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The antigenic structure of poliovirus

By J. M. Hogle and D. J. Filman

Department of Molecular Biology, Research Institute of Scripps Clinic,
10666 *North Torrey Pines Road, La Jolla, California* 92037, *U.S.A.*

[Plates 1 and 2]

We have solved the structure of the Mahoney strain of type 1 and the Sabin (attenuated vaccine) strain of type 3 poliovirus by X-ray crystallographic methods. By providing a three-dimensional framework for the interpretation of a wealth of experimental data, the structures have yielded insight into the architecture and assembly of the virus particle, have provided information regarding the entry of virus into susceptible cells, and defined the sites on the virus particle that are recognized by neutralizing monoclonal antibodies. Thus locating mutations in variants selected for resistance to neutralizing monoclonal antibodies has defined three antigenic sites of the surface of the virion, and provided clues as to the mechanisms by which viruses escape neutralization. Finally, comparison of the structures of the two strains, together with analysis of sequences of many poliovirus strains, have begun to define the structural changes associated with serotypic differences between polioviruses.

Introduction

Determining the nature of the interactions between viral antigens and the immune system is crucial to our understanding of the mechanisms utilized by vertebrates to clear viral infection and to the development of effective immunization strategies for protection against viral diseases. The development of rapid genome-sequencing methods, the introduction of methods for preparing monoclonal antibodies and the demonstration that short synthetic peptides can serve as useful proves of the immune response to protein antigens made it possible to map the important epitopes of a number of viruses on the linear sequences of their constituent proteins. In several instances the three-dimensional structures of the viral antigens have been determined by X-ray crystallographic methods, permitting the antigenic sites to be described in the three-dimensional context of the complete structure.

In the first structural study of this kind, Wilson *et al.* (1981) described the high-resolution structure of the haemagglutinin of influenza virus A/Hong Kong. In a companion paper, they analysed the three-dimensional distribution of sequence differences between natural isolates of influenza virus and of mutations selected by neutralizing antibodies (Wiley *et al.* 1981). They observed that the sequence changes that accompanied escape from immune recognition were located in exposed loops on the outer surface of the haemagglutinin molecule and were clustered in three or four discrete regions of the molecule.

In a similar study, Colman and co-workers determined the structure of the other surface glycoprotein of influenza virus, the neuraminidase, and mapped its antigenic sites (Colman *et al.* 1983; Varghese *et al.* 1983). These authors also found that mutations that accumulated under immune selection were located in exposed loops on the surface of the protein and were clustered in three or four discrete sites on the protein surface. Recently, Colman *et al.* (1987)

have determined the structure of the neuraminidase complexed with a Fab (a Fab is the proteolytically cleaved antigen-binding fragment of an antibody molecule). It was observed that the interaction between the neuraminidase and the Fab involved an extensive area on the surface of protein. The contacts observed involved several exposed loops of the neuraminidase that previously had been identified as components of antigenic sites, as well as all four complementarity determining loops of the Fab molecule. Such extensive contacts between an antibody and its cognate antigen have also been observed in studies of complexes between hen egg-white lysozyme and Fabs from antilysozyme monoclonal antibodies (Amit *et al.* 1986; Sheriff *et al.* 1987).

In addition to the earlier structural studies on the isolated surface glycoproteins of influenza virus, three-dimensional structures have been determined for the complete virions of several members of the picornavirus family, including the Mahoney strain of type 1 poliovirus (Hogle *et al.* 1985), the Sabin strain of type 3 poliovirus (Filman *et al.* 1989), rhinovirus 14 (Rossmann *et al.* 1985) and Mengo virus (Luo *et al.* 1987). For poliovirus and rhinovirus 14 the sequences of a number of variants that are resistant to neutralization by monoclonal antibodies have been determined (Minor *et al.* 1983, 1985, 1986; Diamond *et al.* 1985; Blondel *et al.* 1986; Page *et al.* 1988; Sherry *et al.* 1986), and the locations of the mutations conferring resistance to neutralization have been described in the context of the three-dimensional structure of the intact virions (Hogle *et al.* 1985; Page *et al.* 1988; Rossmann *et al.* 1985). For poliovirus, studies with neutralization-resistant mutants have included representative strains of all three serotypes, and have revealed significant serotype-specific differences in the relative dominance of the antigenic sites. Comparison of the Mahoney 1 and Sabin 3 structures has provided some insight into the structural basis for these serotypic differences.

Poliovirus structure

Poliovirus is a member of the picornavirus family, which also includes the coxsackieviruses, the rhinoviruses, the cardioviruses (such as EMC, Mengo, and Theiler's virus), the aphthoviruses (foot-and-mouth disease virus), and hepatitis A virus. All known poliovirus isolates can be grouped into one of three serotypes by their behaviour with reference panels of neutralizing antisera. The poliovirus particle is approximately 310 Å† in diameter and has a molecular mass of 8.5 MDa (Rueckert 1985). The virion is composed of 60 copies of each of four capsid protein subunits, VP1 ($M_r = 33000$), VP2 ($M_r = 30000$), VP3 ($M_r = 26000$) and VP4 ($M_r = 7400$), arranged on a $T = 1$ icosahedral surface, and a single-stranded messenger-sense RNA genome of approximately 7500 nucleotides.

The molecular structures of the three large capsid proteins (VP1, VP2, and VP3) are generally similar to one another. Each is composed of a conserved core, comprising an eight-stranded antiparallel β-barrel with flanking helices (figure 1*a*). At one end of the β-barrel the individual strands are connected by short loops, giving the barrel the overall shape of a triangular wedge. In contrast to the great structural similarity exhibited by the core regions, each of the three major subunits has a different set of loops connecting the regular secondary structural elements of the cores, and different amino and carboxy terminal extensions (figure 1*b–d*). The conserved cores pack together, forming the closed protein shell of the particle. The amino terminal extensions form a network on the inner surface of the protein shell that we

† 1 Å = 10^{-10} m = 10^{-1} nm.

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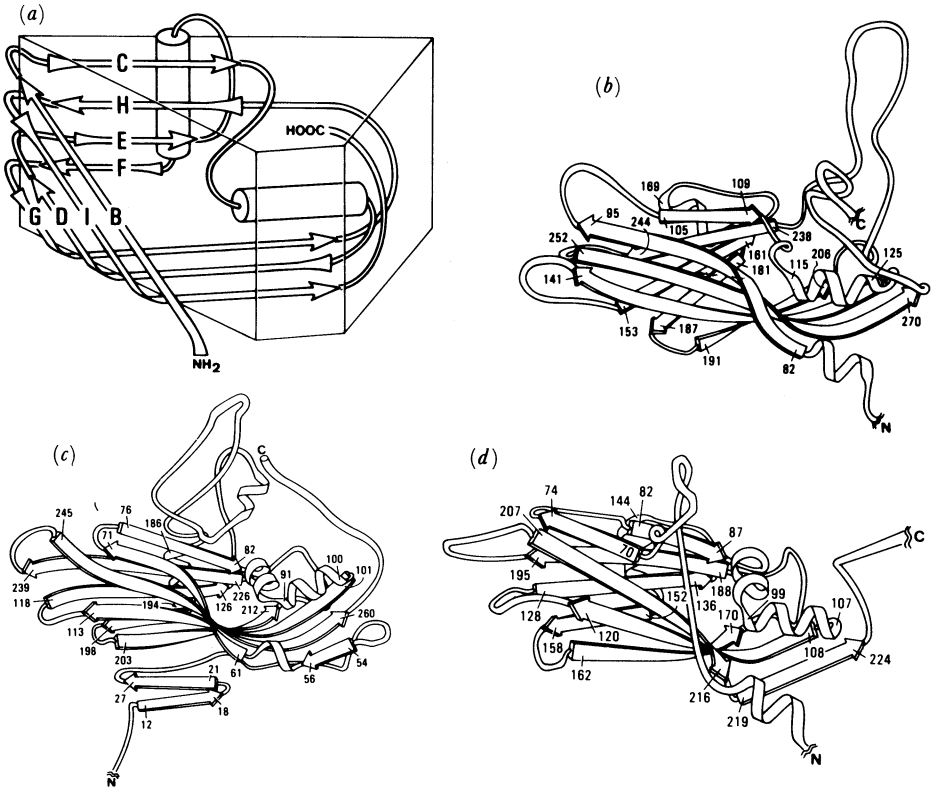

