Solution Structure of Bacteriophage PRD1 Vertex Complex*

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Bacteriophage PRD1 is a prototype of viruses with an internal membrane. The icosahedral capsid and major coat protein share structural similarity with the corresponding structures of adenovirus. The present study further explores similarities between these viruses, considering the 5-fold vertex assemblies. The vertex structure of bacteriophage PRD1 consists of proteins P2, P5, and P31. The vertex complex mediates host cell binding and controls double-stranded DNA delivery. Quaternary structures and interactions of purified spike proteins were studied by synchrotron radiation x-ray solution scattering. Low resolution models of the vertex proteins P5, P2, and P31 were reconstructed ab initio from the scattering data. Protein P5 is a long trimer that resembles the adenovirus spike protein pIV. The receptor-binding protein P2 is a 15.5-nm long, thin monomer and does not have an adenovirus counterpart. P31 forms a pentameric base with a maximum diameter of 8.5 nm, which is thinner than the adenovirus penton pII. P5 further polymerizes into a nonameric form ([P5]3). In the presence of P31, P5 associates into a P5cP31 complex. The constructed models of these assemblies provided support for a model of vertex assembly onto the virion. Although similar in overall architecture, clear differences between PRD1 and adenovirus spike assemblies have been revealed.

Bacteriophage PRD1 is a prototype organism of the Tectiviridae family (1). PRD1-like viruses infect a broad range of Gram-negative bacteria harboring a P-, N-, or W-type conjugative plasmid encoding the phage receptor complex (2, 3). The virion consists of an outer protein shell of about a 65-nm diameter enclosing a protein-rich membrane vesicle (4). The latter contains the phage genome, a linear 14.9-kilobase pair double-stranded DNA molecule with a terminal protein attached to both 5' ends (5–8).

The icosahedral capsid is built from 240 copies of a P3 trimer on a pseudo T = 25 lattice, an arrangement similar to that of the adenovirus capsid (4, 9). Furthermore, the fold of P3 is similar to that of the adenovirus hexon (10, 11), thus suggesting an evolutionary relationship between a bacterial and an animal virus (11–13). In addition to P3, the outer capsid shell also contains a receptor binding complex composed of proteins P2, P31, and P5, located on the 5-fold vertices (14). An outline of the assembly pathway of these vertices has been recently delineated by genetic, biochemical, and electron microscopic methods (14–17) (see Fig. 1). A pentameric protein, P31, anchors the P5 trimer to the vertex (14, 17). The P5 trimer contains two domains separated by a collagen-like sequence (see Fig. 1). The N-terminal domain (P5N, residues 1–135) interacts with protein P31 and is necessary for P5 incorporation into virions (15, 17). The C-terminal domain (P5C, residues 136–340) is necessary for trimerization and P2 binding (15, 17). Based on a 38% sequence identity between P31 and P5N and the ability of P5c to dissociate P31c, a model of the 5-5-fold symmetry adapter has been proposed (17) (see Fig. 1). In this model, P5N domains associating with the remaining P31 molecules form the vertex base. The trimer of P5C constitutes the spike shaft and supports P2 binding (Fig. 1).

All three proteins are essential for controlled DNA delivery. Upon binding to the receptor, P2 is considered to trigger conformational changes within the vertex complex that are transmitted to the internal membrane. The membrane then forms a tubular appendage thought to serve as a conduit for DNA delivery (16). In the absence of P2 the vertex complex becomes unstable, leading to premature release of the packaged DNA (16). This observation suggested that the vertex is in a metastable conformation stabilized by P2 and that the vertex disassembles upon receptor binding.

Further structural investigations of the vertex complex by electron microscopy were precluded because of the symmetry mismatch between P31 (pentamer) and P5 (trimer), and P5 and P2 (monomer), respectively (14). Although protein P2 has yielded diffracting crystals (18), the vertex complex is unlikely to yield to crystallization because of its size and flexibility. Because the vertex complex is essential for host recognition and controlled DNA delivery, it is important to unravel its quaternary structure. X-ray solution scattering is used here to obtain structural information about the arrangement of individual proteins within the complex. The shape of individual vertex proteins and models of their association within the vertex are presented, and some aspects of their interactions in vitro are investigated. The structure of the vertex is also compared with that of the adenovirus, and the implications for PRD1 assembly and DNA release are discussed.

