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Structures of Asymmetric ClpX Hexamers Reveal Nucleotide-Dependent Motions in a AAA+ Protein-Unfolding Machine

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SUMMARY

ClpX is a AAA+ machine that uses the energy of ATP binding and hydrolysis to unfold native proteins and translocate unfolded polypeptides into the ClpP peptidase. The crystal structures presented here reveal striking asymmetry in ring hexamers of nucleotide-free and nucleotide-bound ClpX. Asymmetry arises from large changes in rotation between the large and small AAA+ domains of individual subunits. These differences prevent nucleotide binding to two subunits, generate a staggered arrangement of ClpX subunits and pore loops around the hexameric ring, and provide a mechanism for coupling conformational changes caused by ATP binding or hydrolysis in one subunit to flexing motions of the entire ring. Our structures explain numerous solution studies of ClpX function, predict mechanisms for pore elasticity during translocation of irregular polypeptides, and suggest how repetitive conformational changes might be coupled to mechanical work during the ATPase cycle of ClpX and related molecular machines.

INTRODUCTION

AAA+ molecular machines use the energy of ATP binding and hydrolysis to power the degradation, remodeling, disassembly, or movement of macromolecular complexes in a wide variety of cellular processes (Hanson and Whiteheart, 2005; White and Lauring, 2007). *E. coli* ClpX is a hexameric AAA+ protein-unfolding machine, which can function alone or with the ClpP peptidase (Levchenko et al., 1997; Grimaud et al., 1998). In the ClpXP protease, a ring hexamer of ClpX mediates ATP-dependent unfolding of specific proteins, for example those bearing the 11 residue ssrA tag, and then translocates the denatured polypeptide into the lumen of ClpP for degradation (Figure 1A; Gottesman et al., 1998; Kim et al., 2000; Singh et al., 2000). ClpX can also unfold proteins with specific recognition tags in the absence of ClpP (Kim et al., 2000). Each subunit of the ClpX hexamer is identical in sequence and consists of large and small AAA+ domains and a family-specific N domain (Figure 1B; Schirmer et al., 1996). However, variants lacking the N domain (ClpX-ΔN) can still combine with ClpP to mediate efficient degradation of native ssrA-tagged proteins (Singh et al., 2001; Wojtyra et al., 2003). Structures of the large and small AAA+ domains are known for a subunit of *Helicobacter pylori* ClpX-ΔN, but these subunits do not form hexamers in the crystal, assembling instead into helical filaments that span the lattice (Kim and Kim, 2003).

The axial pore of the ClpX hexamer serves as the translocation channel into ClpP (Figure 1A; Ortega et al., 2000). Moreover, three different pore loops—called "GYVG," "pore 2," and "RKH"—play roles in binding the ssrA tag (Siddiqui et al., 2004; Farrell et al., 2007; Martin et al., 2007, 2008a, 2008b). In addition, some of these loops also mediate binding to and communication with ClpP and are needed for protein unfolding and/or translocation. For example, current models suggest that the GYVG loops grip polypeptide substrates and then pull or drag these molecules into the pore as a consequence of nucleotide-dependent loops movements (Martin et al., 2008b). This pulling mechanism could generate a force to unfold native substrates that cannot enter the pore and provide a way to translocate the polypeptide once unfolding occurs. It is not known if these GYVG loop movements occur in a localized fashion or as part of larger domain or subunit motions. Importantly, ClpX can translocate radically different polypeptides, including homopolymeric blocks of large, small, charged, or hydrophobic amino acids, as well as unnatural sequences with additional methylene groups between successive peptide bonds (Barkow et al., 2009). Moreover, ClpX can translocate disulfide-bonded proteins, which requires simultaneous passage of three polypeptide chains through the axial pore (Burton et al., 2001; Bolon et al., 2004). How the ClpX pore mediates transport of such diverse polypeptide substrates is not known.

ATP binding and hydrolysis fuel protein unfolding and translocation by ClpX. In principle, a ClpX hexamer could bind six ATPs, but solution experiments with saturating ATP show that at least two subunits remain nucleotide free (Hersch et al., 2005). Moreover, these studies reveal a minimum of two classes

