Further Studies on the Interaction between Polynucleotides and Antibodies to Poly(inosinic acid)·Poly(cytidylic acid)†

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ABSTRACT: Interactions between antibodies to poly(inosinic acid)·poly(cytidylic acid) [poly(I)·poly(C)] (or Fab fragments) and double-stranded polynucleotides have been studied by several techniques. In quantitative precipitation tests, the amounts of precipitated antibodies depend upon salt concentration. This effect of salt is reversible. From the comparison of the association constants (deduced from fluorescence measurements) for the binding of Fab fragments to several polynucleotides, a direct interaction between atoms or groups of the bases, which can be involved in hydrogen bonds, can be excluded. The complexes between poly(I)·poly(C) and Fab fragments interact with ethidium bromide. The association constants for the binding of ethidium bromide to poly(I)·poly(C) and to poly(I)·poly(C)–Fab fragments complexes are about the same, but Fab fragments reduce the number of base pairs available to ethidium bromide binding. At saturation of poly(I)·poly(C) by Fab fragments, the number of binding sites of ethidium bromide is only reduced by a factor of two. It is concluded that all the nucleotide residues covered by the Fab fragment binding site do not interact with the amino acid residues. Circular dichroism studies of the complexes between Fab fragments and polynucleotides are in favor of some conformational change of the polynucleotides.

Antibodies to double-stranded polyribonucleotides can be elicited in rabbits by injection of double-stranded polynucleotides adsorbed on methylated bovine serum albumin. Immunochemical studies have led to the conclusion that in the formation of the complex the conformation of the polynucleotide was an important parameter (general reviews, Plescia & Braun, 1968; Lacour et al., 1973; Stollar, 1973, 1975). Recent findings on the complexes between polynucleotides and purified antibodies to poly(A)·poly(U)[1] (studied by microcomplement fixation assays; Johnston et al., 1975) and Fab fragments to poly(I)·poly(C) (studied by fluorescence measurements; Guigues & Leng, 1976) were in agreement with this conclusion. Moreover, from the comparison of the association constants for the binding of the anti-poly(I)·poly(C) Fab fragments and several polynucleotides, it was concluded that the Fab fragments were located in the minor groove of the double-stranded helix. These Fab fragments interact with the sugars of the pyrimidine strand and with the phosphate groups but interactions with the C(2)O group of pyrimidine residues and/or the N(3) group of purine residues had not been excluded. On the other hand, the circular dichroism spectrum of the Fab fragment–poly(I)·poly(C) complex was different from the sum of the spectra of the two components, suggesting a conformation change of poly(I)·poly(C) (Guigues & Leng, 1976a).

In this paper, we report some more experiments on the interaction between antibodies to poly(I)·poly(C) and polynucleotides. It is shown that the atoms of the bases are not directly involved in the binding with the Fab fragment binding site. Some knowledge on the number of nucleotides residues interacting with the Fab fragments was obtained using ethidium bromide as a fluorescent probe.

Materials and Methods

The purification of polynucleotides, calf thymus DNA, antibodies and Fab fragments has been already described in detail (Guigues & Leng, 1976b). The antiserum used in this work was from the rabbit 125, while the one used previously was from the rabbit 121. Bacteriophage f2 double-stranded RNA (50% G·C) was supplied by Dr. Doskocil, poly(3-isoadenylic acid) (poly(isoA)) by Dr. Michelson and poly(I)·poly(s^2C) by Dr. Scheit.

Ethidium bromide was a Calbiochem product. The molar extinction coefficient was taken equal to 5600 M^{-1} cm^{-1} at 480 nm.

Absorption spectra were recorded with a Cary 15 spectrophotometer. Fluorescence measurements were performed with a Farrand MKI spectrophotometer equipped with a thermostated cell holder. The optical path length of the cell was 0.5 cm. The degree of polarization is

$$p = \frac{I_{vv} - I_{vh}\frac{I_{hv}}{I_{hh}}}{I_{vv} + I_{vh}\frac{I_{hv}}{I_{hh}}}$$

the subscripts v and h in I represent the polarization direction of the polarizer and the analyzer, respectively (v = vertical; h = horizontal).

The binding experiments were performed by adding increasing amounts of ethidium bromide to a solution of polynucleotide or polynucleotide–Fab fragments complexes. The total variation of volume was 5%.

Fluorescence decay measurements were done with an apparatus using single photon-counting technique (Wahl, 1975). Wavelengths were selected by interferential filters (excitation 520 nm; emission 615 nm). Data were analyzed by a systematic research of lifetimes (Brochon et al., 1976).

