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# Electron microscopy of the reactions of anti-poly A. poly U and anti-poly I. poly C antibodies with synthetic polynucleotide complexes and natural nucleic acids

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ABSTRACT

The reactions between purified anti-poly A. poly U and-poly I. poly C. antibodies (IgG and IgM), and synthetic and natural polynucleotides were visualized at the molecular level. This was achieved by the use of fine tungsten bidirectional shadowing of molecules adsorbed onto thin carbon films, combined with dark field electron microscopic observation. A progression was observed from monogamous multivalency (binding of a single multifunctional antigen molecule with several combining sites of the same antibody molecule simultaneously) (Crothers and Metzger, 1972, Immunochemistry, 9, 341-357), to aggregation. Different types of figures were observed, among which loops formed by the coiling of the antigen around a single IgM molecule were very frequently seen. The tendency of IgG antibodies to bind cooperatively to certain antigens was also noted. In contrast, cross-links were seldom encountered. The cross-reactivity of different polynucleotides was also assessed by a quantitative analysis. The length of antigen associated to an antibody molecule (either IgG or IgM) was also measured.

## INTRODUCTION

Electron microscopic studies of both immunoglobulins (1, 2) and nucleic acids (3-5) have been numerous, and several antigen-antibody complexes have been observed at the ultrastructural level (6-11).

Immuno-electron microscopy techniques, with antibodies to minor nucleosides or to modified nucleosides have been used to localize the corresponding hapten in the small unit of the E.coli ribosome (12), in modified Col E1 DNA (13) and to determine the site of adenylylation of the glutamine synthetase of E.coli (14).

Since we demonstrated the immunogenicity of double- and triple-stranded synthetic polynucleotide complexes (15, 16), a vast number of studies have been pursued with antibodies thus obtained and synthetic homopolynucleotides, polynucleotide analogues or natural nucleic acids (for reviews see 17-19). However, the interaction between antibodies directed against double-stranded polynucleotide structures and polynucleotides has not yet

been studied at the molecular level.

Our purpose was to observe this particular immunological reaction as a whole, and we therefore did not attempt to separate IgM from IgG antibodies, nor did we try to isolate the antigen-antibody complexes from the unreacted components.

In this paper we visualize the different association forms between purified anti-poly A. poly U and - poly I. poly C IgM and IgG and synthetic polynucleotide complexes (poly A. poly U, poly I. poly C, poly G. poly C, poly dG. poly dC, poly A. 2 poly U, poly A. 2 poly I) and natural nucleic acids as well.

## MATERIALS AND METHODS

1. Antigens. The polynucleotides : poly A ($S_{20}$ = 8.4), poly U ($S_{20}$ = 12.9), poly I ($S_{20}$ = 10.0) and poly C ($S_{20}$ = 11.3 [used for poly I. poly C] or 9.7 [used for poly G. poly C])were purchased from Boehringer Mannheim and poly G ($S_{20}$ = 8.8) from Miles Laboratories. They were further purified by extraction with water-saturated phenol, dialysis against 0.15 M NaCl and precipitation with cold ethanol as previously described (20).

The double- and triple-stranded complexes were prepared as described previously (16,20) except for poly dG. poly dC which was obtained from Miles Laboratories, used without further purification and contained 58.5 % G and 41.5 % C.

The respective sedimentation constants were : poly I. poly C, $S_{20}$ = 16.7 ; poly A. poly U, $S_{20}$= 12.5 ; poly G. poly C, $S_{20}$ = 14.5 ; poly dG. poly dC, $S_{20}$ = 12.2 ; poly A. 2 poly U, $S_{20}$ = 20.7.

All of the antigens used throughout this study, with the exception of viral DNAs, were heterogeneous in size (plate I). This heterogeneity has already been reported for poly I. poly C (21).

Rotavirus RNA, a natural double-stranded ribonucleic acid, was a generous gift of Dr. J. COHEN, Station de Recherches de Virologie et d'Immunologie, I.N.R.A. 78850 Thiverval - Grignon (France) ; it is illustrated in fig. 7, plate I. It should be noted that the macromolecules differ widely in size ; indeed, after polyacrylamide gel electrophoresis, the rotavirus genome is distributed in 4 classes, with MW ranging from $0.2 \times 10^6$ to $2.2 \times 10^6$ daltons (22).

SV40 and phage PM2 circular DNAs (forms I and II) as well as the replicative form of ΦX174 DNA were gifts of Dr. B. REVET, Institut de Recherches sur le Cancer, 94800 Villejuif (France).

