Fractionation and Characterization of Antibodies Elicited by ϕX174 Deoxyribonucleic Acid–Ribonucleic Acid Hybrid†

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ABSTRACT: Antibodies to ϕX174 DNA·RNA hybrid synthesized in vitro were elicited in rabbits. Titration and absorption with double-stranded synthetic homopolymers indicated the presence of three kinds of antibodies, one (15%) specific for single-stranded (ss) DNA, another (71%) was specific for both DNA·RNA hybrids and double-stranded (ds) RNA, and a third (13%) was specific for DNA·RNA hybrids. The double-stranded nucleic acid specific antibodies were separated from ss-DNA-specific antibody and nonimmune immunoglobulin by immunoprecipitation with poly(dT)·poly(A) or poly(dC)·poly(I). A DNA·RNA hybrid specific antibody was then purified by absorbing the DNA·RNA hybrid and ds-RNA-specific antibody to poly(A)·poly(U)–agarose. The characterization of the antibodies and the antigens was performed by a competition radioimmunoassay using ϕX174 DNA·[^{3}H]RNA hybrid as a radiolabeled antigen (Nakazato, H. (1979) *Anal. Biochem. 98*, 74). For the major DNA·RNA hybrid and ds-RNA-specific antibody, the relative potency of the competitors decreased in the following order: poly(dC)·poly(I) > ϕX174 DNA·RNA hybrid > poly(C)·poly(I) > poly(A)·poly(U) > poly(dT)·poly(A) ≃ poly(dI)·poly(C) > *Escherichia coli* RNA > *E. coli* tRNA. Poly(dC)·poly(G) and poly(dG)·poly(C) showed incomplete cross-reactivity. ss-DNA, ds-DNA, ribohomopolymers, deoxyribohomopolymers, double-stranded deoxyribohomopolymers, poly(C)·poly(G), equimolar mixture of poly(dA) and poly(U), heparin, and poly(vinyl sulfate) showed little, if any, competition. The antibody which did not bind to poly(A)·poly(U)–agarose demonstrated almost complete specificity for DNA·RNA hybrid. This was shown by the complete loss of competing activity of poly(A)·poly(U), poly(C)·poly(I), and rRNA with ϕX174 DNA·[^{3}H]RNA antigen. The order of the relative potency of other competitors was similar to that of the DNA·RNA hybrid and ds-RNA-specific antibody. These data indicate that there are sites in the DNA·RNA hybrids of naturally occurring sequences that are more antigenic than poly(dT)·poly(A) and must resemble the structure of poly(dC)·poly(I), since the latter competes even more effectively for antibody than natural DNA·RNA hybrids.

Antibodies to polynucleotides have found multiple application in molecular and cytological studies of nucleic acids (Stollar, 1975; Rudkin & Stollar, 1977; Derrick, 1978; Stumph et al., 1978).

Antibody specific to DNA-RNA hybrid structure was successfully elicited in rabbits using poly(dT)·poly(A) complexed with MBSA[1] as an antigen (Stollar, 1970). Although it was suggested that the antibody recognizes the conformation of DNA·RNA hybrid structure rather than the constituent bases (Stollar, 1975), it has not been well studied whether any difference of conformation of hybrids resulting from the difference in base composition will affect the antigenicity of the structure.

In an attempt to isolate DNA·RNA hybrid from a vast excess of DNA, I produced antibodies against poly(dT)·poly(A) in rabbits as previously described (Stollar, 1970) and found that the antibodies bound poly(dT)·poly(A) far better than the ϕX174 DNA·RNA hybrid, which had been synthesized in vitro (H. Nakazato, unpublished results). Because little is known about the antigenic conformation of the DNA·RNA hybrid of naturally occurring sequences, I have produced, fractionated, and characterized antibodies against the ϕX174 DNA·RNA hybrid, which are reported here.

Materials and Methods

Solutions. PBS is 0.02 M sodium phosphate buffer (pH 7.4) containing 0.15 M NaCl. PBSEtw is PBS containing 5 mM EDTA, 0.1% Tween, 0.02% NaN_3, and 0.067 μM pentachlorophenol. SSC is 0.15 M NaCl and 0.015 M sodium citrate. TNE is 0.02 M Tris-HCl buffer, pH 7.4, containing 0.3 M NaCl and 1 mM EDTA. TES is 0.02 M Tris-HCl buffer, pH 7.4, containing 1 mM EDTA and 0.5% $NaDodSO_4$.

Reagents. Synthetic homopolynucleotides, poly(dC)·poly(dA), poly(C)·poly(I), poly(A)·poly(U), ϕX174 ss-DNA, and *Escherichia coli* tRNA and rRNA were obtained from Miles. A ϕX174 RF ds-DNA was from Bethesda Research Lab. Poly(U)–agarose was obtained from P-L Biochemicals. Purified AMV reverse transcriptase was donated by Dr. M. G. Sarngadharan of Litton Bionectics.

