Specificity of Antibodies to Poly(I)†

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ABSTRACT: Sera of rabbits immunized with poly(I)·poly(C) adsorbed on methylated serum albumin precipitate poly(I)·poly(C) and poly(I). Some properties of the antibodies to poly(I)·poly(C) have been already reported (Guigues, M., and Leng, M. (1976), *Eur. J. Biochem. 69*, 615–624). In this work, we have studied the antibodies which react with poly(I). The antibodies have been purified by affinity chromatography on a Sepharose–oligo(I) column, and the IgG fraction has been isolated by gel filtration. By gel diffusion, in 0.15 M NaCl, a very positive reaction has been found with poly(I) and poly(dI), a faint reaction with poly(U) and no reaction with poly(A), poly(C), poly(I)·poly(C), and poly(A)·2poly(I). The precipitation of poly(I) by the antibodies is inhibited by oligo(I) of degree of polymerization larger than 3. The association constants for the binding of the anti-poly(I) Fab fragments to oligo(I) and polynucleotides have been deduced from fluorescence experiments. With oligo(I), the association constants depend upon temperature and ionic strength. The thermodynamic parameters for Fab fragments–$(Ip)_5I$ complexes are $\Delta H° = -12$ kcal/mol and $\Delta S° = -11$ cal deg^{-1} mol^{-1} at 30 °C. The mean number of nucleotide residues of poly(I) covered by one Fab fragment is 6. The comparison of the association constants of Fab fragments with different polynucleotides shows that the bases, the sugars, and the phosphate groups are involved in the binding to the amino acid residues of the Fab fragment binding site. The antibodies interact with multistranded polynucleotides containing poly(I). In the presence of Fab fragments, the thermal stability of poly(I)·poly(C), poly(A)·2poly(I), poly(I) (in 1 M NaCl) is decreased. These results lead to the conclusion that the antibodies to poly(I) recognize single-stranded poly(I).

Numerous works have been performed to understand the specific recognition of nucleic acids by proteins (Von Hippel and McGhee, 1972; Sobell, 1976). The study of the complexes between nucleic acids and antibodies to nucleic acids can be useful to solve this problem. It is well-established that double-stranded synthetic polyribonucleotides adsorbed on methylated bovine serum albumin are capable of eliciting an immune response in rabbits. In the recognition of the polynucleotides by the antibodies, the conformation of the double-stranded polynucleotides is a fundamental parameter (Plescia and Braun, 1968; Lacour et al., 1973; Stollar, 1973, 1975; Guigues and Leng, 1976a).

It has been also found that sera of rabbits immunized with poly(I)·poly(C)-methylated bovine serum albumin complex precipitate not only poly(I)·poly(C) but also poly(I) (Nahon-Merlin et al., 1973; Negro-Ponzi et al., 1971; Field et al., 1972). In a recent work we have shown that there were three families of antibodies in these antisera, reacting specifically with poly(I)·poly(C), poly(I), and poly(C), respectively (Guigues and Leng, 1976b). It seemed to us of interest to study in more detail the specificity of these antibodies to poly(I).

The elucidation of the specificity of antibodies to poly(I) presents some difficulties because the conformation of poly(I) in solution depends upon the experimental conditions (general reviews, Michelson et al., 1967; Felsenfeld and Miles, 1967; and more recently, Maurizot and Boubault, 1973; Thiele and Guschlbauer, 1973; Cech and Tinoco, 1976). At neutral pH and at low monovalent salt concentration poly(I) behaves as a single-stranded helix. At high ionic strength, a multistranded structure, probably a four-stranded structure, is formed by poly(I) (it has been concluded from the fiber diffraction study of poly(I) that the ordered form is a four-stranded helix (Arnott et al., 1974; Zimmerman et al., 1975). Between these two ionic strengths, mixtures of the two forms are present which evolve differently with experimental conditions.

