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Conformational Shift of a Major Poliovirus Antigen Confirmed by Immuno-Cryogenic Electron Microscopy

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Small, interfacial conformational changes occur in some Ag–Ab interactions. Using cryogenic electron microscopy (cryo-EM), we have demonstrated such changes in a major antigenic site of a poliovirus capsid protein. During cell entry, native human poliovirus (160S particle) converts to a cell entry intermediate (135S particle) and later to an RNA-released (80S) particle. By mixing particles with Fabs of the neutralizing C3 mAb, we labeled the external loop connecting the B and C β-strands (BC loop) of the capsid protein VP1 (residues 95–105) in the 160S and 135S states. We then determined three-dimensional structures by cryo-EM and enhanced their interpretability by fitting high-resolution coordinates of C3 Fab and the capsid proteins into the density maps. Binding of C3 to either 160S or 135S particles caused residues of the BC loop, located on the tip of a prominent peak known as the “mesa,” to move by an estimated 5 Å. C3 Abs are neutralizing and can bind bivalently. The orientation of the bound Fabs in our reconstructions suggests that C3 neutralizes poliovirus by binding two adjacent BC loops on the same mesa and inhibiting conformational changes in the viral capsid. The Journal of Immunology, 2013, 191: 884–891.

Abbreviations used in this article: BC loop, a loop connecting the B and C β-strands of the poliovirus protein VP1; C3, an mAb to poliovirus VP1 protein; cryo-EM, cryogenic electron microscopy; CTF, contrast transfer function; RMSD, root-mean-square deviation; 80S, RNA-released poliovirus particle; 135S, cell entry intermediate poliovirus particle; 160S, native poliovirus virion; σ, SDs above the average.

Materials and Methods

Preparation of viral and Fab complex

Poliovirus virions (160S particles) and C3 Fab were prepared as described previously (12, 14, 15). 135S particles were prepared by heat treatment (50°C for 3 min) of 160S particles in low-salt buffer containing 2 mM CaCl₂ (13, 15). Equal volumes of 160S particle (3.9 mg/ml) and C3 Fab (1.7 mg/ml) solutions were mixed, giving a Fab/virus ratio of 74:1. A similar ratio was used to mix 135S particles and C3 Fabs.

Electron microscopy

Mixtures of poliovirus (160S or 135S) and C3 Fab were suspended over beryllium films, vitrified, and imaged as described previously (16). A CM200 electron microscope (FEI, Hillsboro, OR) equipped with a Gatan 626 cryoholder (Gatan, Pleasanton, CA) was used. Focal pairs of micrographs were recorded at magnifications of 38,000× at 120 kV. The electron dose was ~14 electrons/Å².
Particle images were extracted, processed, and normalized as described (17). Focal settings ranged from 0.73 to 1.77 μm underfocus. Focal-pair images were computationally combined for orientation determination but used separately for origin determination and computation of the three-dimensional (3D) reconstruction. A model determined via common lines (18) and a previous reconstruction (17) were used to begin iterative projection matching to determine particle orientations and origins for the 160S-C3 and 135S-C3 reconstructions, respectively (19, 20). Bsoft routines were used to assess and correct for contrast transfer function (CTF) effects (21) via the algorithm by Conway and Steven (22), but images within a focal pair were not combined during CTF correction. However, for orientation and origin determination, only phase-flipping CTF corrections were performed. Spherically averaged density plots were used to calibrate size (21, 23) against previously solved structures (17) and a poliovirus x-ray crystal structure (2). For surface renderings of 3D maps, contour levels were determined by the number of SDs (σ) above the average map density.