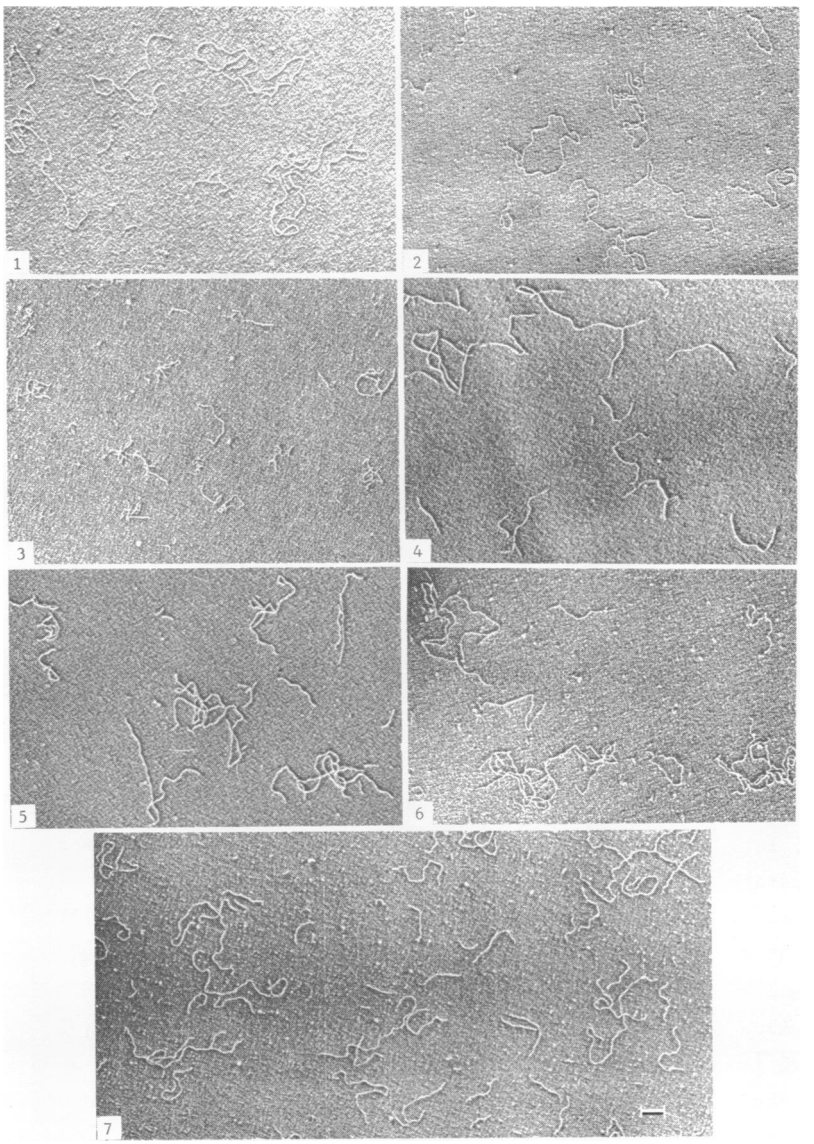



PLATE I . Morphology of different polynucleotides.
Double-stranded synthetic polynucleotide complexes: Fig. 1-4.
Fig. 1: poly A. poly U; Fig. 2: poly I. poly C; Fig. 3: poly G. poly C ;
Fig. 4: poly dG. poly dC.
Triple-stranded synthetic polynucleotide complexes: Fig. 5-6.
Fig. 5: poly A. 2poly U; Fig. 6: poly A. 2 poly I.
Naturally occurring bi-helical rotavirus RNA: Fig. 7.
Scale (Fig. 7) for all figures represents 100 nm.

The Tamm & Horsfall human urinary glycoprotein was prepared in Dr Hartmann's laboratory, Faculté de Médecine des Cordeliers, Paris. A detailed analysis of the chemical composition and ultrastructure of this glycoprotein is described elsewhere (23).

2. Immunization of rabbits was carried out as described previously (20). For isolation of specific antibody, the same bleedings were used throughout these experiments, i.e. after 7 injections of immunogen for the anti-poly I. poly C serum n° 1223, and 6 injections of immunogen for the anti-poly A. poly U serum n° 1172.

3. Specific antibody was isolated as described by Johnston and Stollar (24) with a few modifications : 1 to 3 ml of each antiserum were incubated at their equivalence point, previously determined by quantitative precipitin tests (25), with poly I. poly C at 37°C for 2 h. and at 4°C for 12 h. The resulting precipitates were washed three times with TBS (NaCl 0.15 M, Tris 0.01 M pH 7.4) and suspended in 1 to 2 ml of distilled water. After addition of pancreatic ribonuclease (10 µg/ml) and T1 ribonuclease (5 units/ml) the precipitates were incubated at 56°C for 35 minutes. NaCl was added to 0.15 M, the mixture was spun down to remove insoluble material and the soluble antibodies were precipitated by adding the same volume of saturated ammonium sulfate and leaving the tubes for 1h. at 0°C. The precipitates were washed three times with 50 % saturated ammonium sulfate, and dissolved in TBS.

As controls we used purified immunoglobulins from mouse myelomas RPC 5 (IgG, lot AL 123) and TEPC 183 (IgM, lot AG 089) obtained from Bionetics Laboratory.

4. Sucrose gradient centrifugation. 150 µl to 200 µl of purified antibody (100 to 150 µg) were applied to 5 ml of a 12-36 % sucrose gradient. The tubes were centrifuged at 40,000 rpm for 18 h. at 4°C in a SW65 Ti rotor using a Beckman Model L5-65 ultracentrifuge. The fractions were collected by puncturing the bottom of the tubes. Fifty µl fractions were tested using a modification of the Farr technique (26, 27). Alternatively, the fractions were also tested with the double antibody technique, using a multivalent horse anti-rabbit immunoglobulins serum, with the same results.

5. Antigen-antibody interaction. The antigens (about 1 µg/ml) and the antibodies (about 20 µg/ml) were dissolved in TBS. Ten µl of various dilutions of antibodies were mixed with 10 µl of different antigens (1 µg/ml) and, as a rule, allowed to interact for 15 seconds at room temperature before adsorption on the grids. However, longer times of interaction were

also tried, up to 2 hours at 0°C. The results reported here summarize the experiments done with 5 different preparations of both antibodies.

