ANTIBODIES TO POLYADENYLATE-POLYURIDYLATE COPOLYMERS AS REAGENTS FOR DOUBLE STRAND RNA AND DNA-RNA HYBRID COMPLEXES

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SUMMARY. Rabbits were immunized with complexes of polyA-polyU and methylated bovine serum albumin, and the resulting sera were examined in two dimensional immunodiffusion and quantitative complement fixation. The sera reacted with the inducing copolymer, with polyI-polyC, with the double strand RNA of reovirus, and to a lesser extent with DNA-RNA hybrids. There was no reaction with polyA alone, polyU alone, polyG-polyC, or with a variety of non-duplex RNA or native or denatured DNA samples. Reactivity of double strand polyribonucleotides was abolished by effective thermal denaturation.

Lacour et al. (1) have reported on the immunization of rabbits with double helical copolymers of polyriboadenylate and polyribouridylate (polyA-polyU) in complexes with methylated bovine serum albumin (MBSA). They obtained antibodies which cross reacted with some samples of native and denatured DNA, as well as with ribosomal and transfer RNA; the sera also reacted with polyA alone and with copolymers of polyinosinate and polycytidylate.

In order to compare these reactions with those of various human anti-DNA antibodies which occur in the sera of some patients with systemic lupus erythematosus, SLE, (2,3), we similarly immunized three rabbits with polyA-polyU-MBSA complexes. The sera we obtained differed in specificity from those described by Lacour et al., and did not cross react with DNA. They behaved as specific reagents for multi-strand structures containing polyribonucleotides, including double strand viral RNA and DNA-RNA hybrids.

MATERIALS AND METHODS

Homopolymers of riboadenylate, ribouridylate, riboguanidylate, riboinosinate

and ribocytidylate (polyA, polyU, polyG, polyI and polyC) were purchased from Miles Laboratories. Equal amounts of polyA and polyU (250 μg/ml) were mixed in 0.3M NaCl with 0.03M Na-citrate (2xSSC buffer), boiled for three minutes and slowly cooled to room temperature; the product showed expected hypochromicity. The copolymers polyG-polyC and polyI-polyC were similarly prepared. For immunization, equal weights of polyA-polyU and MBSA (4) were mixed and emulsified in complete Freund's adjuvant. Three doses, each containing 125 μg of polyA-polyU, were given intradermally and subcutaneously at weekly intervals to three New Zealand White rabbits. An intravenous injection without adjuvant was given to each a week later and serum obtained a week after this. A second similar course of immunization was carried out five months later.

Yeast RNA was purchased from Sigma Chemical Co. Soluble RNA of E. coli was provided by W.S. McNutt, reovirus RNA by W.K. Joklik, and the total RNA extracted from KB tissue culture cells by V. Stollar. DNA samples were prepared as described previously (5). R. Jayarman provided samples of DNA-RNA hybrids, which were prepared by heating a mixture of separated light single strands of T_4 bacteriophage DNA and pulse-labeled (^{32}P)-RNA extracted from phage-infected E. coli late in the infection cycle; 20% of the labeled RNA was hybridized, as determined by ribonuclease resistance and binding to nitrocellulose membrane filters. Without the heating step for annealing, no hybridization occurred as judged by these criteria.

Two dimensional immunodiffusion and quantitative micro complement (C') fixation were performed as described previously (6,7).

RESULTS AND DISCUSSION

In two dimensional immunodiffusion, sera from all three immunized rabbits precipitated with polyA-polyU, but not with polyA alone or with polyU alone (Fig. 1). Irregular precipitation also occurred in an angular region between the wells containing serum and the separate homopolymers. This was apparently due to annealing of the polymers

