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P. aeruginosa PilT Structures with and without Nucleotide Reveal a Dynamic Type IV Pilus Retraction Motor

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Type IV pili are bacterial extracellular filaments that can be retracted to create force and motility. Retraction is accomplished by the motor protein PilT. Crystal structures of *Pseudomonas aeruginosa* PilT with and without bound β,γ-methyleneadenosine-5′-triphosphate have been solved at 2.6 Å and 3.1 Å resolution, respectively, revealing an interlocking hexamer formed by the action of a crystallographic 2-fold symmetry operator on three subunits in the asymmetric unit and held together by extensive ionic interactions. The roles of two invariant carboxylates, Asp Box motif Glu163 and Walker B motif Glu204, have been assigned to Mg^{2+} binding and catalysis, respectively. The nucleotide ligands in each of the subunits in the asymmetric unit of the β,γ-methyleneadenosine-5′-triphosphate-bound PilT are not equally well ordered. Similarly, the three subunits in the asymmetric unit of both structures exhibit differing relative conformations of the two domains. The 12° and 20° domain rotations indicate motions that occur during the ATP-coupled mechanism of the disassembly of pili into membrane-localized pilin monomers. Integrating these observations, we propose a three-state "Ready, Active, Release" model for the action of PilT.

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Keywords: type IV pili; motor protein; P-loop ATPase; crystallography

Introduction

Type IV pili (Tfp) are extracellular appendages that are present in a wide variety of Gram-negative bacteria and in some Gram-positive bacteria, including pathogens of plant, fungi and animals, soil-dwelling bacteria, and extremophiles.[1,2] While not necessary for viability, Tfp play an important role in the lifestyle of many bacteria by participating in biofilm formation, cell adhesion, phage uptake, DNA uptake, and a type of flagellar-independent surface motility that is often called twitching motility.[3] Thus, understanding the mechanisms that power pilus assembly and disassembly will yield significant information about how bacteria use pili for virulence and about the mechanisms of molecular motors. Gene products that control Tfp regulation, assembly, and disassembly have been identified, and their roles have been characterized in several model organisms.[1,4] The three-dimensional structure of the pilus filament has been studied using multiple biophysical techniques,[5] and recent work has begun to elucidate the interactions of conserved membrane proteins at the base of the pilus.[6]

The force generated by the retraction of a single pilus is over 100 pN,[7] and the retraction of a Tfp bundle leads to nanonewton forces.[8] PilT, the homohexameric machinery that powers pilus retraction, is thus the strongest known biological motor. PilT belongs to a family of secretion ATPases that are conserved in the Tfp, type II secretion, and type IV secretion systems, and are defined by four signature sequences. Walker A and Walker B motifs are canonical in P-loop ATPases, a group that includes motor proteins F_1-ATP synthase, myosin, RecA, and many helicases. Asp and His Boxes are motifs unique to the secretion ATPase family. As the name implies, the Asp Box contains two conserved carboxylic acid residues. These are likely involved in coordinating active-site geometry. The His Box contains two namesake histidine residues, and substituting one of these residues leads to loss of PilT activity *in vivo*.[9,10]

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Abbreviations used: Tfp, type IV pili; NTD, N-terminal domain; CTD, C-terminal domain; *Pa*PilT, *Pseudomonas aeruginosa* PilT; AMP-PCP, β,γ-methyleneadenosine-5′-triphosphate; *Aa*PilT, *Aquifex aeolicus* PilT; PDB, Protein Data Bank; IMPP, inner membrane platform protein; PEG, polyethylene glycol.

The structures of five secretion ATPases are known.[9,11–14] All share a bilobed architecture consisting of a PAS-like N-terminal domain (NTD) joined by a flexible linker to a RecA fold C-terminal domain (CTD). The ATP binding site lies between the two domains, with all four of the signature motifs found near the nucleotide in the CTD. We have previously reported the structure of PilT from *Aquifex aeolicus*,[9] a hyperthermophile whose PilT is 51% identical with *Pseudomonas aeruginosa* PilT (*Pa*PilT). That structure highlighted another invariant feature of PilT and other secretion ATPases: a pair of clamping arginines at the tips of NTD β5 and β6, which interact with the phosphates of ATP and drive conformational change in the motor.[9,14] In order to understand the detailed steps of pilus retraction, we have now solved both the apo structure and the ligand-bound structure of PilT from *P. aeruginosa*, an important and experimentally amenable pathogen with well-characterized Tfp.