In the present communication, we describe a method of purification of antibodies to poly(I). The study of the complexes antibodies–oligonucleotides and polynucleotides led us to the conclusion that these antibodies to poly(I) recognize single-stranded poly(I).

Experimental Procedure

Polynucleotides were purchased from Miles, P-L Biochemicals, and Choay Chimie. They were purified as already described (Deubel and Leng, 1974). Poly(m^7I,I) containing 30 and 40% m^7IMP were prepared according to the method of Pochon et al. (1968) and characterized as already described (Guigues and Leng, 1976b). Poly(m^1,m^7I) was prepared according to the method of Michelson and Pochon (1966).

Oligo(I) were prepared by alkaline hydrolysis of poly(I) and separated on a DEAE[1]-cellulose column (Simpkins and Richards, 1967). In order to get $(Ip)_nI$, the oligo(I) were treated with alkaline phosphatase before the application of the DEAE-cellulose column. The purity of the oligonucleotides (degree of polymerization, DP, from 1 to 8) was checked by paper chromatography (system solvent ethanol–1 M ammonium acetate, v/v).

The fraction $(Ip)_{10}I$ was a mixture of oligonucleotides of DP larger than 8. The DP (11) was deduced from the ratio (inosine/IMP) after alkaline hydrolysis.

$(Ip)_5Up$ was prepared by treatment of 7 mg of a statistic copolymer poly(I,U) containing 20% uracil residues with pancreatic RNase (0.5 mg) during 1 h at 37 °C. The oligonucleotides were separated by paper chromatography (same

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[1] Abbreviations used: DEAE, diethylaminoethyl; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; DP, degree of polymerization.

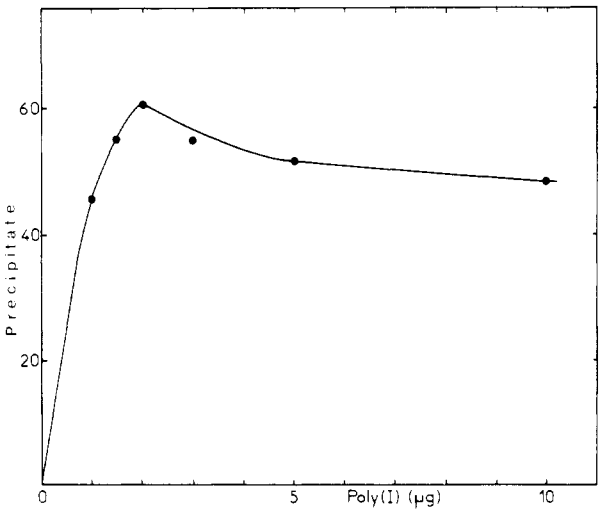

FIGURE 1: Precipitation of poly(I) by antibodies to poly(I). Amount of precipitated antibodies (μg) as a function of the amount of poly(I). Solvent: 50 mM NaCl–5 mM Tris-HCl–0.1 mM EDTA (pH 7.5). Amount of antibodies (65 μg), volume 275 μL, temperature 4 °C.

system as before). It has been verified by analysis of the ultraviolet spectrum that we were dealing with $(Ip)_5Up$.

The sedimentation constant of poly(I) was 5.3 S in 0.15 M NaCl–5 mM Tris-HCl (pH 7.5) at 20 °C. The concentration of poly(I) was determined using an extinction coefficient of 10 200 M^{-1} cm^{-1} at 249 nm in 15 mM Na^+ at 25 °C. The solutions of poly(I) were kept in 15 mM NaCl, pH 7.5 at 4 °C. Before use, they were heated at 60 °C for 3 min and slowly cooled. The extinction coefficients of oligo(I) were those given by Tazawa et al. (1972).

The conjugate between periodate oxidized IMP and bovine serum albumin was prepared according to the method of Erlanger and Beiser (1964).

The preparation of affinity columns has been already described (Guigues and Leng, 1976b). About 0.2 mg of oligo(I) was linked per mL of wet Sepharose and about 0.5 mg of native DNA per mL of wet Sepharose.