6. Electron microscopy. Five µl of the reaction mixtures of antigens and antibodies as defined above were deposited for 1 minute on carbon-coated 600 mesh copper grids previously activated (within 1 hour at the longest) by a glow discharge in the presence of pentylamine (28). The grids were then washed with 4 drops of aqueous 2 % uranyl acetate (0,22 µm Millipore filtered) and dried by direct deposition of the sample-coated side onto filter paper. No chemical fixation was used.

Tungsten-tantale bidirectional shadowing was done at room temperature in a clean high vacuum ($10^{-8}$ Torr) evaporator similar to the Cryofract from Reichert (29). The shadowing angle was about 6° and the thickness of deposits estimated to 5-20 Å, with a Kronos quartz thickness monitor. Pictures were taken with a Philips EM 300 or 400 in dark field (30) (80 KV, objective aperture 40 µm, tilting angle about 14. $10^{-3}$ rad).

7. Determination of the reactivity of different antigens. In order to assess the reactivity of the various antigens, the proportion of IgM bound to the polymers was estimated. This numeration was done on pictures taken after a 15 seconds interaction time between antibodies and all of the antigens (see Materials and Methods, 5).

Free and bound IgM were counted in photograph areas corresponding to about 16 $\mu^2$ on the specimen grid ; the valency of the attachment was not taken into account, nor were IgG molecules considered.

The percentage of bound IgM (Table I) were obtained by pooling the data from several photographs and experiments. A $\chi^2$ test was used to control that the percentages of bound IgM were actually homogeneous in different photographs and experiments.

The non specific binding of either mouse myeloma IgM to poly A. poly U, or of anti-double stranded polynucleotide complexes antibodies to unrelated antigens such as DNAs or Tamm-Horsfall glycoprotein, was evaluated in the same way (Table I). This allowed the calculation of the mean value of all the non specific attachments which was found to be $3.8 \pm 0.9$.

## RESULTS

The specific antibodies used in this study were isolated from two immune sera directed against double-stranded polynucleotide complexes : poly A. poly U (n° 1172) and poly I. poly C (n° 1223).

Both direct observation in the electron microscope and sucrose gradient

analysis showed the existence of two distinct populations in the antibody preparations (fig. 1). The larger molecules, somewhat variable in shape, assumed very often a stellar-like appearance (arrow), with an average diameter of 320-400 Å. The smaller molecules exhibited either a triangular or a globular shape, and measured approximately 160 Å in their larger dimension. These measurements are in accord with those previously published for IgM and IgG (31). It should be emphasized that a peculiarity of these immunological systems is that in spite of the fact that the sera come from hyperimmunized animals (see Materials and Methods), the antibodies are constituted in majority by IgM molecules.

Polynucleotides being macromolecules bearing repeated antigenic determinants, the interaction between antibodies and antigens led very often to the formation of aggregates, as shown in plate II, fig. 1, even though the reaction between antigens and antibodies was allowed to proceed for 15

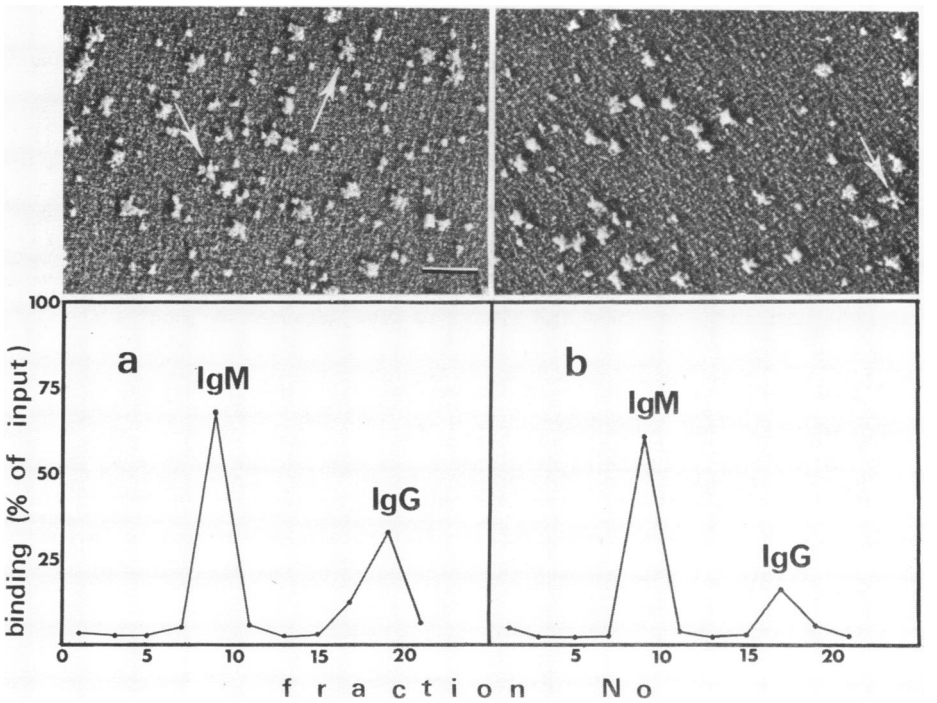



FIGURE 1 : Sucrose gradient analysis of rabbit purified antibodies.
a : n° 1172 anti-poly A. poly U ; b: n° 1223 anti-poly I. poly C.
The fractions were tested by radioimmunoassay.
Above are electron micrographs of the corresponding purified antibodies. Scale = 100 nm.

