Substrate Translocation Mechanism of Membrane-binding ATP-dependent Protease FtsH

ATP-dependent proteases are involved in various cellular processes including cell division, cell differentiation, signal transduction, and stress response [1]. Among them, FtsH is unique, because it is the only protease that is anchored to the cytoplasmic membrane and the only essential protease for bacterial growth [2]. Some closely related homologues of FtsH have been found in eukaryotic mitochondria and chloroplast [3]. FtsH degrades not only misassembled subunits of membrane protein complexes for their quality control but also some short-lived cytosolic regulatory proteins for cellular regulation [2]. FtsH comprises an N-terminal transmembrane segment and a C-terminal cytosolic region, which consists of AAA$^+$ (ATPases associated with diverse cellular activities) and protease domains [2]. The AAA$^+$ domain is responsible for ATPase activity, and has the conserved Walker-A, Walker-B, and second region of homology (SRH) motifs. The arginine residue at the C-terminus of the SRH motif, the so-called "arginine finger," is crucial for ATP hydrolysis [2].

Recently, we successfully crystallized and determined a soluble region of FtsH (sFtsH) from *T. thermophilus* using the data obtained at **BL38B1** and **BL41XU** beamlines [4]. The hexameric structure (Fig. 1(a)) adopts a flat-cylinder-like shape (135 Å diameter, 65 Å height) bearing a non-crystallographic three-fold rotational symmetry. As shown in the middle panel of Fig. 3(a), the cylinder is divided into two disks. The lower disk (the protease domain) forms the six-fold-symmetric structure (Fig. 1(a), the bottom view). The upper disk is composed of six AAA$^+$ domains, and each AAA$^+$ domain contains ADP. Remarkably, the orientation of the AAA$^+$ domain relative to the protease domain is significantly different between adjacent subunits (Fig. 1(b)). One subunit (orange in Fig. 1(b)) exhibits extensive contacts between AAA$^+$ and protease domains, and thus is designated as a "closed" subunit. In the adjacent subunit designated as an "open" subunit (green in Fig. 3(b)), the AAA$^+$ domain rotates by 31° away from the protease domain. Consequently, the architecture of FtsH is a trimer of dimers.

In the hexameric structure, R313 is the residue corresponding to the arginine finger (Fig. 2(a)). In the closed subunit, the distance between the oxygen atom of the γ-phosphate of the bound ADP and the Nη atom of R313 of the adjacent open subunit (Fig. 2(a), lower) would be close enough for R313 to interact with the oxygen atom of the γ-phosphate of ATP, when bound to the active site. In contrast, the corresponding distance in the open subunit is too far for ATP hydrolysis (Fig. 2(a), upper). Thus, only the ATPase catalytic site in the closed subunit should be active.

Analysis of the primary sequence revealed that FtsH carries a zinc-binding sequence (HEXXH, where X is any residue) within the protease domain. Likewise, the structure around the protease active site shows a significant difference between the two subunits. In the closed form, the AAA$^+$ domain pushes down the lid helix toward the protease domain. As a result, the kinked helix occupies the space for the polypeptide substrate just above the protease catalytic site (Fig. 2(b), lower). On the contrary, in the open form, the lid helix becomes straight and moves away from the active site (Fig. 2(b), upper). Then, the active can accommodate the substrate.

FtsH has an alternative pathway for the polypeptide substrate, a tunnel leading to the protease catalytic site of the open subunit

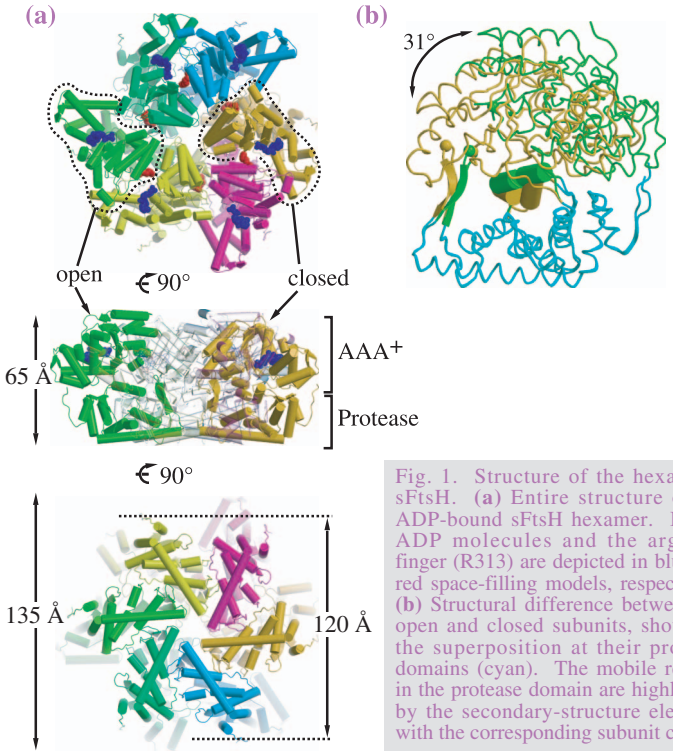

Fig. 1. Structure of the hexameric sFtsH. **(a)** Entire structure of the ADP-bound sFtsH hexamer. Bound ADP molecules and the arginine finger (R313) are depicted in blue and red space-filling models, respectively. **(b)** Structural difference between the open and closed subunits, shown by the superposition at their protease domains (cyan). The mobile regions in the protease domain are highlighted by the secondary-structure elements with the corresponding subunit color.

from the exterior of the molecule via the adjacent closed subunit (Fig. 3(b)). F229 in MFVG motif, which was proposed to be responsible for the substrate binding and translocation [5], is located at the entrance of the tunnel in the closed subunit. On the other hand, F229 in the adjacent open subunit is fully exposed to solvent on the top of the three-fold-symmetric structure.

