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Conformational flexibility of the ATPase SecA enables peptide interaction and translocation

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Abstract

The SecA ATPase forms a functional complex with the protein conducting SecY channel to translocate polypeptides across the bacterial cell membrane. SecA recognizes the translocation substrate and catalyzes its unidirectional movement through the SecY channel. The recent crystal structure of the *Thermotoga maritima* (*T. maritima*) SecA SecYEG complex shows the ATPase in a conformation where the nucleotide binding domains (NBD) have closed around a bound ADP-BeFx complex and SecA's polypeptide binding clamp is shut. Here we present the crystal structure of *T. maritima* SecA in isolation, determined in its ADP bound form at 3.1 Å resolution. SecA alone has a drastically different conformation in which the nucleotide-binding pocket between NBD1 and NBD2 is open and the preprotein cross-linking domain (PPXD) has rotated away from both NBDs, thereby opening the polypeptide-binding clamp. To investigate how this clamp binds polypeptide substrates, we also determined a structure of *Bacillus subtilis* (*B. subtilis*) SecA in complex with a peptide at 2.5 Å resolution. This structure shows that the peptide augments the highly conserved β -sheet at the back of the clamp. Taken together, these structures suggest a mechanism by which ATP hydrolysis can lead to polypeptide translocation.

Keywords

protein translocation; SecA; peptide binding; X-ray structure

The evolutionarily conserved protein-conducting SecY channel allows the translocation of unfolded polypeptide chains across the bacterial cell membrane, an essential process in the biosynthesis of many secretory and integral membrane proteins. SecY partners with either the ribosome to allow translocation during protein synthesis (co-translational translocation) or forms a complex with the SecA ATPase to translocate polypeptides after completion of their synthesis in the cytosol (post-translational translocation)¹. The SecY channel is a heterotrimeric membrane protein consisting of the SecY, SecE and SecG subunits^{2,3}. SecY forms an hourglass shaped translocation pathway with water-filled funnels towards both sides of the membrane. The narrowest point, close to the center of the lipid bilayer, is formed by conserved hydrophobic residues that point radially inwards (the pore ring). The

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ACCESSION NUMBERS: Structure coordinates have been deposited at the protein data bank with accession codes 3JUX for *T. maritima* SecA and 3JV2 for the *B. subtilis* SecA-peptide complex, respectively.

channel is plugged by a short α -helix (plug helix) that is located within the periplasmic funnel and rests against the pore ring residues³.

SecA is a multi-domain protein that belongs to superfamily 2 of DEAD-box ATPases^{4,5}. It contains two RecA-like nucleotide-binding domains (NBD1 and 2) that sandwich the nucleotide at their interface. In addition, SecA contains a pre-protein cross-linking domain (PPXD) that emerges from NBD1, as well as helical scaffold and helical wing domains (HSD and HWD, respectively)⁴. A number of crystal structures of isolated SecA from different organisms in the apo- and ADP bound conformations confirm this overall architecture^{4,6,7}. The structures differ the most with respect to the location of the PPXD. In the closed conformation, the PPXD interacts with the HWD, leading to a compact conformation of SecA⁴. In the open conformation, the PPXD has rotated away from the HWD⁸, generating a groove between the PPXD and NBD2 that is called 'the clamp' ⁹.

The recent crystal structure of the *T. maritima* SecA-SecYEG complex revealed that SecA uses its PPXD and HSD to interact with the SecY channel⁹. The PPXD interacts with the conserved cytoplasmic loops of the SecY subunit (6/7 and 8/9 loops) whereas helices 2 and 3 of the HSD (termed SecA's two helix finger) insert deeply into the cytoplasmic funnel of SecY⁹. As shown by disulfide cross-linking and functional experiments, the tip of the two-helix finger contacts the polypeptide chain right above the entrance to the translocation pore, and a conserved hydrophobic residue (in most cases a Tyr) at the fingertip is essential for protein translocation¹⁰.

Building a model for the SecA-SecYEG complex was challenging, due to the relatively low resolution (4.5Å) of the complex structure and the absence of high resolution structures for *T. maritima* SecA and SecYEG in isolation⁹. The interpretation of the SecA part of the complex was based on a comparison with structures of isolated SecAs from other organisms^{4,8}. Significant differences are observed between the structures of these SecAs and SecA in complex with SecY, but it was unclear whether these changes are induced by complex formation or are due to specific architectural features of *T. maritima* SecA. It was concluded that the PPXD is instrumental in the capture of a translocation substrate. In the SecA-SecYEG complex, the PPXD has rotated all the way towards NBD2, thereby closing the deep groove or clamp between them. In isolation, however, the PPXD opens the clamp by a large rotation towards the HWD. We have proposed that a translocation substrate enters the open clamp and is then captured by the rotation of the PPXD. Our new structures show the opening of the polypeptide binding clamp for *T. maritima* SecA in its ADP bound form and demonstrate that a peptide interacts with the clamp by augmenting a highly conserved β -sheet at its back.