Circular dichroism spectra were recorded with a Jouan II dichrograph. The absorbances of the solutions were smaller than 1.5.

The other techniques have been already described (Guigues & Leng, 1976a,b).

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[1] Abbreviations used: poly(A), poly(C), and poly(I), poly(adenylic acid), poly(cytidylic acid), and poly(inosinic acid); poly(iso A), poly(3-isoadenylic acid); EB, ethidium bromide.

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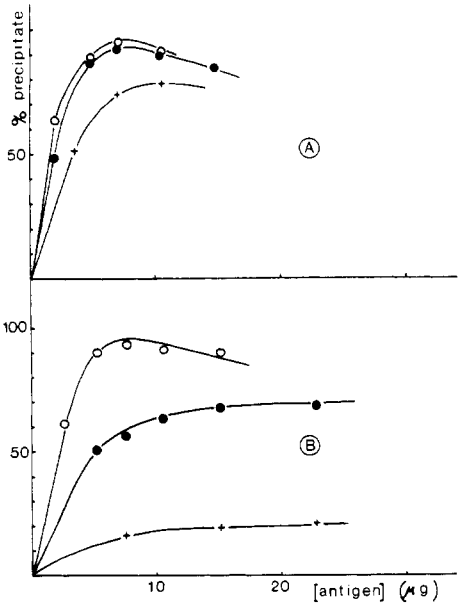

FIGURE 1: Precipitin assays. IgG concentration was 0.35 mg/mL. (A) Precipitation with poly(I)·poly(C); (B) precipitation with poly(I)·poly(dC). Salt concentration: (○) 10 mM NaCl; (●) 0.15 M NaCl; (+) 2 M NaCl. All the solutions contain 5 mM Tris-HCl (pH 7.5). Total volume was 0.2 mL.

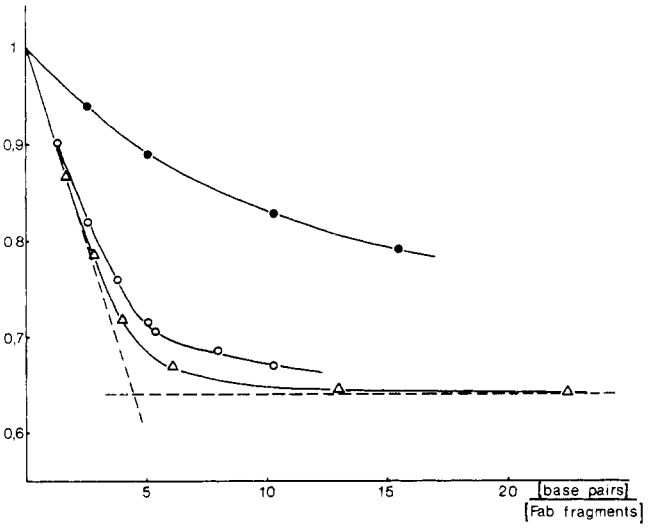

FIGURE 2: Variation of the fluorescence intensity of Fab fragments as a function of poly(I)·poly(brC) concentration, expressed in moles of base pairs. Fab fragments concentration was (△) 2.2 and 5×10^{-6} M and (○) 1×10^{-6} M; salt concentration was 10 mM NaCl, 5 mM Tris-HCl, 0.1 mM EDTA (pH 7.5). (●) Fab fragments concentration was 0.54×10^{-6} M; salt concentration was 0.15 M NaCl, 5 mM Tris-HCl, 0.1 mM EDTA (pH 7.5). Temperature was 5 °C.

Results

Precipitin Tests. The antibodies to poly(I)·poly(C) were purified by affinity chromatography on a Sepharose-poly(A)·poly(U) column and then by gel filtration as already described (Guigues & Leng, 1976b). The interactions between these purified antibodies and several polynucleotides were studied by quantitative precipitation. As shown in Figure 1, in 10 mM NaCl and 0.15 M NaCl, more than 90% of the antibodies are precipitated by poly(I)·poly(C) at the equivalence point (it should be noted that the molar ratio (base pairs/IgG) in the precipitate in the region of antibody excess is 10–12). In 2 M NaCl, in the same range of poly(I)·poly(C) concentration, smaller amounts of antibodies were precipitated. The effect of ionic strength was also observed with poly(I)·poly(dC) (Figure 1) and with poly(I)·poly(br^{5}C) or double-stranded RNA (results not shown).