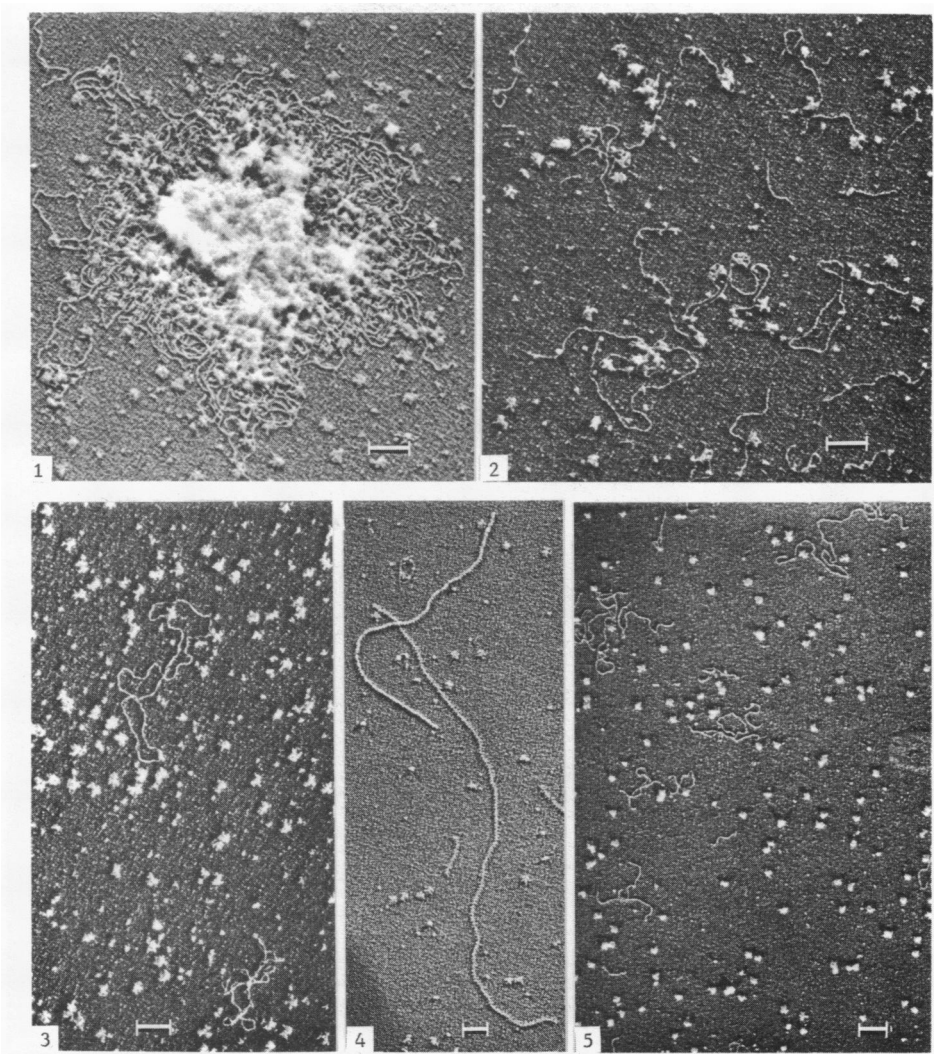



PLATE II.

Fig.1 : Reaction of anti-poly A. poly U with poly I. poly C : aggregation.

Fig.2 : Reaction of anti-poly I. poly C with rotavirus double-stranded RNA. A higher AG/AB ratio than in Fig. 1 allows a better visualization of the interactions between antibodies and antigen.