**Modeling**

Atomic coordinates for the C3 Fab-peptide complex (12) (1FPT in Protein Data Bank) were first fitted "by eye" into the 160S-C3 and 135S-C3 density maps by use of the UCSF (University of California, San Francisco) Chimera package (24) or the program O (25). Next, a core-weighted, rigid body fitting algorithm, implemented in CHARRM (26), was used to refine the fits. In determining placement of the coordinates in the cryo-EM map, the CHARRM routine computed a "theoretical map" from the atomic coordinates at the same resolution as the cryo-EM map and then aligned the internal ("core") density and overall domain shape of the theoretical and cryo-EM structures. The fit was then observed graphically and was judged correct when the overall shape of the coordinates matched that of the observed map. The Fab Ag-binding or variable domain and the Fab non–Ag-binding or constant domain were fitted separately. We included peptide coordinates (corresponding to residues 97–103 of VP1) in our fitting of the variable domain. To fit the Ag-binding domains, five Fabs were fitted separately to the five tips of one mesa. Symmetry was applied so that a single Fab binding site contained five overlapping sets of fitted coordinates. Then, the overlapping coordinates were averaged and used to calculate fitting errors. The root-mean-square deviation (RMSD) of the five fits was computed as the fitting error. In UCSF Chimera (24), the separately fitted constant and variable domains of the Fab were joined and elbow-angle residues were energy-minimized. The program RBOW (27) was then used to calculate elbow angles.

The C3 Fab coordinates included a peptide Ag, residues 93–103 of VP1 (12). Residues 97–103 were visible in the binding cleft of the C3 Ag-binding domain, and we compared the fitted Co positions of these seven residues to the positions of the corresponding residues in the 160S crystal structure (2) and the 135S pseudo-atomic model (13) placed into the 160S-C3 and 135S-C3 maps, respectively. Using model and fitted coordinates, we used the match alignment and measure rotation functions in UCSF Chimera (24) to determine the shift and twist of the BC loop between the 160S and 135S models and the shift and twist between the peptide Ag in the 160S-C3 and 135S-C3 fitted coordinates. This shift and twist was an axis determined by the algorithm. For a pseudoatomic model of the virus-Fab complexes, peptide residues 97–103 from the fitted Fab coordinates were used to replace corresponding residues for VP1 in the 160S crystal structure (2) and in the 135S pseudoatomic model (13). These seven residues were joined to the unaltered B and C β-strands via energy minimization (in UCSF Chimera) (24) of residues 95–96 and 104–105. The UCSF Chimera (24) and Bsoft (21) packages were used to prepare images of modeled coordinates and cryo-EM reconstructions.

**Results**

We computed 3D reconstructions of the 160S-C3 and 135S-C3 complexes by cryo-EM (Figs. 2, 3A, Table I). Their resolutions were 11 and 9 Å, respectively. As expected, C3 Fab binds to the tip of the mesa, which is where the BC loop resides in both the 160S (2) and 135S (13) particle states (Figs. 2, 3). Five C3 Fabs surround each mesa, giving it the appearance of a five-petaled flower, C3 Fab density is as strong as the capsid protein density (Fig. 2C), indicating full or nearly full occupancy of all 60 binding sites per virus. Particularly in the 135S-C3 reconstruction, Fab Ag-binding domains show the appearance of β-sheets (for which ~10-Å resolution is needed; compare with Ref. 33) and indicate that the bound Fabs are firmly held in place (Fig. 2C, white arrows).

Coordinates of the C3 Fab-peptide complex (12) were fitted into the cryo-EM density maps and used to infer the position of the BC loop in Fab-bound 160S and 135S particles. For the Ag-binding (variable) domain, five regions of C3 Fab density around one five-fold vertex were used to fit coordinates, and we report the average (after symmetry operations to bring four of the five fittings to one position) of all five fits. Only one region was used to fit the non–Ag-binding (constant) domain. The elbow angle of C3 Fab varied significantly between the 160S-C3 (168.6°) and 135S-C3 (182.7°) states and both differed from the angle in the Wien et al. (12) crystal structure (132.6°). Coordinates from a previously determined crystal structure of the 160S particle (2) and a pseudoatomic model of the 135S particle (made by fitting 160S coordinates into a 135S cryo-EM map) (13) were placed, initially without adjustment, in the 160S-C3 and 135S-C3 maps and used for comparison. In the 135S preparation used to make the complex, a few of the particles were 80S and were readily recognizable as such from their low-density centers (Fig. 2A, black arrow) (compare with Ref. (15). A reconstruction of the 80S-C3 complex was also computed, although the small number of particles limited the resolution (Table I). As expected, the 80S-C3 reconstruction showed that the C3 Fab bound with high occupancy in a position and orientation similar to that seen in the higher resolution 135S-C3 complex (Supplemental Fig. 1 and Supplemental Video 3).

