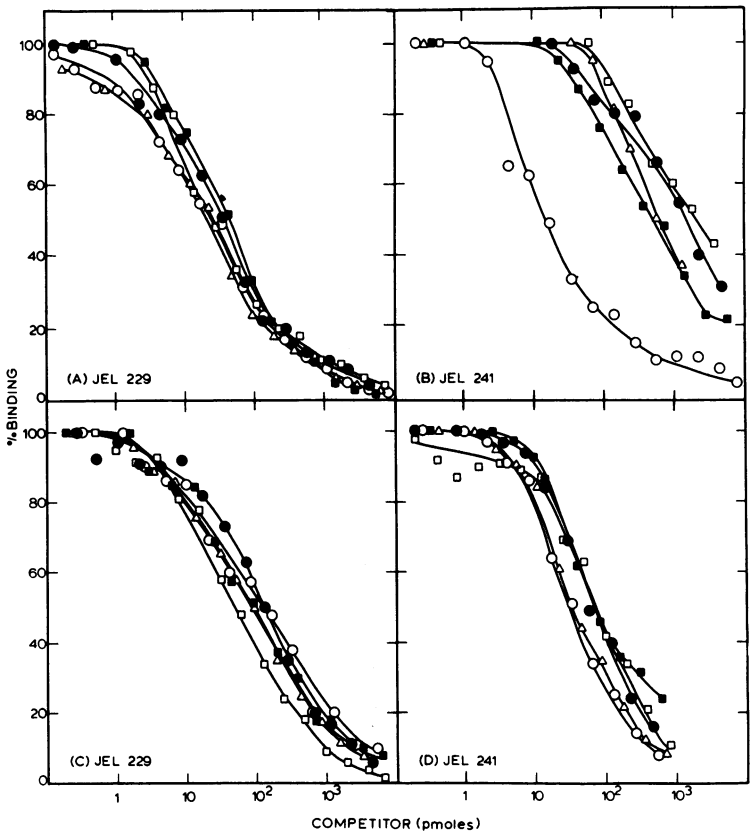

Figure 4. The effects of methylation of pyrimidines on the binding of Jel 229 and Jel 241 to alternating (pyrimidine-purine) DNAs investigated by the competitive SPRIA. O = calf thymus DNA (included as a standard). In (a) and (b) the symbols are ● = poly[d(GC)].poly[d(GC)]; □ = poly[d(IC)].poly[d(IC)]; ■ = poly[d(TG)].poly[d(CA)] and △ = poly[d(AU)].poly[d(AU)]. In (c) and (d) the same symbols are used for the analogues containing methylated pyrimidines where C is replaced by m^5C and U by T.

compared to the unmethylated DNAs, poly[d(GC)].poly[d(GC)], poly[d(IC)].poly[d(IC)] and poly[d(TG)].poly[d(CA)]. In every case the competition curve for the methylated DNAs (Figure 4(d)) follows closely to that of the standard calf thymus DNA while the unmethylated DNAs are all one to two orders of magnitude weaker in their binding (Figure 4(b)). Because the effect of methylation on binding constants is the same for (A-U), (G-C) and (I-C) base-pairs, the most obvious explanation is that the antibody is interacting with the methyl groups directly. However Klug et al. (10) have

Table II: Relative Binding Constants to Synthetic Duplex DNAs[a]