EXPERIMENTAL PROCEDURES

Protein Purification and Sample Preparation—Protein P5 was over-expressed in Escherichia coli cells harboring plasmid pJB51 and purified as described earlier (17). The purification yielded P5c, which was

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further purified by size exclusion chromatography. P5C was prepared by digestion of P5 with collagenase from Clostridium histolyticum (Sigma). The digestion products were separated by size exclusion chromatography on a Hi-Load Superdex 200 26/60 gel filtration (Amersham Pharmacia Biotech) column. Proteins P2 and P31 were overexpressed in E. coli and purified as previously described (14, 16).

Proteins were concentrated to 10–15 mg/ml, and large aggregates were removed by filtration (0.2 μm pore size) and centrifugation (30 min, 95,000 rpm, A-100 rotor, Beckman Airfuge). If required, protein solutions were further purified by size exclusion chromatography on a Superdex 200 (Amersham Pharmacia Biotech) column just before the small angle x-ray scattering (SAXS) experiment. Sample homogeneity and purity was checked by dynamic light scattering and SDS-polyacrylamide gel electrophoresis (17). SAXS data were obtained from proteins in 20 mM HEPES, 150 mM NaCl buffer, pH 7.0, unless indicated otherwise.

A complex with an apparent stoichiometry P5c:P31 was obtained during incubation of complexes P31 and P5 for 48 h at 4 °C, after which apparent equilibration was reached. A noncovalent complex (P5c,P5) was formed during incubation of P5c at 37 °C for 48 h. Because of their slow dissociation rates, these complexes remained in apparent equilibrium with the reactants for the duration of the SAXS experiment. To study complex formation between P5c and P2, the two proteins were mixed at 5 °C and incubated for 24 h. Volume fractions of complexes and reactants were determined by analytical size exclusion chromatography (300 × 10 mm Superdex 200 (Amersham Pharmacia Biotech) equilibrated with 20 mM HEPES, 150 mM NaCl buffer, pH 7.0) using refractive index and absorption detectors.

Data Collection and Processing—The synchrotron radiation x-ray scattering data were collected using standard procedures on the X33 camera (19–21) of the EMBL on the storage ring DORIS III of the Deutsches Elektronen-Synchrotron (DESY) equipped with a multiwire proportional detector with a delay line readout (22). The beam was focused onto the detector placed at distances of 1.4, 2.5, and 4 m from the 120-μl sample cell. The covered range of momentum transfer was 0.1 < s < 5.5 nm⁻¹ = 4 m sinθ/λ, where 2θ is the scattering angle, and λ = 0.15 nm, the x-ray wavelength. The data were normalized to the intensity of the incident beam, corrected for the detector response, the scattering of the buffer was subtracted, and the difference curves were scaled for concentration using the program SAPOKO (22). To check for radiation damage and aggregation during the SAXS experiment, the data were collected in 10 successive 1-min frames. The reduced data sets at low angles were extrapolated to zero concentration after standard procedures (23). The scattering patterns recorded at different sample-detector distances were merged, and the forward scattering I(0), distance distribution functions p(r), and radii of gyration Rg were evaluated with the indirect transform package GNOM (24, 25). The maximum dimensions Dmax of the protein complexes P5, P5c, P2, and P31 were estimated from the experimental data using the orthogonal expansion program ORTOGNOM (26).

