Journal of Structural Biology 179 (2012) 202–210

Contents lists available at SciVerse ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

ClpP: A structurally dynamic protease regulated by AAA+ proteins

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ARTICLE INFO

Article history:
Available online 14 May 2012

Keywords:
ClpP
ADEP
ClpAP
ClpXP
Axial channel
ATP-independent proteolysis

ABSTRACT

Proteolysis is an important process for many aspects of the bacterial physiology. Clp proteases carry out a large proportion of protein degradation in bacteria. These enzymes assemble in complexes that combine the protease ClpP and the unfoldase, ClpA or ClpX. ClpP oligomerizes as two stacked heptameric rings enclosing a central chamber containing the proteolytic sites. ClpX and ClpA assemble into hexameric rings that bind both axial surfaces of the ClpP tetradecamer forming a barrel-like complex. ClpP requires association with ClpA or ClpX to unfold and thread protein substrates through the axial pore into the inner chamber where degradation occurs. A gating mechanism regulated by the ATPase exists at the entry of the ClpP axial pore and involves the N-terminal regions of the ClpP protomers. These gating motifs are located at the axial regions of the tetradecamer but in most crystal structures they are not visible. We also lack structural information about the ClpAP or ClpXP complexes. Therefore, the structural details of how the axial gate in ClpP is regulated by the ATPases are unknown. Here, we review our current understanding of the conformational changes that ClpA or ClpX induce in ClpP to open the axial gate and increase substrate accessibility into the degradation chamber. Most of this knowledge comes from the recent crystal structures of ClpP in complex with acyldepsipeptides (ADEP) antibiotics. These small molecules are providing new insights into the gating mechanism of this protease because they imitate the interaction of ClpA/ClpX with ClpP and activate its protease activity.

1. Introduction

Macromolecular assemblies comprised of multiple protein components perform most enzymatic reactions in bacteria, including proteolysis (Baumeister, 2005). These complexes are dynamic macromolecular machines that continuously change conformation in order to perform their functions. Visualizing these conformational changes and the dynamics of these protein complexes is necessary to understand the molecular mechanisms that allow these enzymes to perform work or catalyze reactions. However, obtaining a three dimensional view of the motions that these complexes undergo during the course of a reaction represents a challenge for X-ray crystallography or cryo-electron microscopy (cryo-EM). These techniques immobilize the macromolecular assemblies in either crystal lattices (Chayen and Saridakis, 2008) or vitreous ice (Dubochet et al., 1988) as a necessary step to obtain their three-dimensional structures. Therefore, most frequently these techniques provide snapshots of macromolecular assemblies that represent ground low-energy conformers of the enzyme. These pictures are insufficient to describe the entire range of motions that macromolecular complexes experience during the course of a reaction. Nuclear magnetic resonance (NMR) spectroscopy is an ideal method to probe protein dynamics but traditionally this technique has been restricted to study small monomeric proteins or protein domains. Recently, impressive advances in instrumentation and experimental tools for the production of appropriately labeled samples (Kay, 2005) have made possible to use NMR to study dynamics in high-molecular weight complexes, which were previously considered to be outside the scope of this technique. Structural biologists have realized that a well-defined average structure obtained by X-ray crystallography or cryo-EM is no longer the endpoint goal, but rather the starting point of additional efforts to uncover the dynamics of macromolecular assemblies and how changes in structure relate to their function (Baldwin and Kay, 2009).