The effect of ionic strength is reversible. Poly(I)·poly(dC) and the antibodies were mixed in low salt concentration (15 mM NaCl). After 3 h, the solution being already very turbid, the salt concentration was increased up to 2 M NaCl. After 24 h, the samples were analyzed. In a control experiment, the antibodies and poly(I)·poly(dC) were directly mixed in 2 M NaCl. In both cases, within the experimental accuracy, the same quantities of antibodies were found in the precipitates.

Ethidium bromide (EB) interacts with poly(I)·poly(C) or poly(I)·poly(br^{5}C). At the equivalence point, the amounts of precipitated antibodies by poly(I)·poly(C) and by poly(I)·poly(C)–ethidium bromide complexes, the molar ratio phosphate/ethidium bromide being equal to 10, were the same. Similar results were obtained with poly(I)·poly(br^{5}C) and poly(I)·poly(br^{5}C)–EB complexes (results not shown). Qualitatively, the dye was in the precipitate because the precipitate was colored and the supernatant colorless.

Association Constants. The interactions between the anti-poly(I)·poly(C)–Fab fragments and several polynucleotides have been studied in solution. The Fab fragments were purified as already described (Guigues & Leng, 1976b). It has been verified that all the purified Fab fragments could still bind to the Sepharose-poly(A)·poly(U) column equilibrated with 0.15 M NaCl, pH 7.5.

In solution, the Fab fragments are fluorescent (λ_{exc} = 295 nm; λ_{em} = 350 nm). The addition of poly(I)·poly(br^{5}C) quenches the fluorescence while the addition of poly(I)·poly(C) does not. The stoichiometry and the association constant for the binding of Fab fragments to poly(I)·poly(br^{5}C) can be deduced from fluorescence intensity measurements assuming that all the Fab fragments are equivalent (Pesce et al., 1971; Molineux et al., 1975; Sage & Leng, 1977).

In Figure 2, we show the variation of the fluorescence intensity as a function of poly(I)·poly(br^{5}C) concentration under different experimental conditions. At low ionic strength and for large quantities of Fab fragments (equal or larger than 2.2×10^{-6} M), the same relative variation of fluorescence was obtained. In these conditions, assuming that the amount of free poly(I)·poly(br^{5}C) is negligible as compared with the amount of bound poly(I)·poly(br^{5}C), the stoichiometry is directly deduced from the amount of added poly(I)·poly(br^{5}C) to saturate the Fab fragments. The number N of base pairs covered by one Fab fragment binding site is about 4.5 (4–5 base pairs).

The association constant at one-half saturation of Fab fragments with poly(I)·poly(br^{5}C) is equal to $1/(\text{polynucleotide})_{free}$ and $(\text{polynucleotide})_{free} = (\text{polynucleotide})_{total} - (N/2)(\text{Fab})_{total}$. In this equation, the overlapping of the binding sites is not taken into account (McGhee & von Hippel, 1974). The mean values of the association constants are 3×10^6 M^{-1} and 2×10^5 M^{-1} in 15 mM NaCl and 0.15 M NaCl, respectively (the experiments were done at two concentrations, 1 and 0.54×10^{-6} M).

The association constants for the binding of Fab fragments to poly(I)·poly(C) and to other polynucleotides have been deduced from competition experiments. In presence of two polynucleotides and in given experimental conditions, one can write (Guigues & Leng, 1976a):

$$\frac{I_2 - I}{I - I_1} = \frac{K_1}{K_2}\frac{P_1}{P_2}$$

where I_1, I_2, and I are the fluorescence intensities of Fab

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

polynucleotides	K(poly(I)·poly(C))/ K(polynucleotides)
poly(I)·poly(C)	1
poly(A)·poly(U)	1.6
poly(I)·poly(br^{5}C)	2.7
double-stranded RNA	4.2
poly(isoA)·poly(U)	2.7
poly(I)·poly(s^{2}C)	30
poly(isoA)·poly(I)	>200
poly(I)·poly(dC)	80
poly(A)·poly(dT)	>200
poly(A)·poly(dU)	>200

[a] Experimental conditions as in Figure 3.