Shape Reconstruction by Simulated Annealing—The shapes of proteins P2, P5c, and P5c were restored from the scattering patterns of monodisperse solution of these proteins using an ab initio shape determination (27) implemented in the program DAMMIN. A sphere of diameter Dmax is filled with a regular grid of points corresponding to a dense hexagonal packing of small spheres (dummy atoms) of radius r0 < Dmax. The structure of the dummy atoms model is defined by a configuration vector X assigning an index to each atom (0 for solvent and 1 for solute particle). In keeping with the low resolution of the solution-scattering data, the method searches for a compact interconnected configuration X minimizing the discrepancy χ between the calculated and the experimental curves,

\[ \chi^2 = \sum \left( \frac{I_{\text{exp}}(s_j) - \sum \alpha(s_j)}{\sigma(s_j)} \right)^2 \]

where N is the number of experimental points, and Iexp(s) and σ(s) are the experimental intensity and its S.D. measured at the momentum transfer s, respectively, and Is is the scattering intensity from the model. Starting from a random configuration, simulated annealing (28) is employed for the minimization. To remove the contribution from the scattering due to internal structure, a constant is subtracted from the experimental data to ensure that the intensity decays as s⁻4 following Porod’s law (29). The shapes of P2, P5c, and P5c were reconstructed with dummy atoms of radius r0 = 0.45, 0.325, and 0.25 nm, respectively. For P5c and P5c, a 3-fold symmetry was assumed.

The models of the pentameric protein P31 were obtained using two ab initio procedures. Because the current version of DAMMIN does not support 5-fold symmetry, the low resolution shape of P31 was restored without applying any symmetry restrictions with r0 = 0.325 nm. A more detailed model has been constructed using a recently developed dummy residues (DR) method (30) implemented in the program GASBOR. This program employs simulated annealing to build a model of the protein structure by finding a chain-like spatial distribution of DRs that fits the experimental scattering data (see Ref. 30 for details). The DR model of P31 was reconstructed assuming a 5-fold symmetry, with 123 residues/monomer corresponding to the primary sequence of the protein (7).

Table I shows the shape parameters and comparison with hydrodynamic data.

| Sample | P5c1 | P5c2 | P2 | P31c | P5c | P5c | P5c | (P5c3)min |
|-------|------|------|----|------|-----|-----|-----|-----------|
| Molecular mass, kDa | 103 | 59.5 | 66 | 68.7 | 240 | 309 |     |           |
| r0 (nm) | 0.325 | 0.25 | 0.45 | 0.19 | 47  | 51  |     |           |
| Dmax (hydro), nm | 27 ± 3 | 12 ± 1 | 21 | 6.5 | 50 ± 10 | 47 ± 10 |     |           |
| Dmax (SAXS), nm | 7.65 ± 0.20 | 3.20 ± 0.09 | 4.15 ± 0.14 | 2.63 ± 0.02 | 11.1 ± 0.4 | 11.4 ± 1.0 |     |           |
| Rg (SAXS), nm | 7.8 | 3.3 | 4.2 | 2.5 | 10.8 | 10.8 |     |           |
| Rg (hydro), nm | 5.5 | 3.5 | 4.1 | 3.1 | 7.8 | 8.6 |     |           |

**Table I.** Summary of shape parameters and comparison with hydrodynamic data.

* Radii used for the ab initio shape reconstruction by DAMMIN.
* Maximum diameters were calculated from hydrodynamic measurements.
* Maximum diameters were calculated from SAXS data.
* Radii of gyration were computed from SAXS data using GNOM (the values for P5c:P31 and (P5c3)min were evaluated from the scattering by the mixtures).
* Hydrodynamic radii were calculated from the low-resolution models derived from SAXS data using HYDRO.
* Hydrodynamic radii were calculated from the models constructed from hydrodynamic measurements using HYDRO. All hydrodynamic measurements for P31 were repeated in this study.

![Image](https://example.com/image.png)
The experimental scattering intensity \( I_{\text{exp}}(s) \) from a mixture of \( K \) different components is written as,

\[
I_{\text{exp}}(s) = \sum_{j=1}^{K} \nu_j \times I_j(s)
\]

where \( \nu_j \) and \( I_j(s) \) are the volume fraction and the scattering intensity from the \( j \)-th component, respectively. Given the scattering curves of the components, program OLIGOMER\(^2\) finds the volume fractions by solving a system of linear equations to minimize the \( \chi^2 \) discrepancy (1) between the experimental and calculated scattering curves. The scattering intensities from the \textit{ab initio} low resolution models of complexes \( P5_3 \) and \( P3 \) were computed using the program CRYSOL (31). The models of complexes \( (P5_3)_{2} \) and \( (P5_3)_{3} \) were constructed interactively from the low resolution models of \( P5_3 \) and \( P3 \) using the program MASSHA (32). The complex \( P5_6:P3 \) was built from two \( P5_3 \), one or two monomers of \( P3 \). The nonameric complex \( (P5_3)_{3} \) was constructed from three \( P5_3 \). The scattering intensities from these models were subsequently computed by CRYSOL.

