Antigenic Structure of Double-Stranded RNA Analogues Having Varying Activity in Interferon Induction†

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ABSTRACT: Antibodies were induced by immunization of rabbits with methylated bovine serum albumin complexes of: poly(I)·poly(BC), an effective interferon inducer; poly(c^7A)·poly(rT), a noninducer that can block induction by active poly(A)·poly(rT); and poly(A)·poly(Um), which has neither inducing nor blocking activity. Similar complexes of f2 phage RNA or tRNA did not induce anti-nucleic acid antibodies. Each anti-polynucleotide serum contained some antibodies specific for double-stranded structure. Antibodies were immunospecifically purified from precipitates made with each serum and homologous or cross-reacting double-stranded polynucleotides. The purified antibodies distinguished among varying helices bearing base or ribose modifications. Anti-poly(I)·poly(BC) specificity paralleled that of the interferon induction system. Anti-poly(A)·poly(Um) specificity favored the 2′-modified polymers. Anti-poly(c^7A)·poly(rT) antibodies were the least discriminating. Cross-reaction results indicated that some antibodies reacted with determinants that included both sugar–phosphate backbones. In far antibody excess, antigen:antibody ratios in precipitating complexes reached a minimum of 7 to 12 base pairs per bivalent IgG molecule. Single antigenic determinants may span about 4 base pairs, with primary contact sites including the phosphate groups and the furanose.

Many biochemical functions involve specific interaction of nucleic acids and proteins. The features of nucleic acids that are important for recognition by proteins vary from system to system and are often difficult to define (Jovin, 1976; Sundaralingam & Rao, 1975). Antibodies to synthetic polynucleotides serve as an informative model for studying the specificity of protein–nucleic acid interactions. The uniform structure of synthetic polynucleotides simplifies the task of defining their conformations as compared with those of naturally occurring RNAs such as mRNA or tRNA. Specific antisera cannot only distinguish RNA from DNA and single-stranded from double-stranded forms, but can also recognize more subtle differences among various helices (Stollar, 1973, 1975; Lacour et al., 1973; Guigues & Leng, 1976a,b). They differentiate among double-stranded DNA, RNA–DNA hybrids, and double-stranded RNA (Stollar, 1970; Lacour et al., 1973) and among various triple-helical structures as well (Stollar & Raso, 1974; Rainen & Stollar, 1977). Once characterized, the antibodies have served as useful reagents for studies of nucleic acids (Silverstein & Schur, 1970; Stollar & Stollar, 1970; Ikegami & Francki, 1974; Rudkin & Stollar, 1977).

We have previously demonstrated the ability of antibodies directed against poly(A)·poly(U) to detect conformational variations among several double-helical polynucleotide analogues of poly(A)·poly(U) and poly(I)·poly(C) (Johnston et al., 1975). Features necessary for recognition of these modified duplexes by purified antibodies were similar to those needed for recognition by cells in the process of interferon induction.

The present work extends these studies by using as immunogens duplex polymers with varying interferon inducing potencies. These polymers were: (1) poly(A)·poly(Um),[1] a noninducer (De Clercq et al., 1972); (2) poly(c^7A)·poly(rT), a noninducer that blocked induction by poly(A)·poly(rT) (De Clercq et al., 1974); and (3) poly(I)·poly(BC), a better inducer than poly(I)·poly(C) in some systems (Torrence et al., 1974; De Clercq et al., 1976). All three duplex polymers reacted poorly with antibodies to poly(A)·poly(U) (Johnston et al., 1975). In the present study, the immunogenic potency of these three polymers was compared with that of poly(A)·poly(U), f2 phage RNA, and transfer RNA. The cross-reactivity patterns of the antibodies to the three polymers were tested reciprocally and with other duplexes modified in either the base or the furanose. In addition, the maximum number of antibodies packed on the duplexes in precipitating complexes was estimated, to provide information on the spacing of antigenic determinants along the helix.

Materials and Methods

Polynucleotides. UmDP, poly(A), poly(U), and poly(I) were purchased from P-L Biochemicals, Inc., Milwaukee, Wis. Radiolabeled poly(A)·poly([^{14}C]U) (control 51-1-328) and poly([^{14}C]I)·poly(C) (control 54-1-335) were obtained from Miles Laboratories Inc., Elkhart, Ind. The c^7ADP, rTDP, and BCDP were the generous gifts of Paul F. Torrence. *E. coli* K12 transfer RNA (stripped) was purchased from General Biochemicals, Chagrin Falls, Ohio. Bacteriophage f2, obtained from M. Malamy, was purified with CsCl density gradient centrifugation. The f2 RNA was purified by the procedure of Gesteland & Boedtker (1964).

