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Structural studies on *Helicobacter pylori* ATP-dependent protease, FtsH

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The ATP-dependent protease, FtsH, degrades misassembled membrane proteins for quality control like SecY, subunit *a* of FoF1-ATPase, and YccA, and digests short-lived soluble proteins in order to control their cellular regulation, including σ32, LpxC and λcII. The FtsH protein has an N-terminal transmembrane segment and a large cytosolic region that consists of two domains, an ATPase and a protease domain. To provide a structural basis for the nucleotide-dependent domain motions and a better understanding of substrate translocation, the crystal structures of the *Helicobacter pylori* (*Hp*) FtsH ATPase domain in the nucleotide-free state and complexed with ADP, were determined. Two different structures of *Hp*FtsH ATPase were observed, with the nucleotide-free state in an asymmetric unit, and these structures reveal the new forms and show other conformational differences between the nucleotide-free and ADP-bound state compared with previous structures. In particular, one *Hp*FtsH Apo structure has a considerable rotation difference compared with the *Hp*FtsH ADP complex, and this large conformational change reveals that FtsH may have the mechanical force needed for substrate translocation.

Keywords: ATP-dependent protease; FtsH; *Helicobacter pylori*.

1. Introduction

ATP-dependent proteases play vital roles in protein quality control and in regulating the levels of certain cellular proteins (Sauer *et al.*, 2004). *Escherichia coli* and other bacteria, including *Bacillus subtilis*, *Staphylococcus aureus* and *Helicobacter pylori*, contain at least five types of ATP-dependent proteases, including ClpXP, ClpAP, HslUV, FtsH and Lon. The first three (ClpXP, ClpAP and HslUV) have a composite structure of the ATPase complex and protease complex. In the case of the last two (FtsH and Lon), ATPase and protease exist in different domains in the same subunit (Gottesman, 1996).

FtsH belongs to the AAA (ATPases Associated with different cellular Activities) protein superfamily, which is characterized by a highly conserved domain comprised of 230–250 amino acid residues. FtsH is divided into an N-terminal transmembrane segment and a large cytosolic region that contains the ATPase and protease domains. The ATPase domain includes the conserved Walker A, Walker B and second region of homology (SRH) motifs. The protease domain contains a zinc-binding motif, which is consistent with the finding that its proteolytic activity is stimulated by a zinc ion (Ito & &Akiyama, 2005).

The crystal structures of the FtsH ATPase domain from *Escherichia coli* (*Ec*) and *Thermus thermophilus* (*Tt*), and the whole cytosolic region from *Thermotoga maritima* (*Tm*) and *Thermus thermophilus* (*Tt*), have been reported previously (Krzywda *et al.*, 2002; Suno *et al.*, 2006; Bieniossek *et al.*, 2006). These structures suggest that substrate polypeptides bind to the lateral portion of a hexagonal plate and are then translocated to the central pore. Moreover, FtsH forms a threefold symmetric hexamer with open and closed subunits, which suggests that the polypeptide substrate isthen delivered from the pore region to the protease catalytic site. However, the mechanism by which FtsH translocates substrate polypeptides is still not fully understood, in part because there is no structural information that suggests how they move from the lateral region of the FtsH hexagonal plate to the central pore region.

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2. Crystal structures of the *Hp*FtsH ATPase domain

2.1. Overall structure

The crystal structures of the ATPase domain of *H. pylori* FtsH in the nucleotide-free state and in complex with ADP were determined by molecular replacement at resolutions of 3.2 and 3.3 Å, respectively. The *Hp*FtsH ATPase domain shows the general structural architecture characteristic of the AAA superfamily and is comprised of two structural subdomains: a large N-subdomain (residues 160–339) and a smaller C-subdomain (residues 344–418). The centre of the N-subdomain contains a 350 sheet made up of five parallel β strands in the order β2–β3–β4–β1–β5 that are surrounded by eight α-helices. The C-subdomain is composed of four helices: α9, α10, α11 and α12 (Fig. 1*a*).

We observed that within the asymmetric unit under nucleotide-free conditions the *Hp*FtsH ATPase domain assumes two different conformations, which hereafter we will refer to as *Hp*FtsH Apo_A

and Apo_B. Although the overall structures of *Hp*FtsH Apo_A and Apo_B are quite similar, a dramatic conformational difference is apparent when their N-subdomains are superimposed: there is a rotational difference of 126.6° in the C-subdomain relative to the N-subdomain, and the root mean square deviation (RMSD) is 9.01 Å.