FIGURE 1. Schematic representation of the capsid proteins VP1, VP2 and VP3. (*a*) A model of the conserved core, which is common to all three major capsid proteins. Strands of the eight-stranded antiparallel β-barrel are shown as arrows and are labelled B–I in a manner consistent with the strand designations in the structurally similar cores of rhinovirus and several plant viruses. Flanking helices are indicated as cylinders. The triangular wedge indicates the overall shape of the cores. (*b*, *c*, *d*) Ribbon diagrams of VP1, VP2, VP3 respectively. Residue numbers have been included as landmarks. Extensions at the amino and carboxy termini of VP1 and VP3 have been truncated for clarity.

believe serves to direct the assembly of the particle. The carboxy terminal extensions and many of the connecting loops decorate the outer surface of the virion. As we shall show, these elaborations on the outer surface contain most of the antigenic sites of the virus.

In forming the protein shell of the virus five copies of VP1 pack around each of the particle fivefold axes, with the narrow ends of their wedge-shaped cores pointing towards the axes (figure 2). Similarly, VP2 and VP3 alternate around the threefold axes with the narrow ends of their cores pointing towards the axes. The tilts of the cores outward along the threefold and fivefold symmetry axes produce prominent radial protrusions at the particle fivefold and threefold axes. These protrusions are separated by deep circular moats located around each of the fivefold peaks. A saddle-shaped depression across the particle twofold axes connects these circular moats.

The protrusions at the fivefold axes are accentuated by three loops which connect the top three pairs of strands in the VP1 beta barrels. Likewise, the smaller protrusions at the particle threefold axes are accentuated by exposed portions of the loops connecting the top two pairs of strands at the narrow end of the β-barrels of VP2 and VP3 and by a small finger-like projection formed by residues 50–70 of VP3. A third major feature located on the outer surface of the particle near the twofold axes is formed by the large double loop that connects the E

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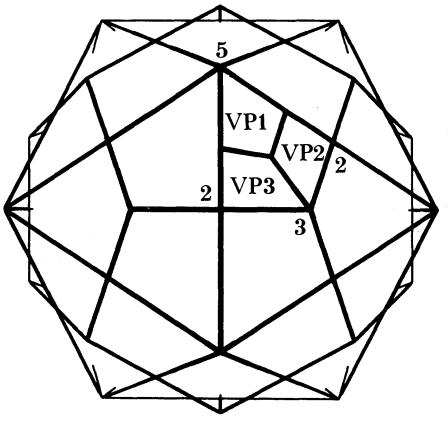

FIGURE 2. A geometric figure representing the overall shape of the poliovirus particle. The figure was generated by superimposing an icosahedron and a dodecahedron. The symmetry axes of the particle and the positions of VP1, VP2 and VP3 within a single icosahedral asymmetric unit are indicated.

and F strands of the β-barrel of VP2 (residues 126–186), by the carboxy terminus of VP2, and by the elongated loop that connects the G and H strands of the beta barrel of VP1 (residues 207–237). These three large features dominate the exposed outer surface of the virus. Each feature is approximately the same size as the antigen binding portion of an Fab, and each is separated from the others by deep depressions that prevent access by large probes such as antibody molecules. The overall appearance of the outer surface of the virion is shown in figure 3, plate 1.

ANTIGENIC SITES

The neutralizing antigenic sites of poliovirus have been characterized by sequencing mutants selected for their resistance to neutralization by monoclonal antibodies (Minor *et al.* 1983, 1985, 1986; Diamond *et al.* 1985; Blondel *et al.* 1986; Page *et al.* 1988). Such studies have been done for all three serotypes of poliovirus, although types 1 and 3 have been more extensively studied than type 2. The mutations that confer resistance to monoclonal antibodies map to a number of discrete regions of the sequence of all three of the large capsid proteins. We have determined the location of each of the mutations in the three-dimensional structure of the virus. Based on the knowledge that the antigen binding site of an Fab fragment of an antibody is approximately 30 Å in diameter, and on the assumption that the binding site is unlikely to span a deep depression on the virus surface, we have grouped the individual mutation sites into three major sites. A very similar distribution of sites has been described for rhinovirus 14 (Rossmann *et al.* 1985; Sherry *et al.* 1986).

Site 1 is located at the top of the large protrusion near the fivefold axes of the particle. This site is defined by mutations in amino acid residues from each of three distinct peptide segments in VP1: amino acids 91–102 (in strand B and the exposed loop connecting strands B and C), amino acid 254 (in strand D), and amino acid 168 in the loop connecting strands E and F (figure 4*a*, plate 1). Site 2 is located at the top of the large protrusion near the particle twofold axes (figure 4*b*). This site is defined by mutations in amino acids from two separate polypeptide segments of VP2: residues 166–170 (in the larger of the twin loops connecting strands E and F) and residue 270 (near the carboxy terminus); and one polypeptide segment of VP1 (residues 221–226, in the large elongated loop connecting strands G and H). Site 3 is located

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in and around the smaller protrusion at the particle threefold axes (figure 4*c*, plate 2). This site is defined by mutations in the finger-like protrusion of VP3 (residues 58–60) from the B strand of VP3 (residues 71 ands 73), by mutations in the loop connecting the B and C strands of VP3 (residues 76, 77 and 79), and by mutations involving amino acid 72 in the loop connecting the B and C strands of VP2. In addition to the above mutation, several mutations have been observed in the polypeptide segment near the carboxy terminus of VP1 (residues 285–289). Because these mutations are located halfway between sites 2 and 3, it has been impossible to assign them to either site on the basis of distance measurements alone. Closer inspection of the structure and of the location of the mutations, however, revealed that this loop was separated from the remainder of site 2 by the ridge of the 164–170 loop of VP2, providing a weak structural argument for the assignment of this peptide segment as a portion of site 3.

The neutralization-resistant mutants of poliovirus have been grouped independently by serological methods, by testing each mutant for neutralization by any of a large number of individual monoclonal antibodies (Minor *et al.* 1985, 1986; Page *et al.* 1988). The grouping of antigenic sites observed in these 'cross-neutralization' studies are, in general, completely consistent with the groupings based on structural considerations alone. The cross-neutralization data have confirmed the identification of the 91–102, 168, and 254 segments of VP1 as components of a single site (that is, that there are monoclonal antibodies that fail to neutralize a variant with mutations in the 91–102 segment of VP1, and that also fail to neutralize variants with mutations in residues 168 or 254 of VP1). Similarly, the cross-neutralization experiments support the conclusion that 164–170 and 270 or VP2 and 221–226 of VP2 are all included in a second site. The behaviour of the variants that map to site 3 is more complex. The cross-neutralization studies have confirmed that residues 285–289 of VP1 (at least in type 3 poliovirus) are included with residues 58–60, 71, and 73 of VP3 in forming part of a third site. These studies have also provided evidence for a link between the portion of site 3 contributed by the B–C loop of VP3 (76–79) and the B–C loop of VP2 (residue 72). Curiously, however, the cross-neutralization studies have so far failed to demonstrate a link between the portions of site 3 contributed by the finger-like insertion and the B strand of VP3 with the portion contributed by the B–C loops of VP3 (residues 76–79) and VP2 (residue 72). The failure to demonstrate a linkage between these two portions of site 3 might be due either to a failure to select the appropriate antibody and mutants, or to the possibility that these two subsites actually function as completely independent antigenic sites. For the present, we have identified the two subsites as site 3A (VP1 285–289, VP3 58–60, 71, 73) and site 3B (VP3 76–79, VP2 72).