Poly(Um), poly(c^7A), poly(rT), and poly(BC) were syn-

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[1] Abbreviations used: poly(Um), poly(2′-*O*-methyluridylic acid); poly(Am), poly(2′-*O*-methyladenylic acid); poly(Ae), poly(2′-*O*-ethyladenylic acid; poly(Cm), poly(2′-*O*-methylcytidylic acid); poly(rT), poly(ribothymidylic acid); poly(c^7A), poly(7-deazaadenylic acid); poly(c^7I), poly(7-deazainosinic acid); poly(BC), poly(5-bromocytidylic acid); poly(BU), poly(5-bromouridylic acid); poly(L), poly(laurusin phosphate); poly(dCz), poly(2′-azido-2-deoxycytidylic acid); poly(dCCl), poly(2′-chloro-2′-deoxycytidylic acid); DEAE, diethylaminoethyl; PBS, phosphate-buffered saline, 0.14 M NaCl, 0.01 M sodium phosphate, pH 7.

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thesized from their nucleoside diphosphates with polynucleotide phosphorylase (lot X-3453A, P-L Biochemicals, Inc.) in a Tris-$MgCl_2$ buffer as described by Torrence et al. (1974). Extensive extraction of the protein with chloroform–isoamyl alcohol was followed by separation of polymers from nucleotides on a Sephadex G-100 column equilibrated and developed with 0.04 M NH_4HCO_3, pH 7. After concentration by lyophilization and solution in SSC buffer (0.15 M NaCl, 0.015 M sodium citrate, pH 7), the homopolymer concentrations were calculated from the following molar extinction coefficients: poly(c^7A), ϵ_{270} = 9000; poly(BC), ϵ_{289} = 5500; poly(rT), ϵ_{266} = 9200; poly(Um), ϵ_{260} = 9400 (Howard et al., 1971; Bobst et al., 1976). Polymer pairs were mixed in equimolar amounts and double-strand formation was monitored spectrophotometrically with mixtures of continuously varying proportions, to ensure that a 1:1 interaction occurred. All polynucleotide spectra and mixing curves agreed with those published by others (Howard et al., 1969; Torrence & Witkop, 1975). Other modified polynucleotides were obtained as described previously (Johnston et al., 1975).

Immunization. Rabbits were immunized by three weekly multiple intradermal injections of 50 to 100 μg of duplex complexed with an equal weight of methylated bovine serum albumin in either complete or incomplete Freund's adjuvant. On the fourth week the same dose, without adjuvant, was injected intravenously. Animals were bled 5 to 7 days later. Subsequent intravenous boosts and bleedings were done at weekly intervals. All studies for each immunogen were done with antisera from a single rabbit. The complement fixation assays were done with antibody purified from a single bleeding or a pool of two bleedings from a single rabbit.

Serological Methods. Two-dimensional immunodiffusion in gels was performed by the method of Ouchterlony (1949) in 0.7% or 0.8% agarose, with 50–70 μL of antiserum and 50–70 μL of polynucleotide solution containing 5–15 μg of polymer. *Counterimmunoelectrophoresis* in agar with a buffer of 0.05 M Tris-HCl (pH 8.0) was done as described previously (Johnston et al., 1975). *Complement fixation* titers of purified antibody with varying polymers were measured as described previously (Johnston et al., 1975) with a micro-complement fixation assay (Stollar, 1977). The titer was defined as the reciprocal of the antibody dilution required for a curve having a maximum of 50% complement fixation. Polymers were compared with a given antibody by an index of dissimilarity (Prager & Wilson, 1971), which is the ratio of the titer obtained with the immunogen to the titer obtained with the cross-reacting duplex. The higher the index, the greater the differences as recognized by the antibodies. Maximal precipitable antibody was measured from *quantitative precipitin* curves. Precipitates were made with 0.05 to 0.2 mL of serum and 2 to 75 nmol of duplex RNA. Protein in washed precipitates was measured by the method of Lowry et al. (1951). To measure maximal antibody packing along the polynucleotide, precipitates were prepared with varying amounts of polymer and 0.1 to 3.0 mL of serum, in proportions from near equivalence to far antibody excess. Mixtures were incubated at 37 °C for 2 h and then at 4 °C for 24 to 72 h. Precipitates were washed twice and redissolved in 1 mL of 0.01 N NaOH. Protein was determined by the method of Lowry et al. (1951) and the antigen:antibody ratio was calculated on the assumption that all of the antigen was precipitated by excess antibody. The assumption was tested by formation of precipitates in antibody excess with ^{14}C-labeled poly(A)·poly(U) or ^{14}C-labeled poly(I)·poly(C) and anti-poly(A)·poly(U) antibody. No radioactivity was left in the supernatant after precipitation; less than 3% was lost during washing of the precipitates.