Preparation of ϕX174 DNA·RNA Antigen. ϕX174 DNA·RNA was synthesized in vitro using ϕX174 circular ss-DNA and *E. coli* RNA polymerase as previously described (Stavrianopoulos et al., 1972). After 6 h at 35 °C, reaction was stopped by adding 0.25 volume of 10 × SSC, and the reaction mixture was dialyzed against TNE at 4 °C overnight. After RNase A was added to 0.5 μg/mL, the mixture was incubated for 30 min at 37 °C. Incubation was continued for another 30 min after adding proteinase K (10 μg/mL) and $NaDodSO_4$ (0.5%). Then 1 volume of TES was added to reduce the NaCl concentration. After phenol extraction and ethanol precipitation, the hybrid was separated from precursors and oligonucleotides by gel filtration on a Sephadex G-50 column that had been equilibrated with TNE. The hybrid eluted in flow-through fractions was pooled, and 2 volumes of ethanol was added. After centrifugation, the precipitate was dissolved in 0.1 × SSC. One-tenth volume of 10 × SSC was added, and the concentration of hybrid was adjusted to

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[1] Abbreviations used: ds, double stranded; ss, single stranded; RF, replicative form; rRNA, ribosomal RNA; tRNA, transfer RNA; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; MBSA, methylated bovine serum albumin; RIA, radioimmunoassay. Noncovalent associations of nucleic acids are indicated by a center dot, such as poly(A)·poly(U).

1 A_{260} unit/mL by adding 1 × SSC.

Immunization. After 25 μL of a 1% solution of MBSA was added to 0.5 mL of φX174 DNA·RNA, the mixture was emulsified in an equal volume of Freund's adjuvant. Three-tenths milliliter of the emulsion was injected into footpads and the rest was given intramuscularly to a New Zealand white rabbit at weekly intervals for 3 weeks. The rabbit was bled weekly starting 1 week after the last injection of the antigen. A second similar course of immunization was carried out 3 months later.

Preparation of Homopolymer Complexes. Double-stranded homopolymers were prepared as described (Nakazato, 1979).

Preparation of φX174 DNA·[^{3}H]RNA, Poly([^{3}H]dC)·Poly(I) and Poly([^{3}H]dT)·Poly(A). Preparation of φX174 DNA·[^{3}H]RNA was reported earlier (Nakazato, 1979). Specific activity was about 360 cpm/pmol for all preparations used. Poly([^{3}H]dC)·poly(I) and poly([^{3}H]dT)·poly(A) were synthesized by using AMV reverse transcriptase, poly(I) or poly(A), and [^{3}H]dCTP or [^{3}H]dTTP, respectively (Baltimore & Smoler, 1971) and purified by the same $NaDodSO_4$–sucrose density gradient procedure as for φX174 DNA·[^{3}H]RNA. Fractions sedimenting at 10 to 18 S were pooled, recovered by ethanol precipitation, and dissolved in 1 × SSC. Specific activity was 2000 cpm/pmol for both hybrids.

Radioimmunoassay. RIA was done as described (Nakazato, 1979).

Preparation of Poly(A)·Poly(U)–Agarose. A small column was packed with 1 mL of poly(U)–agarose and washed extensively with PBSEtw until A_{260} of the wash fell to less than 0.01. About 80 A_{260} units of poly(A) in 0.4 mL of PBSEtw was added to the column and allowed to bind to the column for 10 min. After extensive wash, poly(A)·poly(U)–agarose was transferred to a graduated centrifuge tube and stored at 4 °C until use in the presence of 0.02% NaN_3. About 50 A_{260} units of poly(A) was bound to 1 mL of agarose which originally contained about 70 A_{260} units of poly(U).

Fractionation of Antibody. A crude immunoglobulin fraction was prepared from pooled antisera by 40% ammonium sulfate precipitation (fraction I). Poly(dT)·poly(A) (2.29 μmol) in 1.6 mL of 1 × SSC was added to 66 mg of fraction I in 4 mL of PBSEtw. After the solution was incubated for 3 h at 37 °C, immunoprecipitate was pelleted by centrifugation at 12000 rpm for 20 min in a Sorvall HB-4 rotor. The pellet was washed twice with 0.5 mL of PBSEtw, transferred to a small Potter-Elvehjem homogenizer, and homogenized after adding 0.5 mL of 4.5 M $MgCl_2$. After 10 min at 23 °C, 25 μL of 0.1 M glycine buffer, pH 3.0, was added, and the homogenate was left at 23 °C for another 10 min. The nonsolubilized immunoprecipitate was pelleted by centrifugation for 10 min at 1000 rpm in the homogenizer and reextracted for 10 min at 23 °C with $MgCl_2$ (0.5 mL)–glycine (25 μL) buffer and then with 0.5 mL of glycine buffer alone. All the extracts were combined and pressure dialyzed for 2 h against 120 mL of 0.1 M glycine buffer, pH 3.5, containing 0.01 M EDTA at 4 °C with one buffer change.