Combined with the data supporting the ATP-dependent open-close domain arrangements, the present structural features, including the intriguing tunnel and the catalytic environments, allows us to postulate the following model for the ATPase cycle (Fig. 3(a)). (i) The open subunit exchanges its bound ADP with ATP and a closing motion is triggered. (ii) Closing motion accompanies rearrangement of the small subdomain, which leads to the conformational transition of the whole FtsH molecule. (iii) Upon completion of the closing motion, the ATPase catalytic site becomes activated and ATP is hydrolyzed. (iv) The closed subunit can return to the open form without releasing ADP and the whole FtsH structure also returns to the original state. The ATP binding to other open subunits in the same hexamer might help this transition.

The synchronized open-close motions of subunits in FtsH would drive the translocation of a substrate polypeptide to the protease catalytic sites. In accordance with the above ATPase cycle, (i) a substrate polypeptide binds to the MFVG region of the open subunit. (ii) It should be sent into the tunnel leading to the protease catalytic site of the adjacent subunit, (iii) in which it is degraded, and (iv) the MFVG region relieves the grasp on polypeptide and returns to the original position.

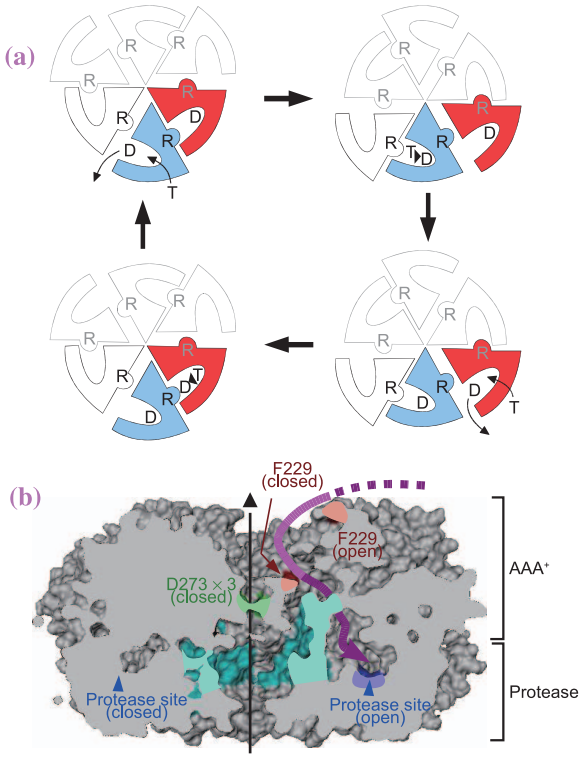

Fig. 3. ATPase cycle and putative polypeptide translocation pathway. **(a)** Schematic diagram of ATPase cycle in the hexameric FtsH. The cycle for the blue subunit is described in the text, where step (i) corresponds to the upper left. T, D, and R represent ATP, ADP, and the arginine finger, respectively. **(b)** A diagram representing a cross section of three-fold-symmetric FtsH, including the three-fold axis (black line) and two protease active sites (blue arrowheads).

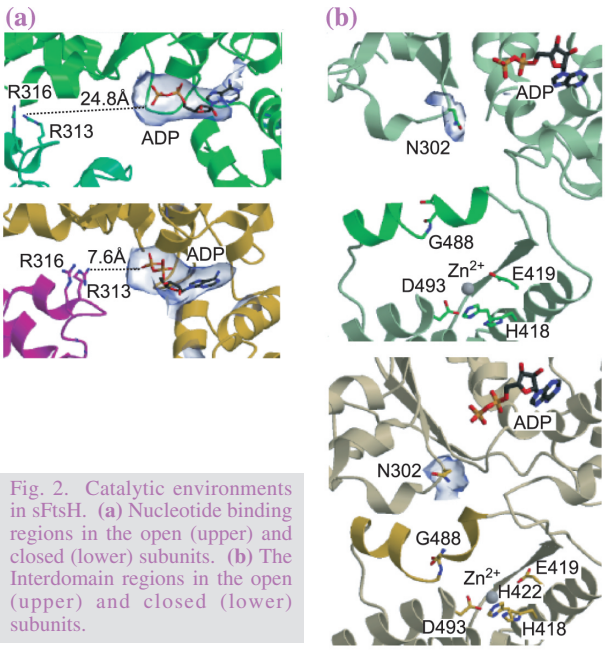

Fig. 2. Catalytic environments in sFtsH. **(a)** Nucleotide binding regions in the open (upper) and closed (lower) subunits. **(b)** The Interdomain regions in the open (upper) and closed (lower) subunits.

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