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Three reversible and controllable discrete steps of channel gating of a viral DNA packaging motor

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ABSTRACT

The channel of the viral DNA packaging motor allows dsDNA to enter the protein procapsid shell during maturation and to exit during infection. We recently showed that the bacteriophage phi29 DNA packaging motor exercises a one-way traffic property using a channel as a valve for dsDNA translocation. This raises a question of how dsDNA is ejected during infection if the channel only allows the dsDNA to travel inward. We proposed that DNA forward or reverse travel is controlled by conformational changes of the channel. Here we reported our direct observation that the channel indeed exercises conformational changes by single channel recording at a single-molecule level. The changes were induced by high electrical voltage, or by affinity binding to the C-terminal wider end located within the capsid. Novel enough, the conformational change of the purified connector channel exhibited three discrete gating steps, with a size reduction of 32% for each step. We investigated the role of the terminal and internal loop of the channel in gating by different mutants. The step-wise conformational change of the channel was also reversible and controllable, making it an ideal nano-valve for constructing a nanomachine with potential applications in nanobiotechnology and nanomedicine.

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1. Introduction

Linear double-stranded DNA viruses package their genomic DNA into a preformed protein shell called the procapsid [1,2]. This DNA encapsulation task is an intriguing step of the viral replication cycle, accomplished by nanomotors using ATP as energy [3–7]. The ingenious design of viral DNA packaging motors and the novel mechanism of action has provoked a broad range of interest among scientists in virology, molecular biology, structure biology, nanotechnology, biophysics, biomaterials, nanomedicine, RNA biochemistry, and therapeutics. In bacteriophage phi29, the nanomotor consists of a protein channel, DNA packaging ATPase gp16, and a ring composed of six pRNA (packaging RNA [8]) to gear the motor [8–10] using one ATP to package 2 [3] or 2.5 [11] base pairs of DNA. The protein hub of this motor is a truncated cone shaped connector [12–14], which contains a 3.6 nm wide central channel that allows the 19.3 kb dsDNA genome to enter during maturation and to exit during the infection process (Fig. 1A–C). The defined phi29 DNA packaging motor, constructed 24 years ago [15], is one of the strongest biomotors [16] assembled *in vitro*. Elucidation of the mechanism of motor action will impact areas of biology, engineering, medicine, and various other nanotechnological fields. The novelty and ingenious design of such machines have inspired the development of biomimetics. *In vitro*, the biomimetic motor could be integrated into synthetic nanodevices [17–21]. *In vivo*, the artificial nanomotors could be used to load drugs, deliver DNA/RNA, pump ions, transport cargos, or drive the motion of components in the heart, eye or other sensing organs in the body. Although the protein sequence of each subunit of the connector holds little sequence homology, and each subunit varies in size [14,22–24], the connector of many viruses displays significant morphological similarity [25]. The structure of the phi29 connector has been previously solved by X-ray crystallography [13,14,26], which shows that it is composed of twelve protein subunits which form a ring with a wider end of 13.8 nm outer diameter buried within the viral procapsid and an extruding narrow end of 6.6 nm. The central channel is 6.0 nm at the wider end and 3.6 nm at the narrow end (Fig. 1C).

Many phenomena concerning procapsid expansion during the life cycle related to DNA packaging of bacteriophage have been reported [27–34]. DNA packaging significantly involves the connector. It has also been reported that the connector is a vital component in the regulation of procapsid shape and size [35,36].

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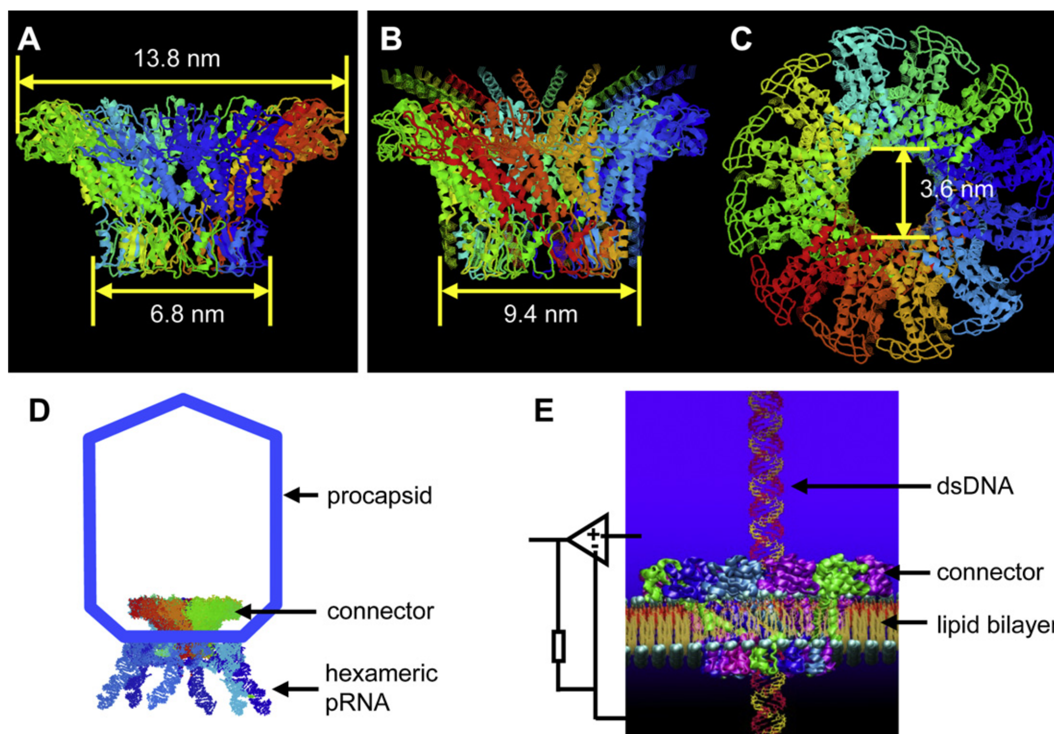