Accessibility of antigenic sites to antibodies

An assumption that is made implicitly in interpreting the neutralization behaviour of monoclonal escape mutants is that the mutated residue is itself a part of the physical site bound by the antibody. Although this is the simplest explanation for the mechanism by which a variant escapes neutralization, there has been some discussion of the possibility that a mutation located outside the binding site might permit an escape from neutralization, either by altering the structure of the binding site or by preventing some structural transition required for neutralization (Diamond *et al.* 1985; Blondel *et al.* 1986).

Examination of the mutations selected by monoclonal antibodies shows that the majority of the altered sidechains are accessible to antibody-sized probes. One very striking example of this property is the VP1 91–102 component of site 1 in type 3 poliovirus. In this region mutations

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are observed at residues 91, 93, 95 and 97–102. The occurrence of mutations only in the odd-numbered positions at the amino terminal portion of this site is related to the structural observation that residues 91–95 are located in the B strand of the VP1 β-barrel. In a β-sheet the side chains always project from alternating sides of the sheet. In the B strand of VP1 the odd-numbered sidechains are exposed on the outer surface of the β-barrel, whereas the even-numbered sidechains are buried inside the β-barrel. In consequence, residues 91, 93, 95 and 97 are accessible to antibodies, whereas residues 92, 94 and 96 are not. In this instance, however, the observed distribution of mutations might not be a simple consequence of the exposure of the odd-numbered residues, considering that the even-numbered residues are packed together in a fairly specific way with other sidechains in the hydrophobic interior of the barrel. Mutations in these interior residues might therefore fail to yield viable virus.

In an effort to put the issue of sidechain exposure on a more objective basis, we have evaluated the degree of exposure of all residues on the outer surface of the type 1 Mahoney structure by using a novel algorithm developed by John Tainer and Elizabeth Getzoff at Scripps Clinic. This algorithm determines the radius of the largest spherical probe tangent to each point on the virus surface that excludes all of the other surface points. This analysis has revealed several interesting trends (Page *et al.* 1988). First, the majority of residues that have been observed as monoclonal release mutations in type 1 poliovirus are exposed to spherical probes at least as large as the antigen-binding portion of an Fab. Second, the mutated amino acids that are exposed to a lesser extent are located close to fully exposed sites and participate in interactions that stabilize the conformation of exposed loops (which form the antigenic sites directly) in an obvious way. In no case have we observed a mutation that would require long-range propagation of a structural alteration to effect escape from neutralization. Third, the calculation has identified several groups of residues that lie close to the known antigenic sites, and that are highly accessible to antibody-sized probes, but have not yet been observed as sites of monoclonal release mutations. These include the loops connecting the H and I strands of VP2 and VP3, the carboxy terminus of VP1 (in or near site 3), and the loop connecting the D and E Strands of VP1 (in or near site 1). The failure to select for mutations in any of these residues may indicate that mutations in the site reduce the viability of the virus. Alternatively, the failure to observe these mutations may simply reflect incomplete sampling owing to the large but finite number of monoclonal antibodies and mutants used in screening.

Use of synthetic peptides to characterize antigenic sites

An alternative and complementary approach to the identification of antigenic sites is to screen antibodies raised against synthetic peptides (which correspond to portions of the linear sequences of the viral proteins) for their ability to bind or neutralize virus. A number

Description of plate 1

Figure 3. A space-filling model of the poliovirus particle. VP1 is blue, VP2 is light yellow, and VP3 is red. The orientation of the particle is the same as that shown for the geometric solid in figure 2.

Figure 4. The antigenic sites of poliovirus. Each panel contains isolated copies of VP1, VP2 and VP3 on the left and an intact particle on the right. VP1 is blue, VP2 is yellow, and VP3 is red. The sites of mutations which confer resistance to neutralizing monoclonal antibodies are highlighted in white. The mutations have been grouped by spatial considerations and by cross-neutralization studies into three general sites as described in the text. (*a*) Site 1; (*b*) site 2; (*c*) site 3; and (*d*) all sites. Section (*d*) illustrates that the mutation sites occupy the outermost features of the particle and are absent from the depressions in the virus surface.

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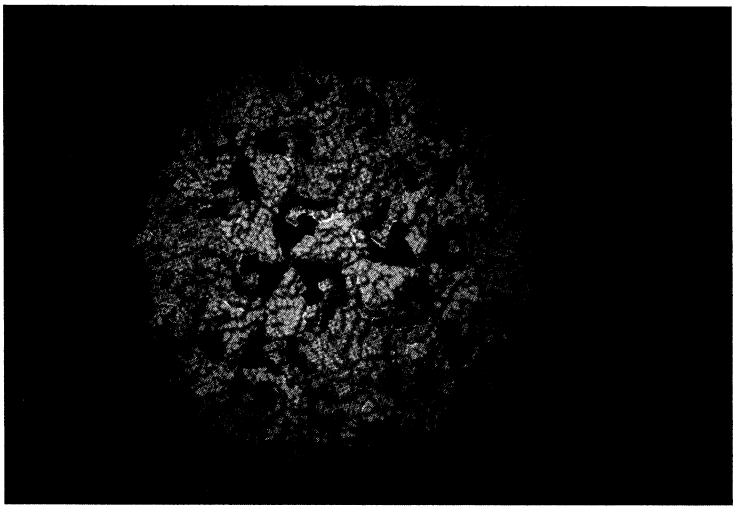
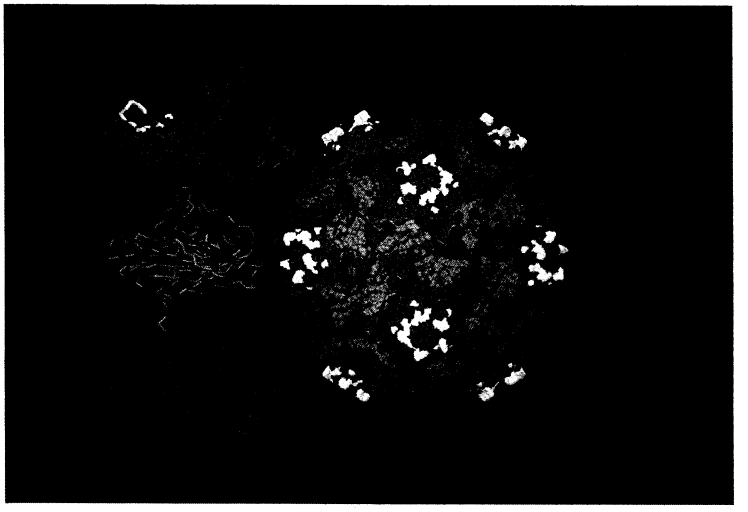
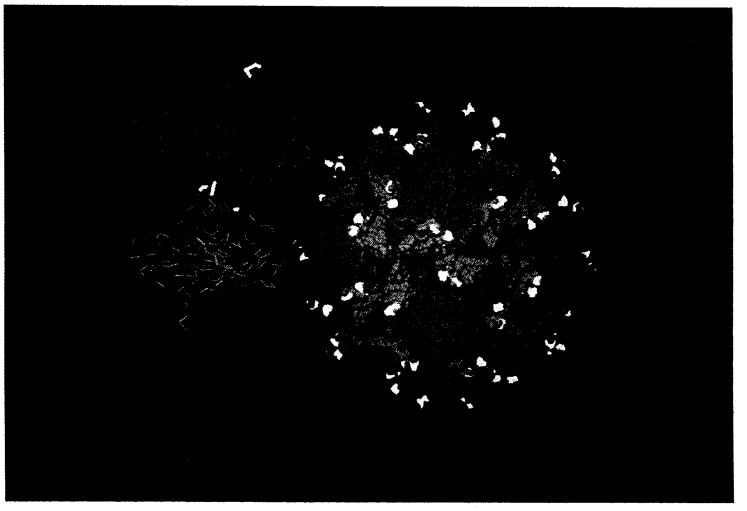


4 *a*



b



FIGURES 3 AND 4*a*, *b*. For description see opposite.