Nucleic Acid	Antibody			
	JEL 242	JEL 239	JEL 229	JEL 241
poly[d(AU)].poly[d(AU)]	-	-	1.0	0.03
poly[d(GC)].poly[d(GC)]	0.37	0.30	0.63	0.01
poly[d(IC)].poly[d(IC)]	0.16	0.08	1.0	<0.01
poly[d(TG)].poly[d(CA)]	0.29	0.21	0.56	0.04
poly[d(AT)].poly[d(AT)]	0.95	1.3	1.5	0.92
poly[d(Gm^{5}C)].poly[d(Gm^{5}C)]	-	-	0.92	0.57
poly[d(Im^{5}C)].poly[d(Im^{5}C)]	-	-	2.2	0.46
poly[d(TG)].poly[d(m^{5}CA)]	-	-	1.5	0.46
poly[d(n^{2}AT)].poly[d(n^{2}AT)]	-	-	0.04	0.10
poly[d(ABr^{5}U].poly[d(ABr^{5}U)]	-	-	0.75	0.18
poly(dA).poly(dT)	0.45	0.58	0.05	<0.01
poly(dG).poly(dC)	0.10	0.10	12.4	<0.01
poly(dI).poly(dC)	0.54	0.13	0.20	<0.01
poly[d(TC)].poly[d(GA)]	0.07	0.09	0.11	<0.01
poly[d(TCC)].poly[d(GGA)]	0.07	0.07	0.11	<0.01
poly[d(TTC)].poly[d(GAA)]	0.29	0.18	0.42	<0.01
poly[d(TTG)].poly[d(CAA)]	0.38	0.20	0.31	0.34
poly[d(ATC)].poly[d(GAT)]	0.24	0.17	0.60	0.04
poly[d(TAC)].poly[d(GTA)]	0.44	0.63	1.2	0.21
poly[d(GGCC)].poly[d(GGCC)]	0.72	0.33	1.1	0.11

[a] Binding constants were determined by competition experiments and measured relative to calf thymus DNA. - = not measured. < 0.01 means that 50% inhibition was not reached at the highest concentration of inhibitor tested.

proposed that methylation improves the stacking of the pyrimidine on the purine below it so that the DNA adopts an alternating or wrinkled conformation. This suggestion has been confirmed for several methylated alternating (pyrimidine-purine) DNAs which show two signals in the phosphorous NMR spectrum (5,6). The latter view (i.e., that Jel 241 recognizes an alternating conformation) is preferred for several reasons.

Firstly, the binding to poly[d(ABr^{5}U)].poly[d(ABr^{5}U)] is less than to poly[d(AT)].poly[d(AT)] (Table II). On the basis of phosphorous NMR spectra