Absorption and Purification of Antibodies. Antibodies reactive with single-stranded components were removed by precipitation with the reactive homopolymer. Equivalence point amounts of polymer and serum, determined from preliminary quantitative precipitation, were incubated at 37 °C for 2 h and at 4 °C for 24 to 48 h. The precipitate was removed by centrifugation and the serum was passed through a DEAE-cellulose (Whatman DE-52) column in a Pasteur pipet to remove any excess polynucleotide. The column was equilibrated and developed with 0.015 M sodium phosphate, pH 8; anti-polynucleotide antibodies passed through directly. In some cases, sera were similarly absorbed with double-stranded polynucleotides.

For immunospecific antibody purification, equivalence precipitates were prepared from 2 to 4 mL of serum with an appropriate amount of polynucleotide as determined from preliminary precipitin tests. The precipitates were washed twice with cold PBS and then suspended in 2 mL of distilled water. Duplex polynucleotides were then denatured on incubation at 52 °C, releasing duplex-specific antibody. The addition of 80 to 160 μg of pancreatic ribonuclease per mL of suspension resulted in digestion of one or both polynucleotide strands and prevented reformation of antigen–antibody complexes when NaCl was added to 0.15 M to dissolve the released antibodies. The antibody-poly(I)·poly(BC) precipitate was digested with a combination of micrococcal nuclease (10 μg/mL), T1 ribonuclease (10 units/mL), and pancreatic ribonuclease (10 μg/mL). Any residual insoluble complexes were removed by centrifugation. The soluble IgG antibody was separated from IgM antibody, enzyme, and oligonucleotide fragments by gel filtration on a Sephadex G-200 column equilibrated and developed with PBS.

Anti-poly(c^7A)·poly(rT) and anti-poly(I)·poly(BC) antibodies were purified from precipitates made with either homologous antigen or with poly(I)·poly(C). Anti-poly(A)·poly(Um) antibodies were isolated only from precipitates made with poly(A)·poly(U) since no detectable protein was released by digestion of antibody–poly(A)·poly(Um) precipitates with either pancreatic ribonuclease (20 μg/mL), micrococcal nuclease (25 μg/mL), or a combination of snake venom phosphodiesterase (200 μg/mL), micrococcal nuclease (20 μg/mL), and alkaline phosphatase (100 μg/mL). This combination had been found to degrade poly(Um) (Zmudzka & Shugar, 1970). The anti-poly(A)·poly(U) sera were purified from precipitates made with poly(I)·poly(C) (Johnston et al., 1975).

Results

a. *Reactions of Whole Sera and Absorbed Sera.* Methylated bovine serum albumin complexes of each of the duplex polyribonucleotides were immunogenic and induced from 0.4 to 1.5 mg of precipitable anti-polynucleotide antibody per mL of serum in early bleedings (Table I). No anti-nucleic acid antibody was induced by similar methylated bovine serum albumin complexes of transfer RNA or f2 phage RNA, even with repeated immunizations.

Although the immunogens all formed double-stranded structures, the whole sera often contained some antibodies that reacted with one of the single-stranded components, as seen in immunodiffusion or counterimmunoelectrophoresis. The reactive homopolymers were: poly(I), poly(c^7A), and poly(Um) for the sera induced by the corresponding duplex immunogens. Absorption with the single homopolymers removed all of the corresponding antibodies and left in the serum antibodies that were specific for double-stranded RNA forms. After absorption with poly(c^7A) and poly(I), respectively, the