**Particle expansion during 160S-to-135S transition**

Our measurements from the 160S-C3 and 135S-C3 maps indicate that during the 160S-to-135S transition, the BC loop moves radially to the same extent than it does in undecorated poliovirus (Fig. 2B, 2C, Supplemental Table I, Supplemental Videos 1, 2). Assuming that the peptide in the C3 Fab crystal structure (fitted into the 160S-C3 and 135S-C3 maps) represents the correct position of the BC loop, the BC loop moved radially outward by 8 Å (5.0%) during the 160S-to-135S transition. Comparison of fitted coordinates of the five C3 densities surrounding a single five-fold vertex showed that the distance between the BC loop and the center of the mesa increased by 1 Å. These movements are consistent with previous observations of the undecorated 160S and 135S particles, where comparisons of cryo-EM reconstructions showed a 4% expansion along the five-fold symmetry axis (from top of the
mesa to center of the particle) (17). Measurements of published models of the BC loops (2, 13) indicated that the distance from the center of the capsid increased by 7 Å (4.5%), and the distance between BC loops and the center of mesa increased by 1 Å. Therefore, positioning of the modeled BC loop in the 160S-C3 and 135S-C3 complexes is consistent with placement of the BC loop in models of 160S (2) and 135S (13) particles.

Ab binding changes BC loop position

One of the issues encountered in fitting atomic-resolution structures into lower resolution cryo-EM reconstructions (∼10 Å in this study) is the need to minimize the number of parameters. Usually, this need is met by using rigid-body approximations for protein subunits, which limits the number to six, that is, three translational and three rotational parameters. In this study, the prominent marker provided by the Fab density and the presence of the BC loop peptide in the C3 Fab crystal structure provided the opportunity to model in greater detail. In particular, we could account for structural changes that take place in the loop and position it more precisely in the 160S-C3 and 135S-C3 complexes. At the present resolutions, no significant changes were observed outside the region of the BC loop.

Using the Fab as a marker, our modeling places the Fab-bound BC loop in a different position with respect to the remainder of VP1 than was observed for the unbound loop in the crystal structure of the 160S particle (2) or in the model of the 135S particle (derived by rigid-body fitting of the capsid proteins to a subnanometer-resolution cryo-EM reconstruction) (13) (Fig. 3B). To measure the deviation, we compared the positions of seven residues in the binding cleft of the fitted C3 Fab coordinates with their corresponding position in the 160S (2) and 135S (13) models. That deviation averages to 5 (±2) Å in both cases (Supplemental Table II). The direction of the Fab-bound deviations is the same for both 160S-C3 and 135S-C3 (Fig. 3B).