Fab fragments were prepared by treatment of the IgG by papain. Fab fragments were separated from Fc fragments on the Sepharose–oligo(I) column. They were further purified on a Sephadex G-200 column.

Association Constants. The solution of anti-poly(I) Fab fragments is fluorescent. In the presence of poly(I) (or oligo(I)), the fluorescence of the Fab fragments is quenched and tends toward a limit value for a large amount of ligand. Assuming that fluorescence quenching is the same for all bound Fab fragments, one can calculate the amounts of free and bound Fab fragments in the presence of ligand. Two cases have to be considered.

Interaction Fab Fragments–Hapten. The determination of the association constants has been already described (Day, 1972; Drocourt and Leng, 1975; Bauvois and Leng, 1976). First, the maximal extent of quenching Q_M was determined: $Q_M = (I_0 - I_m)/I_0$ where I_0 and I_m are the values of fluorescence intensity of Fab fragments in absence and at saturation of hapten. Then, assuming that the Fab fragments are monovalent, one can calculate r and r/c (r is the molar ratio of bound hapten over total antibodies and c the concentration of free hapten). The average value of the association constant is equal to $2r/c$ at $r = 1/2$ or has been obtained from the variation of log $[r/(1-r)]$ as a function of log c, assuming a Sips distribution (Day, 1972). The index of heterogeneity α can also be deduced. The association constants for the binding of Fab fragments to oligo(I) (up to $(Ip)_6I$) were determined by this method. The concentration of oligo(I) was expressed in moles of oligonucleotide.

Interaction Fab Fragments–Polynucleotide. The association constants have been determined at half-saturation of Fab fragments with the polynucleotide (Pesce et al., 1971; Molineux et al., 1975).

First the stoichiometry of the binding was obtained at high concentration of Fab fragments. Under these conditions, it was assumed that the amount of free polynucleotide is negligible as compared with the amount of bound polynucleotide.

At one-half saturation, the association constant K is equal to $1/[\text{polynucleotide}]_{free} = 1/([\text{polynucleotide}]_{total} - n/2[\text{Fab}])$. n is the mean number of nucleotide residues covered by one Fab fragment binding site. The concentration of poly(I) was expressed in moles of nucleotide.

Immunization. Three random bred rabbits were immunized with poly(I)·poly(C) adsorbed on methylated bovine serum albumin according to the procedure of Nahon-Merlin et al. (1973). The sera were collected a week after the intravenous booster.

Methods. Gel diffusion in 0.15 M NaCl–5 mM Tris-HCl, pH 7.5 at 4 °C, and precipitin tests have been carried out as already described (Drocourt and Leng, 1975). Absorption spectra were recorded on a Cary 15 spectrophotometer and fluorescence spectra with a Farrand MKI spectrofluorimeter (Guigues and Leng, 1976a).

Results

Purification. The immunoserum (1.5 mL/mL of wet Sepharose-oligo(I)) was applied on a Sepharose–oligo(I) column equilibrated with 0.15 M NaCl–5 mM Tris-HCl (pH 7.5). The column was washed with this buffer until the absorbance of the effluent at 280 nm was less than 0.04, then with 1 M NaCl–5 mM Tris-HCl (pH 7.5) until again the absorbance was less than 0.04, and then with 1 mM acetic acid (twice the volume of the column). The antibodies were eluted with 2 M acetic acid (all the experiments were performed at 4 °C). After dialysis against 0.1 M Tris-HCl (pH 8) and then against 0.15 M NaCl–5 mM Tris-HCl (pH 7.5), the antibodies were applied on a Sepharose–DNA column (8 mg of protein/mL of wet Sepharose) equilibrated with the same buffer. The proteins not retained on this column (about 80%) were then applied on a Sephadex G-200 column. Two peaks were obtained. Only the proteins of the second peak were kept (about 90% of the total proteins). The yield depends upon the antiserum. It is of the order of 0.3 mg/mL serum.