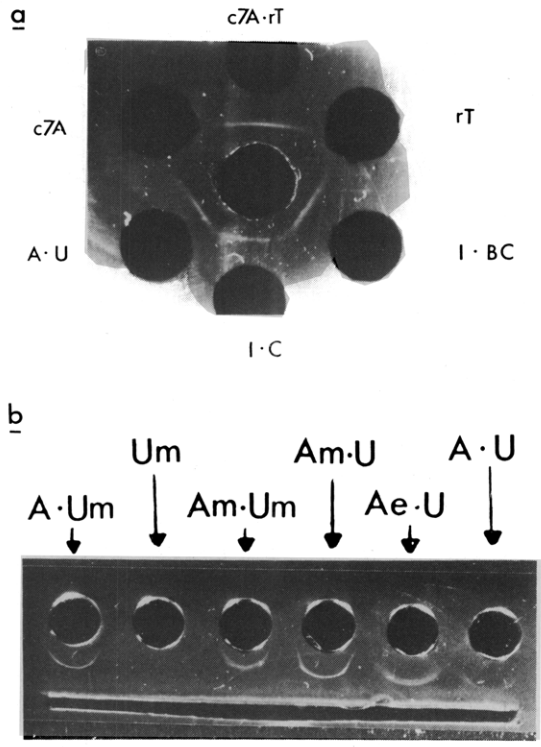

FIGURE 1: (a) Immunodiffusion with anti-poly(c^7A)·poly(rT) serum absorbed with poly(c^7A). Central well contained 50 μL of absorbed serum and peripheral wells contained 50 μL of polymer (0.5 μmol/mL) in PBS. Gels were 0.8% agarose in PBS. (b) Counterimmunoelectrophoresis of antipoly(A)·poly(Um) serum after absorption with poly(Um). The trough contained 150 μL of absorbed serum and the wells contained 50 μL of polymer (10 μg/mL). Electrophoresis was in 0.8% agar in 0.05 M Tris (pH 8.0) at 8 mA per 5 × 7 cm plate for 1 h.

TABLE I: Immunogenicity of Nucleic Acid–Methylated Bovine Serum Albumin Complexes.

Immunizing polymer	Test antigen	Maximal precipitable antibody[a] (mg/mL serum)
Poly(A)·poly(U)	Poly(A)·poly(U)	1.1
	Poly(I)·poly(C)	1.2
Poly(c^7A)·poly(rT)	Poly(c^7A)·poly(rT)	1.5
	Poly(I)·poly(C)	0.68
Poly(I)·poly(BC)	Poly(I)·poly(BC)	0.77
	Poly(I)·poly(C)	0.79
Poly(A)·poly(Um)	Poly(A)·poly(Um)	0.36
	Poly(A)·poly(U)	0.22
f2 phage RNA	f2 phage RNA	0
E. coli tRNA	*E. coli* tRNA	0

[a] Anti-poly(I)·poly(BC) serum was from the first bleeding, anti-poly(A)·poly(Um) and anti-poly(c^7A)·poly(rT) sera were from the second bleedings, and anti-poly(A)·poly(U) serum was a pool of third and fourth bleedings. All sera were from individual rabbits.

anti-poly(c^7A)·poly(rT) and anti-poly(I)·poly(BC) sera gave lines of apparent identity between the immunogen and several cross-reacting double-stranded polymers in immunodiffusion. Results with absorbed anti-poly(c^7A)·poly(rT) are shown in Figure 1a. After the absorption with poly(Um), the anti-poly(A)·poly(Um) serum reacted well with poly(A)·poly(Um) but only very weakly with poly(A)·poly(U) (Figure 1b).

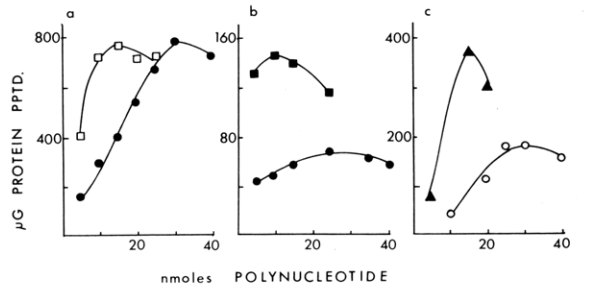

FIGURE 2: Quantitative precipitin curves of (a) anti-poly(I)·poly(BC) serum with poly(I)·poly(BC) (□) or poly(I)·poly(C) (●); (b) anti-poly(c^7A)·poly(rT) serum with poly(c^7A)·poly(rT) (■) or poly(I)·poly(C) (●); and (c) anti-poly(A)·poly(Um) serum with poly(A)·poly(Um) (▲) or poly(A)·poly(U) (○).

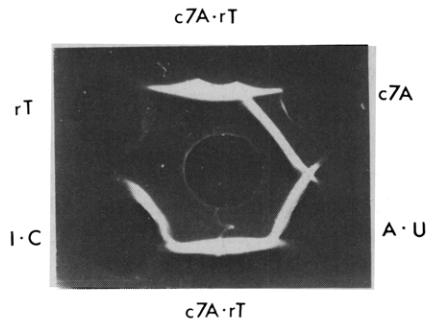

FIGURE 3: Immunodiffusion analysis of anti-poly(c^7A)·poly(rT) whole serum. The center well contained 60 μL of undiluted serum and peripheral wells contained 50 μL of polymer at 0.6 μmol/mL.