TABLE II: Fluorescence Decay Parameters of EB-Poly(I)·Poly(C)-Fab Fragments Complexes.[a]

Fab fragments concn	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	C_1	C_2	C_3
0	1.6	14	28	0.30	0.10	0.60
1.9×10^{-6}	1.6	12	28	0.40	0.06	0.54
5.7×10^{-6}	1.6	13	29	0.50	0.09	0.41

[a] EB concentration = 5.9×10^{-6} M; poly(I)·poly(C) concentration (in base pairs) = 1.5×10^{-5} M. T = 5 °C; solvent was 10 mM NaCl, 5 mM Tris-HCl, 0.1 mM EDTA, pH 7.5. C_1, C_2, and C_3 are the amplitudes of the exponential terms of time constants τ_1, τ_2, and τ_3, respectively.

fragments at saturation of polynucleotide (1) or polynucleotide (2) and in presence of polynucleotide (1) and (2) at concentrations P_1 and P_2, respectively, K_1 and K_2 being the association constants. The experiments were performed by adding to a solution of Fab fragments, first poly(I)·poly(br^{5}C) (P_1) and then increasing amounts of a competing polynucleotide (P_2). Linear variations of $(I_2 - I)/(I - I_1)$ as a function of P_1/P_2 were found (Figure 3) (for sake of clarity, the results are shown for only three polynucleotides). The ratios of the association constants are given in Table I.

Interaction with Ethidium Bromide (EB). EB interacts with poly(I)·poly(C) (Waring, 1966). We have studied the binding of EB to poly(I)·poly(C) in presence of Fab fragments by fluorescence.

The addition of Fab fragments to EB-poly(I)·poly(C) complexes (base pairs/EB equal to 2.55) results in a decrease of the fluorescence intensity and in an increase of the degree of polarization of EB until a molar ratio base pairs over Fab fragments equal to 4.5. There is no more variation for larger additions of Fab fragments (Figure 4).

The fluorescence decays of EB in presence of poly(I)·poly(C) and in presence of poly(I)·poly(C)-Fab fragments complexes have been found identical within experimental accuracy (Table II). One of the lifetimes (τ_1 = 1.6 ns) is attributed to free EB. τ_2 and τ_3 are attributed to EB bound to poly(I)·poly(C). Two lifetimes of bound EB to DNA have been already reported (Genest & Wahl, 1972). The major lifetime τ_3 = 28 ns (which corresponds to the majority of bound EB) is the same as the one obtained with EB bound to poly(A)·poly(U) (Wahl et al., 1977). These results show that the quantum yields of EB bound to poly(I)·poly(C) and to poly(I)·poly(C)-Fab fragments complexes are the same. Thus, the amounts of EB bound to these products can be deduced

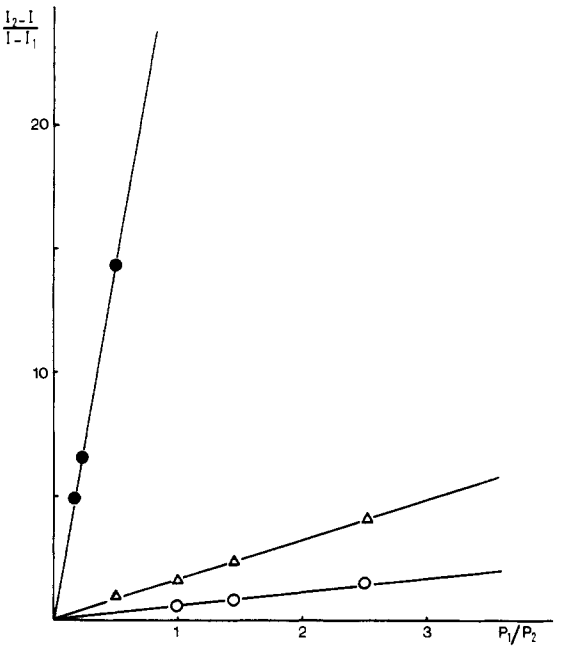

FIGURE 3: Competition experiments. Variation of $(I_2 - I)/(I - I_1)$ as a function of P_1/P_2. P_2 (poly(I)·poly(br^{5}C)) concentration was 5×10^{-5} M; Fab fragments concentration was 10^{-6} M. Competition between poly(I)·poly(br^{5}C) and double-stranded RNA (△), poly(A)·poly(U) (○), and poly(I)·poly(dC) (●). Temperature was 5 °C; solvent was 10 mM NaCl, 5 mM Tris-HCl, 0.1 mM EDTA (pH 7.5).