Hydrodynamic Parameters and Display of Models—The values of the hydrodynamic radius \( R_h \) were calculated from the low resolution models using the program HYDRO (33) in rigid body approximation (34). The bead sizes were 0.45, 0.25, 0.45, 0.19, 0.45, and 0.45 nm for complexes \( P5_3 \), \( P5C_3 \), \( P2 \), \( P31_5 \), \( P5_6:P31 \), and \( (P5_3)_3 \), respectively. The figures displaying the low resolution models of the protein complexes were prepared on an SGI Octane workstation using the program ASSA (35).

**RESULTS**

**Shape Determination of Vertex Proteins**

**Low Resolution Shape of \( P2 \)—**The structural parameters of protein \( P2 \) computed from SAXS and hydrodynamic data are presented in Table I. According to the hydrodynamic data (17), \( P2 \) is an elongated particle with an axial ratio of about 1:10. A low resolution model of this complex in Fig. 3, left column, was restored \textit{ab initio} from the SAXS data using the program DAMMIN, and the fit to the experimental scattering data is displayed in Fig. 2A. The \textit{ab initio} shape reveals an elongated, peanut-shaped particle with an axial ratio 1:7, consistent with both hydrodynamic (17) and crystallization results (18).

**\( P31 \) Modeling—**According to analytical ultracentrifugation (14), \( P31 \) is a pentamer with molecular mass \( = 68.7 \) kDa. The low resolution shape of \( P31_5 \) reconstructed \textit{ab initio} by DAMMIN without symmetry restrictions is displayed in Fig. 3, middle column, and the fit to the experimental scattering data is presented in Fig. 2B, curve 2. A much more detailed model (Fig. 3, right column) is provided by the program GASBOR, which allowed, in contrast to DAMMIN, to impose a 5-fold symmetry and to fit the entire scattering pattern (the fit is presented in Fig. 2B) curve 3). Earlier hydrodynamic measurements suggest that \( P31_5 \) is an elongated particle with \( D_{\text{max}} \) and \( R_h \) equal to 24 and 4.2 nm, respectively (17). These results were in obvious contradiction with the two \textit{ab initio} SAXS models in Fig. 3 (middle and right columns), which indicate that \( P31_5 \) is a globular particle with \( D_{\text{max}} \) and \( R_h \) equal to 8.5 and 2.5 nm, respectively. To resolve this discrepancy, a new series of hydrodynamic experiments was performed yielding a value \( R_h = 3.1 \) nm and \( D_{\text{max}} = 6.5 \) nm, which agreed with the SAXS model. It is conceivable that the samples used in the previous hydrodynamic analysis contained higher oligomers or aggregates\(^4\) of \( P31 \).

**Shape of the \( P5 \) Trimer and \( P5 \) C-terminal Fragment—**According to the biochemical data (17) the smallest form of protein \( P5 \) is an elongated trimer \( (P5_3) \) constructed from three monomers associated via the C-terminal domain with low tendency to further oligomerization. Two constructs of the \( P5 \) trimer were studied, namely the full-length complex \( P5_3 \) and the

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\(^2\) D. I. Svergun, V. V. Volkov, and A. Sokolova, unpublished information.

\(^4\) R. Tuma and J. Caldentey, unpublished information.
C-terminal domain P5C3 obtained by collagenase cleavage of P53. Low resolution shapes of P5C3 and P53 (Fig. 4) were restored using DAMMIN, and the corresponding fits to the experimental data are shown in Fig. 2A. The independently obtained model of P5C3 is very similar to one end of the P53 model, as illustrated by the superposition in Fig. 4, left column. This end of the P53 model was therefore tentatively assigned to the C terminus, although the orientation cannot be distinguished solely on the basis of the SAXS data. Based on such tentative domain assignment, the N-terminal domain assumes highly extended conformation. The values of $D_{\text{max}}$ and $R_h$ computed from the low resolution models of P5C3 and P53 using HYDRO are in a good agreement with hydrodynamic measurements in Table I.