Quantitative analysis of precipitation with whole sera showed there were detectable differences between the immunogens and cross-reacting duplexes, in terms of the amount of antibody precipitated and the amount of antigen required for equivalent precipitation (Figure 2). Cross-absorption studies were done to evaluate further the extent to which some antibody populations were unique for a given duplex structure. In one case, the preference for homologous antigen reflected the presence of antibodies that could react with both homopolymer and duplex polymer. In this example, the homologous reactivity of anti-poly(c^7A)·poly(rT) serum was completely absorbed by a combination of the homopolymer poly(c^7A) and the cross-reacting duplex poly(I)·poly(C). This absorption left behind no antibodies detectably unique to the immunizing helix. Immunodiffusion experiments also showed that the reactivity of this serum could be accounted for as a combination of two antibody populations—one specific for double-stranded structures and one reactive with poly(c^7A) alone (Figure 3). The precipitin line formed with poly(c^7A)·poly(rT) spurred over the lines formed with either poly(c^7A) or poly(A)·poly(U); the latter two lines crossed. Poly(A)·poly(U) showed a reaction of identity with poly(I)·poly(C) in other experiments.

With anti-poly(I)·poly(BC), repeated absorption with poly(I)·poly(C) always left behind antibody to both the poly(I)·poly(C) and the poly(I)·poly(BC), whereas the homologous duplex completely removed antibodies to both helices. This result may have reflected a low avidity of the antibody for the cross-reacting poly(I)·poly(C) but suggested that the same antibody populations were reacting with both helices. This interpretation is consistent with the shift of the precipitation curve to high antigen concentrations for the cross-reaction, with no change in the height of the curve (Figure 2a).

The clearest demonstration of fractionation of anti-helix antibody populations by absorption was seen with anti-

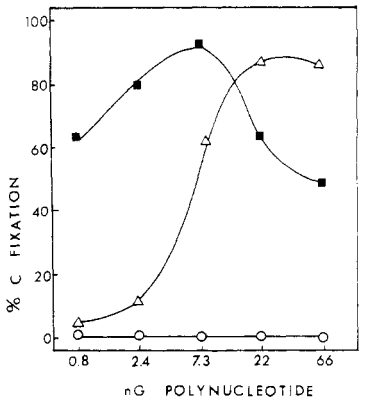

FIGURE 4: Quantitative micro-complement fixation reactions of anti-poly(A)·poly(Um) antiserum (1:600 dilution) after absorption with both poly(A)·poly(U) and poly(Um). Test antigens were: poly(A)·poly(Um) (■), poly(I)·poly(dCz) (△), and poly(A)·poly(U) or poly(Um) (○). The latter two were negative with 1/300 serum.

poly(A)·poly(Um) serum. After effective absorption with both the homopolymer poly(Um) and the cross-reactive poly(A)·poly(U), there remained in the serum antibodies that reacted with other duplex forms, especially those bearing a 2′ substituent in one of the strands (Figure 4).

b. *Reactions of Purified Anti-dsRNA Antibodies.* With anti-poly(c^7A)·poly(rT) or anti-poly(I)·poly(BC) sera, IgG antibodies were immunospecifically purified from washed precipitates made with antiserum and either the immunogen or cross-reactive poly(I)·poly(C). The purified antibodies reacted with only double-stranded polymers. If some antibodies to single homopolymers were included in the precipitates, they were not redissolved under the conditions of purification. Under those conditions, however, the double-stranded polymers were denatured and antibodies that were specific for helical structure were released. The antibodies purified with homologous antigen and with poly(I)·poly(C) showed similar specificities in tests with a variety of modified polymers.

The purified antibodies were tested with modified duplexes in micro-complement fixation assays. Indices of dissimilarity were calculated (Prager & Wilson, 1971) to compare the antibody specificities (Figure 5). As described previously (Johnston et al., 1975), reactivity of polymers with the anti-poly(A)·poly(U) antibodies was reduced to a much greater extent by modifications in the furanose than by changes in the purine or pyrimidine bases (Figure 5). A similar pattern was seen with anti-poly(I)·poly(BC) antibodies. Although poly(I)·poly(C) reacted as well as the homologous duplex, some specificity for the structure dependent on the substituted cytosine was suggested by the observation that poly(A)·poly(BU) and poly(c^7I)·poly(BC) reacted better than their unbrominated analogues.