2.2. The ADP binding sites

The ATPase catalytic site is situated at the interface between the N- and C-subdomains (Fig. 1*b*). This binding site and the conformation of ADP within the structure of the *Hp*FtsH-ADP complex are similar to those seen within the structures of the *Tt*FtsH-ADP and *Tm*FtsH-ADP complexes, which reflects the strict conservation of the active-site residues. The Walker A motif provides the binding site for the ribose and diphosphate parts of the ADP molecule. The diphosphate group interacts with the strictly conserved Gly213, Thr214, Lys216 and Thr217 residues *via* hydrogen bonds, confirming that the *Tt*FtsH-ADP and *Tm*FtsH-ADP complexes have highly conserved fingerprint sequences in this region (Suno *et al.*, 2006). On the other hand, the structure of the adenine and ribose-binding pocket shows greater variability. Gly164 and His343 in the *Tm*FtsH-ADP complex and Ala159 in the *Tt*FtsH-ADP complex each form hydrogen bonds with the adenine ring of ADP. By contrast, the adenine ring of the *Hp*FtsH-ADP complex is stabilized primarily by a hydrogen bond between the N and O atoms of Ala173 and the Nε2 atom of His354, as well as by hydrophobic interactions with Leu218, Ile350 and Ala379.

2.3. Comparison of the FtsH ATPase domain structures

Compared with the *Tt*FtsH-ADP complex [Protein Data Bank (PDB) ID: 2DHR], the differences in the rotation angles of the C-subdomain in the two Apo structures of *Tt*FtsH (PDB IDs: 1IY2, 1IXZ) were 18.8° and 36.4°, respectively, which is similar to the differences seen in HslU. The crystal structures of HslU indicate that it assumes four different conformational states during the catalytic cycle, and that it rotates about 21.5° in going from the nucleotide-free state to the ADP-bound state (Wang *et al.*, 2001). These large motions are often associated with the binding and release of nucleotides and, presumably, generate the force mediating translocation of protein substrates. It is noteworthy that the rotational difference relative to two subdomains within the structures of the *Hp*FtsH Apo_A and other structures is considerably larger than that seen with HslU. Compared with the *Hp*FtsH Apo_A, the rotational differences relative to the N-subdomain between the C-subdomains in the *Hp*FtsH Apo_B, *Hp*FtsH-ADP and *Tt*FtsH-ADP complexes were 126.6°, 134.5° and 126.7°, respectively.

By comparing the Apo structures (1IXZ, 1IY2 and *Hp*FtsH Apo_A) with the structure of the *Tt*FtsH-ADP complex, it is possible to predict the trajectories of the movement during the course of ADP binding. The trajectory of the movement of the *Hp*FtsH ATPase domain was easily constructed by overlapping the Apo structures onto the N-subdomain of the *Tt*FtsH-ADP complex ATPase domain. This analysis showed sequential rotations of 18.8°, 29.9° and 92.1° from the *Tt*FtsH ADP complex to 1IY2, from 1IY2 to 1IXZ, and from 1IXZ to *Hp*FtsH Apo_A, respectively (Fig. 2). This is the first description of the significant movement that occurs when a nucleotide binds to the active site of *Hp*FtsH. It suggests that movement of this subdomain may generate the mechanical force needed for substrate translocation.