The purified proteins have been analyzed by several techniques (ultracentrifugation, immunoelectrophoresis, immunodiffusion, ultraviolet absorption). All the results agree with those expected for immunoglobulins G.

It has been verified by gel diffusion that the unbound proteins to the Sepharose–oligo(I) column (in 0.15 M NaCl) do not react with poly(I). Moreover no precipitation between DNA and the proteins eluted from the Sepharose–oligo(I) column has been found.

Specificity. The specificity of the purified IgG has been studied by gel diffusion and quantitative precipitation.

Gel Diffusion. There was a very positive reaction between the antibodies and poly(I) or poly(dI) and a very faint reaction with poly(U). There was no reaction with poly(A), poly(m^1, m^7I), poly(C), poly(I)·poly(C), and with the conjugate between bovine serum albumin and IMP.

Quantitative Precipitation. The results are shown in Figure 1. At the equivalence point, more than 90% of the antibodies are precipitated. The molar ratio phosphate/antibody in the

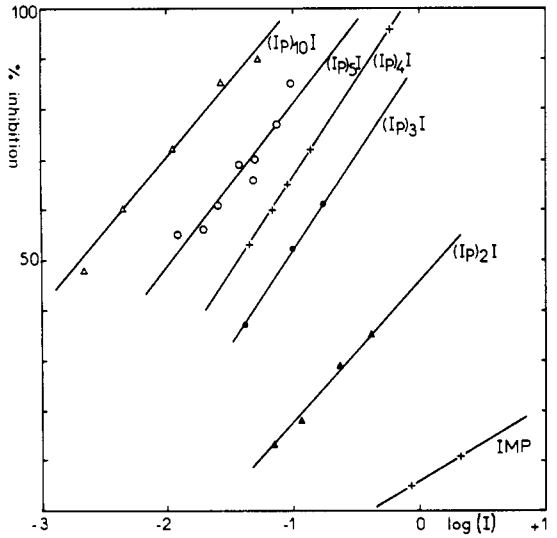

FIGURE 2: Inhibition by oligo(I) of the precipitation of poly(I) by the antibodies. Percent of inhibition as a function of the logarithm of the oligo(I) amount (in μmol). Same experimental conditions as in Figure 1. Amount of poly(I), 2 μg; amount of antibodies, 65 μg; volume, 275 μL.

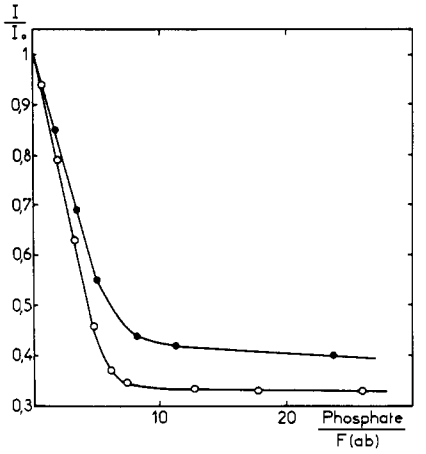

FIGURE 3: Variation of Fab fragments fluorescence intensity as a function of the molar ratio (phosphate/Fab fragments). I_0 and I are the fluorescence intensities of the Fab fragments in absence and in presence of poly(I). λ_{exc} = 295 nm; λ_{em} = 350 nm. Solvent: 0.15 M NaCl-5 mM Tris-HCl-0.1 mM EDTA (pH 7.5). (○) Fab fragments concentration, 1.1 × 10^{-6} M; temperature, 5 °C. (●) Fab fragments concentration, 3.9 × 10^{-7} M; temperature, 30 °C.

TABLE I: Association Constants for the Binding of Fab Fragments to Oligo(I).[a]

	$(Ip)_4I$	$(Ip)_5I$	$(Ip)_6I$
$K_0(M^{-1})$	1.5 × 10^6	1.5 × 10^7	6 × 10^7
α	0.9	0.9	0.8

[a] Solvent: 0.15 M NaCl-5 mM Tris-HCl-0.1 mM EDTA (pH 7.5). Temperature, 30 °C.