The anti-poly(c^7A)·poly(rT) antibodies were the least discriminating and did not show reciprocal distinctions with the other antibody systems. That is, antibodies to the other double-stranded RNAs reacted poorly with poly(c^7A)·poly(rT) but antibodies elicited by poly(c^7A)·poly(rT) recognized all dsRNAs about equally (Figure 5). Even though some features of this helix are quite distinct as recognized by antibodies to the other polynucleotides, the poly(c^7A)·poly(rT) does share some structural features common to all of these helices and these features are involved in the immune recognition of this analogue.

Anti-poly(A)·poly(Um) antibodies could not be released from precipitates made with the homologous antigen itself, but some could be purified from precipitates made with cross-

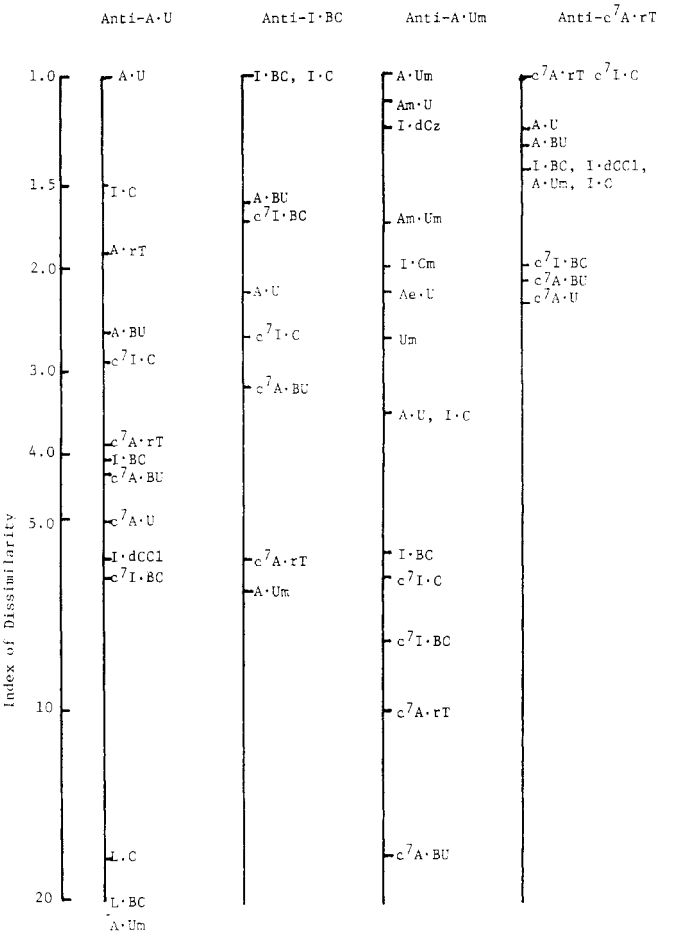

FIGURE 5: Summary of cross-reactions of immunospecifically purified antibodies. Results with anti-poly(A)·poly(U) antibodies purified from precipitates with poly(I)·poly(C) were as described previously (Johnston et al., 1975). Anti-poly(I)·poly(BC) and anti-poly(c^7A)·poly(rT) were purified from precipitates made with homologous polynucleotides. Anti-poly(A)·poly(Um) was purified from precipitates made with poly(A)·poly(U). Quantitative microcomplement fixation assays were done at varying serum dilutions. Indices of dissimilarity were calculated as the ratio of the 50% complement fixation titer for the homologous antigen to the 50% complement fixation titer for the cross-reactive antigen.

reactive poly(A)·poly(U). These purified antibodies still showed strongest reactivity with the immunogen and other 2′-modified polymers (Figure 5); with this specificity, they also reacted with the single-stranded poly(Um). They differed in this latter respect from the populations that were left after absorption with poly(A)·poly(U) and/or poly(Um) and that were strictly specific for double-stranded structure (Figures 1b and 4). Poorest reactivity occurred with polymers that were modified in the bases and not in the ribose.

c. *Antibody Packing on the Helix.* An estimate of the packing of antibodies onto helical polynucleotides when antibodies were in great excess provided insight into the distribution of antigenic determinants. Extrapolated values for the minimal number of base pairs per antibody molecules in the precipitate ranged from 7 to 12 (Figure 6 and Table II). This range is probably greater than the size of a single antigenic determinant, since the binding of one IgG may sterically hinder the binding of another nearby. Furthermore, at least some of the antibodies must have had Fab sites filled since precipitation occurred. If both sites were filled on all IgG molecules, there would be, at most, 4 to 6 base pairs per binding site. These estimations are similar to the values of 3 to 5 base pairs obtained by Guigues & Leng (1976a,b), who used fluorescence