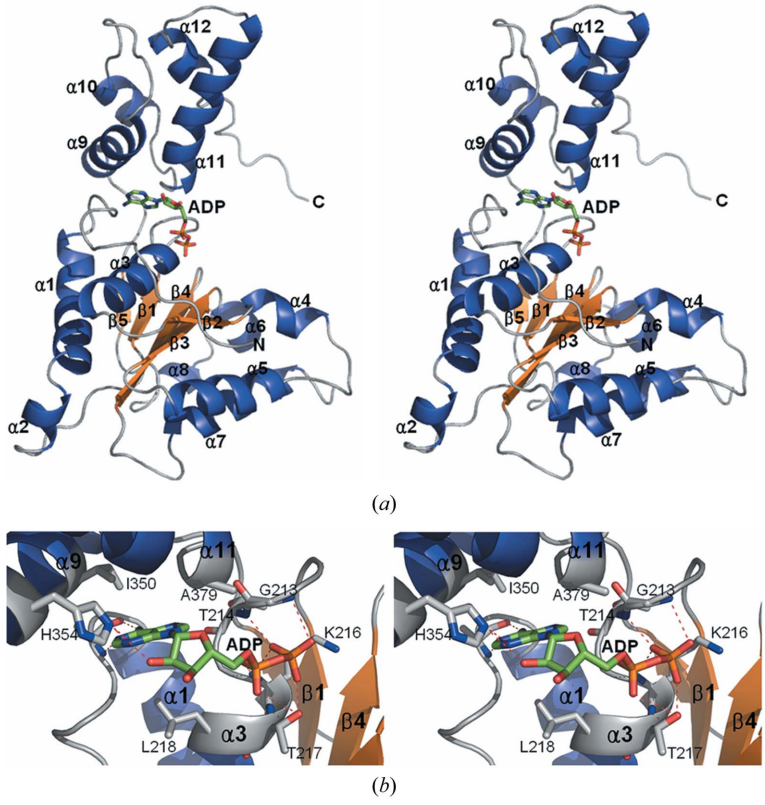

Figure 1
Crystal structure of the *Hp*FtsH ATPase domain. (*a*) Stereoview of a ribbon diagram showing the overall structure of the *Hp*FtsH ATPase. The α-helices are labelled α1–α12, and the β-strands are labelled β1–β5. (*b*) Stereoview of the ADP binding site. The bound ADP molecule is shown as the ball-and-stick model.

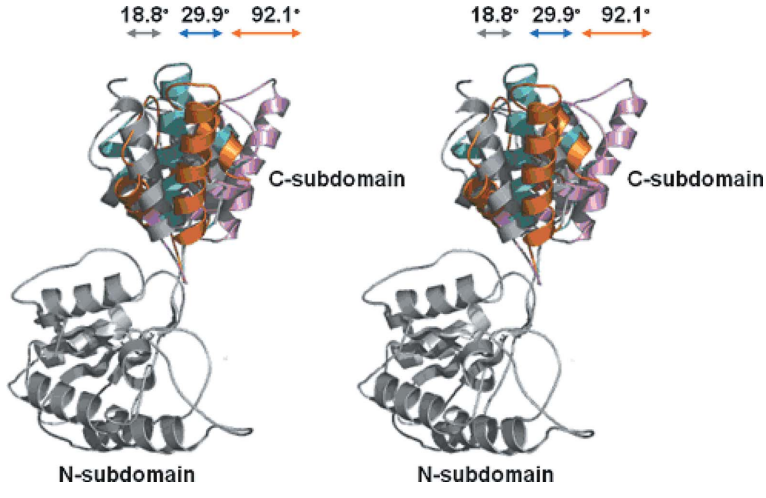

Figure 2
Conformational changes in the Apo structures of *Hp*FtsH (PDB IDs: 1IXZ, 1IY2 and *Hp*FtsH Apo_A) relative to the ATPase domain of the *Tt*FtsH-ADP complex (PDB ID: 2DHR). The *Tt*FtsH-ADP complex and Apo structures (1IY2, 1IXZ and *Hp*FtsH Apo_A) are coloured grey, cyan, orange and pink, respectively. This illustrates the sequential rotations of 18.8°, 29.9° and 92.1° from the *Tt*FtsH-ADP complex to Apo structure 1IY2, from Apo structure 1IY2 to Apo structure 1IXZ, and from Apo structure 1IXZ to *Hp*FtsH Apo_A, respectively.

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References

Bieniossek, C., Schalch, T., Bumann, M., Meister, M., Meier, R. & Baumann, U. (2006). *Proc. Natl Acad. Sci. USA*, **103**, 3066–3071.
Gottesman, S. (1996). *Annu. Rev. Genet.* **30**, 465–506.
Ito, K. & Akiyama, Y. (2005). *Annu. Rev. Microbiol.* **59**, 211–231.
Krzywda, S., Brzozowski, A. M., Verma, C., Karata, K., Ogura, T. & Wilkinson, A. J. (2002). *Structure*, **10**, 1073–1083.
Sauer, R. T. *et al.* (2004). *Cell*, **119**, 9–18.
Suno, R., Niwa, H., Tsuchiya, D., Zhang, X., Yoshida, M. & Morikawa, K. (2006). *Mol. Cell.* **22**, 575–585.
Wang, J., Song, J. J., Franklin, M. C., Kamtekar, S., Im, Y. J., Rho, S. H., Seong, I. S., Lee, C. S., Chung, C. H. & Eom, S. H. (2001). *Structure*, **9**, 177–184.