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A Monoclonal Antibody that Neutralizes Poliovirus by Cross-Linking Virions

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The neutralization of type 1 poliovirus by monoclonal antibody 35-1f4 was studied. The virions were rapidly linked by antibody into oligomers and larger aggregates, followed by slow redistribution of antibody between the immune complexes. The antibody content and infectivity of immune complexes were determined. Remaining single virions were fully infectious and free of antibody. The oligomers and larger aggregates did not significantly contribute to the residual infectivity, which therefore correlated with the number of remaining single virions. Papain digestion of neutralized poliovirus released fully infectious, antibody-free virions from the immune complexes. Anti-immunoglobulin antibodies reneutralized these virions. Polymerization was shown to occur even at virus concentrations of less than 10^3 PFU per ml.

In the past 30 years, the mechanism by which poliovirus is neutralized has received considerable attention. The main line of thought with respect to the virus-antibody interaction was laid down in a classic study by Dulbecco et al. (9), establishing the exponential character of the neutralization; it was concluded in this study that the attachment of a single molecule of neutralizing antibody was sufficient to inactivate a virus particle. In that and all subsequent studies, neutralization was considered to be due to damage inflicted by antibodies onto single virions. On the other hand, Bradish and Crawford (4) reported that the tobacco mosaic and tomato bushy stunt viruses underwent antibody-mediated polymerization and suggested that ". . .the observations on bushy stunt virus set a probable pattern for the antibody-combining properties of other small spherical or polyhedral viruses, such as poliomyelitis or foot-and-mouth disease." However, the implications of these observations were not investigated with animal viruses.

Interest in the subject of poliovirus neutralization was recently revived when monoclonal, neutralizing antibodies became available (2, 6, 10, 13, 14, 19). As was briefly reported elsewhere (5), monoclonal antibody (MoAb) 35-1f4 caused the neutralization of type 1 poliovirus by antibody-mediated polymerization. MoAb 35-1f4 belongs to the G2a class; it binds to N antigen but not to H antigen or to isolated capsid polypeptides (6). In this paper, a more detailed account of the properties of this antibody is given. Whereas in preliminary work the MoAb was labeled with fluorescein, the antibody in this study was labeled with [^{14}C]formaldehyde for precise measurement of the antibody-virion interactions.

We found that the reduction of infectivity (i.e., neutralization) brought about by MoAb 35-1f4 could roughly be equated with the loss of single virions to polymerization, and we propose that the neutralizing characteristics of 35-1f4 derived from the inability of this immunoglobulin G (IgG) to bind bivalently to a single virion.

Several authors have shown the reestablishment of infectivity by papain digestion of neutralized virus (11, 14, 15). It has been suggested that the bivalent binding of immunoglobulin to poliovirus caused a conformational change leading to neutralization; papain reversed this process (11). We show that papain depolymerized virus-35-1f4 complexes and caused the reappearance of free 160S particles.

MATERIALS AND METHODS

Virus cultivation and infectivity titration. Unlabeled or [^{3}H]leucine-labeled type 1 poliovirus (strain 1a/S3) was grown in HeLa cells and purified as previously described (3, 21). The final purification step was the collection of 160S particles after sucrose gradient centrifugation. The preparation contained no measurable polio-related antigens other than N antigen.

The molarity and infectivity of poliovirus were determined as described elsewhere (4a). The ratio of physical particles to PFU was repeatedly determined to be ca. 220.

Purification and labeling of 35-1f4. Mouse ascitic fluids containing this antibody were generated as previously described (6). The immunoglobulins were precipitated twice in 50% ammonium sulfate, and the final precipitate was taken up and dialyzed in 20 mM potassium phosphate–100 mM NaCl (pH 7.2). The protein concentration was determined spectrophotometrically, assuming $E^{1\%}_{280} = 14$ (12).

35-1f4 was labeled by reductive methylation as previously described (20). To 200 μg of ammonium sulfate-purified immunoglobulin in 100 μl of potassium–EDTA buffer (20 mM potassium phosphate, 100 mM KCl, 0.1 mM Na_2EDTA [pH 7.2]) at 4°C, 10 μl of 1 M potassium borate (pH 9.0) and 2 μl of [^{14}C]formaldehyde (New England Nuclear Corp., Boston, Mass.; 60 mCi/mmol, 1% solution) was added, followed by ten 1-μl portions of 33 mM $NaBH_4$ at 30-s intervals. After extensive dialysis, the protein content and specific radioactivity were determined. The recovery of protein was 96%. The specific radioactivity of ^{14}C-labeled 35-1f4 used in this study was 800 cpm/pmol, corresponding to ca. 11 ^{14}C-methyl-labeled groups per IgG molecule. No loss of activity as a result of the labeling procedure could be detected by immunoprecipitation (see Fig. 1) A fluorogram of a sodium dodecyl sulfate-gel of the labeled 35-1f4 showed the heavy and light chains (M_Ws, 55,000 and 25,000, respectively) characteristic of IgG (Fig. 2). Virtually no other proteins were present.