**Association of Vertex Proteins**

**Interaction of P53 with Vertex Protein P31**—The complex P50-P31 was obtained by incubation of complexes P315 and P53. Such preparations of P50-P31 always contained an equilibrium mixture of all three constituents (P50, P31, P315, and P53). Because monodisperse solutions of P50-P31 were not available, its low resolution shape could not be determined directly from the scattering data. The models of the P50-P31 were constructed from two P53 and one or two P31 monomers. The P31 monomer was conveniently taken to be the asymmetric portion of the model of P315 obtained using the program GASBOR in the previous section. The actual shape does not have appreciable influence on the fitting because of the low volume fraction of P31 within the complex. To select a plausible arrangement of the two P53 in (P53)$_2$, previous biochemical results were taken into account; the C-terminal domain of P5 is the trimerization domain, whereas the N-terminal domain is responsible for further oligomerization of P53 and its association with P31 (15, 17). Additionally, preliminary results\(^5\) indicated that solutions of a recombinant N-terminal fragment always contain a mixture of monomers and dimers. These results suggest that oligomerization of P53 proceeds most likely via dimerization of the N-terminal domain.

A set of models was generated in which two P53 trimers associated via their assigned N-terminal domains together with one or two P31 monomers. The models differed in the separation between the P53 molecules in the XY plane (see the orientation in Fig. 6) and in the positions of the monomeric P31. Their scattering patterns were computed and processed using OLIGOMER to fit the experimental data (Fig. 5A). Models

\(^5\) R. Tuma and J. H. K. Bamford, unpublished information.
containing only one monomeric P31 systematically fitted the experimental data better than those incorporating two P31 molecules. Although omitting P31 from the model did not appreciably affect the fit, chromatography indicated that stoichiometric amounts of P31 were present in the complex, and therefore, P31 was included in the modeling. The discrepancy and the volume fraction of the P56:P31 in the mixture as functions of the separation between the two complexes P53 are presented in Fig. 5, B and C, respectively.

The best model of P56:P31 has a separation of 14 ± 1 nm along the x axis and 4.0 ± 0.5 nm along the y axis between the centers of the two trimers and contains one P31 monomer inside (P53)2 as displayed in Fig. 6. The fit to the experimental data with χ = 0.83 (Fig. 5A) corresponds to volume fractions of P56:P31, P53, and P31 of 54 ± 5%, 10 ± 5%, and 36 ± 5%, respectively. Using these values and the molecular masses of the three components, the molar composition of the mixture can be recalculated as 51% P53 + 49% P31, which is indeed very close to the originally prepared equimolar solution. The results from fitting the SAXS data correspond well to those obtained independently by chromatography (volume fractions P56:P31, 57%; P53 + P31, 43%). This agreement and the steep dependence of volume fraction on the dX-dY separation (Fig. 5C) also rules out that other minima could represent the data better that the solution obtained by minimizing the discrepancy. Additionally, overall parameters of the P56:P31 model are in a good agreement with the hydrodynamic data (Table I).

**Self-association of P5—Because monodisperse solutions of P5 nonamers ((P5)9) were not available, a similar approach as used in the P56:P31 case was applied. Fig. 5A shows data recorded from mixtures of (P5)9 and P5. Several tentative models of (P5)9 were built using the program MASSHA, and...**
the scattering data from the mixture were analyzed using OLIGOMER. The initial model was obtained by adding a third P53 to the configuration of two P53 in the complex P53c:P31 (Fig. 6). Two configurations with a triangular or a linear distribution of the P53 molecules in the XZ plane were tested as illustrated in Fig. 6, and the scattering patterns from these models were computed by CRYSTOL. The discrepancy \( \chi \) provided by OLIGOMER was insensitive to the position of the third P53 molecule, but changes in the volume fraction had more significant effects. A linear distribution of the P53 models provides better agreement with the fractions of the different components obtained by chromatography. The best model \( \chi = 1.01 \) and the fit of the experimental curve are presented in Fig. 6, right column, and in Fig. 5A, respectively. The volume fractions of trimer \( (58 \pm 11\%) \) and nonamer \( (42 \pm 11\%) \) agree within the experimental error with the values obtained by chromatography (about 50% trimer and 50% nonamer).