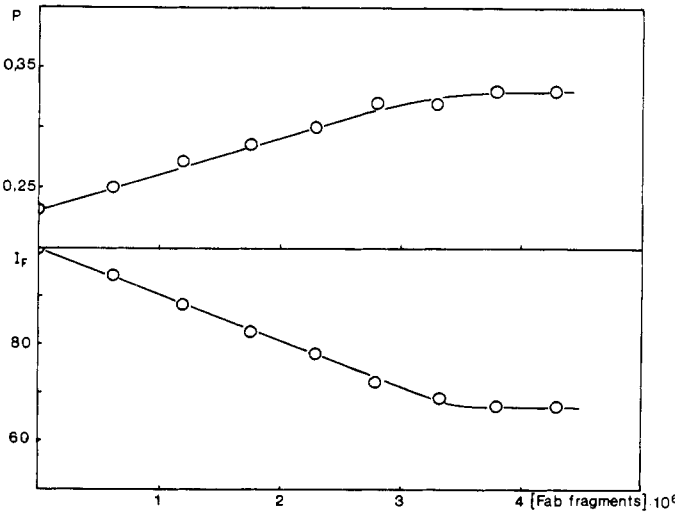

FIGURE 4: Variation of the fluorescence intensity I_F and of the degree of polarization p of ethidium bromide as a function of Fab fragments concentration. Poly(I)·poly(C) concentration, 1.5×10^{-5} M expressed in base pairs; ethidium bromide concentration, 5.9×10^{-6} M. λ_{exc} = 520 nm; λ_{em} = 590 nm. Temperature was 5 °C; solvent was 10 mM NaCl, 5 mM Tris-HCl, 0.1 mM EDTA (pH 7.5).

from measurements of fluorescence intensity (the amounts can also be deduced from the relative amplitudes of the decays and are in agreement with those deduced from measurements of fluorescence intensity). In Figure 5, we have reported the values of r/c as a function of r (r is the molar ratio bound EB per base pair and c the concentration of free EB). The full lines have been calculated according to the equation established by Lawrence & Daune (1976)

$$\frac{r}{c} = K(\alpha - nr)\left(\frac{\alpha - nr}{\alpha - (n-1)r}\right)^{n-1}$$

where K is the association constant, n the number of base pairs

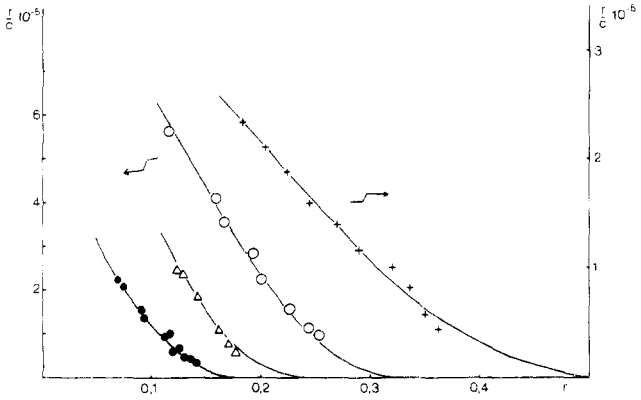

FIGURE 5: Binding isotherms of ethidium bromide to DNA (+), to poly(I)·poly(C) (○), to poly(I)·poly(C)–Fab fragments complexes; the molar ratio poly(I)·poly(C) (in base pairs) over Fab fragments was equal to 6.1 (△), to 3.27 and 2.45 (●). Poly(I)·poly(C) concentration was 1.5×10^{-5} M. Same solvent and temperature as in Figure 4.

per binding site, and α a parameter which takes into account that there are some constraints on the polynucleotide which prevent the binding of EB to some sites. α is equal to 1 in the case of the free polynucleotide and smaller than 1 when some sites are not available to EB binding ($0 \leq \alpha \leq 1$). As a control experiment, the binding of EB to calf thymus DNA has been also studied. First K and n were determined for the binding of EB to poly(I)·poly(C). Then for the binding of EB to poly(I)·poly(C)–Fab fragments complexes, n was assumed to be equal to 3 (value found for the binding of EB to poly(I)·poly(C)), and K and α were determined. The results are summarized in Table III.

To be sure that in curve 3 of Figure 5, poly(I)·poly(C) was saturated with Fab fragments, the same experiment was performed with a fraction of Fab fragments from another lot of antibodies to poly(I)·poly(C). The Fab fragments were fractionated according to the following procedure. To 2 mL of Sepharose–poly(A)·poly(U) equilibrated with 0.2 M NaCl, 5 mM Tris-HCl, pH 7.5, Fab fragments (7 mg) were added (final volume: 12 mL) and the mixture was gently stirred for 4 h. After centrifugation, the supernatant was discarded, the Sepharose–poly(A)·poly(U) was washed with 3 mL of 0.2 M NaCl, 5 mM Tris-HCl (pH 7.5) and the bound Fab fragments (4 mg) were eluted with 1 M acetic acid. The association constant for the binding of these Fab fragments to poly(I)·poly(C) was about 2×10^7 M^{-1} and N was equal to 3 (5 °C, 10 mM NaCl, 5 mM Tris-HCl, pH 7.5). The binding of EB to poly(I)·poly(C)–Fab fragments complexes was studied, the ratio base pairs over Fab fragments being 3 (two experiments were done the concentration of poly(I)·poly(C) being 1.5 and 3×10^{-5} M, respectively). Within the experimental accuracy, the values of r/c as a function of r are on curve 3 of Figure 5. In the same experimental conditions, addition of Fab fragments to a solution of free EB does not change the fluorescence intensity.