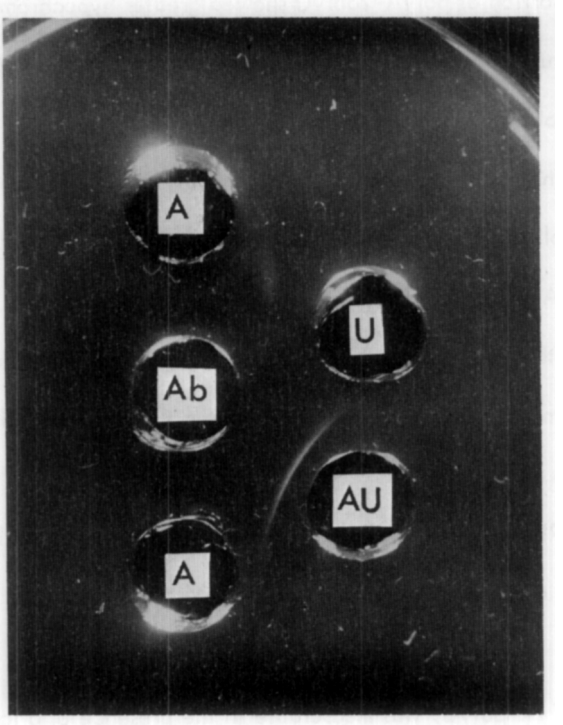

Fig. 1. Two dimensional immunodiffusion of anti-polyA-polyU-MBSA serum 68-170a (Ab) with: polyA (A); polyU (U); and polyA-polyU (AU), in 0.8% agarose in 0.1M borate buffer, pH 8.4, with 1:10,000 merthiolate. Antigens were tested at 200 μg/ml, with undiluted serum.

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where they met in the gel, as neither reacted alone. The sera also precipitated with reovirus RNA and with polyI-polyC, but not with polyG-polyC, and in the latter respect were similar to the sera studied by Lacour et al. (1). The polynucleotides did not precipitate with normal serum.

The anti-polyA-polyU-MBSA sera did not precipitate with E. coli soluble RNA, yeast RNA, the total RNA extracted from KB cells, or with native or denatured DNA of calf thymus, salmon, E. coli or dogfish erythrocytes.

A first course serum (68-170a) was examined in greater detail in quantitative micro C' fixation. PolyA-polyU reacted to give 50% maximal C'fixation with a 1:10,000 dilution of this serum; 0.02 μg of the copolymer was required for equivalence. A copolymer

prepared in the proportion of polyA-2polyU showed greater hypochromicity, as expected for a triple strand structure (7) and was slightly more reactive than the copolymers prepared with equal amounts of polyA and polyU.

With much more concentrated serum (1:100), no C' fixation occurred with any of the following: polyA alone, polyU alone, total yeast RNA, E. coli total or soluble RNA, or native DNA of calf thymus, salmon, or dogfish erythrocytes. These native DNA samples did react with two human SLE sera, while these SLE sera did not react with polyA-polyU. Thus the immunochemical distinction between the ribonucleotide and deoxyribonucleotide structures was reciprocal.

Reovirus RNA, which has been shown to be a double helical structure (8,9), reacted to give 50% maximal C' fixation with serum diluted 1:3500, a reaction similar to that of polyI-polyC. PolyG-polyC was not reactive. The C' fixation of reovirus RNA, as well as that of polyA-polyU, was measurable in the presence of a large excess (up to at least 100-fold) of yeast RNA, E. coli or native DNA, as these materials did not cause more than slight inhibition. With the total RNA of KB cells, some C' fixation was

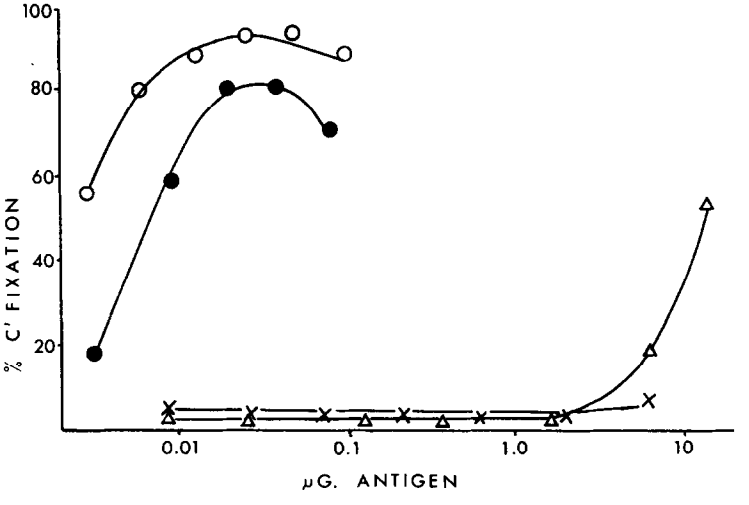

Fig. 2. C' fixation of anti-polyA-polyU-MBSA serum 68-170a (1:2500) and : polyA-polyU (○); reovirus RNA (●); KB cell RNA (△); and yeast RNA (×).