The bacterial Caseinolytic protease (ClpP) clearly exemplifies this scenario. The crystal structures of ClpP proteins from several organisms have been experimentally determined (Bewley et al., 2006; Gribun et al., 2005; Kang et al., 2004; Szyk and Maurizi, 2006; Wang et al., 1997). These structures constitute a starting point in our understanding of the function and regulation of the ClpP protease. They show that ClpP assembles into a tetradecameric barrel-shaped enzyme with an enclosed chamber containing

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http://dx.doi.org/10.1016/j.jsb.2012.05.003

14 proteolytic active sites. Entry of the protein substrates into the ClpP degradation chamber occurs through the two axial pores (Ortega et al., 2000), which are gated by the N-terminal region of the protomers. These protein motifs must adopt multiple conformations to either prevent or permit the entry of protein substrates into the ClpP degradation chamber. However, the structure of the N-terminal region of ClpP in the open and closed state of the gate is unknown since in most of the crystal structures these motifs are partially or completely disordered. Likewise, how the degraded polypeptides are released from the proteolytic chamber also remains largely controversial. This process probably also involves mobile motifs of ClpP, either at the N-terminus or at the 'handle' region located at the equator of the tetradecamer (Sprangers et al., 2005). However, clear structural information on how the movements of these regions regulate the exit of proteolysis products remains to be determined. Consequently, the available structural information about ClpP only provides a partial understanding on how ClpP performs its function and by no means presents a full description on the motions of the protein during important steps of the degradation process including entry of substrates and exit of the products.

In what follows, we will review the available structural information and describe the conformational changes that occur in the structure of ClpP to allow the access of protein substrates to the digestion chamber. Aspects of our research in this area are highlighted. We will also comment on some models proposed by different research groups to mechanistically describe how ClpP regulates substrate entry. The current literature shows that we only have a fragmented understanding of the molecular mechanisms of the ClpP protease.

2. ClpP is structured as a self-compartmentalized protease

Proteases are involved in virtually every aspect of the bacterial physiology, including timing of the cell division cycle and responses to heat shock and other stresses (Gottesman et al., 1997; Sauer and Baker, 2011). Proteases are also key contributors to the maintenance of protein homeostasis, a process that involves removing damaged, denatured and aberrantly folded proteins that are harmful to the cell (Wickner et al., 1999). They are critically important for the overall fitness of bacteria and contribute to virulence (Butler et al., 2006). ClpP carries out a large proportion of protein degradation in the bacterial cell. This enzyme is a highly conserved serine protease present throughout bacteria and is also found in the mitochondria and chloroplasts of eukaryotic cells (Corydon et al., 1998; Katayama-Fujimura et al., 1987; Maurizi et al., 1990).

ClpP orthologs (Bewley et al., 2006; Gribun et al., 2005; Kang et al., 2004; Szyk and Maurizi, 2006; Wang et al., 1997) are structurally very similar (Fig. 1A). In all these species, the 21-kDa ClpP monomer is folded in three subdomains: the "handle', the globular "head" and the N-terminal region (Fig. 1A). ClpP is considered a self-compartmentalized protease because in *Escherichia coli* and many other bacterial species it oligomerizes as two stacked heptameric rings enclosing a central chamber containing 14 proteolytic active sites, each comprised of a canonical Ser-His-Asp catalytic triad (Wang et al., 1997). The handle region of the protomers forms the area of interaction between the two heptameric rings and the heads comprise the main body of the rings (Fig. 1B and C). The N-terminus is located at the axial regions of the tetradecamer (Fig. 1B), but in most crystal structures the electron density of this region has been uninterpretable; hence, they are not modeled (Fig. 1A). Based on these observations, this region of ClpP is believed to be highly flexible.

ClpP on its own can degrade small peptides (<5 amino acids) (Thompson et al., 1994; Woo et al., 1989) and also with low