FIGURE 2. Binding of C3 Fab to poliovirus. (A) Complex of 135S particles and C3 Fab imaged via cryo-EM. Bound Fabs can be seen as fibrous extensions surrounding each round particle. One 80S-C3 complex is seen in this view (arrow). Scale bar, 100 nm. (B and C) C3 Fab bound to 160S (left column) and 135S (right column) particles. For each image pair (same row), maps are viewed from the same vantage point and therefore are to scale. (B) In the top two rows, renderings are viewed along a five-fold symmetry axis. In the bottom two rows, views are 90° rotated from the corresponding view in the top two rows. Within each grouping, the grayscale view shows Fabs bound at one five-fold mesa, and the colored view is a closeup view of one bound Fab (compare with Supplemental Video 1). Coordinates of the Fab and bound peptide (12) were fitted (ribbon) into the cryo-EM density of the mesa-bound Fab (mesh). In the grayscale panels, ribbon is black for both Fab and peptide. In the colorized panels, Fab (variable domain) and bound peptide (VP1 residues 97–103) ribbons are purple and green, respectively. Mesh is surface rendering of 3D reconstruction contoured at 0.5 σ. The modeled 160S-C3 and 135S-C3 peptides are twisted 13° and shifted 8 Å with respect to each other, reflecting the overall twist observed in the Ag-binding domain of the Fab (compare with Supplemental Videos 1, 2). (C) From maps viewed along a two-fold symmetry axis, central density slices from cryo-EM maps of C3 complexed with 160S (left) and 135S (right). Relative density estimates: a sphere of radius of 3 pixels was centered on the designated spots (see Fab and Capsid labels in right panel) and the average density was computed. The density of C3 is almost the same as the density of the poliovirus capsid in each case. β-sheet structure was observed in both the Fab (small white arrows) and capsid (small black arrows) densities. Inset (left panel), a non-central density slice of the 160S-C3 map gives a better view of β-sheets within the Fab density. The β-sheet structure is only seen if the imaged objects are well ordered. Another indication that the Fabs were rigidly ordered was an assessment of the resolution of small volumes of the maps (local-resolution test) (see Ref. 28), where flexible domains have lower resolution than rigid domains. The Ag-binding domains and the adjacent rigid capsid were shown to have the same high-resolution value (data not shown).
FIGURE 3. Interaction of Fabs with BC loops. (A) Elbow axes of bound Fabs. Top row, Stereo view of a surface rendering of the 160S-C3 reconstruction viewed along a two-fold symmetry axis. Darker hues are closer to the center of the particle. Bottom row, Stereo closeup view of region near a two-fold axis of 160S-C3 reconstruction (surface rendering). Lines represent elbow axes estimated for Fabs in virus-Fab complexes, showing their relative relationships: cyan (C3 Fab attached to 160S poliovirus, Fab shown), dark blue (C5 Fab attached to 135S poliovirus, Fab not shown), white (Fab1 attached to human rhinovirus 14, Fab not shown) (29), and green (Fab17-IA attached to human rhinovirus 14, Fab not shown) (30, 29). In contrast to Abs that bind two BC loops in adjacent pentamers across each two-fold axis in human rhinovirus 14 (29, 31), the binding aspect of the C3 Fab suggests that bivalent binding occurs on adjacent BC loops on the same pentamer (compare with Fig. 4). The position and orientation of the C3 Fab elbow can (Figure legend continues)
Although the C3 Fab crystal coordinates included only a peptide of residues 93–103 (12), the position of the seven BC loop residues in the fitted Ag-binding cleft (amino acids 97–103) should mimic the position of the BC loop when C3 Fab is bound to 160S or 135S particles. Therefore, the observed 5 Å deviations indicate that the BC loop moves upon binding of C3 Fab to either 160S or 135S particles. Our modeling is based on the fitting of the Ag-binding domain into five symmetry-related positions in the cryo-EM density. We determined the fitting error (RMSD of five averaged fits) to be 0.5 and 0.4 Å for the 160S-C3 and 135S-C3 fits, respectively. We compared bound residues 97–103 in the fitted Fab structures (Cx coordinates only) to their corresponding residues in the BC loop of the 160S crystal structure (2) or 135S pseudoatomic model (13). We found a deviation per residue between 2.5 and 9.0 Å (Supplemental Table II). Coordinates of bound peptide residues 97–103 were joined to the remainder of VP1 for coordinate models that combined capsid and Fab residues (Fig. 3B).

The Fab-bound C3 epitope, that is, the BC loop, twists more extensively between the 160S and 135S structures than was deduced in the 135S pseudoatomic model (13) (Fig. 2B, Supplemental Videos 1, 2). Comparison (via UCSF Chimera) of the 160S-C3 and 135S-C3 BC loops showed a rotation of 4.6° and a shift of 3 Å. Comparison of the same change in the fitted coordinates of the 160S-C3 and 135S-C3 models showed a rotation of 13° and a shift of 8 Å.