Interaction of P5 with the Receptor-binding Protein P2—Genetic data indicated that the incorporation of P2 is dependent upon the assembly of the full-length P5 onto the virus particle and, in particular, that the presence of the P5 C-terminal domain was required (15). Solutions of monomeric P2 and P5C3 containing up to 20 mg/ml each protein were examined to establish whether P2 forms a complex with the trimer of P5 C-terminal domain. In all cases, the experimental data could be fitted by a linear combination of the P2- and P5C3-scattering curves (data not shown). No increase in the apparent mass and radius of gyration of the solutes were observed. A direct interaction between the P2 and the C-terminal domain of P5 can thus be ruled out, suggesting that incorporation of P2 may require an intact junction between the two domains (e.g. the collagen-like motif (36)) or co-assembly with other constituents of the vertex.

DISCUSSION

SAXS Modeling—As seen from Table I, the values of \( D_{\text{max}} \) from hydrodynamic measurements are systematically higher than those estimated from the SAXS data. This difference is easily explainable by the different influences of the hydration shell surrounding the particles in solution. In hydrodynamic experiments, the entire shell is taken into account, whereas in SAXS, only the most ordered water molecules in the first hydration shell make a significant contribution to the scattering. Additionally, limitations of the hydrodynamic models and the neglect of flexibility during rigid body modeling could be another source of discrepancy between the measured and SAXS-predicted hydrodynamic radii. This may, in particular, be the case with the P5 trimer and its N-terminal domain (Table I).

Self-consistency of the modeling is demonstrated by the similarity between the two P5 C-terminal domain structures obtained by independently fitting the data corresponding to the full-length protein and the P5C3 fragment, respectively (Fig. 4). Because of the intrinsically limited information content of SAXS curves, application of additional constraints enhances the reliability of the modeling. This is exemplified by the restoration of the P515 structure in Fig. 3. Without applying the 5-fold symmetry, the simulated annealing algorithm gives an approximation of the overall dimensions of the assembly. In contrast, application of the appropriate symmetry and utilization of the amino acid sequence significantly improve the model.

Structure and Function of Vertex Proteins and Their Domains—The elongated shape of the P2 monomer is consistent with its role as the receptor binding spike, which is exposed on the surface of the virion (15–17). Similarly, the long P53 is well fit for its role as a shaft of the vertex spike protruding from the virion surface (17). The present results also show that the two
domains of P5₃, which were previously identified by proteolysis (17), also constitute the structural domains of the trimer (Fig. 4). The C-terminal domain is an asymmetric but compact trimer, whereas separation of densities within the middle portion of the N-terminal domain is apparent. This is consistent with the metastable nature of the N-terminal domain association within the trimer and its role in further oligomerization of P5 and association with P31 (17). Furthermore, preliminary characterization of a recombinant N-terminal fragment has suggested that this domain preferentially dimerizes. Arrangement of P5₃ within the model of (P5₃)₃ suggests that the preferred orientation of the N-terminal domains within the dimer is antiparallel. Thus, the parallel association of the N-terminal domains within P5₃ is less favorable and may account for its metastability.

The P31 pentamer (Fig. 3, right column) exhibits a mushroom shape with a star-shaped head (8-nm diameter) and a round neck (5.5-nm diameter). The pentamer is about 4 nm thick. Despite the fact that protein P31 shares 38% sequence identity with the N-terminal domain of P5 (17), its pentameric quaternary structure and globular shape (Fig. 3) are very different from that of the P5 N-terminal domain (cf. Fig. 4). Although both proteins have high amounts of β-strand secondary structure and most likely share the same structural motif, the configuration of their polypeptide chains is solution is different. However, it is conceivable that binding of P5N to P31 within the context of the capsid shell induces conformational changes, and both polypeptides assume similar three-dimensional structure (see below).

