Antibodies specific to two deoxyribotrinucleotide sequences *

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ABSTRACT

Antibodies to the deoxyribotrinucleotides dpApTpA and dpApApT were prepared by injecting the bovine serum albumin conjugates of the respective haptens in rabbits. The specificities of the antibodies were determined by estimating the inhibition of the binding of the tritiated haptens to the immunoglobulins by various nonradioactive mono- and oligonucleotides, using nitrocellulose membrane binding assay. Anti-dpApTpA and anti-dpApApT antisera were found to contain antibodies which were highly specific to the respective hapten sequence.

INTRODUCTION

Anti-oligonucleotide antibodies of defined and narrow specificities are expected to be useful as probes for nucleic acid structures and as specific inhibitors of proteins and enzymes which bind to nucleic acids. They will also serve as models for studying the general problem of nucleic acid-protein interactions.

Antibodies to a few ribodinucleoside monophosphates and ribotrinucleoside diphosphates have been raised (1-5), but detailed data regarding their specificities is not available. Plescia *et al* (6) elicited antibodies to a few ribotrinucleotides, but the degree of specificity with respect to the composition and sequence of the trinucleotides was not established. In fact there were some indications that the anti-trinucleotide sera had antibodies that primarily reacted with the ribose phosphate backbone of the trinucleotides.

After successful elicitation of antibodies to the deoxyribodinucleotide dpApT (7), it was thought worthwhile to try to prepare antibodies to two related deoxyribotrinucleotides dpApTpA and dpApApT to further establish the feasibility of producing anti-oligonucleotide antibodies which are capable of recognizing specific nucleotide sequences.

Antibodies were raised in rabbits by injecting bovine serum albumin conjugates of dpApTpA and dpApApT. The specificities of the antibodies were

established by determining the effectiveness of a number of nonradioactive mono- and oligonucleotides to inhibit the tritiated hapten-antibody binding in the nitrocellulose membrane binding assay system (8).

The results showed that the antisera contained antibodies which were highly specific to the respective deoxyribotrinucleotide hapten sequences.

MATERIALS AND METHODS

^3H -dpApTpA and ^3H -dpApApT were prepared at the Bhabha Atomic Research Center, Bombay, by the Wilzbach procedure (9), by exposing the trinucleotides to tritium gas for a period of two weeks. The specific activity of ^3H -dpApTpA and ^3H -dpApApT was 1200 cpm/pmol and 1300 cpm/pmol respectively (uncorrected for counting efficiency and other sources of errors). The deoxyribooligonucleotides were chemically synthesized and characterized as described in the preceding paper. BSA conjugates of nucleotides were prepared as given earlier (8). Injection and bleeding schedules, double diffusion in agar and quantitative precipitation tests were done as described earlier (8). Gamma globulins from the antisera were prepared by sodium sulphate precipitation (10).

Membrane binding experiments were done as described earlier (8). Tris-HCl buffered saline (TBS or buffer) containing 0.14M NaCl, 0.01M Tris-HCl (pH 7.5) and 0.02% NaN_3 was used in all the experiments. The reaction mixture for the labelled hapten-antibody binding experiments contained 0.1 ml of the labelled hapten (6,000 cpm for ^3H -dpApTpA and 8,000 cpm for ^3H -dpApApT) in buffer, 100 μg of gamma globulins in 0.1 ml buffer and 0.1 ml of buffer or inhibitor in buffer. After incubation at 0°C for 20 minutes, the contents were filtered on prewetted nitrocellulose membrane filters, washed with TBS, dried and counted.

RESULTS

(i) Anti-dpApTpA antibodies : The antiserum from the rabbit (R-14) immunized with BSA-dpApTpA conjugate showed strong precipitation with the homologous antigen when tested by double diffusion in agar. Fig. 1 shows the results of the quantitative precipitation reaction with R-14 antiserum. The zone of maximum precipitation with BSA-dpApTpA is very clear. The titre of precipitable antibody is about 1.6 mg/ml. A very small amount of precipitation is observed with BSA.

Specific binding of ^3H -dpApTpA to anti-dpApTpA antibodies : The R-14 gamma globulin preparations were found to bind the labelled hapten at temperatures ranging from 0°C to 27°C with little evidence of hapten degradation,

Fig. 1. Quantitative precipitation reactions of an anti-dpApTpA antiserum (R-14). $\circ$ — $\circ$, BSA-dpApTpA; $\bullet$ — $\bullet$, BSA.

although at higher temperatures binding was lowered slightly presumably due to degradation. Experimental conditions chosen for all assays (20 min at 0°) described in this paper were such as to minimize or abolish interference from nucleases. Fig. 2 shows the specific binding of ^3H -dpApTpA by the gamma globulins. Binding increases linearly till the saturation point where it levels off to a plateau.