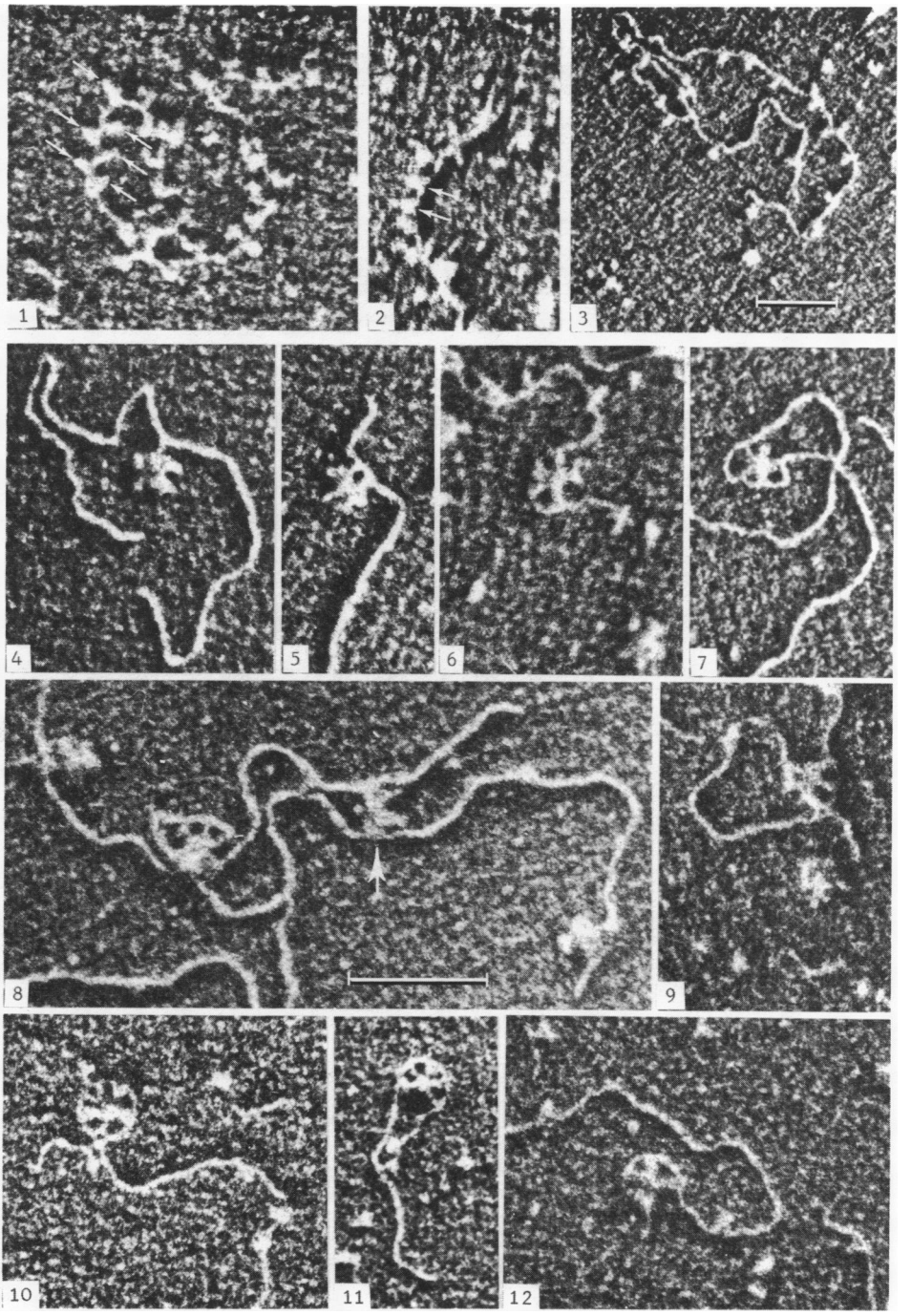
Fig. 3-5 : control experiments

Fig. 3 : anti-poly I. poly C plus $SV_{40}$ circular DNA forms I. and II.

Fig. 4 : anti-poly I. poly C + Tamm-Horsfall glycoprotein.

Fig. 5 : TEPC 183 mouse myeloma IgM + poly A. poly U.

All scales represent 100 nm.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seconds only. The interaction between single antibody molecules and the antigen is more clearly visible in relative antigen excess (plate II, fig. 2).

Varying the proportions of antibody and antigen allows one to observe a progression from monogamous multivalency (binding of a single multifunctional antigen molecule with several combining sites of the same antibody molecule, simultaneously (31) to aggregation where separate antigen molecules are cross-linked by several antibody molecules (31), presumably.

The multisite attachment to a single antigen molecule was observed for both classes of antibody. Fig. 1 (plate III) shows a series of IgG bound to their homologous antigen, poly A. poly U. On the following photograph (fig. 2, plate III) one can see even more clearly anti-poly A. poly U IgG lined up like swallows on a telephone wire along a single macromolecule of a natural double stranded RNA, rotavirus RNA. Wider intervals can be observed between the antibody molecules (fig. 3, plate III). The fact that the two Fab fragments can sometimes be distinguished (fig. 2 : arrows) allowed us to measure first the length of RNA covered by an IgG molecule, which is

PLATE III

Examples of different types of reactions between IgG (Fig. 1-3) or IgM (Fig. 4-12) and various polynucleotides.

Fig. 1 : Reaction of anti-poly A. poly U IgG with poly A. poly U : Cooperative binding with antibody molecules lying alternatively on both sides of the antigen (⟶).

Fig. 2 : Reaction of anti-poly A. poly U IgG with rotavirus double-stranded RNA. The two Fab fragments are sometimes distinct (⟶).

Fig. 3 : Reaction of anti-poly A. poly U IgG with poly I. poly C.

Fig. 4 and 5 : Reaction of anti-poly A. poly U IgM with rotavirus double-stranded RNA.

Fig. 6 : Reaction of anti-poly I. poly C IgM with rotavirus double-stranded RNA.

Fig. 7 : Reaction of anti-poly I. poly C IgM with poly A. 2 poly I.

Fig. 8 : Reaction of anti-poly I. poly C IgM with poly A. 2 poly U : a rare example of cross-link (⟶)

Fig. 9 : Reaction of anti-poly I. poly C IgM with antigenic determinants located far apart on the same rotavirus RNA molecule.

Fig. 10-12 : Reactions of anti-poly I. poly C IgM with double-stranded rotavirus RNA : examples of loops. The Fab fragments can sometimes be resolved.

Fig. 1-3, 5-7 and 9-12 illustrate monogamous multivalent binding.

All figures, except Fig. 3, are at the same magnification (see scale in Fig. 8 ; scales = 100 nm).

110-160 Å, second the distance between two antigenic determinants : 60-90 Å for neighbouring molecules, 70-110 Å for the two combining sites of a single IgG. If the distance between two base pairs in the "A"-RNA conformation is taken to be 2.82 Å (32), one antibody molecule of the IgG class covers 39-57 base pairs, and the distance between two adjacent available epitopes is 21-39 base pairs. However, one must take into account that these distances are overestimated for two reasons, the metal deposits on the preparations and the steric hindrance due to the fact that the bulk of an IgG molecule renders inaccessible all the antigenic determinants lying underneath. A more precise estimation of the distance separating two epitopes would undoubtedly be reached using Fab fragments, although in all probability it would also be overestimated since an X-ray diffraction study showed the dimensions of an Fab' fragment to be 80 x 50 x 40 Å (33).