The dialyzed solution was centrifuged for 10 min at 10000 rpm, and an equal volume of saturated ammonium sulfate was added to the supernatant at room temperature. After the solution was stirred for 20 min, the precipitate was recovered by centrifugation, washed once with 50% saturated ammonium sulfate, and dissolved in 0.5 mL of 0.1 M glycine buffer, pH 3.0. Ammonium sulfate precipitation was repeated once more and final precipitate was dissolved in PBS. After overnight dialysis of PBS, the solution was clarified by centrifugation (fraction II).

The fraction II equivalent to 30 mg of the original immunoglobulin fraction (fraction I) was mixed with 0.15 mL of prewashed poly(A)·poly(U)–agarose. After the solution was shaken for 3.5 h at 23 °C, the agarose was pelleted by centrifugation and washed with 1 mL of PBS.

The combined supernatant was dialyzed against PBS (fraction III). The agarose pellet was transferred to a column by using three 0.5-mL portions of PBS and the wash was dialyzed against PBS (fraction IV).

The antibody bound to the poly(A)·poly(U)–agarose was eluted by treatment with 4.5 M $MgCl_2$–0.1 M glycine buffer, pH 3.0, for 3 h at 23 °C and dialyzed as above (fraction V). All the fractions were clarified by centrifugation and stored at −20 °C.

An additional fractionation was done by using 1.18 μmol of poly(dC)·poly(I) in place of poly(dT)·poly(A) to precipitate antibodies from 73 mg of the immunoglobulin fraction (fraction I) in 5.1 mL of PBSEtw. The fractions corresponding to those described above and designated by Roman numerals were numbered in Arabic numerals for this case.

Definition. One relative unit of antibody is defined as the amount that binds 50% of the input hybrid (usually about 5 pmol). The relative specific activity is therefore the reciprocal of the amount of antibody required to bind 50% of the input hybrid and is expressed in relative units per microgram of protein.

Results

Antibody Production. Antibody production was monitored on a double immunodiffusion plate (pattern D obtained from Cappel Lab., Inc.) using 1 to 4 μg of nucleic acid and 8 μL of serum obtained weekly.

After three weekly inoculations of the antigen, one out of three rabbits produced antiserum which reacted with both φX174 DNA·RNA and poly(dT)·poly(A). The other two rabbits, one of which later died, produced antisera which reacted with only φX174 DNA·RNA. Three months later, another three weekly inoculations of the antigen were repeated. The first animal produced φX174 DNA·RNA and poly(dT)·poly(A) reactive antisera for more than 12 weeks. Sera from another animal were reactive with φX174 DNA·RNA and φX174 ss-DNA, but not with poly(dT)·poly(A), and thus were regarded as ss-DNA-specific antisera.

The sera collected weekly from the first animal after the last booster inoculation were pooled and used throughout this study.

Titration of Antibody. An immunoglobulin fraction obtained by ammonium sulfate precipitation was first titrated with poly(dT)·poly(A), poly(dC)·poly(I), or poly(A)·poly(U), all being chosen for their reactivity in double diffusion tests. Widely different chemical properties of the antibody and the antigens allowed us to quantitate them differentially in the immunoprecipitate as described in the legend to Figure 1. As shown in Figure 1, the pattern of precipitation of both antigen and antibody was quite similar when poly(dT)·poly(A) or poly(dC)·poly(I) was used. The maximum precipitation of antibody was obtained when 12 to 23 nmol of the nucleic acids was added. Addition of more nucleic acids resulted in decreasing recovery of the antibody in the precipitate. The precipitation of the nucleic acids was almost 100% when less than 15 nmol of the nucleic acids was added and became progressively less when increasing amounts of nucleic acids were added. The maximum precipitation of 0.09 to 0.1 nmol of the antibody molecule corresponds to 2.2 to 2.4% of the input protein. (Because most of the antibody activity was found in the IgG fraction in a preliminary experiment, the