Structure of *Thermotoga maritima* SecA

T. maritima SecA crystallized in a broad screen designed to crystallize it in complex with SecYEG in the presence of ADP and BeFx⁹. However, the crystals obtained in the presence of 0.1M HEPES pH 7.5 and 60% MPD only contained SecA. The SecA construct used for crystallization lacks the C-terminal 55 residues (amino acid 817–871) and represents a fully functional SecA protein¹¹. Identical crystallization conditions produced two different crystal forms of SecA, one with space group $P2_12_12_1$ at 3.1Å resolution and one in C2 with 2.65Å resolution. In both cases, *T. maritima* SecA crystallized as a monomer as judged by the extent of interactions between one monomer and either symmetry- or non-crystallographic symmetry related molecules. The PPXD in the C2 crystal form could not be traced, but was well defined in the $P2_12_12_1$ space group. Therefore, the structure presented here is based on the 3.1Å data set (Table 1).

T. maritima SecA has a structure similar to previously studied SecAs^{4,6,7}. However, in contrast to all other SecAs studied to date, *T. maritima* SecA has a 50 residue long insertion in NBD1 (residues 154–204) (Figure 1). This extension forms two additional β -strands at the periphery of NBD1 and two short α -helices connected by a loop. The extension is located on the membrane distal part of SecA and does not interact with the SecY channel⁹.

Despite the presence of ADP and BeFx during crystallization, our structure represents the ADP bound conformation of SecA with no extra density representing the BeFx complex. Accordingly, the nucleotide binding domains are rotated away from one another, thereby opening the nucleotide binding pocket and preventing the arginine finger in NBD2 (Arg570) from contacting the nucleotide. Compared to the channel bound, ADP-BeFx-stabilized state of *T. maritima* SecA, NBD2 is rotated away from NBD1 by about 15° (Figure 2). Whereas similar differences had been observed when *T. maritima* SecA in the SecY complex was compared with *B. subtilis* SecA in isolation^{4,9}, it is now clear that these conformational changes are not caused by species differences. The nucleotide-induced changes are in fact similar to those seen with other DEAD-box ATPases. For example, the two NBDs of the helicase PcrA move towards each other during the transition from the ADP- to the ATP- (or ATP-analogue-) bound state¹², similar to the case of SecA.

Our structure shows SecA in its closed conformation, in which the PPXD contacts the HWD, a conformation that has also been observed in SecAs from other species^{4,6}. Compared to the SecY-bound state of SecA, the PPXD is rotated by approximately 80° towards the HWD (Figure 3). We postulate that this is the resting state of SecA in solution, before it interacts with substrate or SecY channel.

Peptide binding inside SecA's clamp

To analyze how SecA interacts with a translocation substrate, we co-crystallized *B. subtilis* SecA (amino acid 1 to 780) with a hydrophilic peptide. A peptide was chosen that does not resemble a signal sequence because, after initiation of translocation, SecA must interact with any peptide segment of the substrate in a sequence-independent manner. The crystals obtained diffracted to 2.5Å resolution and allowed the tracing of all residues present in the *B. subtilis* SecA construct with the exception of a loop within the HWD (residues 648 to 651). Additional electron density not accounted for by SecA was found at the back of the clamp next to the two-stranded β -sheet connection between NBD1 and the PPXD. The density is consistent with an additional short β -strand extending the sheet next to its N-terminal β -strand (residues 222 to 224) (Figure 4a). The β -strand of the peptide was characterized by backbone density for three residues, but side chain density was observed for only one residue of the peptide. Although the identity of this residue is unclear, it appears to form a hydrophobic stacking interaction with Phe182 of NBD1 (Figure 4b). Weak discontinuous density was also observed on either side of the β -strand formed by the peptide.

SecA interacts with the peptide as a monomer in a conformation that is similar to a previously described splayed open conformation⁸. However, the PPXD has rotated significantly further towards both NBDs, although not as far as in the channel-bound conformation (Figure 4c). The movement of the PPXD is consistent with the proposed capture of a polypeptide inside the clamp.

