Structural Basis for Membrane Anchorage of Viral φ29 DNA during Replication

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The abbreviations used are: ds, double-stranded.

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Prokaryotic DNA replication is compartmentalized at the cellular membrane. Functional and biochemical studies showed that the Bacillus subtilis phage φ29-encoded membrane protein p16.7 is directly involved in the organization of membrane-associated viral DNA replication. The structure of the functional domain of p16.7 in complex with DNA, presented here, reveals the multimerization mode of the protein and provides insights in the organization of the phage genome at the membrane of the infected cell.

DNA replication occurs at specific cellular locations where the nucleic acids and proteins involved in this and related processes are arranged as supramolecular complexes anchored to cellular substructure(s), which in bacteria is the membrane (1, 2). The inherent compartmentalization of DNA replication within such structures, also named replicative complexes, allows surface catalysis, which increases the efficiency of the replicative process (3). Little is known, however, about the architecture of replicative complexes and the role of structural proteins in their formation and/or maintenance. These structural proteins probably provide a framework responsible for recruitment and organization of the essential components involved in the replication process.

DNA replication of the Bacillus subtilis phage φ29 has been studied extensively in vitro (4, 5) and has also been employed as a model system to study membrane-associated DNA replication (6, 7). These latter studies showed that the phage-encoded membrane protein p16.7 is required for efficient in vivo φ29 DNA replication. The dimeric p16.7 protein contains an N-terminal transmembrane domain (residues 1–20), a coiled-coil region (residues 30–60), and a functional dimeric C-terminal domain (residues 63–130, named p16.7C) (8). p16.7C can bind unspecifically to both single-stranded and double-stranded (ds) DNA. In addition to dimers, p16.7C can form multimers. Multimerization is important for its DNA binding mode in vitro and is probably required for proper compartmentalization and membrane anchorage of viral φ29 DNA during replication in vivo (8, 9). Structural studies revealed that a p16.7C dimer is an elongated molecule defined by a dimeric six helical fold that is able to form larger oligomeric structures (10).

EXPERIMENTAL PROCEDURES

Protein Crystallization and Data Collection—Protein p16.7C was overexpressed and purified to homogeneity as described previously (10). Crystallization experiments were carried out with a p16.7C solution at 10 mg/ml containing 50 mM phosphate buffer, pH 7.5, 200 mM NaCl. Protein crystallization was achieved by microbatch techniques under oil. 1 μl of the protein solution was mixed with a solution containing 1 mM 16-mer annealed complementary oligonucleotide with sequence 5’–CCGGTTGATGCCACCGG–3’. DNAs of different lengths with or without cohesive ends were also tried for crystallization. However, DNA molecules with sizes longer than 16 base pairs produced amorphous solids or severe twinned crystals and DNA molecules shorter than 14 base pairs preclude crystallization. Prismatic crystals (0.1 x 0.1 x 0.2 mm) appeared after 2 or 3 days of incubation at 20 °C. Crystals were transferred to a solution containing 30% glycerol and mounted in a fiber loop and frozen at 100 K in a nitrogen stream. X-ray diffraction data were collected in a CCD detector using the European Synchrotron Radiation Facility Grenoble synchrotron radiation source at wavelength 0.91 Å at the BM16 beam-line. Diffraction data were processed using MOSFLM (11). Structure Determination and Refinement—The x-ray structure of p16.7C in complex with DNA was solved by molecular replacement at 2.9 Å with the coordinates of the x-ray structure of the unbound p16.7C (12). The electron density map calculated with the phases of this model was good enough to manually refine the residues of the six asymmetric peptide chains (protein p16.7 residues 66–129). This model was then refined using the simulated annealing routine of CNS (13). Several cycles of restrained refinement with REFMAC5 (14) and iterative model building with O (15) were carried out. The electron density map for the DNA was poor but allowed the model of the asymmetry moiety of the oligonucleotide used in the crystallization experiments. DNA structure was first considered as a rigid body and refined manually on a graphic station and was, afterward, included in a restrained refinement. Although both the R and Rmerge dropped by the inclusion of the DNA model on the refinement, only slight improvement of the electron density is observed after the refinement. Calculations were performed using CCP4 programs (16). The final model was refined by iterative maximum likelihood positional and translation, libration, and screw rotation displacement refinement (14). Tight non-crystallographic restraints between different protein chains were applied throughout the refinement but they were loosened in the last cycle of refinement. The stereochemistry of the model was verified with PROCHECK (17). Ribbon figures were produced using MOLESCRIPT (18) and RASTER (19). The accessible surface area of p16.7C dimer and protomer was calculated with the program “access” from LIGPLOT package (20). Phasing and refinement statistics are summarized in TABLE ONE.