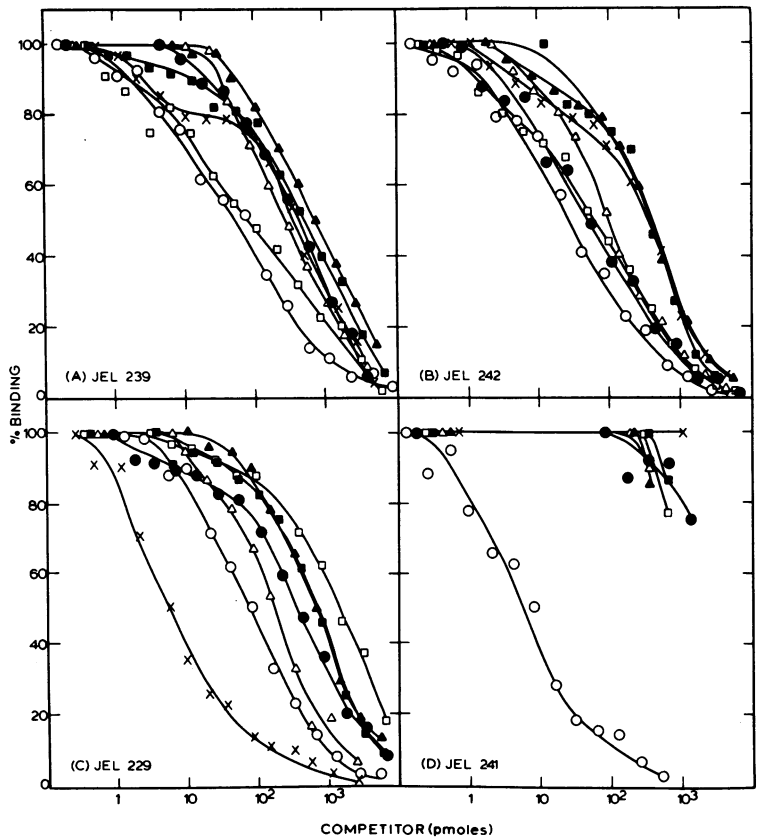

Figure 5. The binding of (a) Jel 239, (b) Jel 242, (c) Jel 229 and (d) Jel 241 to (pyrimidine).(purine) DNAs investigated by the competitive SPRIA. O = calf thymus DNA (as a standard); ● = poly(dI).poly(dC); □ = poly(dA).poly(dT), ■ = poly[d(TC)].poly[d(GA)]; △ = poly[d(TTC)].poly[d(GAA)]; ▲ = poly[d(TCC)].poly[d(GGA)] and X = poly(dG).poly(dC).

it was shown that the brominated polymer adopts a conformation with a less pronounced alternating structure than poly[d(AT)].poly[d(AT)] (33). Moreover, if the 5-position of pyrimidines was being recognized directly, then one would expect the brominated polymer to have the higher binding constant since bromine, although the same size, is more polarizable than a methyl group (21,34). Secondly, the weak binding to poly[d(n^2AT)].poly[d(n^2AT)] also tends to suggest that Jel 241 demonstrates analogue recognition. In this case, we would postulate that the 2-amino group interferes with the alternating structure by altering the base-stacking characteristics of the purine. It

seems unlikely that the 2-amino group in the minor groove is involved directly, since the binding constants to poly[d(AT)].poly[d(AT)], poly[(Gm^5C)].poly[d(Gm^5C)], poly[d(Im^5C)].poly[d(Im^5C)] and poly[d(TG)].poly[d(m^5CA)] are all comparable. Thirdly, recognition of an alternating conformation provides a simple explanation for the weak or negligible binding to (pyrimidine).(purine) DNAs which do not have pyrimidines and purines on the same strand (Figure 5(d)). Finally, the preference of Jel 241 for poly[d(TAC)].poly[d(GTA)] compared to poly[d(ATC)].poly[d(GAT)] can be explained by differences in stacking interactions between the two polymers which have been noted previously (24). That is, the conformation due to TpA is recognized but ApT is not (10).

Synthetic nucleic acids - Jel 229. In contrast to Jel 241, Jel 229 shows only a small preference for methylated DNAs (Figure 4, Table II). That is, the competition binding curves in Figure 4(a) and (c) show only small differences. Moreover, for the other synthetic DNAs, there is no consistent correlation between sequence and binding constant and therefore we conclude that Jel 229 is another example of pure analogue (conformational) recognition. The weak binding to poly[d(n^2AT)].poly[d(n^2AT)](which was also noted with Jel 241) and other evidence suggests that this polymer adopts an unusual conformation. (Personal communication, Dr. J.H. van de Sande)

The most interesting differences, however, are revealed by the relative binding constants to (pyrimidine).(purine) DNAs (Table II and Figure 5(c)). For example, the binding constant to poly(dG).poly(dC) is an order of magnitude greater than to calf thymus DNA while that to poly(dA).poly(dT) is twenty-fold lower. On the basis of DNase I cleavage studies and a recent model for poly(dG).poly(dC), it has been suggested that poly(dG).poly(dC) has a large minor groove whereas that of poly(dA).poly(dT) is narrower than that found in the standard B conformation (3,9,35). Also, poly(dI).poly(dC) which binds weakly may adopt a conformation closer to that of poly(dA).poly(dT) than to poly(dG).poly(dC) (35). The implication, therefore, is that Jel 229 provides a "yardstick" for the width of the minor groove. In this regard, the relatively good binding to poly[d(TTC)].poly[d(GAA)] is important. One might, on the basis of sequence, expect its binding constant to be similar to that of poly(dA).poly(dT). As it is, the order of preference in this series of DNAs is poly(dG).poly(dC) > poly[d(TTC)].poly[d(GAA)] > poly[d(TCC)].poly[d(GGA)] = poly[d(TC)].poly[d(GA)] > poly(dA).poly(dT). In other words, the binding constants to (pyrimidine).(purine) DNAs cannot be correlated with base composition. Consequently, we must conclude that, as a group, these DNAs are conformationally diverse.