TABLE II: Association Constants for the Binding of Fab Fragments to Oligo(I).[a]

	K_0 (M^{-1})				
	$(Ip)_2I$	$(Ip)_3I$	$(Ip)_4I$	$(Ip)_5I$	$(Ip)_6I$
15 mM NaCl	$<10^5$	$\simeq 10^5$	8 × 10^6		
1 M NaCl			2 × 10^5	2 × 10^6	5 × 10^6

[a] Solvent: NaCl (concentrations given in the table) plus 5 mM Tris-HCl-0.1 mM EDTA, pH 7.5. Temperature, 30 °C.

precipitate, in the zone of antibody excess, is about equal to 12. At the equivalence point, the precipitation of poly(I) by the antibodies can be inhibited by oligo(I) as shown in Figure 2. The inhibition is very small with IMP and increases as the degree of polymerization becomes larger.

Association Constants. Association constants for the binding of Fab fragments to oligo(I) or to poly(I) have been deduced from fluorescence experiments. In solution the Fab fragments are fluorescent. The emission spectrum has an uncorrected maximum at 355 nm (λ_{exc} = 295 nm). In presence of poly(I) or oligo(I), the fluorescence intensity of the Fab fragments decreases and tends toward a limit for large amounts of ligand. The maximum of the spectrum is blue shifted (at saturation, λ_{max} = 344 nm). The same extent of maximal quenching Q_M = 0.66 has been found in the presence of poly(I) and oligo(I) of DP larger than 5. We assumed that Q_M is equal to 0.66 even for ligands having a low affinity for the Fab fragments. The interactions between Fab fragments and oligo(I) have been first studied.

Interactions Fab Fragments–Oligo(I). The association constants for the binding of Fab fragments to $(Ip)_4I$, $(Ip)_5I$, and $(Ip)_6I$ have been determined at 30 °C. The results are given in Table I. We also indicate the values of the index of heterogeneity α.

The same values of the association constants have been found between pH 6 and pH 8. On the other hand, the association constants depend upon temperature. In 1 M NaCl, pH 7.5, K_0 values for $(Ip)_5I$ are 1.5 × 10^7, 6.5 × 10^6, and 1.7 × 10^6 M^{-1} at 15, 20, and 30 °C, respectively. The variation of log K as a function of $1/T$ is linear. The following thermodynamic parameters have been calculated: $\Delta H° = -12$ kcal/mol and $\Delta S° = -11$ eu at 30 °C.

The terminal (2′ or 3′) phosphate group does not play a role in the binding. The association constants for the binding of Fab fragments to $(Ip)_n$ or $(Ip)_{n-1}I$ are the same ($4 < n < 6$), with experimental accuracy.

The substitution of the terminal hypoxanthine residue by a uracil residue decreases the affinity of the oligonucleotides to Fab fragments. The ratio $K_0[(Ip)_5Ip]/K_0[(Ip)_5Up]$ is about 25 and $K_0[(Ip)_4Ip]/K_0[(Ip)_5Up]$ about 2.5.

The conformation of poly(I) depends upon the salt concentration which is not the case of the oligo(I) (Maurizot, 1970). We have studied the interactions with oligo(I) of different DP at two salt concentrations. Because of the inaccuracy of the experiments for small or large values of the association constants, it has not been possible to obtain the association constants of all the oligo(I) at one salt concentration. The results are given in Table II. These results and those given in Table I clearly show that the association constants decrease as the ionic strength increases indicating a contribution of electrostatic interaction in complex formation (Record et al., 1976).

