New model systems to study DNA-protein recognition mechanisms

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dpG antibodies were fractionated on a cellulose-double-stranded DNA column. The flow-through fraction bound denatured DNA but not double-stranded DNA (dsDNA). The dpG-eluted fraction bound dsDNA preferentially. The results show that proteins can recognise dpG in DNA by different mechanisms, some involving DNA unwinding.

dpG antibody *Fractionation* *dsDNA binding* *dsDNA-cellulose chromatography* *dpG conformation*

1. INTRODUCTION

Protein-DNA interactions play a key role in the regulation of gene expression [1]. There is a view [2,3] that for a protein to bind to specific nucleotide units or nucleotide sequences on double-stranded DNA (dsDNA), at least local melting of the dsDNA will be beneficial as the main instruments of recognition, i.e. hydrogen bond donors and acceptors of DNA bases, are largely engaged in hydrogen bonding between the two strands in dsDNA. It has been pointed out by others [4,5] that there is a sufficient number of hydrogen bond donors and acceptors available in the grooves of dsDNA to interact with a protein without unwinding of the DNA. Our studies on the binding of a population of dpG antibodies to *E. coli* dsDNA show that these proteins bind to dpG units on dsDNA and denaturation of the DNA only hinders the binding. Our results also indicate that the conformations of some dpG residues in native double-stranded *E. coli* DNA are different from those in denatured DNA (dnDNA).

It is known [6] that the major population of antibodies elicited against nucleotides and nucleosides do not bind to dsDNA but do bind after denaturation of the DNA. From this it is argued that the antigenic determinants are buried inside the DNA double helix and become available for antibody binding only on unwinding of the helix [7]. Some of these antibodies have been used as specific reagents for single-stranded regions of DNA [8,9]. These antibodies appear to be good model systems for protein-DNA recognition involving unwinding of DNA helix. We had shown that in addition to the above types of single-stranded DNA (ssDNA) binding antibodies there are populations of antibodies which bind to dsDNA, in antisera raised against the haptens dpG and dpC [10]. Here we report the purification of these antibodies and show that they bind to dpG residues preferentially on dsDNA.

2. MATERIALS AND METHODS

2.1. *Preparation of dpG antibodies*

dpG-specific antibodies were raised in rabbits against bovine serum albumin (BSA) conjugates of dpG and IgG were prepared from the antisera by the combined use of Na_2SO_4 precipitation and DEAE-cellulose column chromatography [10].

2.2. *Preparation of* E. coli *[^{3}H]dsDNA and dnDNA*

E. coli [^{3}H]DNA (New England Nuclear, spec. act. 2500000 cpm/μg) was sheared and treated

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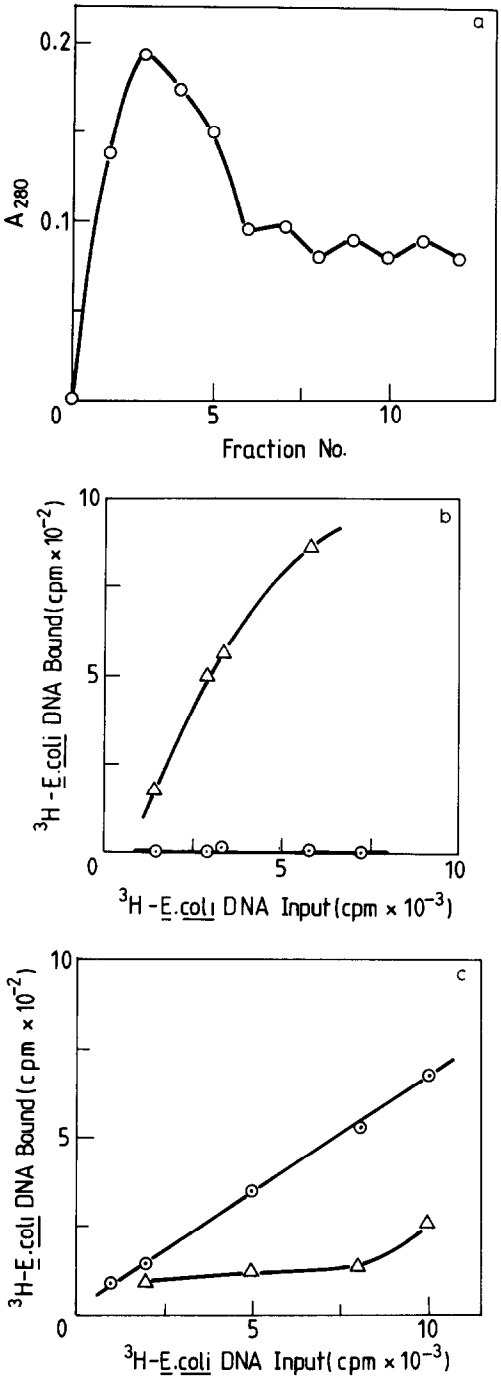