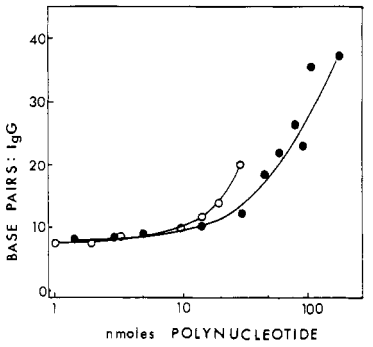

FIGURE 6: Antigen:antibody ratios in antibody excess precipitates as a function of the amount of antigen added per 0.1 mL of serum. Anti-poly(A)·poly(U) serum was tested with poly(A)·poly(U) (○) and poly(I)·poly(C) (●). Values were extrapolated to a minimum base pair; IgG value to estimate the closest packing of antibodies along the helix (Table II).

quenching measurements to estimate the packing of anti-poly(I)·poly(C) Fab fragments onto poly(I)·poly(BC). These data indicate that there is more than one effective determinant per helix turn and suggest that steric hindrance of IgG binding may be minimized by a spiral stacking of antibody along the helix.

The packing of antibody onto the immunizing duplex was about the same as onto a cross-reacting duplex such as poly(I)·poly(C), and the packing values for all of the antibody–antigen systems were similar. The differences in cross-reactivities observed in these systems probably arose from differences in avidities of the antibodies for various helices rather than in the number of determinants per length.

Discussion

Immunological distinctions among nucleic acid secondary structures occur at the levels of both immune responsiveness and of antibody specificity. All of the analogues of double-stranded RNA elicited antibody formation, whereas the secondary structure of tRNA or f2 phage RNA did not. Distinctions in responsiveness occur even among strictly helical forms, since native DNA and closely related helices have not been immunogenic, whereas double-stranded RNA, RNA–DNA hybrids, triple helical polynucleotides and even double-stranded poly(dG)·poly(dC), which differs from native DNA structure, are immunogenic (Stollar, 1973, 1975; Lacour et al., 1973). Experimentally induced antibodies that are formed against helical nucleic acids distinguish sharply among classes of helices (Stollar, 1975) and, as noted in the present quantitative assays, they also recognize structural differences among helical forms within a given class such as double-stranded RNA. The studies of poly(c^7A)·poly(rT) showed that the precise distinctions made in recognition for responsiveness and in antibody specificity are not always parallel.

Relationship to Specificity in Interferon Induction. The specificity of antibodies to double-stranded polyribonucleotides and that of interferon induction show several parallel properties. As previously described (Johnston et al., 1975) and further verified in this study, the antibody systems are like interferon induction in that recognition depends on secondary structure and the nature of the sugar–phosphate backbone but not on a specific base content or sequence of the double-stranded RNA. In both systems, modification of the bases of component nucleotides affects fine recognition.

Poly(I)·poly(BC) is as good an inducer of interferon as poly(I)·poly(C) in some systems (Torrence et al., 1974; De Clercq et al., 1976b). The antibodies directed against the brominated duplex showed strong reactivity with both of these helices and much weaker reactivity with the noninducers poly(c^7A)·poly(BU), poly(c^7A)·poly(rT), and poly(A)·poly(Um). Like the anti-poly(A)·poly(U) antisera, these antibodies recognized the structural features required for induction, but also reacted well with a noninducer, poly(A)·poly(BU); this polymer may rearrange to a triple-stranded structure under conditions of the interferon induction assay (DeClercq et al., 1974).

TABLE II: Antibody Packing Along Helical Polynucleotides.[a]

Antiserum to:	Test antigen	Extrapolated minimum value of base pairs/IgG molecule
Poly(A)·poly(U)	Poly(A)·poly(U)	7
	Poly(I)·poly(C)	8
Poly(c^7A)·poly(rT)	Poly(c^7A)·poly(rT)	12
	Poly(I)·poly(C)	10
Poly(I)·poly(BC)	Poly(I)·poly(BC)	8
	Poly(I)·poly(C)	10
Poly(A)·poly(Um)	Poly(A)·poly(Um)	12

[a] Antigen:antibody ratios for precipitates made in far antibody excess were calculated for several amounts of antigen added to 0.1 to 3.0 mL of serum for each polymer–serum combination. The ratios, expressed as the number of base pairs per IgG molecule, were plotted and extrapolated to a minimum value as shown in Figure 5, for estimation of the closest packing of antibody along the helix.