Interactions Fab Fragments–Polynucleotides. As described in Experimental Procedure, first the stoichiometry of the association poly(I)–Fab fragments has been determined. At 5 °C and at different Fab fragments concentrations (10^{-6} to 5 × 10^{-6} M), the same variations of I_0/I as a function of P/Fab were obtained (Figure 3). It can be deduced that about 6 nucleotide residues are covered by one Fab fragment. The association constants were calculated at one half saturation of Fab fragments. The experiments were performed at 30 °C and at different Fab fragments concentration (5 × 10^{-8} to 10^{-6} M).

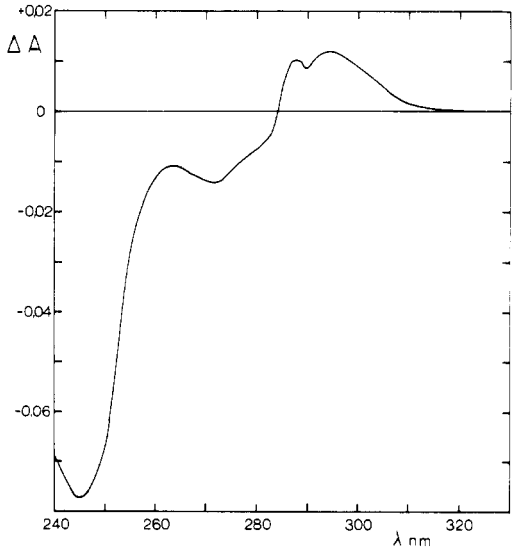

FIGURE 4: Ultraviolet difference spectrum between Fab fragments-oligo(I) mixture and Fab fragments plus oligo(I). Solvent as in Figure 3; temperature, 30 °C. Fab fragments concentration 10^{-5} M; $(Ip)_5I$ concentration, 10^{-5} M.

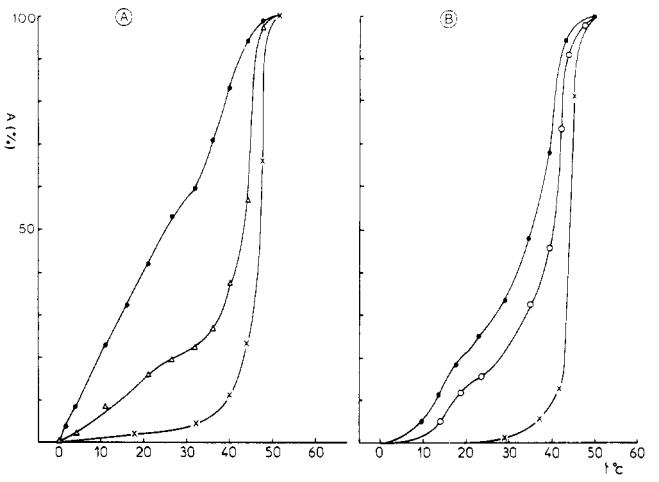

FIGURE 5: Thermal stability of poly(I)·poly(C) and poly(A)·2poly(I). Variation of the absorbance (%) at 260 nm as a function of temperature. (A) Poly(I)·poly(C) (×—×) concentration 9.4×10^{-5} M. Poly(I)·poly(C) plus Fab fragments, (●—●) P/Fab = 15, (Δ—Δ) P/Fab = 30. Solvent: 17 mM NaCl-5 mM Tris-HCl-0.1 mM EDTA, pH 7.5. (B) Poly(A)·2poly(I) (×—×) concentration 8×10^{-5} M. Poly(A)·2poly(I) plus Fab fragments; (●—●) P/Fab = 15; (○—○) P/Fab = 20. Solvent: 0.15 M NaCl-5 mM Tris-HCl-0.1 mM EDTA, pH 7.5.