Fig. 1. Illustration of the phi29 connector channel structure. (A) Side view without showing the C- and N-terminal fragment [14]; (B) Side view and (C) bottom view with complete protein sequence [51]; (D) Model of phi29 DNA packaging motor within the procapsid; (E) Illustration of dsDNA translocation through a connector channel reconstituted in an artificial lipid bilayer for the measurement of conductance.

Based on logical analysis, it is reasonable to believe that procapsid expansion is linked to the connector conformational change. However, direct evidence of a conformational change of the connector has never been reported.

We have recently inserted the reengineered connector into a lipid bilayer [20,37] (Fig. 1E). The translocations of ions and dsDNA through the channel demonstrate the potential to use the connector for DNA sensing and fingerprinting at the single-molecule level. We also demonstrated that the phi29 motor channel serves as a valve and exercises a one-way dsDNA traffic mechanism [38]. The direction of DNA trafficking is from the N-terminal narrower end to the C-terminal wider end. This raises a question of how dsDNA is ejected during infection if the channel only allows dsDNA to travel one way. It has been proposed that the motor connector adopts a conformational change after DNA packaging is completed [29]. Such a conformational change renders the channel to allow the dsDNA to come out of the viral procapsid. Here we report that the connector channel indeed exercises conformational change during gating or motor function stimulated by a variety of factors. The conformational change exhibited three discrete identical steps, with each step reducing the size of the channel by one third.

Gating phenomenon has been observed in various protein pores or ion channels [39] and plays a key role in regulating ion transportation through a membrane. Ion channels may be classified by the nature of their gating [40], such as voltage-gated [41], ligand-gated [42], stretch-gated [43], or other gating [40]. Voltage-gated ion channels are activated by changes in electrical potential difference near the channel, while the ligand-gated ion channels are opened or closed in response to the binding of a chemical messenger (i.e., a ligand). There is also recent progress on synthetic channels, which are sensitive to the environmental stimuli, such as temperature [44], voltage [45], pH [46,47], or their combination [48]; but they have a gating mechanism different from protein channels. Here we report a real-time direct observation of the gating of the phi29 DNA packaging nanomotor connector protein channel. It is also interesting to find that this viral protein channel gating can be induced by both voltage and ligand binding, which is similar to the other ion channels.

2. Materials and methods

2.1. Materials

The phospholipid, 1,2-diphytanoyl-sn-glycerol-3-phosphocholine (DPhPC) (Avanti Polar Lipids, Alabaster, AL), Nickel-NTA nanogold (1.8 nm; Nanoprobes), n-decane (Fisher), chloroform (TEDIA) were used as instructed by the vendor. All other reagents were from Sigma, if not specified. The construction and purification of phi29 C-terminal tagged connectors have been reported previously [20,49].

2.2. Regineering, expression and purification of phi29 connector

The construction of the plasmid harboring the gene coding for gp10, the overexpression of gp10 and the purification of phi29 connector have been reported recently [50,51]. The deletion of the tunnel loop (N229–N246) of gp10 was performed by two-step PCR. First, primer pair F1–R1 and primer pair F2–R2 were used to amplify the DNA sequence coding for gp10 (1–228) region and gp10 (247–309) respectively. In the second round of PCR, F1 and R2 were used as primer pairs to link and amplify the PCR product in the first round. The second PCR product was digested with NdeI/XhoI and ligated into NdeI/XhoI sites of the vector pET-21 a(+) (Novagen). The deletion of the N-terminal (1–14) of gp10 was performed by PCR. The sequence coding for gp10 (15–309) region was amplified by a pair of primers. The forward primer contained NdeI restriction site; the reverse primer contained XhoI restriction site and a 6-histidine affinity tag. The PCR product was digested with NdeI/XhoI and ligated into NdeI/XhoI sites of the vector pET-21 a(+) (Novagen).

The connector mutants constructed were expressed, and then were purified with Nickel affinity chromatography [52]. Cells were resuspended with His Binding Buffer (15% glycerol, 0.5 M NaCl, 5 mM Imidazole, 10 mM ATP, 50 mM Na_2HPO_4–NaH_2PO_4, pH 8.0), and the cleared lysate was loaded onto a His•Bind® Resin Column (Novagen) and washed with His Washing Buffer (50 mM Na_2HPO_4–NaH_2PO_4, 15% glycerol, 0.5 M NaCl, 50 mM Imidazole, 10 mM ATP, pH 8.0). The His-tagged connector was eluted by His Elution Buffer (50 mM Na_2HPO_4–NaH_2PO_4, 15% glycerol, 0.5 M NaCl, 0.5 M Imidazole, 50 mM ATP, pH 8.0).

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