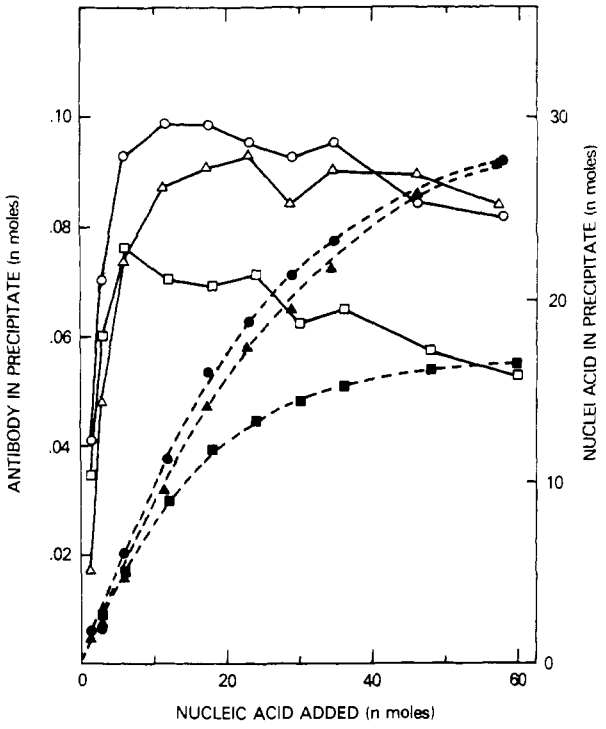

FIGURE 1: Titration of antibody with nucleic acid. PBSEtw (80 μL) containing 616 μg of immunoglobulin fraction of the antibody and varying amounts of nucleic acid, as indicated, was incubated for 30 min at 37 °C and then for 4 days at 4 °C. After centrifugation, the precipitate was washed three times with 80 μL of cold 1 × SSC and once with acetone and dried. The precipitate was dissolved in 40 μL of 0.1 N NaOH and incubated at 37 °C overnight. H_2O (300 μL) was added before measuring absorbance at 260 nm to quantitate nucleic acids. The absorbance attributable to protein was less than 15% of the total at most and was subtracted from it to obtain the nucleic acid content. An aliquot of the solution was used to measure protein content (Lowry et al., 1951): (○—○) antibody coprecipitated with poly(dC)·poly(I) (●---●); (△—△) antibody coprecipitated with poly(dT)·poly(A) (▲---▲); (□—□) antibody coprecipitated with poly(A)·poly(U) (■---■).

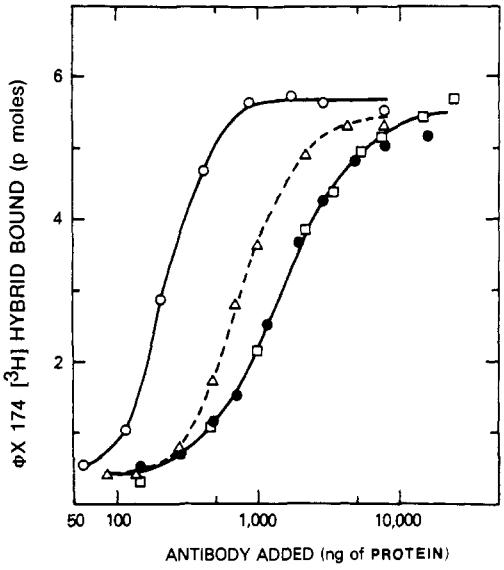

FIGURE 2: Antibody activity remaining after absorption with double-stranded nucleic acid. Immunoglobulin fraction (616 μg) was mixed with either 22.9 nmol of poly(dT)·poly(A), 17.3 nmol of poly(dC)·poly(I), or 11.9 nmol of poly(A)·poly(U) in 80 μL of PBSEtw. A control was treated similarly without adding nucleic acids. After the solution was incubated for 30 min at 37 °C, absorption was continued for 4 days at 4 °C with occasional shaking. The supernatant obtained after centrifugation for 20 min at 10000 rpm was removed and used to measure the remaining unabsorbed antibody activity. RIA was done as described under Materials and Methods using 5.6 pmol of φX174 DNA·[^{3}H]RNA and the supernatant obtained after absorption with (●—●) poly(dT)·poly(A); (□—□) poly(dC)·poly(I); (△---△) poly(A)·poly(U); and (○—○) none.

antibody molecule was tentatively regarded as IgG throughout this work.) On the other hand, the poly(A)·poly(U) recovery started to decrease from 100% when more than 10 nmol was added. Also, the antibody precipitation reached a maximum when less than 10 nmol of the poly(A)·poly(U) was added. In all cases, 20 to 30 base pairs per one antibody molecule were found in the precipitates in the antibody excess regions where less than 6 nmol of the nucleic acid was added. The maximum amount of antibody precipitated with poly(A)·poly(U) was about 80% of that precipitated with poly(dT)·poly(A) or poly(dC)·poly(I). These differences seemed to suggest that there were at least two populations of antibodies.

Most likely, about 80% of the antibodies precipitated with either hybrid of homopolymers was reactive with DNA·RNA hybrid and ds-RNA and about 20% was specific for only the DNA·RNA hybrid. It was also possible that the antibody specific for the DNA-RNA hybrid and the one specific for ds-RNA were different entities and precipitated differentially. Although less likely, it is possible that the φX174 DNA·RNA inoculated into the rabbits contained some ds-RNA (Tabak & Borst, 1971), which elicited the ds-RNA-specific antibody.