The immunoglobulin was mixed with an excess of poliovirus, and the mixture was ultracentrifuged in a sucrose density gradient to determine the fraction of radioactivity

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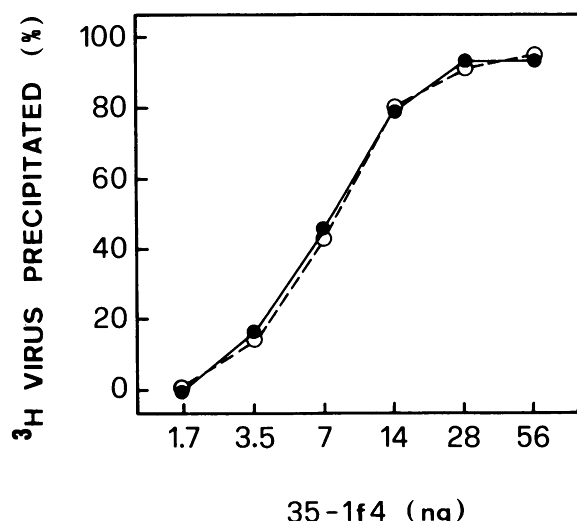

FIG. 1. Effect of reductive methylation on the antigen-binding capacity of MoAb 35-1f4. Antibody 35-1f4 was labeled with [^{14}C]formaldehyde as previously described. A 36 nM solution of unlabeled or labeled 35-1f4 was serially diluted twofold. Of each dilution, 10 μl was tested for its ability to bind 16 fmol (1,000 cpm) of N-antigenic poliovirus, by using a *Staphylococcus aureus*-mediated immunoprecipitation method (22). ●, Unlabeled 35-1f4; ○, 35-1f4 after reductive methylation.

which cosedimented with poliovirions and their polymers. This fraction varied from 50 to 90% of the immunoglobulin purified from different ascitic fluids. This percentage times the protein concentration was considered to represent the concentration of polio-specific antibody and was used to compute antibody/virion molar ratios. The nonbinding proteins were immunoglobulins, as determined by sodium dodecyl sulfate-gel electrophoresis, and presumably admixtures of serum globulins contaminating the ascitic fluids.

Analysis of virus-35-1f4 mixtures by ultracentrifugation. ^{3}H-labeled poliovirus in 50 μl of buffer A (20 mM Tris-hydrochloride, 5 mM EDTA, 150 mM NaCl, 0.05% Nonidet P-40, 0.1 mg of bovine serum albumin per ml [pH 7.2]) was mixed with ^{14}C-antibody in an equal volume of buffer A under constant stirring. After 1 h at 37°C and, as stated below, overnight at 4°C, samples were taken for infectivity titration. The remainder was layered onto a 14.5-ml sucrose gradient (15 to 30%) in 20 mM Tris-hydrochloride (pH 7.2)–120 mM KCl–3 mM magnesium acetate. After 150 min at 85,000 × g_{av} and 4°C, fractions were collected, and the pellet was suspended in 0.3 ml of water. All fractions were assayed for radioactivity and for infectivity as described below. If necessary, the antibody/virus ratio was corrected for spillover of trailing monomeric virions on the basis of the sedimentation profile of the virions in the absence of antibodies.

The neutralization and polymerization results were similar when phosphate-buffered saline, complete or minus divalent cations, was used.

Preparation of Fab fragments. 35-1f4 was digested with 1% (wt/wt) mercuripapain essentially as described previously (23). The buffer used was 0.1 M sodium phosphate (pH 7.0)–10 mM cysteine–2 mM EDTA. After 16 h of incubation at 37°C, the Fab fragments were isolated by Sephadex G-100 gel filtration. Fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the absence of 2-mercaptoethanol. The fractions containing Fab fragments were pooled and stored at −20°C in small samples until further use.

RESULTS

Formation of oligomers and larger aggregates. Samples of purified MoAb 35-1f4 were labeled with [^{14}C]formaldehyde by reductive methylation (see above). This procedure did not cause a detectable reduction in the antigen-binding capacity of the antibody (Fig. 1).