RESULTS AND DISCUSSION

To gain insight in the molecular function and the DNA binding mode of p16.7 we have determined the crystal structure of p16.7C in complex with dsDNA (see “Experimental Procedures”). Interestingly, three p16.7C dimers, arranged side by side defining a deep dsDNA binding cavity, form a functional dsDNA binding unit (Fig. 1A). To our knowledge, the arrangement of three dimers forming a roughly half circular DNA binding site has not been reported before. There are only few structural differences between the DNA bound and unbound p16.7C dimers, which are mainly confined to the C-terminal tails (Fig. 1B). These tails, which are disordered in the apo form, become ordered in the p16.7C-DNA complex due to intermolecular contacts between p16.7C dimers. The specific organization of the p16.7C dimers in the dsDNA binding unit is therefore probably due to its particular self-complementary shape. The tridimeric unit formed in the presence of dsDNA most likely displays a high stability because there is a 20% decrease of the solvent accessible area in the central p16.7C dimer of the complex, and more than 68% of this buried surface area is formed by non-polar atoms (511 Å2 of a total of 746 Å2). These values are similar to those observed for the formation of other non-transient
stable oligomers (21). The electron density map at the central cavity forms as a continuous envelope running parallel to a crystallographic axis that allows the localization of the dsDNA (Fig. 1C). The dsDNA fits remarkably well in the concave cavity formed by the three p16.7C dimers. In addition, this concave surface, which has a strong positive electrostatic potential (Fig. 2A), does not present edges or structural elements that can penetrate the DNA grooves. This indicates that dsDNA binding is purely driven by electrostatic forces, which is in agreement with the unspecific dsDNA binding activity observed for p16.7C (8, 10). In fact, the observed structural features at the protein p16.7C-DNA interface are shared by some unspecific dsDNA binding domains of processive enzymes that are able to hold and track DNA. In particular, there is a remarkable similarity between the DNA binding surface of p16.7 and that of the processivity factors or sliding clamps (22). As the tridimeric p16.7C unit partially does, these molecules encircle DNA in a central ring formed by antiparallel α helices. Interestingly, the cavity is lined by a striped pattern of positively charged and hydrogen bond donor side chains above which the DNA phosphate backbone lays (Fig. 2B). Whereas these interactions would restrict a pure translational diffusion of the DNA, they may allow a screw-like displacement of the DNA helix, maintaining the DNA backbone at rather fixed positions within the complex. In accordance with this model, the electron density map of the DNA is good enough to locate the phosphate backbone but insufficient to determine the precise position and the chemical nature of the base pairs. This is a consequence of unspecific DNA binding of p16.7C and is inherent to complexes of proteins that bind DNA at multiple positions along a DNA fragment (23).

In summary, the structure of the p16.7C-dsDNA binding unit described in this work provides insights in the process by which p16.7 anchors dsDNA to the bacterial membrane. The N-terminal coiled-coil and transmembrane domains, present in full-length p16.7 protein, are located opposite to the DNA

### TABLE ONE

| Data collection and refinement statistics | p16.7C-DNA |
|-------------------------------------------|-----------|
| **Data collection**                       |           |
| Space group                               | P2₁2₁2₁   |
| Cell dimensions                           |           |
| a, b, c (Å)                               | 65.51, 72.11, 127.36 |
| α, β, γ (°)                               | 90.0, 90.0, 90.0 |
| Resolution (Å)                            | 50.0–2.9 (3.06–2.90) |
| R<sub>free</sub> or R<sub>merge</sub>     | 0.057 (0.364) |
| R<sub>f</sub>                               | 8.0 (2.1) |
| Completeness (%)                          | 96.2 (90.7) |
| Redundancy                                | 4.9 (4.1) |
| **Refinement**                            |           |
| Resolution (Å)                            | 50.0–2.9 (2.98–2.90) |
| No. reflections                           | 12,368 |
| R<sub>work/R<sub>merge</sub>               | 25.3/29.2 (31.7/38.8) |
| No. atoms                                 | 3198 |
| Protein                                   | 328 |
| Ligand/ion                                | 52 |
| Water                                     | 90.3 |
| B-Factors                                 |           |
| Protein                                   | 51.7 |
| DNA                                       | 52.1 |
| Water                                     | 90.3 |
| Root mean square deviations               |           |
| Bond lengths (Å)                          | 0.028 |
| Bond angles (°)                           | 2.244 |

*FIGURE 1. Crystal structure of the tridimeric p16.7C-DNA complex.* A, two views of a ribbon representation of the structure of p16.7C in complex with dsDNA. Individual p16.7C dimers are shown in red, yellow, and blue; DNA is shown in green. B, superimposition of the Cα traces of the three p16.7C dimers forming the complex with DNA (protomer chains are colored red and green) onto the structure of the unbound form of p16.7C (gray). α helices are labeled. The maximum root mean squared deviation between structures for the superimposed Cα atoms is 0.55 Å. C, a section of the 2F<sub>o</sub> – F<sub>c</sub> electron density map contoured at 1 σ showing the DNA. The map is calculated using phases from a model that never had a nucleotide included.
binding cavity (Fig. 1A). This configuration is compatible with the native protein being attached to the membrane and with its proposed role of anchoring φ29 DNA to the membrane of the infected cell (Fig. 3).

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