To test these possibilities, fraction I antibody was absorbed with poly(dT)·poly(A), poly(dC)·poly(I), or poly(A)·poly(U) and the remaining antibody activity in the supernatant was analyzed by RIA as described earlier (Nakazato, 1979). As can be seen in Figure 2, the remaining activity was almost the same after absorption with poly(dC)·poly(I) or poly(dT)·poly(A), and more activity remained unabsorbed with poly(A)·poly(U). Relative antibody units in the unabsorbed fractions were calculated to be 470, 470, and 880, respectively. A control antibody which had not been treated with any nucleic acid had 3070 relative antibody units.

Absorption of 71% of the antibody activity by poly(A)·poly(U) strongly suggested that the antibody precipitated with poly(A)·poly(U) (Figure 1) binds both ds-RNA and the DNA·RNA hybrid, since the antibody activity was measured by binding of antibody and φX174 DNA·[^{3}H]RNA, which contained little, if any, ds-RNA (Nakazato, 1979).

Therefore, it seemed likely that the difference in the relative antibody units absorbed with the hybrids of homopolymers and the poly(A)·poly(U) is the amount of the antibody activity that is specific only to the DNA·RNA hybrid.

By competition RIA, antibody activity which remained unabsorbed by the hybrids of homopolymers was shown to be ss-DNA specific, because φX174 ss-DNA was a better competitor than φX174 DNA·RNA itself (the index of relative potency (see Table II) of ss-DNA being 0.3) and poly(dC)·poly(I), poly(dT)·poly(A), poly(A)·poly(U), and *E. coli* rRNA were very poor competitors, if at all (data not shown). The presence of ss-DNA regions in the φX174 DNA·RNA synthesized in vitro (Bassel et al., 1964) may explain the production of the antibody and binding of the φX174 DNA·[^{3}H]RNA to it. From the above data, it was calculated that, of all the antibodies detectable by the RIA, 15.3% is ss-DNA specific, 71.3% is a DNA·RNA hybrid and ds-RNA specific, and 13.3% is specific only for the DNA·RNA hybrid. This indicates that only 15.8% of the antibodies reactive with double-stranded nucleic acids were DNA·RNA hybrid specific, a number in good agreement with that obtained from titration experiments described above.

Fractionation of Antibodies. Since the presence of at least three antibody populations was indicated, I attempted to

Table I: Fractionation of Antibody

antibody fraction	rel sp act.[a] (units/μg of protein)	total rel units
experiment 1		
(I) $(NH_4)_2SO_4$ precipitation	6.66	440 000
(II) poly(dT)·poly(A) precipitation	217	274 000
(III) poly(A)·poly(U)–agarose, first wash	22.7	6 300
(IV) poly(A)·poly(U)–agarose, second wash	62.5	7 000
(V) eluate	217	58 000
experiment 2		
(1) $(NH_4)_2SO_4$ precipitation	6.25	455 000
(2) poly(dC)·poly(I) precipitation	217	343 000
(3) poly(A)·poly(U)–agarose, first wash	35.7	3 500
(4) poly(A)·poly(U)–agarose, second wash	104	3 100
(5) eluate	217	101 000

[a] Calculated from RIA data similar to those shown in Figure 2 as described under Materials and Methods.

fractionate and purify them. First, the antibodies were precipitated by poly(dT)·poly(A) from the ammonium sulfate purified crude immunoglobulin fraction to get rid of nonimmune proteins and ss-DNA-specific antibody. Extraction of the immunoprecipitate with 4.5 M $MgCl_2$ at low pH followed by dialysis against low pH buffer allowed the separation of antibodies from most of the nucleic acids which precipitated at low pH. The antibodies were separated further from the residual nucleic acid by ammonium sulfate precipitation (fraction II). The recovery of antibody protein was 1.9% of the total protein which had been purified from antisera by ammonium sulfate precipitation (Table I) and was in good agreement with the titration data described above. The recovery of activity was 62% of the total activity or 74% (Table I) if one takes the ss-DNA-specific activity into account. The fraction II was then absorbed with poly(A)·poly(U)–agarose. The two unbound fractions obtained as described under Materials and Methods (fractions III and IV) were kept separate because competition RIA showed that fraction IV, but not fraction III, still had a low level of affinity for poly(A)·poly(U) (see below).

The recoveries of protein and antibody activity in fraction III were 22 and 2.3% and, in fraction IV, 8.9 and 2.6% of those of fraction II, respectively. A recovery of activity lower than that expected from titration and absorption experiments might be explained by the poor dissociation and recovery of high avidity antibody specific for DNA·RNA hybrids from the earlier immunoprecipitation step. The poor recovery of the DNA·RNA hybrid specific antibody might have been concealed by the good recovery of the major antibody specific for DNA·RNA hybrid and ds-RNA, which may have less affinity for the hybrid poly(dT)·poly(A). Inactivation of the antibody in the previous step is another possible explanation. Although high recovery of protein and low recovery of activity seem to support this explanation, it is less likely when one finds a high recovery of activity for the major DNA·RNA hybrids and ds-RNA-specific antibody population in this step (see above). Low relative specific activity of these antibodies could be explained either by low avidity of the antibodies or by contamination by nonimmune or inactivated IgG or by both. No further experiment was done to clarify this point.