Different amounts of antibody were mixed with a constant amount of ^{3}H-labeled poliovirus. After incubation, the residual infectivities and the sedimentation profiles of the virus-antibody mixtures were determined (Fig. 3). In the presence of antibody, the virus was distributed over a peak of 160S and additional peaks corresponding to virion oligomers (dimers, trimers, etc. [5]). A comparison of Fig. 3A and B indicates that, as more ^{14}C-labeled 35-1f4 was added, (i) the degree of neutralization increased, (ii) the number of unpolymerized virions decreased, and (iii) the amount of virus recoverable from the pellet increased. The amount of virus present as oligomers was strongly dependent on the antibody/virion ratio. These observations suggest that the virions underwent antibody-mediated polymerization resulting in their incorporation into large aggregates.

The polio-specific antibody sedimented in association with the virion oligomers and large aggregates (Fig. 3 and Table

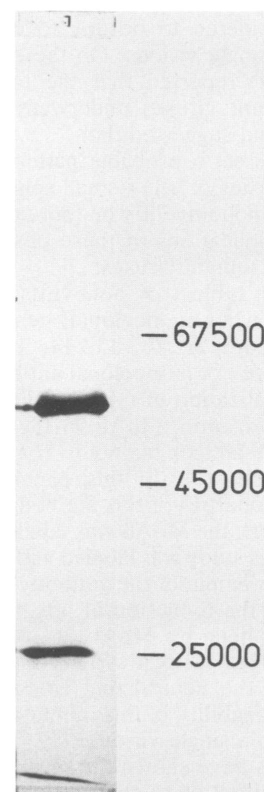

FIG. 2. Gel electrophoresis of ^{14}C-labeled 35-1f4 antibodies. ^{14}C-labeled 35-1f4 was denatured and analyzed by sodium dodecyl sulfate-gel electrophoresis (16). The fluorogram was compared with the stained gel showing the following molecular weight standards: bovine serum albumin, 67,500; ovalbumin, 45,000; and chymotrypsin, 25,000.

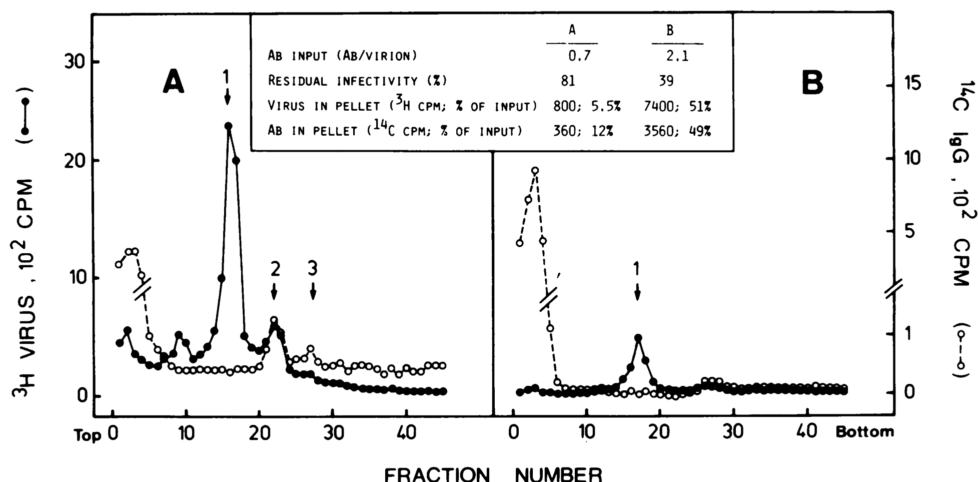

FIG. 3. Sucrose gradient sedimentation profiles of ^{3}H-labeled poliovirus mixed with ^{14}C-labeled MoAb 35-1f4. ^{14}C-labeled 35-1f4 was added at molar ratios shown in the inset to ^{3}H-labeled poliovirus at a final concentration of 25 nM (4.6×10^{10} PFU per ml) in buffer A (unspecific antibody was excluded from the input ratios [see text]). After 1 h at 37°C and overnight at 4°C, a sample was taken to determine the residual infectivity. The remainder was analyzed by sucrose gradient centrifugation (see text). The amount of virus and antibody in the suspended pellet is represented in the inset. The percentage of antibody in the pellet is represented as a fraction of the total ^{14}C radioactivity applied onto the gradient, of which 50% was polio specific. Arrows 1, 2, and 3 indicate the positions of the monomeric, dimeric, and trimeric virions, respectively (5).