Implications for protein translocation

Based on our SecA-peptide structure, we propose that SecA initially binds its substrates in the open clamp; the two β -strands linking the NBD1 and PPXD would interact with the backbone of a polypeptide chain by inducing a β -strand conformation. The extension of the

β -sheet, also called β -strand augmentation¹³, would be sequence-independent, consistent with the requirement that SecA interacts with a broad range of polypeptide segments. Additional support for this model comes from two earlier structures of *B. subtilis* SecA. One structure shows full-length SecA (containing additional 70 residues at its C-terminus) with a short region of its C-terminal domain (residues 794 to 796) interacting in a β -strand conformation with the same residues that bind the peptide in our structure (residue 222 to 224)⁴. The other example is a crystal form of *B. subtilis* SecA with two copies of the ATPase in the asymmetric crystallographic unit¹⁴. Here, the clamp of one SecA copy made crystal contact with NBD2 of the other SecA copy, thereby converting a short α -helix of NBD2 into a β -strand that augments the β -sheet connecting NBD1 and the PPXD. Again, residues 222 to 224 are involved in this interaction. Interestingly, in all cases the curvature of the β -sheet would direct the additional β -strand into the clamp. Recent disulfide bridge cross-linking experiments confirm that a translocating polypeptide chain moves through the clamp¹⁵. These cross-linking experiments and the fact that other crystal structures of full-length SecA show the C-terminus disordered⁸ argue against the possibility that the peptide employed in our studies mimics interactions of the C-terminus in full-length SecA. Rather, the C-terminal segment of SecA would normally interact with SecB^{16, 17}, thus keeping SecA's clamp accessible for a translocating polypeptide.

We propose that the β -strand interaction would also be important when SecA has bound to the SecY channel and actively moves a polypeptide chain. The clamp probably widens and tightens during the ATP hydrolysis cycle, and the β -strand interaction might prevent complete dissociation of the polypeptide. Because the β -strand involves only 3-4 residues, the interaction is likely weak enough to allow polypeptide movement during translocation.

NMR data indicated that a synthetic signal peptide binds to the outside of the clamp¹⁸, suggesting that SecA has distinct binding sites for signal sequences and polypeptide segments that follow them. How a signal sequence would be transferred from the outside of the clamp into the SecY channel is unclear, particularly because the SecA-SecYEG structure shows that a direct path is blocked by the interaction of the PPXD with loops of SecY⁹. Perhaps the signal sequence is released from its binding site before SecA interacts with SecY, or the hydrophobic interior of the clamp provides an additional or alternative binding site for the signal sequence.

A comparison of the *T. maritima* SecA structures in complex with the SecY channel and in isolation shows that the NBDs move relative to one another during the ATP hydrolysis cycle. This movement could be propagated to SecA's two-helix finger via the long, bent α -helix that interacts with both NBDs and the two-helix finger ("transducer helix", Figure 1)¹⁹. In the ATP bound state, the fingertip would move towards the channel and drag the polypeptide chain with it, a movement that requires a weakened interaction of the clamp with the substrate. After ATP hydrolysis and release of inorganic phosphate, the finger would retract from the channel pore, while the clamp tightens its grip on the polypeptide.

Acknowledgments

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Figure 1.

Structure of *T. maritima* SecA. NBD1 and 2 are shown in blue; the two helix finger as part of the HSD is shown in brown; the transducer helix in orange; the PPXD is shown in yellow; and the HWD in gray. The insertion in NBD1 not present in other structures of SecA is shown in purple. The bound ADP molecule is shown in a stick representation.

Figure 2.

Movement of the nucleotide binding domains. The structure of isolated *T. maritima* SecA in its ADP bound conformation is superimposed with the channel bound, ADP-BeFx stabilized state (pdb entry 3DIN). The alignment is on NBD1 (residues 20-200) and shows the opening of the nucleotide-binding cleft in the ADP conformation. NBD1 is shown in dark blue for both nucleotide bound states, NBD2 is in bright and dark cyan for the ADP- and ADP-BeFx bound states, respectively. ADP is shown as sticks.

Figure 3.

The rotation of the PPXD. Surface representation of *T. maritima* SecA in its isolated (a) and channel-bound conformation (b). The NBDs are colored in dark and light blue and the PPXD in yellow as in Figure 1. Between the free and SecY-bound conformations, the PPXD undergoes a large rotation of approximately 80°.

Figure 4.

Binding of a peptide to SecA's clamp. (a) and (b) SigmaA weighted, 2mFo-DFc electron density map obtained for *B. subtilis* SecA crystallized in the presence of peptide, but calculated with phases exclusively from SecA (contoured at 0.8). A short stretch of additional density was observed adjacent to the two-stranded β -sheet connecting NBD1 with the PPXD. A poly-Ala model was built in the electron density and is shown as red sticks. (b) A side view of the β -sheet suggests that Phe182 of NBD1 interacts with a side chain of the bound peptide. (c) Localization of the peptide (shown as red spheres) at the entrance of SecA's clamp. The PPXD is shown in yellow, NBD1 and 2 are shown in dark and light blue, respectively. (d) Side view of the clamp. The path of a translocating polypeptide chain is shown as a red broken line. Parts of the PPXD are shown as a cartoon and a loop has been deleted for clarity. The tip of the two-helix finger is shown in brown.