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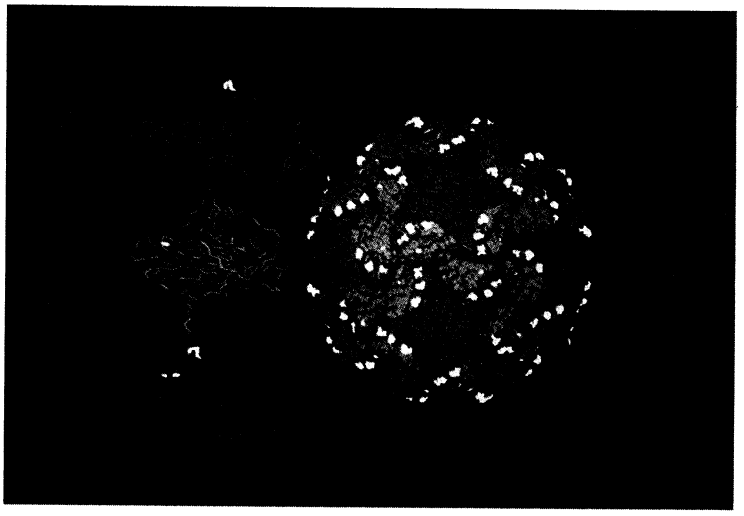
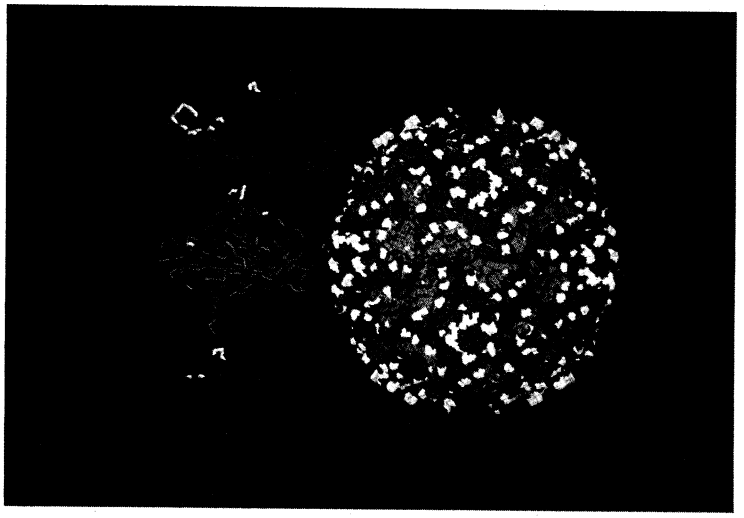
4 *c*



d



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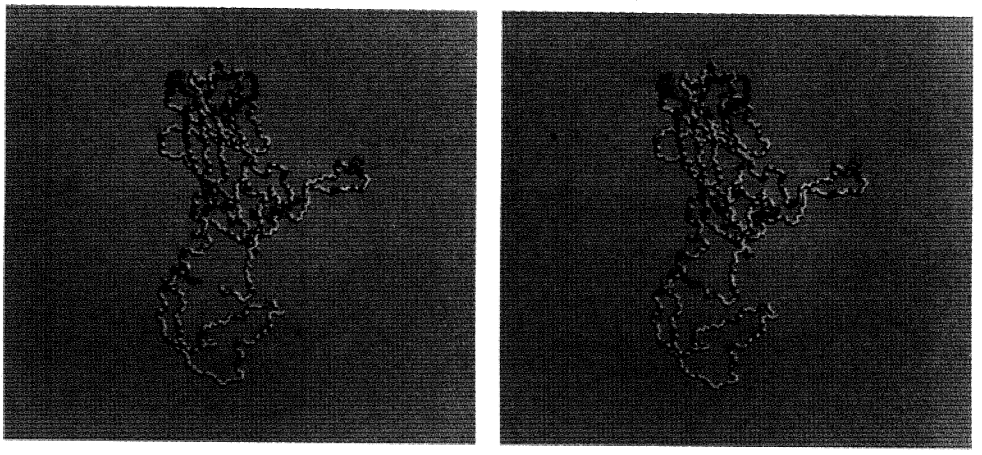

FIGURES 4*c*, *d* AND 5. For description see facing plate 1.

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of investigators have used this method to characterize the antigenic sites of poliovirus (Emini *et al.* 1983, 1984; Blondel *et al.* 1986; Chow *et al.* 1985; Ferguson *et al.* 1985; Minor *et al.* 1986; Diamond *et al.* 1985). Many of the antigenic sites identified by these studies correspond to the sites defined by the monoclonal antibody studies. Specifically, synthetic peptides corresponding to the B–C loop (Emini *et al.* 1983; Chow *et al.* 1985; Ferguson *et al.* 1985) and H–I loop at the narrow end of the β-barrel of VP1 and the E–F loop of VP1 (site 1) (Chow *et al.* 1985), the G–H loop of VP1 (Chow *et al.* 1985) and the E–F loop of VP2 (site 2) (Emini *et al.* 1983), and the carboxy terminal loop of VP1 (site 3) (Chow *et al.* 1985) have been shown to induce a neutralizing response to poliovirus or to prime for a subsequent neutralizing response upon subsequent inoculation with a sub-immunizing dose of virus.

The general similarity between many of the sites identified by synthetic peptide antibodies and those identified by monoclonal escape mutations has interesting implications for the classical distinction between sequential (linear) and conformational (three-dimensional) epitopes. Historically, synthetic peptide antibodies have been used to define sequential epitopes. In contrast, the antigenic sites of poliovirus, as identified by collecting the results of the monoclonal release studies, are clearly conformational. The correspondence between the sites identified by the two operational definitions suggests that, at least in the case of poliovirus, the antipeptide antibodies recognize sequentially contiguous portions of larger conformational sites, and that the distinction between sequential and conformational sites may be largely semantic.

Several of the peptides that have been shown either to elicit or to prime for a neutralizing response against poliovirus have not yet been observed in any of the monoclonal escape experiments. In particular, a peptide corresponding to the third loop (D–E loop) at the narrow end of the barrel of VP1 has been shown to prime for a neutralizing response (Jameson *et al.* 1985). This loop is highly exposed in the virion and is located close to the residues that form site 1. Once again, the failure to observe monoclonal escape mutations in this loop might reflect either the limited (albeit large) sampling of mutations or restrictions on the viability of virus having mutations within this loop. Indeed, this loop has been identified as a site of monoclonal escape mutations in rhinovirus 14 (Rossmann *et al.* 1985).