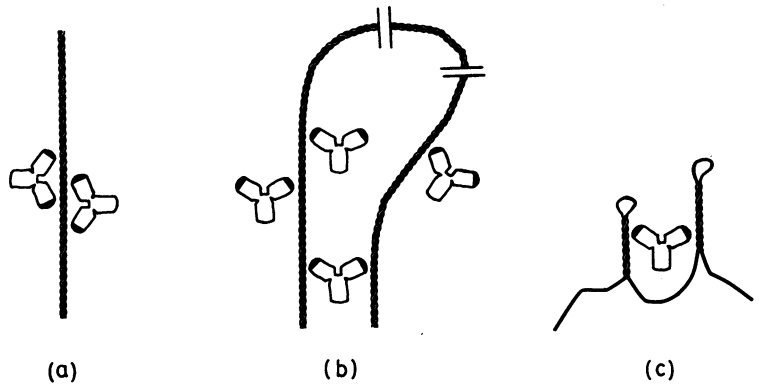

Figure 6. Schematic view of antibodies binding to DNA. (a) Interdigitation as proposed by Burdick and Emlen (40). (b) Because of the topology of the interaction, only one arm of the IgG can be bound to the same piece of DNA, unless the DNA loops back on itself. Thus very high molecular weight or flexible DNA will have a higher binding constant. (c) This type of interaction may be favoured in heat-denatured DNA where two stretches of duplex can readily be brought into favourable juxtaposition.

Synthetic Nucleic Acids - Jel 242 and 234. As was the case with Jel 229, these antibodies do not demonstrate a consistent variation between binding constant and base sequence and these differences tend to be small (Table II and Figure 5). Therefore, they are probably rather similar to many other autoimmune duplex specific antibodies which have been reported previously (12,13,14). However a careful study of the relative binding constants (Table II) reveals two important conclusions.

Firstly, Jel 242 and 239 are different. The more than two-fold variation in binding constant to poly[d(IC)].poly[d(IC)], poly(dI).poly(dC) and poly[d(GGCC)].poly[d(GGCC)] cannot be explained by experimental error. Since to date we have only examined four autoimmune duplex specific antibodies, it seems probable that the repertoire is very large and many other diverse specificity patterns will be revealed in the future.

Secondly, although the differences are smaller than with Jel 229 and 241, the (pyrimidines).(purine) group of DNAs show the greatest variability. In particular, the binding constants to poly(dA).poly(dT) are greater than to poly(dG).poly(dC) and the other (pyrimidine).(purine) DNAs. These antibodies therefore, are also probably capable of measuring the width of the minor groove but unlike Jel 229, a large minor groove is not preferred.

DISCUSSION

Analogue and digital recognition. Previous authors who have studied autoimmune duplex specific antibodies have concluded that they interact primarily with the phosphodiester backbone of DNA (15,36). It is precisely this feature which allows these antibodies to be used to study DNA conformational variations. A two fold difference in binding constant can be measured reliably by this technique yet this corresponds to a very small difference in the ΔG between two conformations. The antibodies described here show analogue recognition. That is, the conformation of the duplex is being recognized rather than functional groups on the bases. Alternatively, the antibodies may alter the conformation to one which can only be adopted by some sequences. Although at present these two possibilities cannot be distinguished they can both be encompassed by the term analogue recognition.

Until the structures of many more synthetic nucleic acids are analysed, the details of this analogue recognition are difficult to visualize. For Jel 242, 239, 229, a reasonable hypothesis is that the antibodies are measuring the width of the minor groove; Jel 229 prefers a wide minor groove while Jel 242 and 239 show less discrimination and probably bind to a minor groove of intermediate width. In some ways, therefore, Jel 242 and 239 behave like DNase I (8,9).