1), but not with single virions. Obviously, polymerization must have started with binding of 35-1f4 to individual virions; however, the observation that the 160S material remained free of antibody suggests that MoAb 35-1f4 did not form permanent complexes with single virions.

Table 1 shows the infectivity of the various antibody-virus complexes obtained after sucrose gradient centrifugation. Whereas the specific infectivity (PFU per mole of virus) of di- and trimers was between one-half and one-third of that of the native virions, the specific infectivity of the large aggregates was much lower.

Composition of oligomers and large aggregates. The antibody content of the large aggregates in successive experiments varied as a function of the initial antibody-virion input. With an input of 0.7 antibody per virion, the aggregates contained about 10 antibodies per virion after 1 h at 37°C (Table 1, experiment 1). In other experiments, the antibody content of the aggregates was found to increase to 24 antibodies per virion as the input molar ratio was increased to 160 (data not shown).

The composition of the oligomers could be measured only at molar ratios ranging from ca. 0.5 to 1.5 antibodies per virion. In this range, their antibody content was relatively constant as indicated by the results in Table 1. For the dimers, the ratio was 1.4 to 1.5 antibodies per virion or ca. 3 molecules of antibody per dimer. This was three times more than the single antibody molecule minimally required to link two virions into a dimer. When sufficient radioactivity was present in trimer and tetramer fractions, the antibody content of the oligomers was also calculated. Trimers were found to contain an average of five molecules of antibody, whereas only two are theoretically required; the corresponding figures for the tetramers were 7.5 and 3. Again, the observed values were 2.5 times higher than the theoretical minima.

Time dependence of polymer composition. Identical mixtures of poliovirus and MoAb 35-1f4 were incubated at 37°C for different times ranging from 0 to 17 h and were analyzed simultaneously by ultracentrifugation. At all times, patterns were obtained which were similar to that in Fig. 3A, except for the amount of aggregated virus. The amounts of virus present as unpolymerized virions, dimers, trimers and larger oligomers, and aggregates are shown in Fig. 4A. As can be seen, the amount of virus sedimenting as 160S material did not change significantly with the incubation time, and these unpolymerized virions remained free of antibody (Fig. 4B). The amount of virus in the dimers and larger oligomers decreased slightly with time, although their antibody content tended to increase. It is worth noting that, even after the

TABLE 1. Infectivity and antibody content of virus-35-1f4 complexes[a]

Expt.	Ab/virion input (molar ratio)	Residual infectivity (%)	Ab content of mono- and polymers		Specific infectivity[b] (%)
			Fraction	Ab/virion molar ratio	
Control	0.0	100	Monomers	0.00	100
1	0.7	70	Monomers	<0.02	101
			Dimers	1.4	54
			Trimers	1.6	37
			Large aggregates[c]	10	1.5
2	0.9	ND	Monomers	<0.02	ND
			Dimers	1.4	ND
			Trimers	1.6	ND
	1.5	ND	Monomers	<0.02	ND
			Dimers	1.5	ND
			Trimers	1.7	ND
			Tetramers	1.9	ND

[a] Concentration of poliovirus, 20 nM (4×10^{10} PFU per ml); ND, not determined; Ab, antibody.
[b] Ratio of PFU to radioactivity.
[c] The pellet was suspended after centrifugation; the infectivity and antibody content were determined as described in the text.