Circular Dichroism. In Figure 6 are presented the circular dichroism spectra of Fab fragments and of poly(I)·poly(C), poly(I)·poly(dC), and double-stranded RNA. We also show the spectra of the mixtures of Fab fragments and these polynucleotides. In the three cases, the experimental spectra are different from the sum of the spectra of the two components.

Discussion

This work was undertaken to get complementary knowledge on the mechanism of recognition between polynucleotides and antibodies to poly(I)·poly(C).

TABLE III: Binding Parameters for Ethidium Bromide and DNA, Poly(I)·Poly(C), Poly(I)·Poly(C)–Fab Fragments Complexes.

	K (M^{-1})	n	α
DNA	4.6×10^6	2	1
poly(I)·poly(C)	1.25×10^6	3	1
poly(I)·poly(C)–Fab fragments			
P/Fab = 6.12	1.3×10^6	3	0.73
P/Fab = 3.27	1×10^6	3	0.54
P/Fab = 2.45	1×10^6	3	0.54

The purified antibodies can be precipitated by several polynucleotides. The shape of the precipitin curves depends upon the salt concentration. This effect can be attributed to the variation of the association constants, which has been already observed with other antigen–antibody complexes (Eisen, 1974). We had found that the association constants for the binding of the Fab fragments to several polynucleotides decreased as the ionic strength was increased (Guigues & Leng, 1976a). It was deduced that 1–2 phosphate groups interact with the binding site by charge–charge interactions (using the theoretical results of Daune (1972) or Record et al. (1976). In the precipitin assays reported in this paper the effect of the ionic strength seems more important with poly(I)·poly(dC) than with poly(I)·poly(C). This is apparent and it is due, in our experimental conditions, to the relative values of the association constants. From the fluorescence experiments, we deduce that the association constants for the binding of poly(I)·poly(C) to Fab fragments are about 8×10^6 M^{-1} and 5.4×10^5 M^{-1} in 0.015 M NaCl and 0.15 M NaCl, respectively. Assuming a linear variation we can calculate that K[poly(I)·poly(C)] in 2 M NaCl is about 1.1×10^4 M^{-1}. K[poly(I)·poly(C)]/K[poly(I)·poly(dC)] is equal to 80 (see Table I). Assuming the same dependence of the two association constants with the ionic strength, K[poly(I)·poly(dC)] in 2 M NaCl is of the order of 1.4×10^2 M^{-1}. In our experimental conditions, almost no formation of complex is expected when the association constant is so small. It should be noted that about the same amount of precipitate was formed with poly(I)·poly(C) in 2 M NaCl and poly(I)·poly(dC) in 0.15 M NaCl (the values of the association constants are of the same order of magnitude) which in first approximation confirms our study in solution with the Fab fragments.

About the same amount of precipitated antibodies were obtained by reacting the antibodies and poly(I)·poly(dC) in 2 M NaCl or first in 10 mM NaCl and then increasing the salt concentration to 2 M. These results differ from those found by Aarden et al. (1976a) in their study of the complexes between double-stranded DNA and antibodies to double-stranded DNA. The amount of precipitated antibodies depends upon salt concentration, but, once the complex is formed, they observed almost no dissociation of the complex by increasing the salt concentration. Their study led them to the conclusion that the antibodies to double-stranded DNA bind to DNA in a monogamous bivalent way (Aarden et al., 1976b). Thus our results suggest that the antibodies to poly(I)·poly(C) do not behave as monogamous antibodies. However, one has to be cautious because of the differences in the molecular weights of the antigens. It is known that double-stranded polynucleotides of molecular weights smaller than 10^6 behave as rods and large molecular weight DNAs as coils (Eigner & Doty, 1965; Deubel & Leng, 1974; Godfrey & Eisenberg, 1976).