On the other hand, Jel 241 probably approaches the helix from the major groove and evidence has been presented which suggests that the antibody recognizes an alternating backbone conformation rather than methylation of the 5-position of pyrimidines. There are, however, several apparent inconsistencies to this view. For example, the binding to poly[d(GGCC)].poly[d(GGCC)] is ten fold higher than to poly[d(GC)].poly[d(GC)]. But in the repeating tetramer one would predict that the GpG sequence is strongly stacked and therefore the GpC sequence may adopt the preferred alternating sequence (3). Similar arguments may be applied to explain the higher binding constant to poly[d(TTG)].poly[d(CAA)] compared to the alternating duplex poly[d(TG)].poly[d(CA)]. Thus, although a different hypothesis may be preferred when further structural studies on these synthetic polymers have been performed, for the moment we postulate that Jel 241 shows analogue recognition of an alternating backbone conformation in the major groove.

Protein recognition and conformational families. This study has revealed that protein recognition of duplex DNA can occur without specific interaction with the functional groups on the base-pairs. Taking Jel 229 and 241 as examples

two methods of analogue recognition have been elucidated.

The width of the minor groove, as revealed by the binding of Jel 229, tends to be rather constant except in the case of (pyrimidine).(purine) DNAs where considerable variation exists. The binding of Jel 229 to poly(dG).poly(dC) is 240 fold greater than to poly(dA).poly(dT) and only 30 fold greater than to poly[d(TTC)].poly[d(GAA)]. Thus (pyrimidine).(purine) DNAs of different sequences can readily be distinguished by DNA-binding proteins. In this regard, it is intriguing that these unusual sequences are frequently found in the 5'-flanking regions of many eucaryotic genes (37,38). We intend to use Jel 229 to discover how long these sequences must be in order to adopt this recognizable conformation.

Secondly, methylation predisposes duplex DNA to adopt an alternating conformation. Previously, the presence of m^5C has been correlated in some instances with gene expression and since this normally occurs at CpG sequences it seemed possible that a flip to the 'Z' conformation was somehow involved (39). However, the fact that Jel 241 binds 50 fold better to poly[d(Gm^5C)].poly[d(Gm^5C)] compared to the unmethylated polymer suggests that the role of m^5C need not involve 'Z' DNA. In general, the adoption of an alternating conformation may be quite sufficient for specific recognition.

Autoimmune antibodies. Although this paper has concentrated on DNA structure and protein binding, the results may also have some significance for our understanding of autoimmune disease.

Firstly, the effects of length on binding constants is rather curious. Although interdigitation of both arms of IgGs to the same DNA duplex (40) appears to be the simplest mode of binding, this cannot explain the increased binding observed with very high molecular weight DNAs. A plausible explanation is that the tertiary structure of DNA is involved (Figure 6). If the two fold axis of symmetry of the antibody is aligned with the two fold axis of DNA then both arms of the antibody can find equivalent binding sites (Figure 6(a)). However, especially for a specific recognition process, the two axes of symmetry may not be aligned so that bifunctional binding of the antibody to the same DNA molecule can only occur if the DNA loops back on itself (Figure 6(b)). The effect of tertiary structure may also explain the preference of Jel 241 for heat-denatured DNA. Although the antibody is duplex specific, the duplex regions in heat-denatured DNA may be brought into close proximity by the presence of intermittent single-stranded regions (Figure 6(c)). This would not be possible except in a very long or flexible DNA duplex. If interdigitation is not the preferred mode of binding to low

molecular weight DNA then immune complexes may result. This might explain the propensity of duplex specific autoimmune antibodies to cause tissue damage via immune complex formation (19).

Finally, the preference of Jel 229 for poly(dG).poly(dC) is intriguing. It has long been thought that the antigenic stimulus for the production of these autoimmune antibodies in the disease SLE cannot possibly be DNA since most duplex DNA is not immunogenic (36). However, to date, poly(dG).poly(dC) is the only right-handed duplex DNA which is highly immunogenic (20,41). It seems possible that Jel 229 was produced after exposure of the immune system to naturally-occuring poly(dG).poly(dC) and, being non-specific, it cross-reacts with other duplexes. Thus DNA may yet be responsible for the production of many of the DNA-binding antibodies seen in SLE.

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