On plate III (fig. 4-12) are illustrated different types of binding of IgM molecules to various antigens. One can observe a progression from monovalent or bivalent (although one can note a few exceptions, the two Fab fragments are hardly distinct, in general) to multivalent binding.

It should be noted that when multivalent binding occurs, it is usually with the same macromolecule of antigen (i.e. monogamous). In very few instances were we able to detect cross-linking of two separate antigen molecules (fig. 8, arrow). Alternately, we could observe several examples of a single IgM molecule binding several antigenic determinants located very far apart on the same macromolecule of antigen (fig. 9) thereby inducing the formation of a loose loop in the polymer.

It should be pointed out that among the figures encountered most often were, indeed, loops formed by the coiling of the antigen around a single IgM molecule (plate III, fig. 10-12 ; text figure 2). In contrast to the slack loops mentioned previously, which could almost be considered as cross-links, the repeated antigenic determinants reacting with the antibody combining sites, are, in this instance, much closer to each other ; their distance, measured on 12 different figures, varied from 120 to 280 Å, that is from 42.5 to 99 base pairs.

The pictures change if the antibodies are reacted with triple-stranded polynucleotide complexes. Then the antigen is not tightly coiled around the antibody molecule (plate III, fig. 7), in opposition to very regular loops where the wound antigen makes an almost perfect circle (text figure 2 ; plate III, fig. 10). This is probably due to the fact that poly A. 2 poly U and poly A. 2 poly I are more rigid than bihelical polynucleotides whether

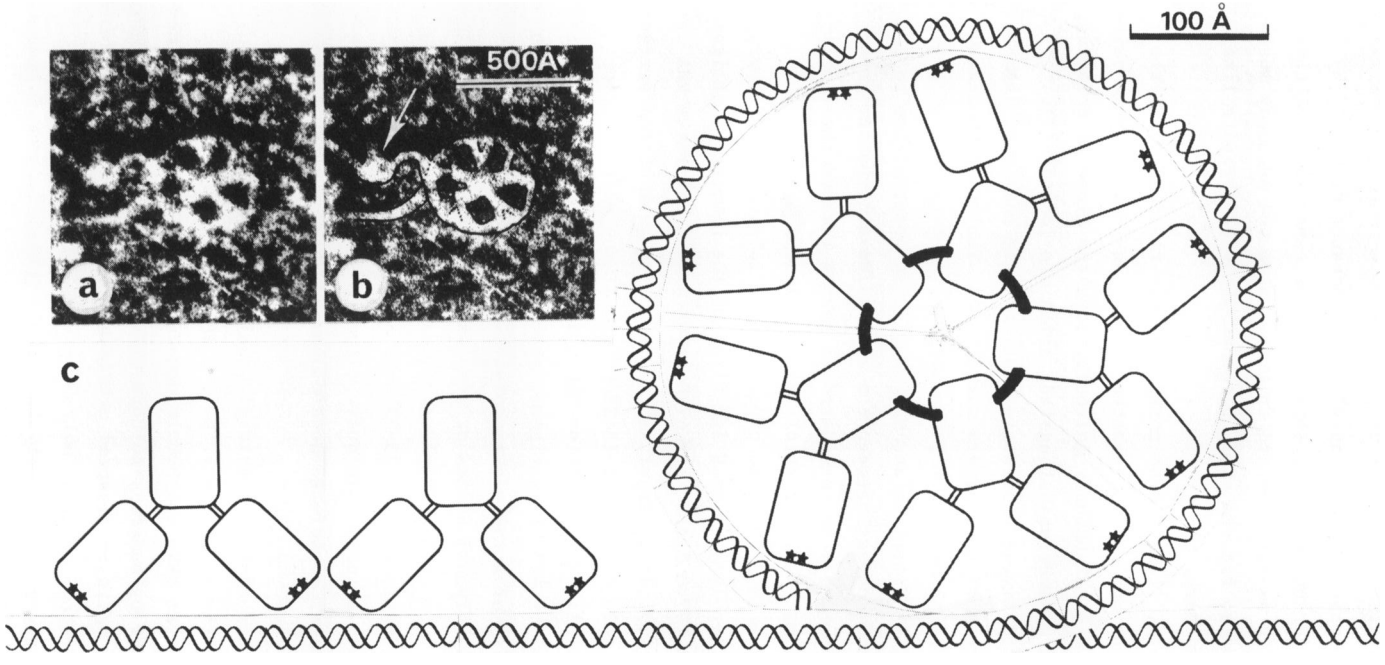


FIGURE 2 : a) High magnification of a loop formed by a naturally occurring double-stranded RNA molecule wound around an anti-poly I. poly C IgM molecule.

b) The contours have been lined for clarity. An IgG molecule is indicated by an arrow.

c) Schematic drawing at scale of a RNA molecule (about 28 Å per turn = "A" conformation) and IgM and IgG molecules. IgG molecules have been drawn according to the dimensions found in the literature (33). The two stars indicate the approximate position of the binding site at the extremity of the Fab fragments. The IgM molecule has been drawn as being constituted by 5 monomers equivalent to IgG.

synthetic or natural.

We were unable to observe the "staplelike" appearance described by Feinstein et al. (6) for bovine IgM ; this was to be expected since they used bacterial flagella as antigens and in this system the antibody must adapt its conformation to fit the antigen rather than the opposite like in the systems studied here.