Fig.1. dsDNA-cellulose chromatography of dpG antibodies. (a) Protein distribution in the dpG-eluted fractions. (b) Binding of flow-through fraction to *E. coli* [^{3}H]DNA (ds and dn). 300 μg flow-through protein and varying amounts of *E. coli* [^{3}H]dsDNA or 200 μg flow-through protein and varying amounts of *E. coli* [^{3}H]dnDNA were used in the assay. (⊙—⊙) dsDNA, (△—△) dnDNA. (c) Binding of dpG-eluted fraction to *E. coli* [^{3}H]DNA (ds and dn). 0.5 μg protein from pooled (a) fractions 2–5 and 7, 100 μg normal IgG and varying amounts of *E. coli* [^{3}H]DNA (ds or dn) were used in the assay. Symbols as for (b).

with S_1 nuclease [10,11] and phenol extracted [12]. It did not bind to the nitrocellulose filter (25 mm diameter, 0.45 μm pore size, Advanced Microdevices, Ambala, India) [10] at 0.14 or 1.14 M NaCl whereas ssDNA is retained 100% on this filter at 1.14 M NaCl [10]. Antibodies that bind to ssDNA [13] did not bind to the dsDNA preparation. By these criteria the dsDNA was free of single-stranded regions.

E. coli [^{3}H]dnDNA was prepared from *E. coli* [^{3}H]dsDNA by heating at 98°C for 30 min and chilling in an ice-salt mixture [10]. It bound to the nitrocellulose filter at 1.14 M NaCl but not at 0.14 M [10]. The [^{3}H]dnDNA is likely to contain some double-stranded regions.

2.3. *Protein-DNA binding assay*

The binding of the antibodies to [^{3}H]dsDNA and [^{3}H]dnDNA was studied by the nitrocellulose filter assay as in [14]. The incubation was in 0.3 ml TBS [10 mM Tris-HCl (pH 7.5), 0.14 M NaCl] at 0°C for 30 min [10]. Control was with normal rabbit IgG instead of dpG antibodies.

3. RESULTS

3.1. *dsDNA-cellulose chromatography of dpG antibodies*

dsDNA-cellulose was prepared according to Alberts and Herrick [15]. Anti-dpG IgG (19.6 mg) was loaded on a dsDNA-cellulose (8 mg calf thymus DNA and 2 g cellulose) column (8 mm diameter) and washed with 100 ml TBS. The column was then eluted with 10 ml of 1% dpG in TBS and 0.75 ml fractions collected. The protein distribution in dpG-eluted fractions is given in fig.1a. Flow-through and dpG-eluted fractions contained 17.6 and 0.19 mg protein, respectively. The flow-through fraction bound to [^{3}H]dpG (not shown) and *E. coli* [^{3}H]dnDNA but not to *E. coli* [^{3}H]dsDNA (fig.1b). Fig.1c shows the binding of dpG-eluted fractions (pooled fig.1a fractions 2–5

and 7) to *E. coli* [^{3}H]DNA (ds and dn). There is much higher binding to dsDNA than to dnDNA, showing that unwinding of the DNA only hinders the binding of antibodies.