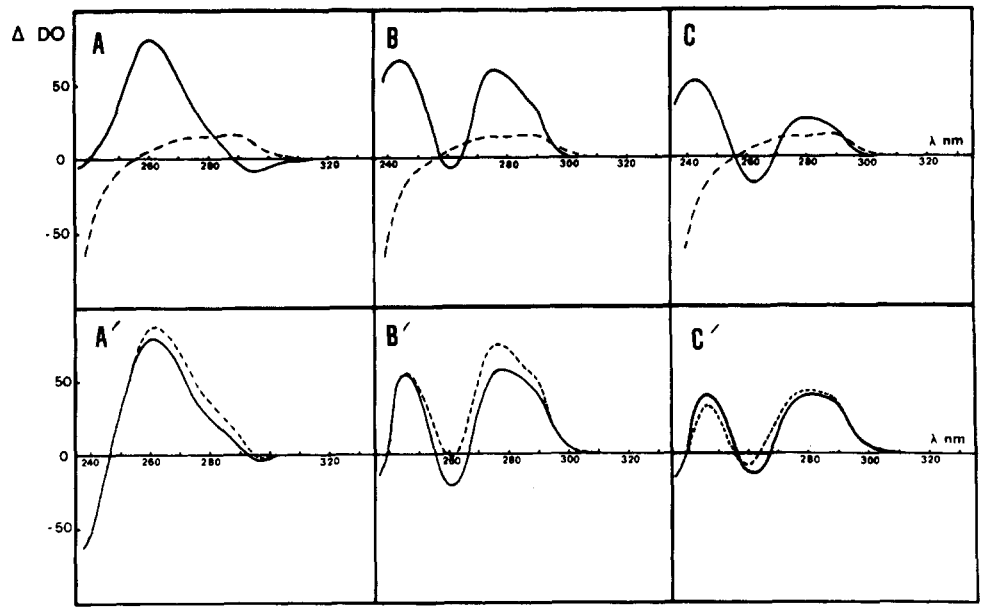

FIGURE 6: Circular dichroism spectra of (A-C) Fab fragments (- - -); concentration was 1.1×10^{-5} M, A double-stranded RNA, concentration 9.2×10^{-5} M, B poly(I)·poly(C) concentration 8.55×10^{-5} M, C poly(I)·poly(dC), concentration 8.9×10^{-5} M. A′: (—) spectrum of double-stranded RNA-Fab fragments mixture, concentration as in A; (- - - -) algebraical sum of the spectra of the two components. The same symbols have been used in B′ and C′ for the mixtures of Fab fragments and poly(I)·poly(C) or poly(I)·poly(dC), respectively. Solvent and temperature as in Figure 4.

Association constants for the binding of the Fab fragments to several polynucleotides have been deduced from competition experiments. K[ds RNA], K[poly(isoA)·poly(U)], and K[poly(I)·poly(C)] are of the same order of magnitude. Thus the C(2)O of pyrimidine residues and the N(3) of purine residues are not directly involved in the binding (in fact the role of the C(2)O groups cannot be completely excluded because a hydrogen bond can still be formed, but it seems unlikely). These results, those in Table III, and those reported previously (Guigues & Leng, 1976a) show that several polynucleotides have about the same affinity for the Fab fragments [poly(I)·poly(C), poly(A)·poly(U), poly(I)·poly(ac^{4}C), poly(isoA)·poly(U), double-stranded RNA, poly(I,7meI)·poly(C), poly(A)·2poly(U)]. Substitutions on all the atoms or groups of the bases which can form hydrogen bonds do not decrease significantly the association constants and it can be concluded that these atoms or groups are not involved in the binding with the Fab fragments. It should be noted that there is some discrepancy in the ratios of the association constants reported here and previously (for example, K[poly(I)·poly(C)]/K[poly(A)·poly(U)] are 1.6 and 6, respectively). We think that these differences reflect the variability of different sera (the serum used in this study was not the same as the one previously used) but they do not change our conclusion; i.e., the bases are not bound by hydrogen bonds to the amino acid residues of the binding site of the Fab fragments. However, the bases might participate in the formation of the complexes by stacking interactions with the aromatic amino acid residues. Studies on complexes between oligopeptides containing aromatic acid residues and nucleic acids have shown that the oligopeptides interact with nucleic acids but they bind preferentially to single-stranded nucleic acids (Brun et al., 1975; Toulmé & Hélène, 1977).