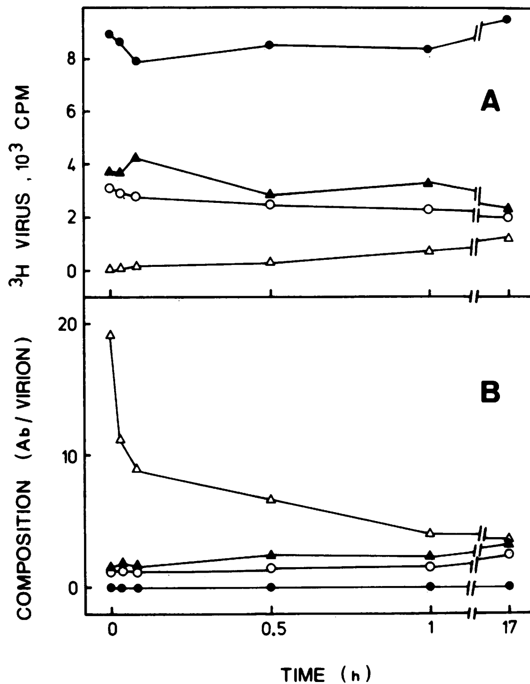

FIG. 4. Time dependence of virion polymerization by MoAb 35-1f4. Two stock mixtures were prepared in buffer A: ^{3}H-labeled poliovirus (87 nM) and ^{14}C-labeled 35-1f4 (59 nM); 50-μl portions of antibody were added with constant stirring to equal volumes of virus at various times before ultracentrifugation. The mixtures were incubated for 1 h at 37°C and 16 h at 4°C, or for 60, 30, 5, 2, or 0 min at 37°C, and layered onto ice-cold sucrose gradients. The centrifugation was started 4 min after layering the 0-min sample. The ultracentrifugation was performed at 0°C, but otherwise it was as described in the text. (A) Amount of virus and (B) relative antibody content of fractions containing single virions (●), dimers (○), and trimers and larger oligomers excluding pellet fraction (▲) and of the aggregates recovered from the pellet (△).

shortest incubation, the antibody content of the dimers was well above the theoretical minimum of 0.5 antibody per virion (the average value after 0, 2, and 5 min of incubation was 1.4 antibodies per virion). The only important changes occurred with the large aggregates, the virus content of which increased threefold during the first minutes, leading to a decrease in their relative antibody content from 19 to 9 antibodies per virion. The relative antibody contents of all polymerized complexes tended eventually to converge to ca. three antibodies per virion.

In summary, at the molar ratio of 0.7 antibody per virion, MoAb 35-1f4 mediated a rapid formation of oligomers and aggregates, followed by a slow redistribution of antibody.

Infectivity of monomeric virions. The specific infectivity of the remaining, unpolymerized virions was indistinguishable from that of native virions (Table 1, experiment 1). However, since the loss of infectivity amounted to only 30% in this experiment as well as in those reported previously (5), the status of the unpolymerized virions after a more extensive neutralization remained unknown. Therefore, a neutralization mixture was made up with two 35-1f4 molecules per virion, which resulted in 80% neutralization (virus concentration, 7×10^9 PFU per ml). After sucrose gradient isolation, the unpolymerized (160S) virions retained the same specific infectivity as untreated virus, whereas 19% of virus was present as 160S material, i.e., almost equal to the residual infectivity. To find out whether this correlation held true in general, the results of six independent experiments were regrouped in Fig. 5. The number of remaining 160S particles was plotted against the residual infectivity. The full line was based on the assumption that 160S particles were fully infectious, whereas all polymerized forms were noninfectious. As can be seen, the experimental results roughly followed this line, and the downward deviations could be explained as the contribution of the oligomers to the infectivity. On the other hand, the results were totally at variance with the view that neutralization was unaccompanied by polymerization.

Neutralization by 35-1f4 Fab fragments. Poliovirus (concentration, 3×10^6 PFU per ml) was incubated with Fab fragments of 35-1f4 (prepared as previously described [23]) at various molar ratios up to more than 10^5 Fab fragments per virion. Even in the presence of this huge excess of Fab fragments, the virus still sedimented at 160S and remained free of Fab fragments (data not shown), whereas the infectivity was essentially unchanged (residual infectivity, 85%). Even though the Fab fragments were apparently inactive, they were still able to recognize poliovirus epitopes, as the virus was neutralized by the joint action of Fab fragments and antiglobulin (residual infectivity, 0.5%). Similar observations of secondary neutralization were reported by Keller (15). They may be explained by considering the action of Fab fragments in association with anti-immunoglobulin as equivalent to that of bivalent antibody.

Effect of papain on poliovirus-35-1f4 complexes. As first reported by Keller (15) and confirmed by others (11, 14), a partial restoration of infectivity occurs when neutralized virus is treated with papain, which cleaves bivalent antibody molecules into monovalent Fab and Fc fragments.

Virus treated with MoAb 35-1f4 at the input ratio of 10 antibodies per virion was completely aggregated (Fig. 6A), and neutralized to 99% (Table 2, line 4). The papain treatment caused the reappearance of 160S particles (Fig. 6B)