observed, but this required amounts some 2000 to 5000-fold greater than the quantities of polyA-polyU required for the same level of C' fixation (Fig. 2). This indicated that 0.02% to .05% of the RNA in the KB sample may be multistrand, either naturally or as a result of the isolation procedure; this question is under further study.

Both polyA-polyU and reovirus RNA lost serological reactivity when they were denatured under appropriate conditions. The polyA-polyU (25 µg/ml) was heated for 10 minutes at increasing temperatures in 0.15M saline-Tris buffer containing 3.7% formaldehyde, which was required to prevent renaturation; samples were diluted into cold buffer for C' fixation assay. The immunochemically measured profile or irreversible denaturation of polyA-polyU under these conditions is shown in Fig. 3. This copolymer also lost reactivity after exposure to ribonuclease at 60°C, though it was unaffected at room temperature. The denaturation of reovirus RNA (Fig. 3) was carried out with 1:10 SSC buffer, without formaldehyde.

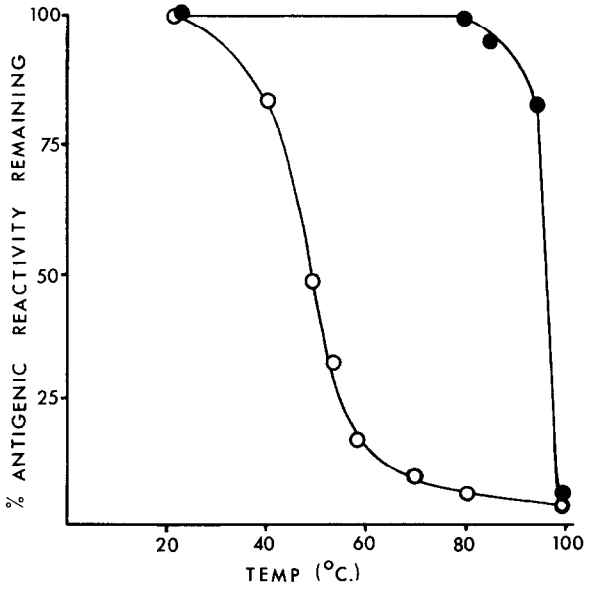

Fig. 3. Immunochemically measured thermal denaturation of polyA-polyU in the presence of 3.7% formaldehyde (O); and of reovirus RNA in 1:10 SSC buffer (●), as determined by loss of C' fixation reactivity with serum 68-170a.

DNA-RNA hybrids, prepared by heating denatured T_4 bacteriophage DNA with T_4-induced RNA, reacted to give 90% C' fixation with a serum dilution of 1:500 but not at all with a dilution of 1:2500. There was no reaction at either dilution with the same RNA alone, with denatured T_4 DNA alone, or with a combination of the same RNA and DNA which were mixed at 4°C immediately before assay and therefore not allowed to anneal.

The sera we have obtained differed from those studied by Lacour et al. (1968) in two major ways, as they did not cross react with DNA and did not react with polyA alone or with non-duplex RNA. In view of the lack of either direct reaction or inhibition by DNA or other RNA, the sera can measure the presence of double strand RNA in the presence of a large excess of other nucleic acids, and preliminary experiments indicate they should be useful for studying the appearance of replicative forms of RNA viruses without the need for artificially stopping host cell RNA synthesis. For this purpose, the background of serologically identifiable multistrand RNA was 0.02% to 0.05% with KB cell RNA and less for E. coli RNA. In the absence of double strand RNA, the sera can measure DNA-RNA hybrids directly; in a mixture, comparative measurements with serum dilutions of 1:500 and 1:2500 may also allow estimation of both structural forms.

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