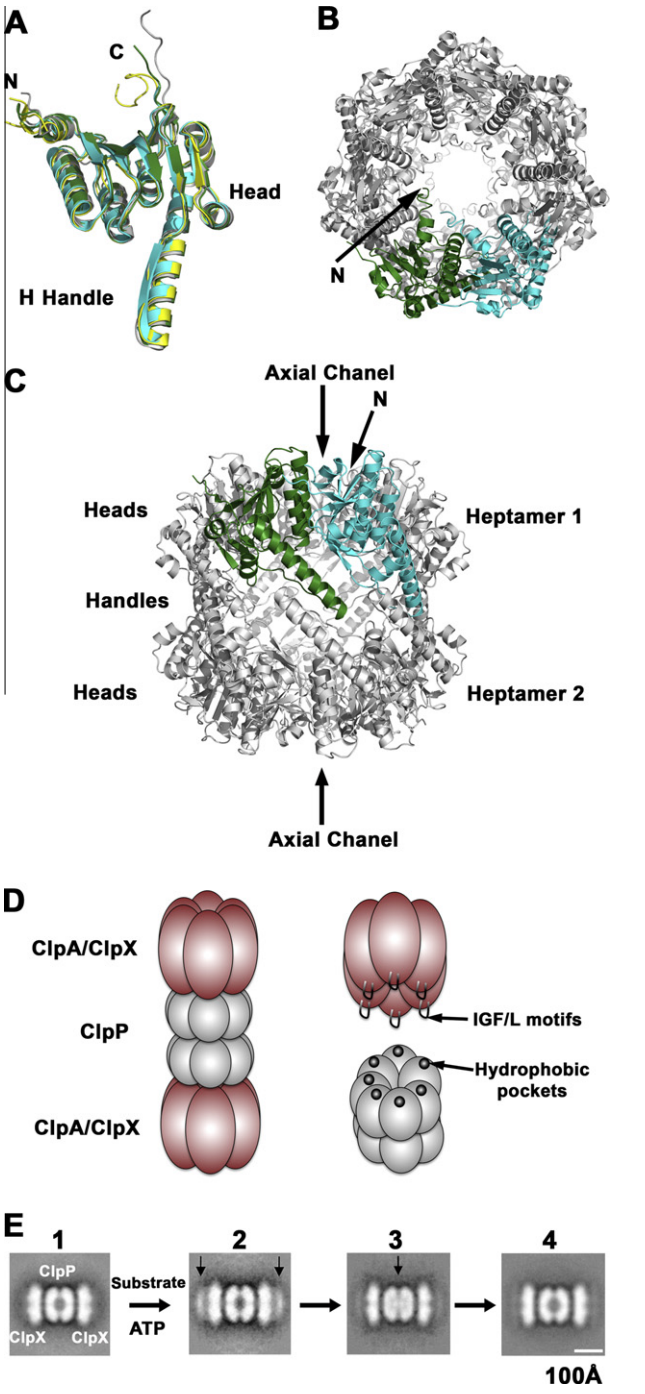

Fig.1. Structure and oligomeric form of the ClpP protease and ClpA/XP complex. (A) Structure-based alignment of ClpP monomers from *E. coli* (green PDB ID: 1TYF), human (gray; 1TG6), *Helicobacter pylori* (cyan; PDB ID: 2ZLO) and *B. subtilis* (yellow; PDB ID: 3KTG). The first 11–20 N-terminal residues are unstructured in these models. The letter 'N' and 'C' indicate the location of the N-terminal and C-terminal end of the proteins, respectively. Top view (B) and side view (C) of the ClpP tetradecamer from E. *coli*. Two adjacent protomers are colored in cyan and green. The location of the N-terminal end of one ClpP protomer in the tetradecamer is indicated by arrows in panel (B) and (C). (D) ClpP in bacteria assembles as ClpAP and ClpXP complexes upon binding to hexameric ClpA or ClpX ATPases. Interaction between the two components of the complex is mediated by six IGF/L loops in the ClpA and ClpX hexamer that dock in hydrophobic pockets located near the outer edge of the ClpP apical surfaces. (E) The substrate translocation process visualized by electron microscopy. ClpX recognizes and unfolds the substrate (1& 2). Then, it is unfolded and threaded into the digestion chamber of ClpP (3) and hydrolyzed (4). Arrows indicate the initial binding site of substrate in ClpX (2) and substrate accumulated in the digestion chamber (3). Figure is modified from (Ortega et al., 2000).