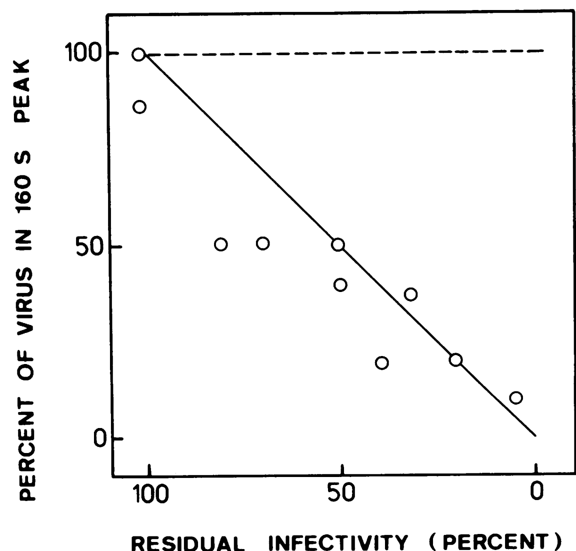

FIG. 5. Relationship between the residual infectivity and the amount of residual 160S virions after neutralization. The data were compiled from six independent neutralization experiments, including those analyzed in Table 1, Fig. 3, and Fig. 7. ——, Theoretical expectation assuming that the infectivity was associated exclusively with the remaining, unpolymerized virions; ----, theoretical expectation assuming no polymerization.

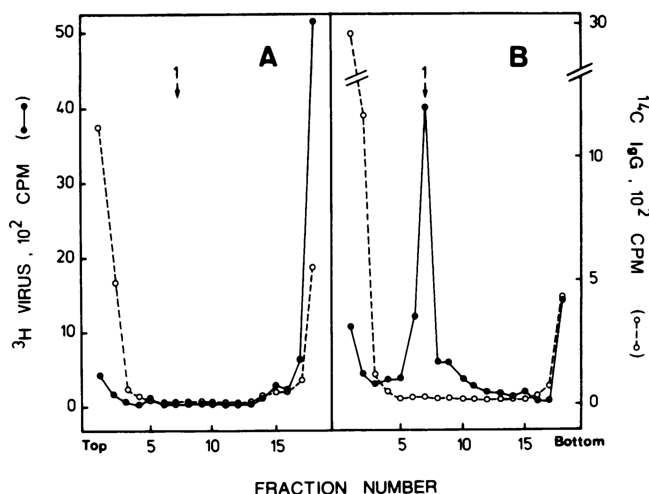

FIG. 6. Effect of papain on poliovirus previously neutralized by MoAb 35-1f4. ^{14}C-labeled 35-1f4 was added in the molar ratio of 0 or 10 antibodies per virion to ^{3}H-labeled poliovirus in a final volume of 200 μl of phosphate-buffered saline, supplemented with 10 mM EDTA and 2 mM cysteine. The concentration of the virus in this mixture was 5 nM, and its infectious titer was 9×10^9 PFU per ml. After 1 h at 37°C, 100 μl of the same buffer containing either 0.0 or 2.5 μg of papain was added, and the incubation was continued for 3 h at 37°C and overnight at 20°C. Then 100 μl of buffer supplemented with 40 mM $HgCl_2$ to inactivate the papain activity and containing either 0 or 10 μg of rabbit anti-mouse antibodies was added, followed by incubation for 1 h at 25°C. At each stage, a sample was taken to determine the residual infectivity (see Table 2). The virus 35-1f4 mixture (A) and the papain-treated virus-antibody mixture (B) were analyzed by sucrose gradient centrifugation. Arrows indicate the positions to which the virions sedimented in a control gradient without antibody.

and restored 30% of the original infectivity (Table 2, line 5). Moreover, the single virions released from the aggregates by papain were fully infectious, and no detectable amount of ^{14}C-radioactivity was present at the 160S position. The latter result indicates that papain caused the Fab fragments to detach from the virions.

Antiglobulin antibodies fully reneutralized the virus (Table 2, line 6), indicating that the Fab fragments had retained their binding capacity. The reneutralization by antiglobulin antibodies can be explained in the same way as the secondary neutralization by Fab fragments and antiglobulin antibodies (see above).

TABLE 2. Effect of papain and antiglobulin antibodies on poliovirus previously neturalized by MoAb 35-1f4

Additions[a]	Residual infectivity of mixture (%)	Specific infectivity[b] of 160S material (%)
None	100	100
RAM	103	ND
Papain	93	110
35-1f4[c]	1.2	—[d]
35-1f4 + papain[c]	30	125
35-1f4 + papain + RAM	0.3	ND

[a] See Fig. 6 for technical details; RAM, rabbit anti-mouse Ig.
[b] Ratio of PFU to radioactivity assayed after sucrose gradient isolation; ND, not determined.
[c] Sedimentation profile shown in Fig. 6.
[d] No 160S peak was present.