**Vertex Model**—A pseudo-atomic model of the P3 shell has recently been obtained by fitting the x-ray coordinates of the P3 trimer into the electron microscopy reconstruction of the PRD1 virion (37). Atomic coordinates belonging to a set of five peripentonal trimers were utilized to position the SAXS structures within the context of the virion (Fig. 7). The hole in the center of the peripentonal trimers has a diameter of ca. 5.5 nm, which is large enough to fit the neck of the P31 pentamer but not the star-shaped protrusions. These protrusions are accommodated on the outer surface of the P3 shell. This configuration is consistent with the electron density assigned to P31₅ in the difference map of San Martín et al. (37).

Subsequently, one P5₃ was placed within P31₅, deriving...
their relative positions from the \( \text{P}_5 \)-\( \text{P}_{31} \) model (Fig. 6). In this configuration, the C-terminal domain of \( \text{P}_5 \) extends about 16 nm outside the virion surface (Fig. 7). The N-terminal domain penetrates the \( \text{P}_{31} \) pentamer and extends about 4 nm into the virion interior (Fig. 7A). Such penetration into the virion is inconsistent with the difference map of San Martín et al. (37), in which no substantial density is found underneath the penton. In the present configuration, \( \text{P}_5\text{N} \) also creates steric clashes with both the \( \text{P}_{31} \) base and the inner membrane. Therefore, we conclude that the extended solution configuration of \( \text{P}_5\text{N} \) cannot be accommodated within the context of the virion, and \( \text{P}_5\text{N} \) must undergo conformational change upon assembly of the vertex. Taking into account the 38% sequence identity between \( \text{P}_5 \) and \( \text{P}_{31} \), it is conceivable that \( \text{P}_5\text{N} \) adopts a compact fold similar to that of \( \text{P}_{31} \) and forms the heteromultimeric base with \( \text{P}_{31} \) as previously proposed (17) (Fig. 1).

The asymmetric receptor-binding protein \( \text{P}_2 \) is likely to extend from the surface of the virion. Although there is unequivocal genetic evidence that incorporation of \( \text{P}_2 \) depends on the presence of full-length \( \text{P}_5 \) (15) (Fig. 1), the present results show that there is no direct association between the C-terminal domain of \( \text{P}_5 \) and \( \text{P}_2 \). The association perhaps takes place before the formation of a stable \( \text{P}_5\text{P}_2 \), or it involves co-assembly of \( \text{P}_{31}, \text{P}_5 \), and \( \text{P}_2 \) within the context of \( \text{P}_3 \) shell. The former is unlikely because \( \text{P}_5\text{C}_\text{P} \) forms a protease-resistant stable trimer, leaving no segment of the chain to be stabilized by \( \text{P}_2 \) binding, and co-expression of \( \text{P}_2 \) and \( \text{P}_5 \) in \( \text{E. coli} \) did not yield a stable complex. In the second case, \( \text{P}_2 \) may wedge into the cavity created between the \( \text{P}_5\text{P}_{31} \) penton and \( \text{P}_5\text{C}_{\text{P}} \) (Fig. 7B). This arrangement may further stabilize the vertex until release of \( \text{P}_2 \) upon binding to the receptor. It has been shown that in the absence of \( \text{P}_2 \), the vertex is labile and leads to membrane and DNA release (16). Receptor binding may trigger \( \text{P}_2 \) release followed by vertex destabilization and disassembly and trigger DNA delivery.

Comparison between \text{PRD1} Vertex and Adenovirus Spike—A striking feature of bacteriophage \text{PRD1} protein \( \text{P}_5 \) is the presence of a collagen-like motif (36, 38), which may be an evolutionary relict from an ancestral virus and is shared with other phage tail proteins (39). On the other hand, the structure of the coat protein and the viral shell has clear relationship to the adenovirus architecture despite any apparent sequence homology between the two proteins (11, 13). Our present data indicate that the vertex arrangement of these two viruses is also similar, but there are clear structural differences even at this resolution.