TABLE III: Association Constants for the Binding of Fab Fragments and Polynucleotides.[a]

Polynucleotide	Conditions	K_0 (M^{-1})
poly(I)	30 °C, 0.15 M NaCl, pH 7.5	7×10^6
poly(dI)	30 °C, 0.15 M NaCl, pH 7.5	3×10^4
poly(I, m^7I) (40% m^7I)	30 °C, 0.15 M NaCl, pH 6	6×10^5
	30 °C, 0.15 M NaCl, pH 8	3×10^5
poly(I, m^7I) (30% m^7I)	30 °C, 0.15 M NaCl, pH 6	1×10^6
	30 °C, 0.15 M NaCl, pH 8	6×10^5
poly(I, U) (20% U)	30 °C, 0.15 M NaCl, pH 7.5	6×10^5
poly(m^1, m^7I)	5 °C, 15 mM NaCl, pH 7.5	10^3
poly(U)	5 °C, 15 mM NaCl, pH 7.5	3×10^3
poly(G)	5 °C, 15 mM NaCl, pH 7.5	10^3
poly(C)	5 °C, 15 mM NaCl, pH 7.5	$<10^3$
poly(A)	5 °C, 15 mM NaCl, pH 7.5	$<10^3$

[a] The concentrations of polynucleotides were expressed in mol of nucleotide residues. Solvent: NaCl (concentrations given in table) plus 0.1 mM EDTA-5 mM Tris-HCl at pH 7.5 and 8 or 5 mM sodium cacodylate-HCl at pH 6.

The mean value of the association constant is $(7 \pm 3) \times 10^6$ M^{-1}. The association constants for the binding of Fab fragments to several polynucleotides are given in Table III. In the calculations, the number of nucleotides covered by one Fab fragment has been assumed to be 6. It should be noticed that the values of the association constants are only approximate since we did not take into account the problem of overlapping binding of large ligand to one-dimensional homogeneous lattice (McGhee and von Hippel, 1974). The antibodies are heterogeneous as found by isoelectric focusing experiments (results not shown); K and n have to be considered as mean values. Also there is some uncertainty on K(poly(I, m^7I)) at pH 8 because poly(I, m^7I) is fluorescent and its fluorescence intensity is not negligible as compared with that of Fab fragments.

In these fluorescence experiments, the excitation wavelength was 295 nm. The screening effect of oligonucleotides and polynucleotides has been corrected (Guigues and Leng, 1976). There are some effects of the oligo(I) binding on the near ultraviolet spectrum of Fab fragments as shown in Figure 4. However, at the excitation wavelength (295 nm), the absorbance changes due to complex formation are small ($\simeq$ 5%).

Thermal Stability. By gel diffusion, no reaction was found between the anti poly(I) antibodies and poly(I)·poly(C) or poly(A)·2 poly(I). However, the Fab fragments interact with these polymers. In presence of Fab fragments, the thermal stability of poly(I)·poly(C) or poly(A)·2 poly(I) is decreased, the decrease of thermal stability depending upon the ratio P/Fab as shown in Figure 5. Some experiments have been also performed with poly(I) in 1 M NaCl (results not shown). The decrease of T_m is about 8 °C for a ratio P/Fab = 20 (the experiments are inaccurate because of the small value of the hyperchromicity).

Discussion

Sera of rabbits immunized with poly(I)·poly(C) adsorbed on methylated bovine serum albumin react not only with poly(I)·poly(C) but also with poly(I). We have purified by affinity chromatography the antibodies which precipitate poly(I). All the anti-poly(I) antibodies of these sera bind to the Sepharose-oligo(I) column. No reaction between the unbound proteins and poly(I) has been found by gel diffusion. The proteins have been applied on a Sepharose-native DNA column in order to remove the nonspecific proteins and the more basic antibodies. After that column, all the antibodies are precipitated by poly(I) but they are still heterogeneous. The immunoglobulins G have been isolated by gel filtration.

Our results show that these antibodies interact specifically with single-stranded poly(I). The precipitation of poly(I) can be inhibited by oligo(I) (oligo(I) do not form multistranded structure under our experimental conditions; Maurizot, 1970); the association constants for the binding of Fab fragments to poly(I) or to $(Ip)_5I$ are of the same order of magnitude and the Fab fragments decrease the thermal stability of multistranded poly(I), of poly(A)·2 poly(I) and of poly(I)·poly(C).