Discussion
C3 Fabs bound to ~60 mesa tips per particle

In the 160S-C3, 135S-C3, and 80S-C3 reconstructions, the Fab was visualized bound to the tip of the five-fold mesa (Figs. 2, 3, Supplemental Fig. 1). This was expected from previous crystal and cryo-EM studies of 160S, 135S, and 80S particles (2, 13, 17, 34, 35) and from the structure of the C3-peptide and Fab complex (12).

Because the Fab and capsid densities are similar (Fig. 2C, Supplemental Fig. 1), all or almost all of the 60 epitopes per particle bound a Fab. This finding is consistent with the previous observation that ~60 Fabs can bind per virion (12).

BC loop shifts upon Fab binding

Residues in the fitted BC loop peptide deviate 2.5–9.0 Å (average, 5 ± 2 Å; RMSD, 5.7 Å) from their positions in the crystal structure of the 160S particle (2) and the model of the 135S particle (13) (Fig. 3B, Supplemental Table II). This shift is consistent with the previous observation that, in the Fab-peptide complex, the structure of the peptide differs significantly from its structure on the surface of the 160S particle (12). Taken together, these two studies indicate that the loop is sufficiently flexible to change structure upon Ab binding.

The C3 Fab coordinates fit snugly within the Fab density in the cryo-EM 160S-C3 and 135S-C3 maps (Figs. 2B, 3B), giving confidence in our fitting and in the inference of Fab-induced BC loop movement. Some algorithms for fitting coordinates within cryo-EM density maps rely only on the overall shape of the macromolecule, but we used a fitting algorithm that emphasized internal density as well as the molecular envelope (26). Because Fabs have a pseudo–two-fold axis along their longest dimension, fitting may appear comparably good when the domains are rotated 180° about this axis. Remarkably, in our core-weighted fittings for both reconstructions (160S-C3 and 135S-C3), L chains for the only all the constant domain of the Fab to bend toward or away from an adjacent Fab on the same pentamer. In contrast, the binding aspect of Fab17-IA on human rhinovirus 14 (green line) allows bending of the elbow to bring two constant domains closer to the two-fold axis, facilitating binding of an Ab across the two-fold axis (31). The binding aspect of Fab1 (white line) is intermediate between Fab17-IA and C3 and, as suggested by binding experiments with Ab (29), likely means monovalent attachment of Abs. (B) C3 Fab binding changes the conformation of poliovirus 160S (top) and 135S (bottom) BC loops. Views are shown as stereo pairs. Mesh is surface rendering of cryo-EM reconstructions at relatively high contour levels (160S-C3 at 1.5σ and 135S-C3 at 2.5σ) to emphasize the core structure and the connection between Fab and virus. Fitted Fab (magenta ribbon), peptide residues 97–103 (green sticks), and VP1 (blue and cyan ribbons) coordinates are shown. Residues within the BC loop (95–105) were adjusted to the Fab-bound position and are shown in blue. The non–Fab-bound position of the BC loop (2) is shown in cyan. (C) Alignment of poliovirus (dark blue) and rhinovirus (light blue) B and C β-strands and BC loops shown as an amino acid sequence (top) and a 3D structure (bottom). Coordinates from the VP1 protein from crystal structures of poliovirus (dark blue) (2) and human rhinovirus 14 (light blue) (30) were aligned using the MatchMaker function in UCSF Chimera (24). (Only the BC region is shown.) The sequences were then aligned to the 3D structure alignment (dark blue, poliovirus; light blue, HRV14). Because the poliovirus BC loop is four residues shorter than the HRV14 loop, some gaps were artificially placed in the poliovirus sequence to emphasize the alignment at the N- and C-terminal ends of the loop and align other residues within the loop (although the 3D structure shows the midloop sequence alignments are insignificant to the 3D structural alignment). Poliovirus secondary structure assignments are shown (top right).
separately fitted constant and variable domains were consistently placed on the same side of the Fab. (Therefore, H chains were also consistently placed.) This consistency suggests that our data have sufficient resolution for the core-weighting algorithm to distinguish L and H chains and would, therefore, place the Ag-binding cleft in the correct position.