For some of the antipeptide antibodies, however, the neutralizing response is more difficult to explain because the immunogenic peptides correspond to portions of the capsid protein that are located in the interior of the native virus. In particular, two of these peptides correspond to sequences in the amino-terminal extension of VP1 (Emini *et al.* 1983; Chow *et al.* 1985) and to the F–G loop of the β-barrel of VP1 (Chow *et al.* 1985). The ability of antibodies raised against these peptides to neutralize poliovirus may be related to conformational alterations in the capsid structure which are induced or, more probably, trapped by the antibodies.

Description of plate 2

Figure 4 (*c–d*). For description see page 472.

Figure 5. Significant differences in the main chain conformation of VP1 between the Mahoney strain of type 1 and the Sabin strain of type 3 poliovirus. In this stereo pair, structurally conserved main chain atoms are white. Differences, which are observed only in the loops and terminal extensions of the capsid proteins, are shown in colour. The B–C loop (yellow in Mahoney 1 and dark blue in Sabin 3) involves the replacement of proline residues in a loop having substantial sequence differences. The D–E loops (magenta in Mahoney 1 and green in Sabin 3) are significantly different in the two structures despite their general similarity in sequence. The highly localized structural changes due to one-residue insertions or deletions are indicated in red in Mahoney 1 and cyan in Sabin 3. The residues indicated in orange represent a portion of the amino terminal extension (residues 20–23) which is ordered in Mahoney 1, but which is disordered in Sabin 3.

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When poliovirus attaches to susceptible cells its capsid proteins undergo a concerted conformational rearrangement that results in the release of the internal protein VP4, a decrease in sedimentation coefficient from 160 S to 135 S, changes in antigenicity, and a loss of infectivity due to a loss of the ability to attach to cells. A similar if not identical form of the virus is the predominant species found inside cells soon after infection, and the conformational change is widely believed to constitute an early step in the uncoating of the virus. We have shown that this conformational rearrangement results in the extrusion of the amino terminus of VP1 (Fricks & Hogle 1989), and that (human) oral vaccinees produce significant levels of antibodies that are specific for the amino terminus of VP1 in cell-released particles (M. Kubitz, C. Fricks & J. M. Hogle, unpublished data). Although we have been unable to determine if these antibodies are capable of neutralizing virus, we believe that it is relevant that the immunization of non-permissive hosts (rabbits and mice) fails to elicit antibodies that bind specifically to the exposed amino terminus. Thus experiments using non-permissive hosts may fail to identify antigenic sites whose exposure depends on conformational changes (or even chemical modifications) that take place during the normal replication cycle in a susceptible host.

Serotype-specific differences in antigenic sites

In the discussion above, our description of the structures of the antigenic sites of poliovirus has ignored strain and serotypic differences. Although this assumption is useful in a general outline of the antigenic structure of the virus it is, in fact, a serious over-simplification. Monoclonal escape experiments reveal a substantial serotype specificity in the extent to which each of the three general sites is represented in the immune response to each of the three serotypes of poliovirus.

Early observations with the Leon strain of type 3 poliovirus and with its attenuated derivative Sabin 3 strain have indicated that the vast majority of monoclonal antibodies to the Leon-derived lineage of type 3 poliovirus were specific for site 1 (Minor *et al.* 1983, 1985). Indeed, because the systematic studies of type 3 poliovirus predated the studies of type 1, this site became widely known as the immunodominant site of poliovirus. In contrast, subsequent experiments with the Mahoney lineage of type 1 poliovirus have found that the vast majority of the murine neutralizing-antibody response is directed against sites 2 and 3 (Diamond *et al.* 1985; Minor *et al.* 1986; Blondel *et al.* 1986; Page *et al.* 1988).

Structural basis for serotypic differences

We have now solved the structures of two strains of poliovirus, namely the Mahoney strain of type 1 and the Sabin (attenuated vaccine) strain of type 3 poliovirus. A comparison of the two structures provides some insight into the reasons for the difference in the relative importance of site 1 in type 1 and type 3 polioviruses and into the more general question of the structural basis for serotypic differences in polio and related viruses.

The Sabin 3 and Mahoney 1 strains of poliovirus exhibit 83% sequence identity at the amino acid level in the capsid protein. Consistent with the high degree of sequence homology between the two strains, the structures of P3/Sabin and P1/Mahoney are strikingly similar. This structural similarity is especially pronounced in the cores of the capsid-protein subunits, but is also seen in the amino-terminal extensions and in may of the connecting loops (see figure 5, plate 2). Significant differences in the conformation of the main chain are observed only in

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the exposed loops and in the terminal extensions of the virus. These structural differences fall into three general categories: (1) differences in loop conformation due to insertions in one strain relative to the other; (2) loops with several sequence differences, *including the replacement of a proline residue*; and (3) differences observed at points of transition between ordered and disordered structure.

All of the insertions occur in exposed loops (VP1 221, 289; VP2 138), or in the disordered amino terminus of VP1. The observable insertions/deletions cause limited, highly localized structural perturbations, which have little effect further than one or two residues on either side of the insertion (figure 5).

The most significant conformational difference between the Sabin 3 and Mahoney 1 structures occurs in the loop that connects the B and C strands of the β-barrel of VP1 (residues 95–104), which constitutes the major portion of antigenic site 1 (figure 5). In this loop the difference between equivalent α-carbon positions is as large as 8Å. Of the six sequence differences in this loop, the most obvious causes of the conformational difference are the substitution of Glu (Sabin 3) for Pro (Mahoney 1) at position 95 and the substitution of Pro (Sabin 3) for Ser (Mahoney 1) at position 97. Consistent with the greater importance of this loop as an antigenic site in type 3 poliovirus, the B–C loop is considerably more exposed in the Sabin 3 structure than it is in the Mahoney 1 structure. A large structural difference associated with the replacement of a proline residue in an exposed loop having several other sequence changes also occurs in the B–C loop of VP3, and in the H–I loop of VP2. In contrast, the E–F loop of VP2 (residues 160–170, part of antigenic site 2), which is an area of high sequence variability, but which does not involve the substitution of a proline, is nearly identical in structure in the two poliovirus strains.

It may be relevant that significant structural differences occur in all three of the major antigenic sites of the virion (Hogle *et al.* 1985; Page *et al.* 1988). In particular, structural changes are seen in the B–C loop of VP1 (which constitutes a major portion of antigenic site 1), in the insertion in the G–H loop of VP1 (site 2), in the insertion at position 289 of VP1 (site 3A), and in the B–C loop of P3 (site 3B). This suggests that differences in three-dimensional structure, as well as simple sequence changes, might play an important role in determining serotypes.

Because of its importance as an antigenic site, the B–C loop of VP1 has been the focus of considerable attention. The sequence of this loop has been determined for a number of strains of all three serotypes of poliovirus (Minor *et al.* 1987). Recently, three research groups have reported the construction of viable intertypic chimeras in which the B–C loop from one strain of poliovirus has been replaced by the corresponding sequence from a different serotype. These chimeras include two examples in which a type 3 loop (Burke *et al.* 1988; Murray *et al.*1988) and one example in which a type 2 loop (Martin *et al.* 1988) has been constructed into a type 1 background. In every case, the hybrid displays the appropriate mosaic antigenicity and is able to induce neutralizing antibodies against both parental serotypes. In the type 2/type 1 chimera, replacement of the B–C loop in the primate-specific Mahoney 1 strain with the corresponding loop from the mouse-adapted Lansing 2 strain produces a hybrid that is able to cause fatal paralysis in mice (Martin *et al.* 1989). This demonstrates that the replacement of this ten-amino-acid loop (which includes only six sequence changes) is sufficient to confer mouse adaptation on the Mahoney 1 strain.