The adenovirus protein pIII forms a penton base, which anchors the spike trimer (pIV) (40–43). The penton base of the adenovirus is significantly larger (10-nm diameter, ca. 12 nm in height (43)) than the corresponding \( \text{P}_{31} \) pentamer, reflecting the larger size of the adenovirus capsid. Additionally, the adenovirus penton base binds to cell surface integrins to elicit internalization of the virion during infection. No such binding to a secondary receptor has been demonstrated for \( \text{P}_{31} \), presumably because of differences in the DNA delivery mechanism.

The adenovirus spike (pIV) is composed of three domains, an N-terminal tail, a shaft, and a globular knob (44). Both adenovirus pIV and \text{PRD1} \( \text{P}_5 \) spike N-terminal domains interact with the penton base (15, 17, 45). The clear distinction between the long shaft and the globular C-terminal knob domain, which is observed in the adenovirus fiber structure, however, is not apparent in the \( \text{P}_5 \) structure. Additionally, the adenovirus fiber shaft and knob are extremely stable and protease-resistant,

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6 P. S. Rydman and D. H. Bamford, unpublished information.

**FIG. 7.** Structural model of the \text{PRD1} vertex assembly in the context of the viral shell. \( A \), view from the inside of the virion. \( B \), top view. The putative attachment site of \( \text{P}_2 \) is indicated by an arrow. \( C \), side view. Only a layer of major coat protein (P3) trimers immediately adjacent to the vertex (peripentonal trimers, individual subunits shown in green, blue, and orange) was considered. Atomic coordinates corresponding to these trimers were obtained from the pseudo-atomic model of the P3 shell (37). The SAXS model of the \( \text{P}_{31} \) pentamer (red) was visually aligned with P3 trimers using the program MASSHA (32) to minimize steric clashes. The SAXS model of the \( \text{P}_5 \) trimer (dark blue) was placed within the \( \text{P}_{31} \) to reproduce the \( \text{P}_5\text{P}_{31} \) position found in the SAXS model of \( \text{P}_5\text{P}_{31} \) (Fig. 6). Final figures were displayed using RasMol (R. A. Sayle, Glaxo Wellcome and the University of Edinburgh, UK).
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whereas only the C-terminal domain of P5 is stable. The main function of the adenovirus knob is binding to its receptor (Coxackie adenovirus receptor, CAR) (46). No receptor binding activity has so far been identified for P5, and the host cell recognition is mediated by P2. Given the elongated nature of P5, one could argue that P5 constitutes the shaft domain, whereas the knob has been reduced to a small but stable trimerization domain, e.g. P5C3.

The receptor-binding protein of PRD1 (P2) is monomeric and elongated and therefore different from the adenovirus knob domain. The present data also indicate that P2 does not form a stable complex with P5C3. Therefore, despite a similar function, P2 is structurally different from the adenovirus knob domain. Such structural divergence probably reflects different modes of vertex disassembly and release of viral DNA. Adenovirus vertex is destabilized by low pH within endosomes (46). In contrast, disassembly of the PRD1 vertex takes place outside of the host cell and is most likely triggered by binding of P2 to the host cell receptor (16). If indeed the adenovirus and PRD1 share a common viral ancestor (13), then the appearance of a new protein P2 in the context of the vertex assembly provides an example of constrained evolution and a good model for further study.

In conclusion, solution structures of PRD1 vertex complexes reveal an overall architecture similar to that of the adenovirus spike, although there are clearly structural differences between the vertex assemblies of the two viruses. In the light of the clear evolutionary relationship between PRD1 and adenovirus (11–13), unraveling such structural and functional differences between these two representatives of prokaryotic and animal viruses will provide a better understanding of viral evolution in general.

The present study also underlines the potential of solution scattering for the structural analysis of large macromolecular complexes. Modern methods of data analysis permit the construction of ab initio low resolution models of individual proteins and complexes thereof from the scattering of monodisperse solutions. Moreover, by combining solution scattering with other physico-chemical and biochemical methods, it becomes possible to quantitatively characterize the structure and composition of mixtures containing different types of particles. This is especially important for the structural analysis of complex and equilibrium systems.

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