Some polynucleotides interact less strongly than poly(I)·poly(C) (poly(I)·poly(s^{2}C)) or do not interact (poly(G)·poly(C), poly(X)·poly(U), poly(A)·2 poly(I), poly(isoA)·poly(I)). Since the atoms of the bases were not involved, we have to conclude that the geometry of the phosphodiester backbone of these polymers and/or their ability to be distorted are different from those of poly(I)·poly(C). X-ray diffraction studies have shown that the double-stranded polyribonucleotides have the A form (general review, Rich & RajBhandary, 1976; Jovin, 1976) but there are several A-type conformations (A as poly(A)·poly(U), A′ as poly(I)·poly(C), A″ as poly(G)·poly(C)). As it has been suggested by Arnott et al. (1973), one can think of a family of A structures, characterized by the C(3′) endo conformation, but differing by the base tilt. In the reaction with the antibodies, these slight changes can be sufficient to differentiate poly(I)·poly(C) and poly(I)·poly(s^{2}C). Another important factor might be the deformation of the polynucleotide. The circular dichroism spectra of mixtures of Fab fragments and several polynucleotides are different from the sum of the spectra of the two components. The analysis of the results is difficult because the protein and the polynucleotides absorb in the range 240-320 nm. The differences between the calculated and the experimental spectra are of the same order of magnitude and even larger than the signal due to the Fab fragments alone. This suggests that the contribution of the polynucleotide is dominant which can be understood assuming some distortion of the polynucleotide. This distortion cannot occur on more residues than those covered by one Fab fragment binding site because the binding isotherms, the melting curves of poly(I)·poly(C) in presence of Fab fragments, and in general the results on antibody-antigen complexes are those expected for a noncooperative process.

The ratio of the association constants K[poly(I)·poly(C)]/K[poly(I)·poly(C)] is 80. X-ray diffraction patterns have shown that the structures of poly(I)·poly(C) and poly(I)·poly(dC) are similar (O'Brien & MacEwan, ; 1970; Arnott et al., 1973). This strongly suggests that the C(2′)OH groups of the sugars of the pyrimidine strand interact with the amino acid residues of the Fab fragment binding site. On the other hand, poly(A)·poly(dT) and poly(A)·poly(dU) interact less strongly. DNA-RNA hybrids are assumed to have the A conformation. Our results suggest that several A-type conformations can be found with hybrids as well as double-stranded polyribonucleotides.

We deduced from the fluorescence experiments that about

3-5 base pairs are covered by one Fab fragment binding site (5-6 base pairs from the precipitin assays). These values are equal or larger than the number of base pairs really interacting with the proteins. We tried to verify this point by using ethidium bromide (EB) as a fluorescent probe.

EB interacts with nucleic acids (Waring, 1965; Le Pecq & Paoletti, 1967). It is generally thought that EB intercalates between base pairs which distorts the double-stranded helix (Fuller & Waring, 1964). X-ray diffraction studies on ethidium-5-iodouridylyl(3′-5′)adenosine complex have allowed us to visualize the intercalative binding and in particular have shown changes in ribose sugar ring puckering (iodouridine residues have C(3′) endo conformation and adenosine residues have C(2′) endo conformation; Tsai et al., 1975).

The binding isotherms of EB to DNA can be analyzed according to the excluded site model (Crothers, 1968) with n, number of base pairs per site, equal to 2. The binding isotherms of EB to poly(I)·poly(C) can also be analyzed according to the excluded site model but in that case $n = 3$. This is surprising since $n = 2$ with poly(A)·poly(U) (Wahl et al., 1977) but in agreement with the results of Waring (1966).

EB does not prevent the binding of antibodies or Fab fragments to poly(I)·poly(C). The same amount of antibodies are precipitated by poly(I)·poly(C) or by poly(I)·poly(br^{5}C) in absence or in presence of EB (base pairs/EB $\simeq$ 5). In solution in presence of EB, poly(I)·poly(C) is saturated by Fab fragments at a ratio of base pairs to Fab fragment equal to 4.5, a value identical with that found in absence of EB. Thus, we assumed that the addition of EB to the Fab fragment-poly(I)·poly(C) complexes does not lead to any release of Fab fragments.

The lifetimes of EB bound to poly(I)·poly(C) and to poly(I)·poly(C)-Fab fragment complexes are the same. For the fluorescence intensity measurements, there are two sorts of EB molecules, the free and the bound EB. The binding isotherms of EB to poly(I)·poly(C)-Fab fragments complexes have been analyzed assuming that $n = 3$. The fraction of poly(I)·poly(C) contributing to the binding decreases as the amount of bound Fab fragments increases. At saturation with Fab fragments, α is equal to 0.5. Only half of the binding sites are blocked by the Fab fragments. On the other hand, the association constants for the binding of EB to poly(I)·poly(C) and to poly(I)·poly(C)-Fab fragment complexes are about the same. This strongly suggests that all the nucleotide residues covered by the binding site of the Fab fragment do not interact with the amino acids residues.

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