Inhibition of ^3H -dpApTpA-antibody binding by various mono- and oligonucleotides: The specificity of the antibodies was characterized by determining the inhibition of the binding of ^3H -dpApTpA to the gamma globulins by various nonradioactive mono- and oligonucleotides. Fig. 3 shows the inhibition of ^3H -dpApTpA-antibody binding by a few oligonucleotides. The hapten dpApTpA is the best inhibitor of the system. The antibodies have a decreased affinity towards the tetranucleotide dpApApTpA (which contains the dpApTpA sequence in its structure) probably because of the steric as well as other changes produced by the presence of an extra mononucleotide. The reaction of antibodies with dpApApT, dpApT and dpTpA is of a very weak order (they are required in more than 1,000 fold molar excess as compared to dpApTpA). The dephosphorylated dinucleotide dApT failed to react with the antibodies. Table 1 summarizes the inhibition data obtained with various mono- and oligonucleotides. The constituent mono- and dinucleotides (dpT, dpA, dpApT and dpTpA) have a

Fig. 2. Specific binding of ^3H -dpApTpA by anti-dpApTpA gamma globulins, as measured by membrane binding technique. The reaction mixtures (0.3 ml) containing 0.1 ml of buffer, 0.1 ml of labelled ^3H -dpApTpA and 100 μg of gamma globulins in 0.1 ml buffer, were incubated for 20 min at 0°C . Blanks contained the labelled trinucleotide in 0.3 ml buffer. $\circ$ — $\circ$, binding by anti-dpApTpA gamma globulins; $\bullet$ — $\bullet$, binding by normal rabbit gamma globulins.

Fig. 3. Inhibition of ^3H -dpApTpA-anti-dpApTpA binding by various nonradioactive deoxyribonucleotides. The reaction mixtures contained 100 μg of R-14 gamma globulins in 0.1 ml buffer, 0.1 ml of ^3H -dpApTpA (6,000 cpm) in buffer and 0.1 ml of buffer or inhibitor in buffer. Control (no inhibitor) bound 1 pmol (1,200 cpm) above a normal rabbit gamma globulin blank, which bound 250 cpm. Each value represents the average of two experiments. $\circ$ — $\circ$, dpApTpA; $\bullet$ — $\bullet$, dpApApTpA; $\square$ — $\square$, dpApApTp; $\triangle$ — $\triangle$, dpApTp; $\blacktriangle$ — $\blacktriangle$, dApTp; $\blacksquare$ — $\blacksquare$, dApTp.

TABLE 1. Inhibition of ^3H -dpApTpA-antibody and ^3H -dpApApT-antibody binding by various mono- and oligonucleotides.

Inhibition curves were constructed for all nucleotides and the concentration of each compound giving 50% inhibition deduced from them.

No.	Inhibitor	Concentration required for 50% inhibition (M)	
		Anti-dpApTpA antibodies	Anti-dpApApT antibodies
1.	dpApTpA	2.0×10^{-9}	2.50×10^{-5}
2.	dpApApT	3.33×10^{-6}	2.70×10^{-7}
3.	dpApApTpA	3.70×10^{-8}	3.33×10^{-6}
4.	dpTpTpT	3.33×10^{-6}	2.80×10^{-5}
5.	dpApT	1.80×10^{-5}	1.50×10^{-5}
6.	dpTpA	6.70×10^{-5}	3.33×10^{-4} @
7.	dpApG	3.70×10^{-5}	3.33×10^{-4} @@
8.	dpTpT	4.0×10^{-5}	3.33×10^{-4} ‡
9.	dpTpC	3.33×10^{-5} *	3.33×10^{-4} ‡‡
10.	dApT	—	3.33×10^{-4} \$
11.	dpA	2.70×10^{-4}	3.33×10^{-3} \$\$
12.	dpT	2.30×10^{-3}	3.33×10^{-3} #
13.	dpG	3.33×10^{-3} **	3.33×10^{-3} ##

* 20% inhibition at this concentration; ** 18% inhibition; @ 26% inhibition
 @@ 25% inhibition; ‡ 48% inhibition; ‡‡ 2% inhibition; \$ 6% inhibition
 \$\$ 28% inhibition; # 15% inhibition; ## 8% inhibition.

very low affinity for the antibodies showing that the antibodies are directed towards the whole trinucleotide molecule. The oligonucleotides dpTpT, dpTpC, dpApG and dpTpTpT are poor inhibitors of the ^3H -dpApTpA-antibody binding and are required in more than 1,000 fold molar excess as compared to dpApTpA. The inhibition data clearly show the high degree of specificity of the antibodies to dpApTpA.

Inhibition by polynucleotides : Fig. 4 gives the inhibition of the ^3H -dpApTpA-antibody binding by polynucleotides. Denatured calf thymus DNA is able to inhibit the binding, although at high concentrations whereas native calf thymus DNA is unable to compete in the binding. Yeast RNA is able to react with the antibodies at still higher concentrations but rat liver tRNA failed to react with the antibodies.

(ii) Anti-dpApApT antibodies : The antiserum from the rabbit (R-17) injected with BSA-dpApApT conjugate gave a strong precipitation with the homologous antigen when tested by double diffusion in agar gel. Fig. 5 gives the quantitative precipitation curve of R-17 antiserum with BSA-dpApApT. There is a fairly well defined zone of maximum precipitation. The titre of antibody precipitable with the homologous antigen is about 2 mg/ml.