However, as a whole, it should be noted that the flexibility of the hinge region allows the antibodies - whether IgG or IgM - to take on quite variable appearances : "The antibody molecule, like the cloud of Polonius, was sometimes globular, sometimes elongated, sometimes looped" (1).

In order to quantitatively assess the reaction, the number of IgM molecules fixed on the various antigens was counted. Table I summarizes the results of experiments done with five different preparations of antibodies. As emphasized in Materials and Methods, these observations were made after a 15 seconds contact between the antibodies and the antigens, when the reactants can hardly be considered as having reached the equilibrium, notwithstanding the rapidity of immunological reactions (34). Indeed, if the reaction is allowed to proceed for a longer time (2 hours), the percentage of anti-poly A. poly U IgM fixed to the homologous antigen increases from 57.6 to 77.0 % and from 47.0 to 62.4 % if rotavirus RNA is considered (Table II). In contrast, there is no increase of the percentage of anti-poly I. poly C attached to SV40 DNA.

Although the percentages of fixation shown in Table I were not obtained in optimal conditions for all the antigens, they can be taken as a rough estimate of the avidity of anti-poly A. poly U and anti-poly I. poly C for different natural polynucleotides or complexes of synthetic polynucleotides.

As was noted previously (15-19, 35-37) the cross-reactivity between poly A. poly U and poly I. poly C is quite extensive, although the anti-poly I. poly C recognizes better the heterologous complex than does the anti-poly A. poly U.

It should be emphasized that a natural double-stranded RNA, rotavirus RNA, is recognized by the anti-poly I. poly C and anti-poly A. poly U almost to the same extent than the homologous antigens. This confirms previous observations made by numerous authors (17-19).

In contrast, and again as observed previously (17, 19) another double-stranded polyribonucleotide complex, poly G. poly C, reacts poorly with either anti-poly A. poly U or anti-poly I. poly C. The difference in immuno-

TABLE I.

All antigens were allowed to react for 15 seconds at room temperature with anti-poly A. poly U (n° 1172) or anti-poly I. poly C (n° 1223). The Table represents the results of five separated experiments. For calculation of the percentage of bound IgM, see Materials and Methods.

| Antibodies anti-poly A. poly U | | | Antibodies anti-poly I. poly C | |
|---|---|---|---|---|
| Antigens | Total nb of IgM counted | Mean percentage of bound IgM $\pm \frac{2s}{\sqrt{n}}$ | Mean percentage of bound IgM $\pm \frac{2s}{\sqrt{n}}$ | Total nb of IgM counted |
| Poly A. poly U | 464 | 57.6 ± 3.9 | 48.1 ± 7.0 | 581 |
| Poly I. poly C | 507 | 36.9 ± 6.8 | 43.7 ± 4.9 | 668 |
| Rotavirus ds RNA | 1519 | 47.0 ± 3.3 | 47.5 ± 3.1 | 1393 |
| Poly G. poly C | 789 | 10.5 ± 1.4 | 18.3 ± 3.3 | 517 |
| Poly dG. poly dC | 2516 | 5.8 ± 1.4 | 5.9 ± 1.4 | 2072 |
| Poly A. 2poly U | 685 | 39.4 ± 3.4 | 14.4 ± 3.9 | 422 |
| Poly A. 2poly I | 1661 | 5.9 ± 1.9 | 11.7 ± 3.1 | 1279 |
| PM2 DNA | 2152 | 4.7 ± 3.0 | 5.2 ± 1.2 | 734 |
| SV 40 DNA | 546 | 1.1 ± 0.7 | 4.2 ± 2.7 | 244 |
| ϕX 174 RF DNA | 977 | 2.1 ± 1.3 | 4.5 ± 3.1 | 1315 |
| Tamm & Horsfall glycoprotein | 333 | 4.7 ± 1.2 | 3.8 ± 1.5 | 887 |
| Poly A. poly U + Mouse Myeloma (TEPC 183) IgM : | | | 4.0 ± 1.3 | 1693 |

TABLE II

| Antibodies + Antigens | Percentage of bound IgM |
|---|---|
| Anti-poly A. poly U + Poly A. poly U | 77.1 |
| " + Rotavirus ds RNA | 62.4 |
| Anti-poly I. poly C + Poly I. poly C | 69.1 |
| " + Rotavirus ds RNA | 69.1 |
| " + SV40 DNA | 4.3 |

Percentage of bound IgM after long incubation times (1 to 2 hours) between antigens and antibodies.

chemical reactivity of poly G. poly C has been attributed to its different sterical conformation (38).

In contrast to what we reported earlier, using a method of inhibition of quantitative precipitin reaction (17) the antibodies used in this study do no react with the polydeoxyribonucleotide complex poly dG. poly dC.

The extent of cross-reactivity of the triple-stranded complexes poly A. 2poly U and poly A. 2poly I varies according to the antibody and the antigen.

It should be pointed out that the different modes of fixation of the antibodies mentioned above do not occur equally with all of the antigens. There seems to be a correlation between high fixation percentages and monogamous multivalent attachments and/or aggregates, while low fixation percentages usually correspond to mono- or bi-valent attachments. In practice, all the loops were observed with poly A. poly U, poly I. poly C and the naturally occurring double-stranded RNA. In all probability, this reflects different antigenic determinant densities along the various macromolecules.