Results

Tertiary and quaternary structures of β, γ-methyleneadenosine-5′-triphosphate-bound PilT

*Pa*PilT was crystallized in the presence of the nonhydrolyzable ATP analog β,γ-methyleneadenosine-5′-triphosphate (AMP-PCP). The *Pa*PilT structure was solved by molecular replacement using *A. aeolicus* PilT (*Aa*PilT) as the model and refined to 2.6 Å resolution (Table 1). The structures presented in this work are the first structures of a retraction motor with relevance to *in vivo* pathogenicity. Unlike the previously solved *Aa*PilT structures,[9] the *Pa*PilT structure has active sites that are in the correct conformation to bind and hydrolyze nucleotide, as well as a sufficiently high resolution to warrant interpretation of side-chain and ligand positions. The three subunits in the asymmetric unit (subunits A, B, and C) conform to the two-domain structure (Fig. 1a), and application of the crystallographic 2-fold axis forms the biologically relevant hexamer (Fig. 1b). As expected, each domain maps well onto the *Aa*PilT structure, with RMSDs of 1.2 Å or 1.3 Å for superposition of the NTD or the CTD, respectively. The NTD (residues 1–98) is a six-stranded anti-parallel β-sheet flanked on one side by three α-helices. The CTD (residues 105–344) is a curved seven-stranded β-sheet sandwiched between α-helices. Strong electron density for the extended β-hairpin between anti-parallel β-strands 12 and 13 is only observed for the C subunit. One of these is the AIRNLIRE helix, which is necessary for pilus retraction *in vivo*[15] (Fig. 1a).

PilT forms a closed hexameric toroid in the crystal lattice with an outer diameter of 115 Å and a height of 60 Å (Fig. 1b). The inner pore is 40 Å in diameter at the N-terminal opening and tapers to a 13. 1-Å outlet constricted by Glu247, located on a loop between α9 and α10. Overall, the hexamer surface displays

Table 1. Data collection and refinement statistics

	AMP-PCP-bound PilT	Unliganded PilT
Data collection		
Wavelength (Å)	0.900	0.979
Space group	$C222_1$	$C222_1$
Unit cell parameters *a*, *b*, *c* (Å)	108.5, 119.6, 185.5	108.2, 121.4, 184.4
Resolution (Å)	30.0–2.60 (2.69–2.6)	30.00–3.10 (3.21–3.1)
Number of unique reflections	35,387 (3452)	21,079 (1652)
R_{merge}	0.052 (0.422)	0.098 (0.384)
I/σ_I	32.6 (2.8)	17.3 (3.1)
Completeness (%)	94.4 (93.6)	94.9 (76.1)
Redundancy	7.4 (4.1)	7.0 (5.6)
Wilson *B*-factor (Å^2)	68.8	59.8
Refinement		
Resolution	29.1–2.60 (2.66–2.60)	23.3–3.10 (3.26–3.10)
Reflections	33,594 (2319)	20,121 (2376)
R_{work}/R_{free}	0.244/0.291 (0.352/0.416)	0.229/0.282 (0.331/0.442)
Number of atoms	7939	7643
Protein	7741	7686
Ligand/ion	109	15
Water	89	4
B-factor (Å^2)	61.0	63.6
AMP-PCP (A/B/C)	70.4/60.9/70.7	NA
RMSD		
Bond lengths (Å)	0.010	0.014
Bond angles (°)	1.434	1.477
Ramachandran plot		
Most favored (%)	95.8	86.3
Outliers (%)	0.41	0.82
ESU (maximum likelihood) (Å)	0.30	0.42

Values in parentheses are for the highest-resolution shell.
NA, not applicable.