The type 2/type 1 chimera grows to very high titre in cultured cells. We have received seed stocks of this virus from M. Girard (Pasteur Institute), and have produced crystals that are

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highly suitable for high-resolution crystallographic study. This structure has recently been solved by molecular replacement methods, and a preliminary atomic model has been built to fit the electron-density map. The 95–104 loop has a significantly different conformation from that observed in either the Mahoney 1 or the Sabin 3 structure (Yeates *et al.* 1989).

Other factors that contribute to observed serotypic differences

Although structural differences may provide a partial explanation for serotype-specific differences in the degree of immunodominance of site 1, studies in which this site is selectively destroyed by proteolysis have indicated that other factors must be important as well (Icenogle *et al.* 1986). A number of strains of poliovirus contain a trypsin-sensitive cleavage site in the 95–104 loop of VP1 (Fricks *et al.* 1985). Cleavage at this site has no effect on the infectivity of the virus *in vitro*, but it prevents monoclonal antibodies that recognize site 1 from binding or neutralizing the virus. Evaluation of the binding or neutralization titres of polyclonal sera against both native and cleaved virus has provided a convenient assay for the percentage of the response that is directed against site 1.

These assays have confirmed the results of monoclonal escape mutation experiments, by showing that site 1 dominates the immune response of several inbred strains of mice to the Leon-derived strains of type 3 poliovirus, but not to the Mahoney-derived strains of type 1 poliovirus. The degree of dominance, however, varies reproducibly with the strain of mouse immunized, ranging from 75% in the SJL/J strain to 95% in the C57BL/6 strain. Studies with congenic strains of mice have failed to reveal any clear-cut relation between the degree of dominance of site 1 in the anti-type 3 response and the major histocompatibility complex type of the mouse immunized. Similar studies with outbred species, including horse, rabbit and monkey, have shown that the degree of dominance of site 1 in the anti-type 3 response varies with the species immunized (30% in the horse and 10% in monkeys) and perhaps with the route of administration.

It may be especially significant that site 1 appears to play little role in the anti-type 3 response in oral (trivalent Sabin) vaccinees (Icenogle *et al.* 1986). Indeed, several lines of evidence, including studies which follow the mutations accumulated during the course of virus replication in oral vaccinees, studies of the relative titres against cleaved and uncleaved virus, and direct studies of the susceptibility of excreted virus to site-1-specific monoclonal antibodies, suggest that the virus produced in the intestinal tract is itself proteolytically processed (Minor *et al.* 1987; Roivainen & Hovi 1987).

Relation between antigenic sites and possible receptor binding sites

When the sites of neutralization-resistant mutants are considered together, it is apparent that collectively they occupy the protrusions that constitute the outermost surface features of both poliovirus and rhinovirus. These protrusions are separated by depressions in which escape mutations have not been observed, and that exhibit a relatively greater degree of sequence conservation in different strains of each type of virus.

Rossmann and his colleagues have suggested that the largest of the surface depressions (namely the 'canyon' surrounding the protrusion at the fivefold axis) probably contains the receptor sites of rhinovirus and poliovirus (Rossmann *et al.* 1985). They argue that this depression is unique in that it is sufficiently deep to prevent antibodies from binding to the

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residues at the bottom of the depression, and they emphasize that the protection from antibody binding, and hence from immune selection, makes this site a particularly attractive candidate for a receptor site.

We believe, however, that the focus on the 'canyon' to the exclusion of other portions of the surface may be premature. Like all RNA viruses, the picornaviruses exhibit a high rate of error during replication, producing occasional mutations distributed through the genome. Lethal mutations in the receptor-binding site are, in fact, made frequently. Because these mutations are lethal, they can neither be selected for nor against by immune pressure. Furthermore, it is not necessary that the receptor site be entirely inaccessible to antibodies. For example, although the receptor site of the influenza haemagglutinin is located in a depression (Weiss *et al.* 1988), the depression is too shallow to protect completely the residues involved in sialic acid binding from antibody binding. Instead it appears that the receptor site is protected by surrounding it by loops that can accommodate mutations (Wiley & Skehel 1987; Weiss *et al.* 1988). The distribution of antigenic sites in both poliovirus and rhinovirus 14 suggests that a similar mechanism of protection may occur in the picornaviruses. Decoration of the outer surface of the virion with mutable, flexible loops may thus represent a common mechanism by which animal viruses maximize their survival in the presence of a neutralizing humoral immune response.