In order to appreciate the non specific binding of IgM molecules to the different polynucleotides, we used two different types of controls : non specific IgM from mouse myeloma on the one hand, and macromolecular antigens as widely different as DNA and Tamm-Horsfall glycoprotein on the other (Table I ; plate II, fig. 3-5). In general we observed only mono- or bi-valent binding in these preparations. All of these controls allowed an assessment of the non specific background ($3.8 \pm 0.9$ % of bound IgM) above which value the reactions were considered as positive.

## DISCUSSION

The aim of this work was to analyze at the molecular level the immunological recognition of conformational determinants in polynucleotides. To do this we used specific antibodies directed against double-stranded polynucleotide complexes, the specificity of which has been well documented (15-19).

We have shown that owing to the multivalency of both antigens and antibodies, recognition leads to aggregation, unless defined experimental conditions are chosen : short interaction time between antibodies and antigens, reactions at 20°C, high antigen/antibody ratios. When these conditions are elected, several observations can be made : in the first place the majority of the reactions with homologous or very close cross-reacting antigens are due to monogamous multivalent binding of either IgG or IgM antibodies (31). Cross-linking is very rarely seen. Both these observations are in accord with those made by Aarden et al. (39) who analyzed the composition of DNA/anti-DNA complexes by equilibrium density centrifugation in CsCl and by zonal centrifugation in sucrose gradients.

In view of the very numerous pictures of monogamous multivalent binding we observed with IgM molecules, we think that the almost complete absence of cross-links is due to the fact that the 10 antibody-combining sites of the IgM molecule are readily attached to the repeated antigenic determinants of the multifunctional polynucleotide ; this multiple site attachment which provides an energetic advantage (40) is made possible here by the flexibility of the antigen and by the closeness of the antigenic determinants. The predominance of multisite attachment to a single antigen particle over cross-linking when both situations are sterically possible has been predicted by Crothers and Metzger (31). When the epitopes are situated further apart on the helix, cross-links may form, and indeed, it was only with polynucleotide complexes cross-reacting poorly with anti-poly I. poly C that we obtained the very few cross-links we were able to observe.

That the flexibility of the antigens - and therefore their ability to react with most or all of the antibody combining sites - plays an important role in the kind of attachment obtained is further demonstrated by the fact that cross-links were observed very frequently when similar studies were done using rigid antigens (6, 11).

We also noted the tendency of IgG antibodies to bind cooperatively when they reacted with homologous or close cross-reacting antigens.

We found (see Results) that in average one IgG molecule covers 48 base pairs, with a distance between two antibody molecules of 26.5 base pairs. Again this observation is in accord with the findings of Aarden et al. (39) who showed that 100 to 200 antibody molecules could bind to one PM2 DNA molecule (about $10^4$ base pairs).

When measured with IgM antibody, the shortest distance separating two antigenic determinants is about 42.5 base pairs. The distance between two epitopes, estimated with this technique, seems therefore to be about 4 helix turns (fig. 2). This distance is obviously overestimated, especially if one takes into account the results of Guigues and Leng (37) who, using precipitin curves as well as fluorescence with anti-poly I. poly C antibody, estimated that 3-5 base pairs are covered by one Fab binding site. This value was in agreement with the findings of Johnston and Stollar (24) who used a precipitin assay and studied the reaction of anti-poly A. poly U with the homologous complex or poly I. poly C. They estimated that there were, at most, 8-12 base pairs per IgG molecule, and 4 to 6 base pairs per combining site if all Fab sites were filled. Although the antibody combining sites vary in dimensions and shapes according to their specificity (33, 41), some of them would undoubtedly correspond to an antigenic determinant of 4 to 6 base pairs. However, the antibody combining site appears like a groove or pocket surrounded by the Fab molecule whose overall dimensions are 80 x 50 x 40 Å (33). It seems therefore that most of the epitopes along the helix will not be available for reaction, because of the steric hindrance due to the presence of whole antibody molecules (fig. 2). In addition steric constraints are imposed on the antibody molecule when multiple site attachment occurs (31).

The specificity of anti-poly A. poly U and anti-poly I. poly C antibodies assessed by electron microscopy is in accord with results obtained with different techniques (15-19, 35-37). Numerous authors have observed cross-reactions between anti-poly A. poly U antibodies and poly I. poly C or *vice-versa* ; both antibodies were also found to extensively react with naturally occurring double-stranded viral RNAs, while there was an agreement on the fact that poly G. poly C, although a double-stranded polyribonucleotide complex, cross-reacted poorly with either antibody (17, 19).

We found in this paper that in contrast to our previous report (17), poly dG. poly dC reacts only slightly better than the controls with anti-poly A. poly U and - poly I. poly C. This discrepancy may be attributed to the polymorphism of this polydeoxyribonucleotide complex. Indeed,

it has been observed that various samples of poly dG. poly dC can exhibit different physicochemical properties. This polymorphism has been attributed not only to the ability of each of the constituent homopolymers to form self-associated helices, but also to their capacity to form two hetero-complexes (42-44). Any given preparation of poly dG. poly dC could then adopt a variety of molecular forms, and the immunological reactivity of poly dG. poly dC would therefore vary accordingly.

In conclusion, using purified antibodies directed against double-stranded polynucleotide complexes of known conformations, we were able to visualize at the molecular level the recognition of synthetic or naturally occurring polynucleotides. The use of these antibodies, as well as other antibodies to various polynucleotide complexes of different sterical geometries should make possible the recognition of local conformations in natural nucleic acids whose configuration is not known as yet.

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