The resolution of the cryo-EM maps (9–11 Å) was sufficient to allow precise fitting of the Fab coordinates within the cryo-EM density. The low error of the core-weighted fitting (26) for the Ag-binding domains implies that the 160S-C3 (RMSD uncertainty, 0.5 Å) and 135S-C3 (RMSD uncertainty, 0.4 Å) reconstructions were sufficiently detailed to allow such precise fitting and measurement of the BC loop deviation. This may at first seem counterintuitive given the limited resolution of the reconstructions, but the confusion stems from a common misconception of resolution. The resolution of a structure is the minimum distance between two objects where the two objects are seen to be separate. In contrast, the fitting error is a measure of how precisely properly constrained models can be placed into a map, and is always much better than the nominal resolution of the map.

Our results are consistent with a two-step binding model proposed previously (12). Wien et al. (12) fitted their crystal structure of the C3 Fab-peptide complex onto the poliovirus crystal structure and found that their fit was incompatible with their observation that 60 C3 Fabs were capable of binding to one virus particle. Their fit showed steric overlap for adjacent Fabs in the outermost β-strands of the Ag-binding domains. They proposed a two-step binding model where initial binding of the V region induced a conformational change in the BC loop. Our results are consistent with this model because we showed 1) the loop conformation is changed (Fig. 3B) and 2) ~60 Fabs did bind per virion (Fig. 2C).

Although the BC loop is readily accessible to Abs, its apparent flexibility may facilitate similar binding to conformationally distinct poliovirus particles. As can be inferred from Ab titer experiments, binding avidities of C3 Abs for 160S, 135S, and 80S particles are nearly identical (11). Additionally, we observed similar Fab/capsid density ratios in the 160S-C3, 135S-C3, and 80S-C3 3D maps (Fig. 2C, Supplemental Fig. 1). Therefore, if the conformation of the BC loop differs among the 160S, 135S, and 80S states, the BC loop appears to have enough flexibility to change conformation to optimally bind the C3 Ab.

Paratope and epitope flexibility

To make the binding interaction stronger and more specific, the paratope (of the Ab) and the epitope (of the Ag) may change conformations during binding (1). For example, the binding of Ab to lysozyme induced conformational changes in the Ag at a demonstrably flexible region (1). For the interaction of HIV-1 capsid protein p24 with Fab13B5, conformational changes were observed in both Ab and Ag (36). In the Fab, shifts were observed in complementarity-determining regions and in an 8° relative rotation between the H and L chain variable domains. In the epitope, Pro207 and Ala208, located in a turn between two helices, moved after Fab binding. In the present study, we showed epitope flexibility by cryo-EM.

The calculated deviation of the fitted, bound epitope is identical (within experimental error) for the 160S-C3 and 135S-C3 structures (Supplemental Table II), further strengthening the conclusion that binding of C3 Fab causes the BC loop to change conformation. Although the Fab-induced movement of the BC loop is not to the same relative position for the two particle states (data not shown), the movement is in the same general direction (Fig. 3B). The consistent deviation and direction for the 160S-C3 and 135S-C3 structures suggest that the Fab interacts in a similar manner with the two particle states and that the neighboring VP1 structure is also similar for the 160S and 135S particles.

The C3 paratope may also shift, but we cannot determine such a shift from our experiment. The previously published crystal structure of C3 Fab with a bound antigenic peptide also did not explore the question of paratope shifts because the only structure determined was that of Fab with Ag bound (12). Of course, differences may exist between the way the Fab binds free peptide and the constrained BC loop (on a virus particle). Such differences, if they occur, are probably found in minor stabilizing interactions,
and the binding cleft in the Fab is unlikely to be in a different position. Because the major Fab–virus interaction is between the binding cleft and the epitope, any major Fab changes, either in the complementarity-determining regions or between H and L chain variable domains (36), should be represented in the model of C3 Fab bound to the BC loop peptide (12).