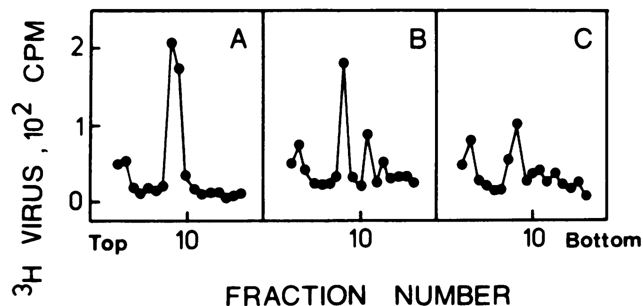

FIG. 7. Sucrose gradient sedimentation profiles of ^{3}H-labeled poliovirus mixed with MoAb 35-1f4. To 4 pM poliovirus (final concentration) in 1 ml of buffer A was added (A) no antibody, (B) 0.6 nM 35-1f4, and (C) 1.0 nM 35-1f4. After incubation for 1 h at 37°C and 16 h at 4°C, the mixtures were analyzed by sucrose gradient centrifugation in 20-ml tubes. After 135 min at 110,000 × g_{av}, 1.2-ml fractions were taken to determine radioactivity. The infectious titer of the mixture as determined before centrifugation was 7.5×10^6 PFU per ml in (A), 50% of this value in (B), and 30% in (C).

Neutralization at low virus concentrations. It might be argued that the high virus concentrations (3.3 to 25 nM or 7×10^9 to 5×10^{10} PFU per ml) used in the experiments reported above may have favored virion polymerization over other neutralization mechanisms. Therefore, it was important to find out whether polymerization also occurred at lower virus concentrations at which poliovirus neutralization is known to obey the percentage law (1). In agreement with this law, the degree of neutralization with MoAb 35-1f4 is independent of the amount of challenge virus, provided that the initial virus concentration is less than $10^{8.5}$ PFU per ml (4a). MoAb 35-1f4 still polymerized the virus at the initial concentration of 10^7 PFU per ml (Fig. 7).

The latter virus concentration was near the lower limit for the determination of virus radioactivity profiles (and too low already to determine the accompanying antibody). On the other hand, the biological assay of the virus PFU was far more sensitive. The method could also be used for the quantitation of virus in immune complexes, provided that these could be dissociated and the infectivity could be regained. Mandel (17) reported that the infectivity of neutralized poliovirus could be restored by a brief treatment at low pH. When virus was neutralized to 98% by MoAb 35-1f4 and treated with citric acid at pH 2.0 (Table 3, experiment 1), 81% of the original infectivity was indeed restored. As expected, very little virus sedimented as 160S material,

TABLE 3. Sedimentation of low concentrations of poliovirus neutralized by MoAb 35-1f4

Expt.	Virus input (PFU per ml)	Residual infectivity of virus-35-1f4 mixture[a] (%)		Infectivity recovered after sucrose gradient centrifugation (% input)	
		Not reactivated	Reactivated at pH 2.0[b]	160S	Aggregates[c] reactivated at pH 2.0[b]
1	5.7×10^7	2	81	0.05	42
	5.7×10^2	1	ND	19	63
2	4.5×10^4	<0.1	ND	<0.3	98

[a] Concentration of 35-1f4, 15 nM; ND, not determined.
[b] Reactivation was carried out by adding 1 M citric acid to a final pH of 2.0, followed by an incubation at 20°C for 30 min.
[c] Aggregates were collected from a CsCl cushion under the sucrose gradient. The CsCl did not interfere (after dilution) with the plaque assay.

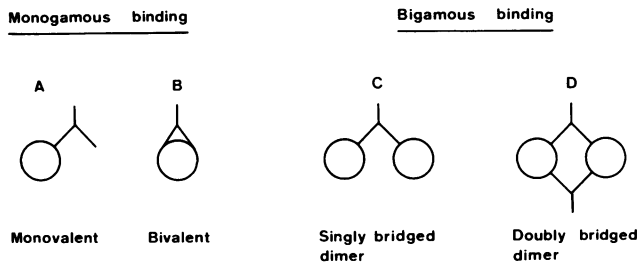

FIG. 8. Different modes of attachment of IgG antibodies to poliovirus. See the text for details.

whereas 42% of the input infectivity could be recovered from the aggregates by acid treatment. Simultaneously, the same experiment was performed with the same antibody concentration and a 10^5-times-lower virus input (570 PFU per ml). Even though partial restoration of the infectivity (from 1 to 19%) was observed, 63% of the input infectivity could still be recovered from the aggregates after treatment at pH 2.0. In a separate experiment with the same antibody concentration, virus at 4.5×10^4 PFU per ml was used; in this case, no virus sedimented as 160S particles, and 98% of the input infectivity was recoverable by acid treatment of the aggregates (Table 3, experiment 2). The results clearly indicate that antibody-mediated aggregation occurred over a wide range of poliovirus concentrations.