^3H -dpApApT-antibody binding : Fig. 6 shows the specific binding of ^3H -dpApApT to R-17 gamma globulins. Binding increases linearly until a saturation point is reached. Normal gamma globulins do not bind the labelled hapten.

Fig. 4. Inhibition of ^3H -dpApTpA-antibody reaction by polynucleotides. Each point represents the average of two experiments. Assays were conducted as given in legend for fig. 3. $\circ$ — $\circ$, dpApTpA; $\bullet$ — $\bullet$, calf thymus DNA denatured by heating and rapid chilling; Δ — Δ , native calf thymus DNA; $\blacktriangle$ — $\blacktriangle$, yeast RNA extensively dialyzed to remove oligonucleotide contaminants; $\square$ — $\square$, rat liver tRNA (dialyzed).

Fig. 5. Quantitative precipitation reactions of anti-dpApApT sera (R-17). Δ — Δ , reaction with BSA-dpApApT; $\blacktriangle$ — $\blacktriangle$, reaction with BSA.

The specificity of the antibodies : The specificities of the antibodies were determined by measuring the inhibition of ^3H -dpApApT-antibody binding by various mono- and oligonucleotides (Table 1). It is clear that the homologous hapten dpApApT is the best inhibitor of the binding. The tetranucleotide dpApApTpA containing the dpApApT sequence in its structure is the next best

Fig. 6. Specific binding of ^3H -dpApApT by anti-dpApApT antibodies. Assays were conducted at different concentrations of ^3H -dpApApT by the usual membrane binding assay. Each point represents the average of two experiments. $\circ$ — $\circ$, binding by anti-dpApApT gamma globulins; $\bullet$ — $\bullet$, binding by normal rabbit gamma globulins.

Fig. 7. Inhibition of ^3H -dpApApT-antibody binding by polynucleotides. $\circ$ — $\circ$, dpApApT; $\bullet$ — $\bullet$, heat denatured calf thymus DNA; Δ — Δ , yeast RNA; $\blacktriangle$ — $\blacktriangle$, native calf thymus DNA; $\square$ — $\square$, rat liver tRNA.

inhibitor. The reaction of the antibodies with the mononucleotides dpT, dpA and dpG is of a very weak order. The antibodies did not react with dpC at the highest concentration tested. The oligonucleotides dpApTpA, dpTpTpT, dpApT, dpTpA, dpTpT, dpTpC, dpApG and dApT have very low affinity for the antibodies. The results of the inhibition analysis with dpApT and dpTpA are worth noting. dpApT which is a constituent sequence in dpApApT is a much better inhibitor of the binding as compared to the dpTpA sequence, which is not present in the hapten. The results show that the anti-dpApApT antibodies are specific for the dpApApT sequence.

Fig. 7 shows the inhibition studies with DNA and RNA. As with anti-dpApTpA antibodies, denatured calf thymus DNA and yeast RNA but not native calf thymus DNA or rat liver tRNA, are able to inhibit the ^3H -dpApApT-antibody binding.

DISCUSSION

The specificity of the anti-dpApTpA and anti-dpApApT antibodies for the respective hapten is clear from the studies on the comparative inhibition of the binding of the labelled haptens to the antibodies by different mono- and oligonucleotides (Table 1). Inhibition studies with the constituent mono- and dinucleotides show that the antibody population under study are directed against the whole molecules of dpApTpA and dpApApT respectively. The inhibition data also show that the antibodies are capable of recognizing the dpApTpA and dpApApT sequences respectively. The tetranucleotide dpApApTpA which contains the hapten sequences in its structure is a good inhibitor of the labelled hapten-antibody binding, showing that the antibodies can recognize the hapten sequences present in the tetranucleotide. The sugar phosphate backbone of the oligonucleotides do not seem to play a dominant role in determining the specificity of the antibodies (cf. 6).

The trinucleotides dpApTpA and dpTpTpT inhibit the ^3H -dpApApT-antibody binding to an equal extent. The former contains the sequence dpApT which is common to dpApApT and the extent of inhibition can be understood, but it is not clear why dpTpTpT is an equally good inhibitor. Also in the case of anti-dpApTpA antibodies, it was observed that dpApApT and dpTpTpT are almost equal in their inhibition of the binding of ^3H -dpApTpA to the antibodies. Until we test more trinucleotides in the inhibition analysis, it will be difficult to offer a reasonable explanation for these observations. The ability of the anti-dpApTpA and anti-dpApApT antibodies to react with denatured calf thymus DNA is assumed to be due to the presence of dpApTpA and dpApApT sequences in the DNA.

The results presented show that it is possible to produce antibodies which are specific for dpApTpA and dpApApT sequences respectively. Our current efforts are directed towards purifying these antibodies in order to use them in some of the investigations mentioned in the introduction.

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