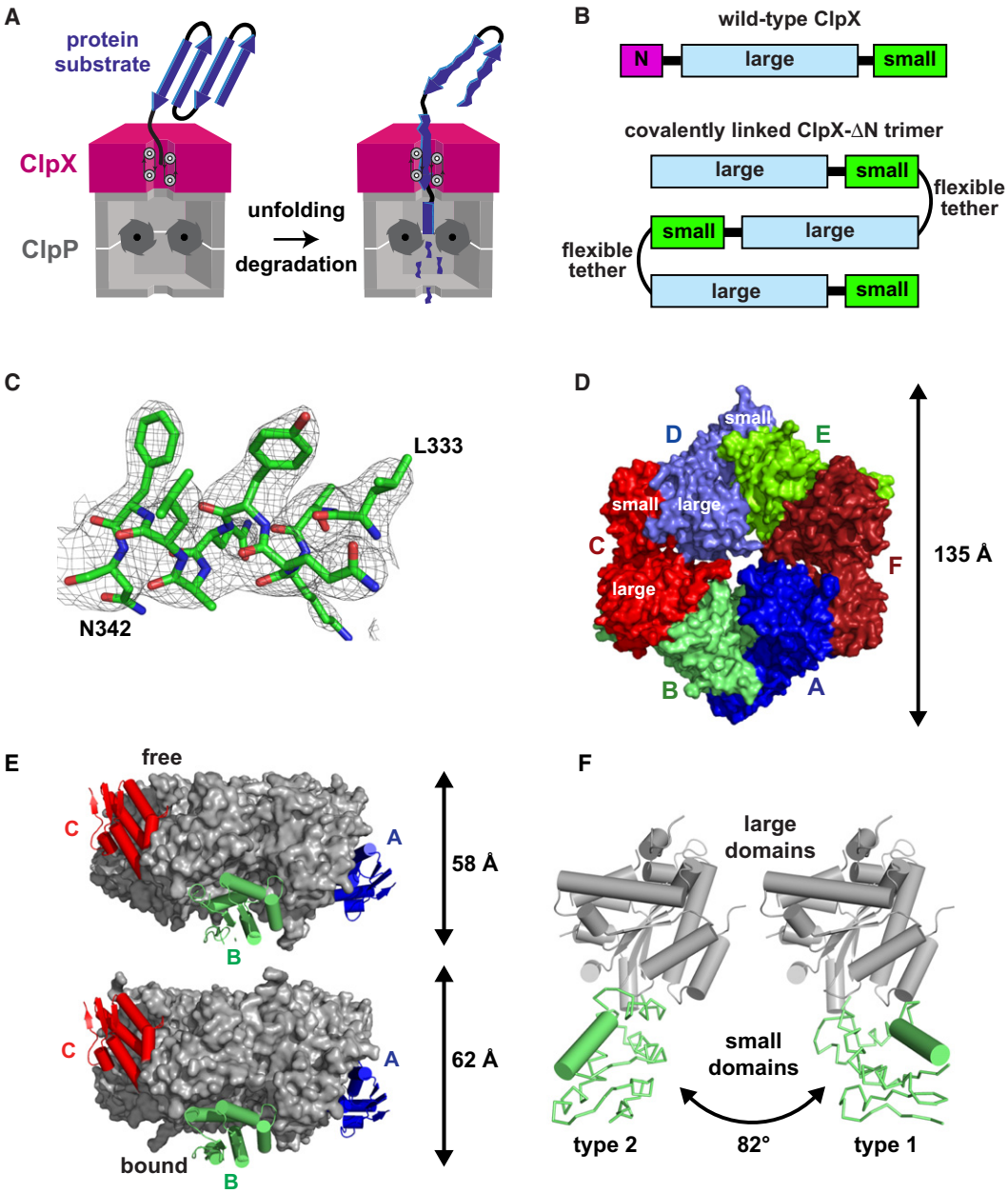

Figure 1. Asymmetric Structures of ClpX Hexamers

(A) Model for protein unfolding and degradation by the ClpXP protease. Cutaway view showing how the degradation tag of a protein substrate could initially bind in the pore of ClpX. ATP-dependent translocation could then lead to unfolding and degradation of the polypeptide by ClpP.

(B) Domain structures of wild-type ClpX and the covalently linked ClpX-ΔN trimer.

(C) Portion of the refined $2F_o$-F_c electron density map (contoured at 1σ) for the nucleotide-bound hexamer.

(D) Surface representation of the nucleotide-bound ClpX hexamer, viewed from the top or ClpP distal face. Each subunit is a different color. The large and small AAA+ domains of two adjacent subunits are labeled.

(E) Side views of the nucleotide-free (top) and nucleotide-bound (bottom) hexamers in mixed surface/cartoon representation. The staggered positions of the small AAA+ domains in chain A (blue), B (green), and C (red) are shown.

(F) The large AAA+ domains of a type 1 subunit (chain A) and type 2 subunit (chain C) from the nucleotide-bound hexamer are shown in the same orientation, revealing a large change in the relative orientation of the attached small AAA+ domains. In the small domains, only the helix formed by residues 333–344 is shown in cartoon representation.

of ATP-binding subunits that differ in the kinetics of nucleotide release. In addition, single-chain ClpX hexamers with covalently linked subunits retain the ability to unfold and translocate substrates efficiently, even when only a few subunits are able to hydrolyze ATP (Martin et al., 2005). These results suggest that ClpX hexamers function asymmetrically, with specific

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