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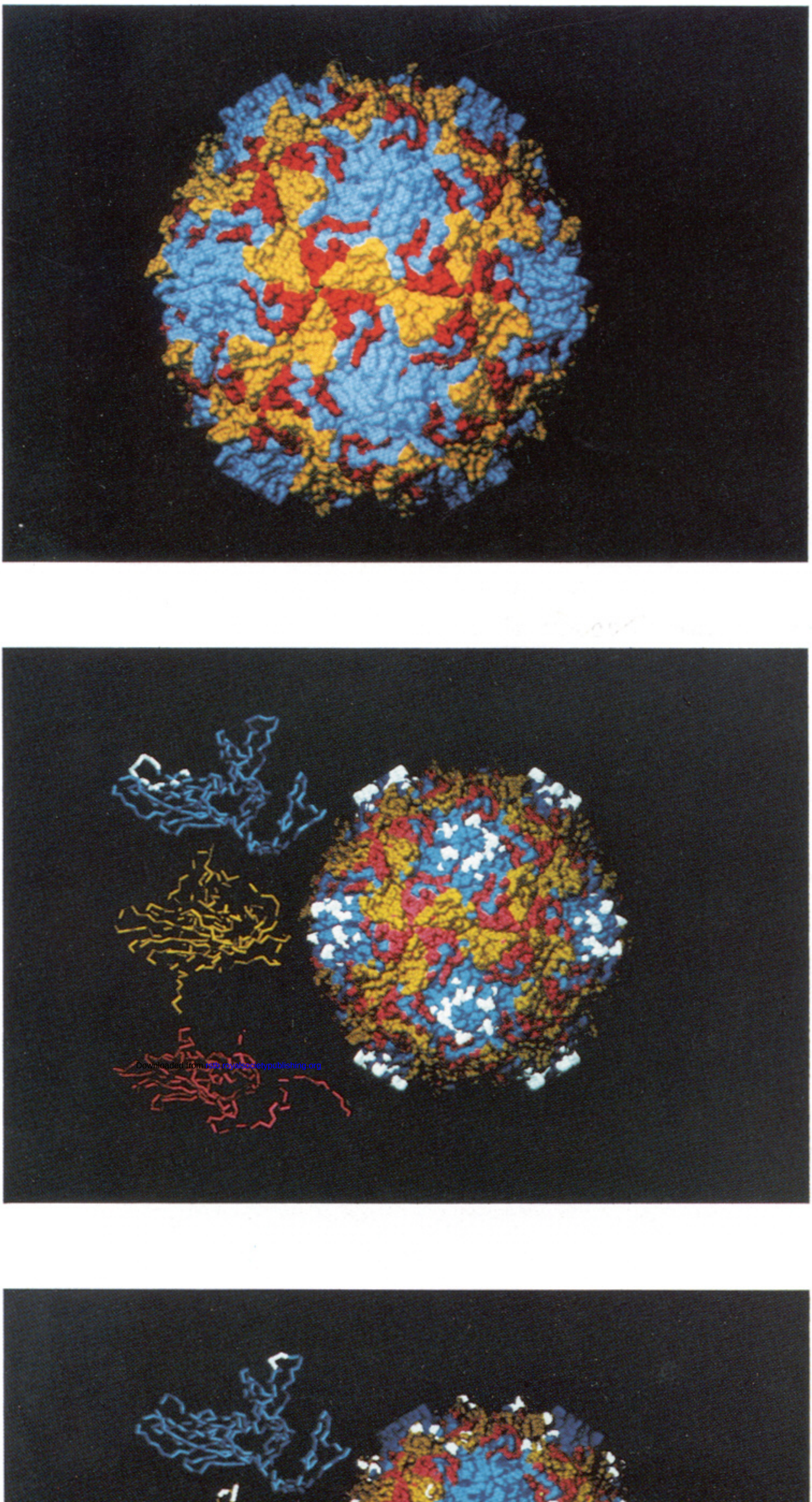
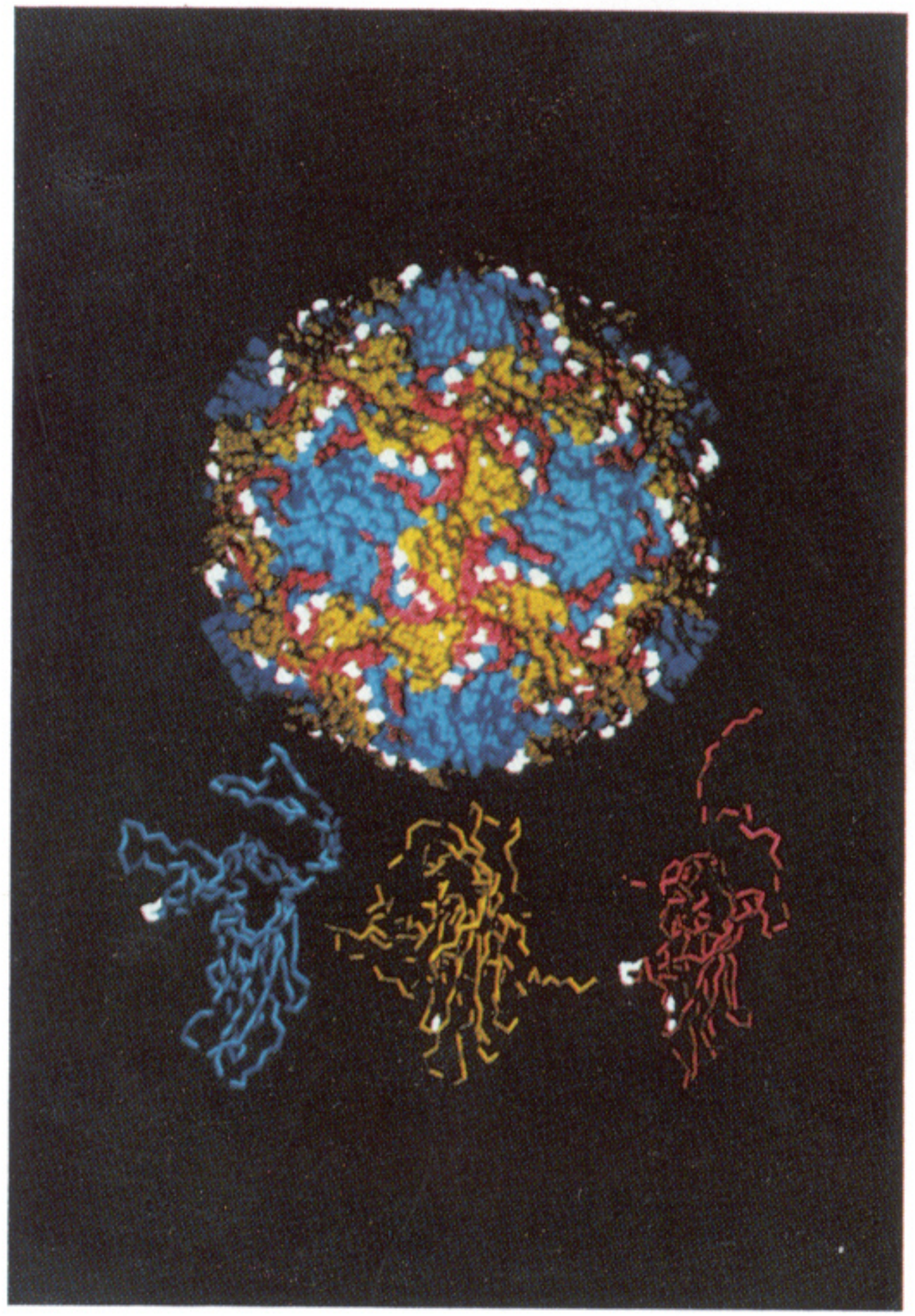
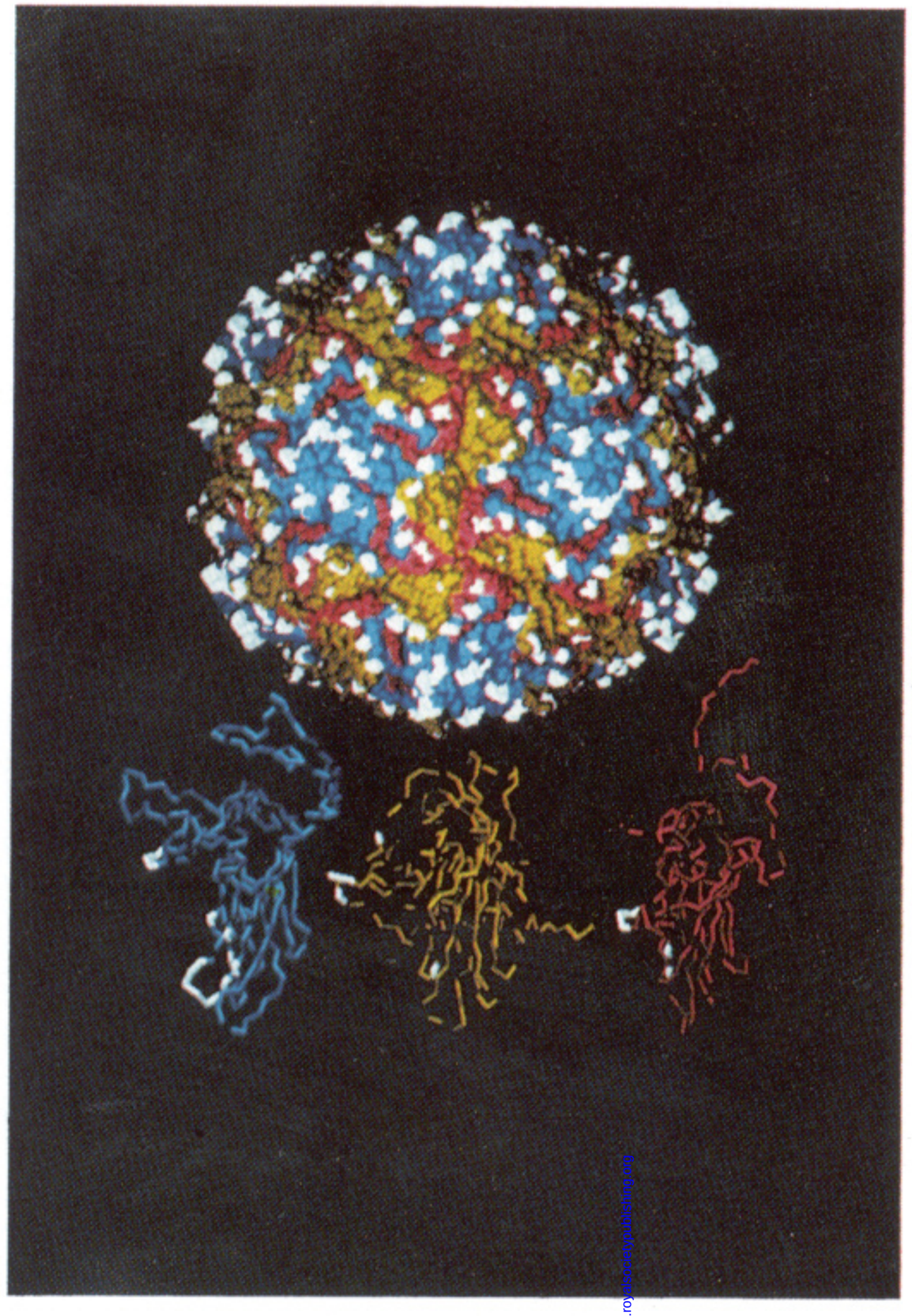


FIGURES 3 AND 4*a*, *b*. For description see opposite.