The recovery of antibody activity and protein which had been bound to poly(A)·poly(U)–agarose by elution with 4.5 M $MgCl_2$ at pH 3.0 was 21% of those of the input fraction

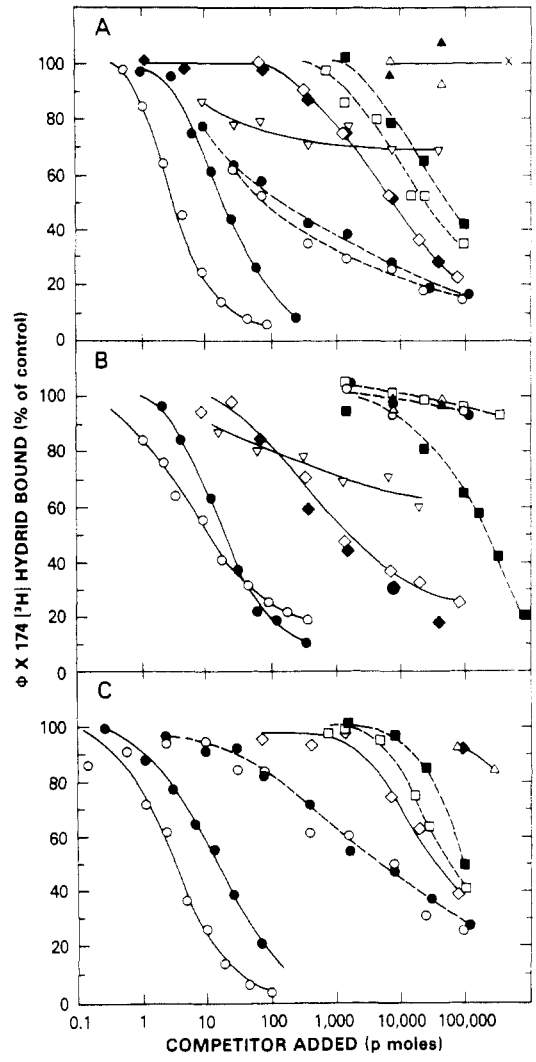

FIGURE 3: Characterization of antibody fractions by competition RIA. Competition RIA was done as described under Materials and Methods: (A) 4.6 ng of fraction II; (B) 36 ng of fraction III; (C) 5.4 ng of fraction 2 was used as an antibody. Input of ϕX174 DNA·[^{3}H]RNA was 5.4 pmol. Binding of ϕX174 DNA·[^{3}H]RNA without competitor was 49.8, 42.2, and 58.5%, respectively. Competitors were (●—●) ϕX174 DNA·RNA; (△—△) ϕX174 ss-DNA; (▲—▲) ϕX174 RF ds-DNA; (×) calf thymus ss- and ds-DNA; (○—○) poly(dC)·poly(I); (◇—◇) poly(dT)·poly(A); (◆—◆) poly(dI)·poly(C); (▽—▽) poly(dG)·poly(C); (●---●) poly(A)·poly(U); (○---○) poly(C)·poly(I); (□---□) *E. coli* rRNA; and (■---■) *E. coli* tRNA.

II, again reflecting the difficulty of recovering high avidity antibody from immunoadsorbent (Parikh & Cuatrecasas, 1977).

When poly(dC)·poly(I) instead of poly(dT)·poly(A) was used to precipitate double helical nucleic acid specific antibodies, essentially the same results were obtained as above, except that the recovery of protein and antibody activity in fractions 3 and 4 were substantially lower than in the corresponding fractions in experiment 1 (Table I).

Characterization of the Antibody Fractions by RIA. Competition RIA was performed to characterize the antibody fractions using ϕX174 DNA·[^{3}H]RNA as an antigen as described previously (Nakazato, 1979). Figure 3 depicts the competition pattern between ϕX174 DNA·[^{3}H]RNA and nucleic acids for the antibody fraction. The numerical expression of the competition is shown in Table II.

It is clear that antibodies recovered after precipitation with poly(dT)·poly(A) or poly(dC)·poly(I) had no reactivity to ss- and ds-DNA (Figure 3, A and C, and Table II).