3.2. *Specificity of dsDNA binding activity*

The specificity of dsDNA binding activity was tested by competition experiments. Fig.2 shows that the DNA binding is inhibited 100% by dpG at 1.1×10^{-7} M. Table 1 gives the effectiveness of compounds related to dpG in inhibiting the dsDNA binding. Inhibition by dpG and the lack of inhibition by dpA, dpT and dpC show the base specificity of antibody binding to dsDNA. The higher effectiveness of dpG compared to dG in inhibition shows the involvement of the phosphate group also in antibody binding. The positive involvement of deoxysugar phosphate in antibody recognition is further evident from the lack of inhibition by guanine itself. These data clearly indicate that the antibodies recognise the total structure of dpG.

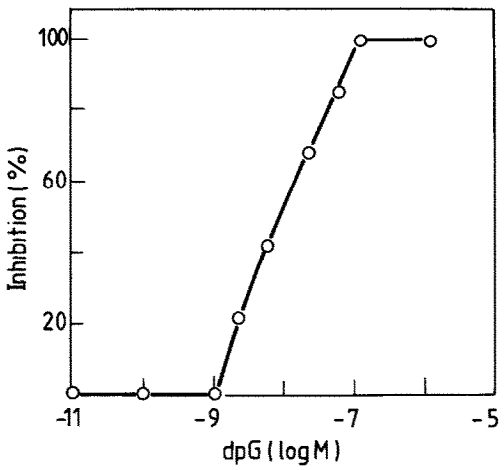

Fig.2. Specificity of the binding of dpG antibodies to [^{3}H]dsDNA. Specificity was studied by competition experiments using a nitrocellulose filter assay as in fig.1. 0.5 μg dpG-eluted proteins (pooled fig.1a fractions 2–5 and 7), 100 μg normal IgG, 2500 cpm *E. coli* [^{3}H]dsDNA and varying amounts of dpG were used. Net DNA bound in the absence of dpG was 243 cpm. DNA bound to normal IgG control was 21 cpm.

Table 1

Binding specificity of dpG antibodies to [^{3}H]dsDNA

Competitor (2.33×10^{-7} M)	Inhibition (%)
dpG	100
dpA	0
dpT	0
dpC	0
dG	43.2
G	0
Guanine	0

Inhibition was studied as in fig.2

4. DISCUSSION

The preferential binding of a population of dpG antibodies to dsDNA compared to dnDNA has important implications. First of all it shows that in dsDNA there is sufficient exposure of nucleotide units for base-specific binding of proteins.

For maximum efficiency of interaction, disposition of interacting atoms and charge distributions are important [16]. Different conformations of dpG are possible due to differences in sugar puckering and glycosyl torsion as well as rotation about P-O bond [17]. The dsDNA-binding population of antibodies that we have isolated are expected to be specific to a certain conformation of dpG. Our results show that this conformation of dpG is present to a much smaller extent in the dnDNA than in the dsDNA. It is expected that these antibodies will be useful in studying the conformational status of dpG in DNA of different biological systems like eukaryotic chromosomes [18], viruses [19], etc. The dsDNA-binding dpG antibodies bound to fixed native human metaphase chromosomes [13] as revealed by indirect immunoperoxidase staining [9]. As in the case of *E. coli* dsDNA, denaturation of the chromosomes by the formamide method [20] led to considerably reduced binding of the antibodies [13].

It may be asked, how the different populations of dpG antibodies arose. The immunogen used to raise the antisera, was a dpG conjugate of BSA which had on average 21 dpG molecules per BSA. As a consequence of protein-nucleotide interactions several conformations of dpG could have

been stabilized on the conjugate and antibodies would then have been formed against at least a few of them.

Nucleotide-specific dsDNA-binding antibodies are not peculiar to dpG alone. Antibodies raised against A, dpC, dpm^{5}C, dpA and dpApT also contain populations that bind to dsDNA [13]. Antibodies which bind to dsDNA with a specificity for base and base sequences can be expected to serve as good model systems for protein-nucleic acid recognitions which do not involve unwinding of the DNA helix.

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