Poly(c^7A)·poly(rT) seemed quite different from helices such as poly(I)·poly(C) and poly(I)·poly(BC) when tested as a cross-reactant with several anti-dsRNA antibodies, but it elicited antibodies almost exclusively to features that it shared with the other polymers. These findings correlate with results of interferon induction assays. Unlike other noninducers, poly(c^7A)·poly(rT) can block induction by its unmodified analogue poly(A)·poly(rT) (DeClercq et al., 1974); thus, it must have the minimal structure required for binding to cell receptors if not for triggering the responsive cell. In the immune response, it may be recognized by a subset of the total population of cells that can recognize dsRNA. Poly(c^7A)·poly(rT) may therefore serve as a probe for identifying the most basic recognition features for both the immune and the interferon responses.

The poly(A)·poly(Um) antibody system displayed a specificity reciprocal to that of both the previously described antibodies and the interferon induction system, since the 2′-modified noninducers were preferred by these antibodies.

The Nature of Antigenic Determinants in Double-Stranded Polyribonucleotides. Our previous and present results support the suggestion that antibodies can recognize the pentose–phosphate backbone of the double helix and that both strands play a role in the formation of a single antigenic determinant since: (1) each of these sera contained some antibodies that reacted with double-stranded but not single-stranded polynucleotides; (2) in no instance was the presence of a particular base necessary for recognition by a given antibody, whereas antibodies to the bases themselves are highly specific (Erlanger & Beiser, 1964; Ungar-Waron et al., 1967; Seaman et al., 1965); (3) in most systems marked changes of reactivity occurred on modification of the furanose, even when only one strand of the helix was altered; (4) the antibodies were sensitive to changes in either strand, and greater changes in reactivity occurred when both strands were modified than when only one was altered.

These findings are consistent with the concept that an antibody binding site spans a groove of the helix and contacts the furanose or phosphate of both strands, and that specificity depends on the conformation-dependent orientation of these groups in a precise geometry. The structure at the 2′ position of the sugar may serve as a portion of the determinant directly (as a contact site), since substituents at this position so markedly reduced reactivity with anti-poly(A)·poly(U) serum and enhanced reactivity with anti-poly(A)·poly(Um) serum. Alternatively, the 2′ substituent may act indirectly through effects on helix conformation, since antibodies induced by the methyl-containing polymer reacted well with ethyl- or azido-substituted analogues. The 2′ methylation can affect the geometry of the helix through its influence on the orientation of the 3′-phosphate residue and therefore on base stacking interactions and helical shape (Alderfer et al., 1974; Singh et al., 1976). Similarly, a change from N to C at the 7 position of the purine may increase the positive tilt of the base in the poly(A)·poly(U) analogue and decrease the base tilt in the poly(I)·poly(C) analogue (Bobst et al., 1976). This could explain the findings that poly(c^7A)·poly(U) and poly(c^7I)·poly(C) differ quite markedly from their unmodified parent molecules both serologically and in interferon induction. As noted with anti-poly(A)·poly(U) serum especially, they also differ substantially from each other. If the furanose and bases do act indirectly, then the phosphate groups may provide the major contact points. Guigues & Leng (1976a) estimated, from the effect of salt concentration on antibody binding, that two phosphates acted as contact sites for a given determinant, even though seven may be encompassed within one antibody combining region.

This view of the antigenic structure of the helix is also consistent with the measurements of packing of antibodies on the helix. There were, at most, 8–12 base pairs per antibody molecule; if all Fab sites were filled, the value would be 4 to 6 base pairs; Guigues & Leng (1976a,b) estimated it as 3 to 5 pairs. X-ray diffraction studies of myeloma protein MOPC 603 showed its combining site to be about 15 × 20 × 12 Å deep (Segal et al., 1974). A span including the backbones of both strands over 4 to 6 base pairs would require a binding site area of about 10 to 15 × 11 to 22 Å. Examination of a three-dimensional model of a helical nucleic acid indicates that the antibody could make contact with the backbones of both strands over 3 to 4 base pairs with a binding site of reasonable depth.

Such a determinant, with the major binding involving the phosphates of both strands, could explain the conformational dependence of recognition for both the antibodies and for interferon induction. As models of protein–nucleic acid interaction, these systems differ from recognition by *lac* repressor protein (Gilbert & Maxam, 1973; Bourgeois & Pfahl, 1976) or the mechanism proposed by Seeman et al. (1976), in which specific base sequences are recognized. Experiments are currently under way to test more directly the involvement of the phosphate in binding by measurements of nuclear magnetic resonance spectra.

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