In the recognition of poly(I) by the antibodies, the hypoxanthine residues play an important role. Modifications of the bases decrease the interaction. Poly(I, m^7I) and poly(m^1, m^7I) interact less strongly with the Fab fragments than poly(I). The small effect of pH on the binding of poly(I, m^7I) to Fab fragments is surprising since the pK of m^7I residues is 6.5 (Pochon et al., 1968). This can be due to a shift of the pK of the bound m^7I residues as compared with that of free m^7I residues (this

suggests that protonated m^7I residues interact strongly). On the other hand, hypoxanthine or IMP are not sufficient to get a stable complex. There is no precipitation of the IMP-bovine serum albumin conjugate by the antibodies and the inhibition of the precipitation of poly(I) by IMP is very small. These results differ from those of Seaman et al. (1965) who found that the precipitation of poly(I) by antibodies of sera of rabbits immunized with poly(I) adsorbed on methylated serum albumin was partially inhibited by IMP.

The phosphodiester backbone alone cannot form a stable complex. Poly(A) is not precipitated by the antibodies and the association constants for the binding of Fab fragments to poly(A), poly(U), poly(m^1, m^7I), and poly(C) are of the same order of magnitude. However, the phosphodiester backbone interacts with the amino acid residues of the binding site. The association constants of the oligo(I) decrease as the ionic strength increases which indicates that charge-charge interactions are involved in the binding. The C(2')OH groups of ribose also interfere since $K[\text{poly(I)}] \simeq 230K[\text{poly(dI)}]$. It is assumed that poly(dI) and poly(I) at 30 °C in 0.15 M NaCl, pH 7.5, are single strands (the circular dichroism spectra of poly(dI) in 15 mM Na^+ (pH 7.5) and 0.2 M Na^+ (pH 7.5), at 30 °C, are identical; results not shown).

From fluorescence experiments, it can be deduced that the mean number of nucleotide residues covered by one Fab fragment is 6. This value agrees with that obtained from the precipitation curve. The molar ratio phosphate/IgG is about 12 and therefore phosphate/Fab fragments is about 6. On the other hand, $(Ip)_{10}I$ is a better inhibitor of the precipitation of poly(I) by the anti-poly(I) antibodies than $(Ip)_5I$. Also, the association constants of $(Ip)_6I$-Fab fragments is larger than that of $(Ip)_5I$-Fab fragments. This can be due in part to the heterogeneity of the antibodies and also to a nonspecific interaction between the nucleotide residues outside of the binding site and the amino acid residues of the antibodies.

Among the six nucleotide residues covered by one Fab fragment binding site, the role of all the hypoxanthine residues might not be the same. Substitution of terminal hypoxanthine by uracil results in a large decrease of the association constants ($K[(Ip)_5Up] \simeq K[(Ip)_4Ip] < K[(Ip)_5Ip]$). For several antigen-antibody complexes, there is a primary point of binding of the antigen to the antibody binding site (Kabat, 1968). Our results suggest that the last nucleotide is the immunodominant group.

In conclusion, it is tempting to speculate on the presence of three families of antibodies in the sera of rabbits immunized with poly(I)·poly(C). This can be due to a partial degradation of poly(I)·poly(C) by nucleases of the serum. However, this is not necessary. Surface receptors of the immunocompetent cells bind poly(I)·poly(C) and this leads to the synthesis of antibodies to poly(I)·poly(C). Some receptors leading to the synthesis of antibodies to poly(I) can behave as the anti-poly(I) antibodies. They act as melting proteins destabilizing poly(I)·poly(C) (Guigues and Leng, 1976; this work) which also explains that the antibodies to poly(I) recognize single-stranded poly(I). The same mechanism can explain the synthesis of antibodies to poly(C). Thus, one antigen, the double-stranded polyribonucleotide poly(I)·poly(C), can induce the synthesis of three families of antibodies.

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