Table II: Competing Activity of Nucleic Acids with φX174 DNA·[^{3}H]RNA for the Antibodies

competitor	index of rel potency of a competitor[a] for antibody fraction[b]		
	II	III	2
φX174 DNA·RNA	1	1	1
poly(dC)·poly(I)	0.17	0.55	0.23
poly(dT)·poly(A)	400	75	2143
poly(dI)·poly(C)	400	50	N.T.[d]
poly(dC)·poly(G)	>>1900[c]	>>950[c]	N.T.
poly(A)·poly(U)	7.5	>>12 000	286
poly(C)·poly(I)	4	>>12 000	286
rRNA	1300	>>12 000	3570
tRNA	2900	11 500	6430
others[e]	>>5000	>>5 000	>>5000

[a] [Amount of a competitor needed to inhibit the binding of φX174 DNA·[^{3}H]RNA by 50%] /[amount of unlabeled φX174 DNA·RNA needed to inhibit the binding of φX174 DNA·[^{3}H]-RNA by 50%]. [b] See Materials and Methods and Table I. [c] Incomplete cross-reaction. [d] Not tested. [e] φX174 ss-DNA, φX174 RF ds-DNA, poly(A), poly(C), poly(G), poly(U), poly(I), poly(dA), poly(dC), poly(dG), poly(dT), poly(dI), poly(C)·poly(G), poly(dA)·poly(dT), poly(dC)·poly(dG), poly-(dC)·poly(dI), equimolar mixture of poly(dA) and poly(U), poly-(vinyl sulfate), or heparin did not inhibit the binding more than 10% when added at about 100 000 pmol.

The most striking results were that poly(dC)·poly(I) was a better competitor than φX174 DNA·RNA itself and poly-(dT)·poly(A) was a very weak inhibitor. This was true for both antibodies recovered by precipitation with poly(dT)·poly(A) and poly(dC)·poly(I) (Figure 3, A and C). This clearly indicated that the residual nucleic acid which might have remained in the antibody preparation after the purification procedure was not responsible for the observed results. Poly(dI)·poly(C), the twin hybrid of poly(dC)·poly(I), had similar cross-reactivity as poly(dT)·poly(A). Poly(dG)·poly(C) showed a typical incomplete cross-reactivity (Figure 3A), suggesting only a fraction of the antibody population is reactive with this hybrid. Poly(dC)·poly(G) showed similar competition as poly(dG)·poly(C) (not shown). Double-stranded ribohomopolymers poly(A)·poly(U) and poly(C)·poly(I) were good competitors and showed almost complete cross-reactivity, indicating that the majority of the antibodies recognized both DNA·RNA hybrids and ds-RNA as well (Figure 3A). Competition by rRNA and tRNA at high concentration (Figure 3A) may result from the double-stranded regions known to exist in these RNAs. As is shown in the legend to Table II, ribohomopolymers, deoxyribohomopolymers, double-stranded deoxyribohomopolymers, poly(vinyl sulfate), or heparin did not show competing activity at very high concentrations. Also, an equimolar mixture of poly(dA) and poly(U) which does not have double-stranded structure under normal conditions (Riley et al., 1966) showed no competing activity. The lack of competition by poly(C)·poly(G) may reflect quite different antigenicity of this nucleic acid from that of poly(A)·poly(U) or poly(C)·poly(I) (see Stollar, 1975).

After absorption of poly(dT)·poly(A) purified antibodies with poly(A)·poly(U)–agarose, cross-reactivity with poly-(A)·poly(U), poly(C)·poly(I), and rRNA diminished almost completely (Figure 3B). Transfer RNA still showed competition, although relative cross-reactivity decreased fourfold (Table II). The possibility that the tRNA preparation was contaminated by trace amounts of DNA·RNA hybrid was tested. The tRNA preparation was heated for 5 min at 98 °C in a 0.01 M Tris-HCl buffer, pH 7.4, and then rapidly cooled on ice. The heat-treated preparation retained the competing activity unchanged, indicating the presence of heat denaturable hybrid was not responsible for the competition. Contrary to the synthetic ds-RNAs, synthetic DNA·RNA hybrids showed increased cross-reactivity, except for poly-(dC)·poly(I), whose relative potency as a competitor was reduced to about twofold of φX174 DNA·RNA, and the competition curve showed a shallower slope than that of φX174 DNA·RNA (Figure 3B and Table II). Fraction IV, which had been washed off the poly(A)·poly(U)–agarose later than fraction III, showed an almost similar competition pattern to fraction III antibodies except that poly(A)·poly(U) or poly-(C)·poly(I) showed slight competition (ca. 20%) at 10 000 pmol. These results indicated some weak affinity of this fraction to ds-RNA. Fraction V showed an almost similar competition pattern to that of fraction II (data not shown), further indicating that the majority of the fraction II antibodies had affinity to ds-RNA, as well as to DNA·RNA hybrid.

Antibodies purified by precipitation with poly(dC)·poly(I) showed essentially similar competition patterns to that of poly(dT)·poly(A) purified antibodies (Figure 3, A and C), although the cross-reactivities of the nucleic acids tested, except poly(dC)·poly(I), were considerably lower (Table II). Fraction 3 antibodies which did not bind to poly(A)·poly(U)–agarose showed competition patterns almost identical with that of fraction III (data not shown). Fraction 5 had similar characteristics to fraction 2.