4 *c*



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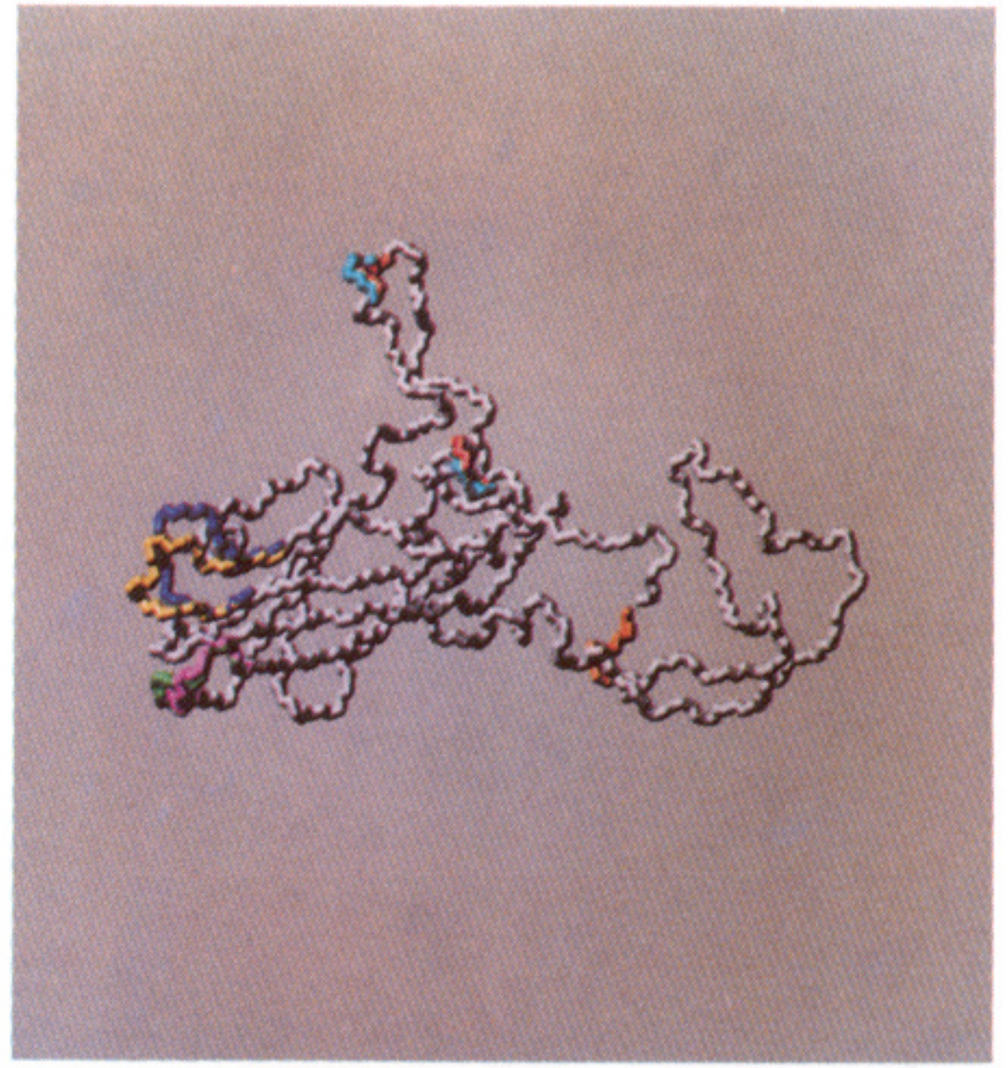

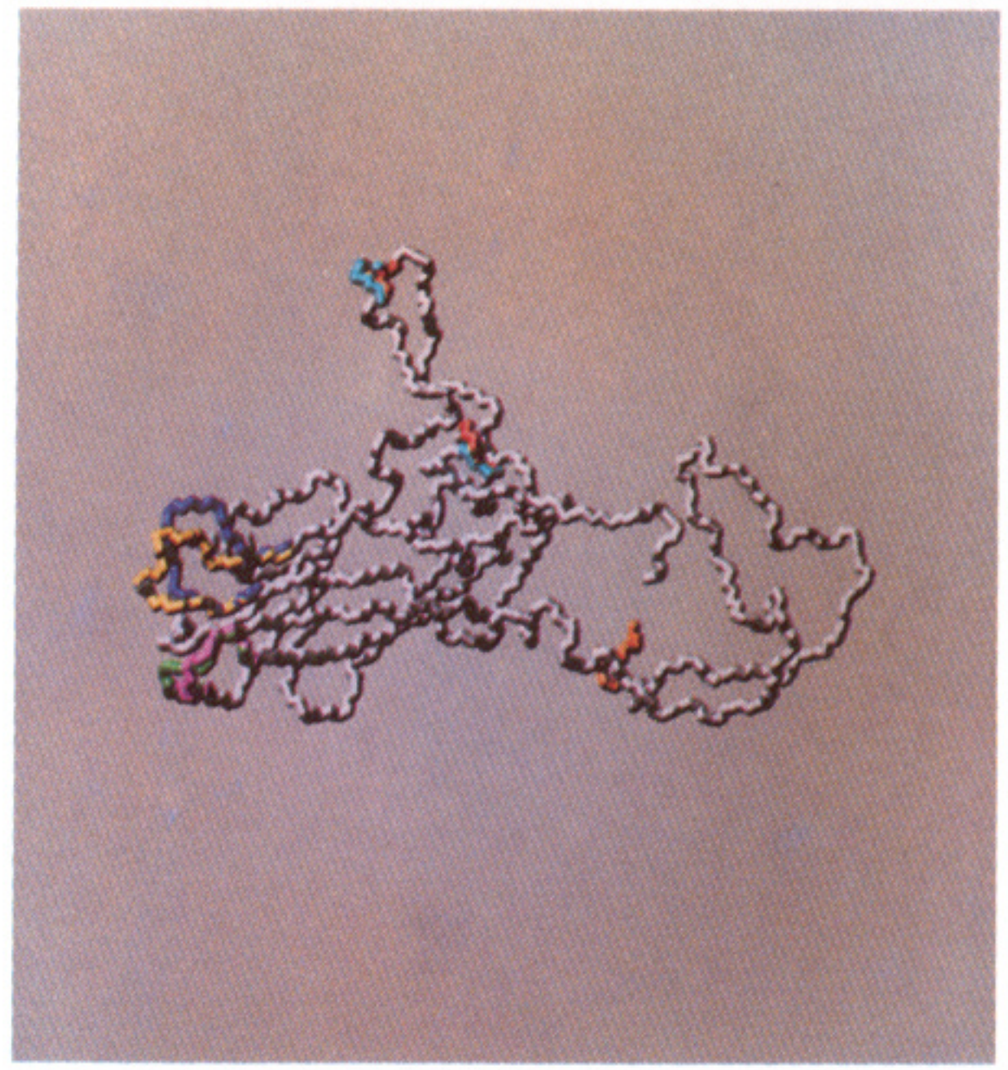

5

FIGURES 4*c*, *d* AND 5. For description see facing plate 1.