

Conformational flexibility of pore loop-1 gives insights into substrate translocation by AAA+ protease FtsH

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AAA+ proteases are hexameric barrel shaped proteases, whose active sites are buried within the so-called proteolytic chamber. The entrance is controlled by six AAA proteins important for substrate recognition, unfolding and translocation. As a homohexamer, FtsH is one of the simplest AAA+ proteases containing one AAA and one protease module, as well as two transmembrane helices and a small periplasmic domain per polypeptide. As the sole membrane-associated AAA+ protease in most bacteria, mitochondria, and chloroplasts, FtsH plays a crucial role in membrane homeostasis. Several substrates are membrane-bound but also soluble often membrane related proteins are processed.

Two crystal structures of a transmembrane helix-lacking FtsH construct from *Aquifex aeolicus* have been determined at 2.9 Å and 3.3 Å resolution in space groups R32 and P312. The typical FtsH hexamer is formed from the two different subunits in the asymmetric unit around the three-fold symmetry axis of the crystals. Similar to other published structures, all subunits are loaded with six ADPs and the two subunits resemble the already known open and the closed conformations. Within the ATPase cycle, while a subunit switches from the opened to the closed state, its pore loop-1 interacts with the substrate and translocates it into the proteolytic chamber. Unique to our models is the inactive conformation of the pore loop, which allows the closed conformation to switch back to the opened state without pushing the substrate out again. Our structures give further insights on how this fold is probably induced and linked to the intersubunit signalling network.