Discussion

It has been shown that φX174 DNA·RNA hybrids synthesized in vitro and complexed with MBSA elicited at least three kinds of antibody when injected into rabbits. The antibody specific to ss-DNA was most likely elicited by ss-DNA regions which are known to occur in the φX174 DNA·RNA preparation (Bassel et al., 1964). The molecule of an antibody fraction that reacts with ds-RNA reacts with DNA·RNA hybrids as well, since the same antibody molecules precipitated by poly(dT)·poly(A) were also bound to poly(A)·poly(U)–agarose.

This is evidence that ds-RNA and the DNA·RNA hybrid share some common immunologically discernible structures. Antibodies against poly(A)·poly(U) or poly(C)·poly(I) showed cross-reactions with DNA·RNA hybrids to varying extents, although poly(dT)·poly(A)-elicited antibodies were usually specific only to the DNA·RNA hybrid structures (Stollar, 1970).

Although titration and absorption experiments suggested the presence of a reasonable amount of hybrid specific antibody, its recovery during this purification was low. The most likely explanation for this is that the dissociation of this antibody fraction from poly(dT)·poly(A) or poly(dC)·poly(I) was low because it had high avidity to these hybrids. The low recovery of this fraction may be hidden by the high recovery of the major antibody of a broader specificity that includes both hybrid and ds-RNA. This may explain why the recovery of the hybrid specific antibody from the poly(dC)·poly(I) purified fraction was even less than from the poly(dT)·poly(A) purified fraction. The affinity of the antibody for poly-(dC)·poly(I) is far greater than for poly(dT)·poly(A). This was shown not only by competition RIA (Figure 3) but also by titration of 1.5 pmol of poly([^{3}H]dT)·poly(A) and poly-([^{3}H]dC)·poly(I) of similar size with the fraction II antibody. The amounts of the antibody required to bind 50% of input poly([^{3}H]dC)·poly(I) and poly([^{3}H]dT)·poly(A) were 1.2 and 50 ng, respectively.

Competition RIA revealed some interesting characteristics of the antibodies and nucleic acids. As has been anticipated from the experience with poly(dT)·poly(A)-elicited antibodies

(see introductory statement), poly(dT)·poly(A) was a fairly weak competitor of ϕX174 DNA·[^{3}H]RNA. On the other hand, poly(dC)·poly(I) was a better competitor than unlabeled ϕX174 DNA·RNA itself. This was rather unexpected because, while we can expect many oligo(dT)·oligo(A) stretches of four to seven base pairs in ϕX174 DNA·RNA hybrid (see Sanger et al., 1977), there are no stretches of oligo(dG)·oligo(I) of these or even shorter lengths. One possible explanation for these results is that there are sites in the hybrids of naturally occurring sequences (similar results as above were obtained when fd viral DNA·[^{3}H]RNA was used as an antigen; Nakazato, 1979; unpublished observations) which are more antigenic than poly(dT)·poly(A), and stretches of poly(dC)·poly(I) resemble such sites more than poly(dT)·poly(A). Since poly(dC)·poly(I) has more such sites than a naturally occurring DNA·RNA hybrid, the former is more potent as a competitor than the latter itself.

It is of interest to know whether poly(dC)·poly(I) elicits antibody as potent as the anti-ϕX174 DNA·RNA antibody in recognizing DNA·RNA hybrids of naturally occurring sequences. If it does, such an antibody will be a useful probe for the study of the antigenic structures of the hybrids of naturally occurring sequences.

The widely different potency of the twin hybrids, poly(dC)·poly(I) and poly(dI)·poly(C), as competitors reflects the different conformations of these hybrids as shown by differences in thermal stability (Chamberlin & Patterson, 1965) and X-ray diffraction patterns (Langridge, 1966; O'Brien & MacEwan, 1970).

It has been well established that poly(C)·poly(G) and poly(dC)·poly(dG) are serologically different from poly(A)·poly(U) and poly(C)·poly(I) (Lacour et al., 1973; Stollar, 1975). The competition RIA results of Figure 3 further confirmed this and also suggested that poly(dC)·poly(G) and poly(dG)·poly(C) are also serologically quite different from poly(dT)·poly(A), poly(dI)·poly(C), and poly(dC)·poly(I), as well as ϕX174 DNA·RNA. The competing activity of the tRNA preparation for the apparently DNA·RNA hybrid specific antibody was shown not to be because of contaminating DNA·RNA hybrid. Whether this activity is because of some conformation of tRNA whose tertiary structure is fairly complex (see Kim, 1976) or because of some other contamination remains to be seen.

Although the major antibody population has broad specificity reacting with both ds-RNA and DNA·RNA hybrid, it may be useful in the study where ds-RNA reactivity does not interfere, e.g., DNA·RNA hybrid isolation from an excess of DNA and/or RNA. On the other hand, the minor DNA·RNA hybrid specific antibody may be used in cytological work where more rigorous specificity is required.

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