However, our fitting results suggest that the BC loop has a slightly different environment in the 160S and 135S particles. Deviations of the fitted BC loop from the previously modeled loops (2, 13) are slightly different in the 160S-C3 and 135S-C3 structures (Fig. 3B, Supplemental Table II). (For the 135S-C3 experiment, C3 Fab was not added until 135S particles had been made.) For example, during the 160S-to-135S transition, the BC loop in 160S-C3 and 135S-C3 complexes rotates 13° and shifts 8 Å, but in the unbound models (2, 13) the BC loop only rotates 4.6° and shifts 3 Å between the 160S and 135S structures.

The observed shift of the BC loop suggests that the loop is not fixed in a certain conformation, but, given the appropriate conditions, can easily adjust. The B factors (temperature factors) for the BC loop are relatively high, suggesting that the loop is flexible (Supplemental Table II). However, this flexibility is probably not random, but is dictated by interactions with other proteins. Binding of Fab to the BC loops may simply trap the BC loop at one extreme of its normal deviation. In our structures, the conformational change appears to be induced by the Ab–Ag binding interaction (compare with Ref. 1). This inducible flexibility may be important in other viral processes, including cell entry. For example, poliovirus receptor (CD155) is known to also interact with the mesa variable domains (36), should be represented in the model of C3 Fab and poliovirus 14 (29, 31) (Fig. 3A). However, attempts to model interpentamer C3 binding, based on the poliovirus C3-Fab complexes reported in the present study, were unsuccessful. Despite the flexibility of the hinge and elbow regions, the C3 Ab simply cannot bind to one epitope and then reach across the two-fold axis and contact a second binding site (compare with Fig. 3A). C3 Ab could bind two five-fold-related BC loops within a single pentamer. This could involve binding to two adjacent loops or two loops separated by one neighboring loop. In our modeling of the Ab-virus complex, an Ab easily bound two adjacent five-fold related BC loops in both the 160S-Fab complex and the 135S-Fab complex (Fig. 4A), but no permissible bending of the elbow or hinge regions was consistent with bivalent attachment to nonadjacent loops (data not shown).

If a C3 Ab binds bivalently to a given five-fold mesa, then the binding may prevent expansion of that mesa (to the 135S state) because the two Fab arms are constrained by linkages in the intact Ab. Adjacent BC loops (on the same mesa) separate by an additional 1 A˚ in the 160S-to-135S transition (Supplemental Table I). Although the Ab can clearly bind to either state (11) (Fig. 4A), the energy required to change the hinge linking the Fabs to the Fc domain may be sufficient to inhibit that slight expansion (4%) and twist (see Results). Alternatively, flexible portions of the Ab may be able to accommodate these slight conformational changes (Fig. 4A, Supplemental Video 4), and the bivalently bound Ab may neutralize by inhibiting the formation of a transient state (between 160S and 135S) in which the particle expands beyond the modest 160S-to-135S expansion (Fig. 4B). Indeed, because the expansion observed in the 135S particle does not indicate a clear path for the N terminus of VP1, initially inside, to reach its location on the outside surface of the 135S particle (43), a transition state that is larger than the 135S state is plausible. In any case, our data and modeling suggest that the 160S-to-135S expansion is not simply a radial shift of a static pentameric mesa (Supplemental Video 4).

**Exit of N terminus of VP1**

During the cell entry process, receptor binding induces native poliovirus (160S) to convert to the 135S intermediate particle (11, 44). In this transition, the virion changes conformation, externalizing the membrane-binding entities VP4 and the N terminus of VP1. Competing models have suggested that the N terminus of VP1 exits through a channel along the five-fold axis (45–47) or at the base of the canyon (13, 17). Our results support the latter model.

Although the BC loop moves as the 160S particle transitions to the 135S particle form, the extent of this movement is inconsistent with the “fivefold exit” model for VP1 and VP4 (45–47). The BC loop of VP1 moves 1 Å from the center of the mesa (Supplemental Table I). If the N terminus of VP1 exits the capsid through the five-fold axis, the BC loop of VP1, and hence the position of C3 Fab, must change significantly. The lack of significant movement supports a model in which the N terminus of VP1 does not exit through the five-fold axis.
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