Table 1

Crystals of *T. maritima* SecA were grown at room temperature (RT) from a mixture of *T. maritima* SecA and SecYEG in 1mM Cymal 6 detergent in the presence of ADP and BeFx by the sitting drop vapor diffusion technique⁹. 2μl of a 100μM protein solution were mixed with an equal volume of well solution containing 0.1M HEPES pH 7.5 and 60% MPD (v/v). Crystals were directly frozen in liquid N₂. *B. subtilis* SecA was co-crystallized at RT by mixing equal volumes of well solution (0.1mM Na₃-citrate pH 5.6, 8% PEG 4000, 100mM NaCl) with *B. subtilis* SecA at 100μM in the presence of 100μM peptide (sequence IRKYGGYIPGLRPGRSTEQYLHR) and 1mM ATPγS. Crystals were cryo-protected in 10% butanediol (v/v) and frozen in liquid N₂. Diffraction data were collected at beamline 19-ID at the Argonne National Laboratory and at X29a at the Brookhaven National Laboratory. Data were processed in Denzo and Scalepack as part of the HKL2000 software package²⁰. Both structures were solved by Molecular Replacement (MR) using Phaser²¹ with the *B.*

subtilis SecA structure lacking its PPXD as search model. The position of the PPXD in *T. maritima* SecA was identified after rigid body refinement in a 2FoFc electron density map. An averaging mask²² was calculated for the full length *T. maritima* SecA based on a manually docked PPXD followed by NCS- and cross-crystal averaging using DMM^{23,24} with the C2 and P2₁2₁2₁ crystal forms. The *T. maritima* SecA structure in its SecY-bound conformation was used as a template for model building in COOT²⁵. The structure was refined in Refmac⁵²⁶ using TLS (Translation/Libration/Screw) parameters. The initial MR phases for *B. subtilis* SecA lacking its PPXD produced an electron density map of sufficient quality to manually dock the PPXD. The model was refined by rigid body as well as restrained refinement with TLS in Refmac⁵. For both structures the electron density in the nucleotide binding pockets suggested the presence of an ADP molecule. Water molecules were placed automatically into the *B. subtilis* SecA structure by COOT using difference map peaks greater than 3σ. The positions of the water molecules were checked manually for chemical plausibility. All figures were prepared in Pymol²⁷.

Data Collection			
Data set	<i>Tm</i>^S SecA	<i>Tm</i> SecA	<i>Bs</i> SecA-polypeptide
Resolution limit [Å]	3.1	2.65	2.5
Space group	P2 ₁ 2 ₁ 2 ₁	C2	P4 ₁
Cell constants [Å]/[°]	64.3, 119.4, 120.8	211.98 101.06, 147.5	106.7, 106.7, 175.6
(a, b, c, / α, β, γ)	90, 90, 90	90, 133.25, 90	90, 90, 90
Unique reflections	17570	58506	67745
Mean I/σ(I)	13.6 (2.5) [#]	18.8 (1.5)	26.4 (2.8)
R _{sym} ^a	0.173 (0.79)	0.11 (0.74)	0.069 (0.46)
Completeness (%)	99.8 (100.0)	93.3 (81.8)	99.7 (98.3)
Redundancy	6.8 (6.7)	6.7 (3.1)	5.4 (4.5)
Wavelength [Å]	1.29	1.29	0.97924
Refinement			
Resolution range [Å]	30 – 3.1		30 – 2.5
NCS ^b	1	2	2
R _{work} (%) ^{//}	22.7		20.8
R _{free} (%) ^{//}	30.3		25.8
Number of Reflections			
Total	15619		64171

Data Collection			
Data set	Tm^S SecA	Tm SecA	Bs SecA-polypeptide
R_{free}	1560		3658
<u>Model geometry</u>			
r.m.s.d. bond length [Å]	0.01		0.0099
r.m.s.d. bond angle [°]	1.36		1.77
<u>Average B-factor [Å²]</u>			
Main chain	42.3		19.7
Side chain	46.6		23.2
<u>Ramachandran analysis^c</u>			
Most favored	89.7%		92.9%
Allowed	9.6%		6.8%
Generously allowed	0.5%		0.3%
Disallowed	0.1%		0%

^S_{Tm}: *T. maritima*, Bs: *B. subtilis*,

[#]Values in parenthesis refer to the highest resolution shell.

^a $R_{sym} = \sum_{hkl} \sum_i |I_i(hkl) - I(hkl)| / \sum_{hkl} \sum_i I_i(hkl)$, where $I(hkl)$ is the integrated intensity of the reflection.

^bNumber of SecA molecules per crystallographic asymmetric unit.

^cPerformed in PROCHECK.

^{//} $R_{work} = (\sum |F_o - F_c|) / \sum F_o$, where F_o and F_c are observed and calculated structure factors.

[¶] R_{free} : is the same as R_{work} but over randomly selected reflections excluded from data refinement.