DISCUSSION

For the sake of clarity, we will recall the definition of virus neutralization by Dulbecco: "Virus neutralization consists of a decrease in the infectious titer of a viral preparation following its exposure to antibodies. . ." (8). Within the framework of this definition, the loss of infectivity can result from (i) the inactivation of individual virions by the antibodies, (ii) an altered relationship among the virions, such as their cross-linking into polymers by antibodies, or both.

Neutralization of poliovirus by MoAb 35-1f4 is due to the polymerization of the virions and to their incorporation into aggregates of low specific infectivity. Papain, which cleaves bivalent IgG into monovalent fragments, both depolymerized (Fig. 6) and reactivated the virus (Table 2).

Monovalent Fab fragments, either premade or generated in situ by papain digestion of immune complexes, failed to form complexes with poliovirus which were of sufficient stability to be detected by sucrose gradient centrifugation. This suggests that monovalent binding of 35-1f4 fragments is unstable. If so, the monovalent binding of monogamous antibody (Fig. 8A), as well as its bivalent, bigamous binding (Fig. 8C), is expected to also be unstable. Dimers formed as in Fig. 8C will exist only transiently, and stability will be dependent on the formation of multiple antibody bridges (Fig. 8D). The observation that the antibody content of the oligomers was well in excess of the theoretical minima strongly suggests that they may indeed possess multiple bridges.

Bivalent 35-1f4 molecules were never found in association with monomeric virions, thus suggesting that bivalent, monogamous binding (Fig. 8B) is unstable or impossible. The geometry of the viral surface may prevent the simultaneous binding of the two paratopes of a single IgG molecule to epitopes located on the same virion. Interestingly, some of the binding sites for neutralizing antibodies on foot-and-mouth disease virus are located at the apices of the icosahedral virions (7), and these point away from each other by more than 60° (the diagonals of an icosahedron form angles of 63°26′). Since the bivalent, monogamous binding of an IgG requires that its two paratopes be aligned with the epitopes, this kind of bivalent binding may be impeded by the divergence of apical epitopes.

The essential features of 35-1f4 which set this antibody apart from the MoAb described by others are (i) the linking of virions into distinct oligomers and (ii) the lack of binding of the antibody to unpolymerized virions. MoAb F7.12 described by Icenogle et al. (14) exhibited feature (i) but not (ii). We performed sucrose gradient centrifugation analyses of Mahoney virus after neutralization with MoAb F7.12. Ascitic fluid of F7.12, a sample of which was kindly provided by R. Rueckert, was purified and labeled as described above for 35-1f4. The crucial observation, i.e., the binding of the antibody to both 160S particles and oligomers (14), was easily reproduced (data not shown). Thus, the discrepancies noted between 35-1f4 and F7.12 are inherent to the antibodies and independent of the techniques used in different laboratories.

The MoAbs described by Emini et al. (11) may represent a third class of poliovirus neutralizing antibodies. Although poliovirus was aggregated by saturating amounts of antibody, no relationship between aggregation and neutralization was established.

The virions remaining after partial neutralization by MoAb 35-1f4, as well as those released from immune complexes by papain digestion, were fully infectious, meaning that they were not permanently inactivated by previous binding of antibody. This rules out hit-and-run mechanisms as an explanation for neutralization by 35-1f4. The only remaining possibility is that the infectious titer of the virus population was reduced because the virions were cross-linked into clusters of reduced infectivity. This simple mechanism does not require any antibody-induced conformational changes in the virions, such as would be revealed by the pI shift reported by Mandel, from pH 7 to pH 4 upon neutralization of poliovirus (18). Actually, MoAb 35-1f4 failed to induce any pI shift (P. Brioen, A. Thomas, and A. Boeyé, J. Gen. Virol., in press), and there have been other reports of MoAbs which failed to cause the pI shift reported by Mandel (11, 14).

One might argue that polymerization as a neutralization mechanism will be operative only at high virus concentrations, i.e., under laboratory conditions involving the use of concentrated virus. However, virion polymerization was still observed at a concentration of less than 10^3 PFU per ml, i.e., 10^{-16} M virus, thus showing that 35-1f4-like antibodies may well be protective in vivo.

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