Crystal Structure of ClpX Molecular Chaperone from Helicobacter pylori*

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Dong Young Kim and Kyeong Kyu Kim‡
From the Department of Molecular Cell Biology, Center for Molecular Medicine, SBRI,
Sungkyunkwan University School of Medicine, Suwon 440-746, Korea

ClpX, a heat shock protein 100 chaperone, which acts as the regulatory subunit of the ATP-dependent ClpXP protease, is responsible for intracellular protein remodeling and degradation. To provide a structural basis for a better understanding of the function of the Clp ATPase family, the crystal structures of Helicobacter pylori ClpX, lacking an N-terminal Cys cluster region complexed with ADP, was determined. The overall structure of ClpX is similar to that of heat shock locus U (HslU), consisting of two subdomains, with ADP bound at the subdomain interface. The crystal structure of ClpX reveals that a conserved tripeptide (LGF) is located on the tip of ClpP binding loop extending from the N-terminal subdomain. A hexameric model of ClpX suggests that six tripeptides make hydrophobic contacts with the hydrophobic cleft of the ClpP hexamer asymmetrically. In addition, the nucleotide binding environment provides the structural explanation for the hexameric assembly and the modulation of ATPase activity.

ATP-dependent proteases, such as protease La, two-component protease ClpAP or ClpXP, the heat shock locus HslU1 (ClpYQ) and FtsH, have essential roles in protein quality control, by removing misfolded protein and modulating the cellular concentration of important regulatory proteins (1–5). ClpA and ClpX are the ATPase components of ClpAP and ClpXP proteases, which can form a hexameric ring shaped complex, while the other component ClpP exists as a proteolytic complex with 14 identical subunits in two stacked heptameric rings (6–8). ClpA and ClpX, members of the Clp/Hsp100 ATPase family, participate in the selection, unfolding, and translocation of specific target proteins into the proteolytic chamber of the ClpP for further degradation (9, 10). In addition, they show the independent chaperone activity by catalyzing the protein remodeling and disassembly (11). ClpA and ClpX belong to the AAA+ (ATPases associated with various cellular activities plus) superfamily, which is involved in a variety of cellular function, contributing to the proper function and maintenance of the cell (5). Structural studies have revealed that the members of this family have two subdomains, a core ATPase domain with mixed α/β structures containing nucleotide binding loop and a C-terminal α-helical domain containing a sensor motif (5). Sometimes, another extra domain is found in the N-terminal region.

Like other proteins in the AAA+ family, each subunit of ClpX is composed of an ATPase core domain in the N terminus and a SSD (sensor and substrate discrimination) domain of the C terminus (5, 12). There is an additional small Cys cluster domain at the N terminus. The ATPase core domain of ClpX contains a tripeptide (LIV-G-(FL) that is responsible for ClpP recognition and unique to ClpA and ClpX (13). The C-terminal SSD domain is known to be involved in substrate binding specificity (14). The N-terminal Cys cluster domain was proposed to contribute to the initial substrate binding, or perform a gating function, to allow the access of the substrate to other binding sites (10). However, it is considered that the Cys cluster domain is not necessary for the proteolytic activity of ClpX protease, or for hexameric assembly, since Escherichia coli ClpX, devoid of the Cys cluster domain, can assemble into a hexamer, form a stable complex with ClpP and show protease and ATPase activities (10).

Unlike ClpX, ClpA has two ATPase domains, which play different roles. The first ATPase domain (D1) is involved in hexameric assembly and the second (D2) is essential for ATPase activity (15, 16). Their functional difference was explained by the different nucleotide binding environments in the crystal structure of E. coli ClpA (17). However, in the current crystal structure of E. coli ClpA, the tripeptide responsible for ClpP binding, and its surrounding loop, are not visible (17). The crystal structures of HslU(ClpxY) complexed with HslV(ClpxQ) revealed that it contains one ATPase domain with similar structural architecture to ClpA D2 (18). Despite the structural resemblance of the ATPase domains, it is expected that HslU has a different structural feature, as it binds to HslV hexamer through the C-terminal loop, while ClpA or ClpX binds to the ClpP heptamer through the ClpP binding loop (13).

Although plenty of structural evidence has been obtained from studies of ClpA and HslU that explains the function of the regulatory component of ATP-dependent protease, several questions still remain; how does the ClpA or ClpX hexamer bind to the ClpP heptamer, or what are their structural differences? To provide a structural basis for a better understanding of the function of the Clp ATPase family, the crystal structure of Helicobacter pylori ClpX, lacking the N-terminal Cys cluster domain (ClpX-ASD, ClpX containing ATPase and SSD domains), was solved. Although attempts were made to express and crystallize ClpX proteins from several bacteria, only the H. pylori (Hp) ClpX-ASD was crystallized and diffracted suffi-
efficiently to solve the structure. Since Hp ClpX shares a 54.3% sequence identity with \textit{E. coli} ClpX (Fig. 1), the biochemical data of \textit{E. coli} ClpX can be useful in the interpretation of the structural implication of Hp ClpX. The crystal structure newly reveals the tripeptide in ClpP binding loop that could mediate the asymmetric binding of the ClpX hexamer to the ClpP heptamer.

**EXPERIMENTAL PROCEDURES**

**Protein Preparation and Crystallization—**Hp ClpX (gene number HP1374), lacking the N-terminal Cys cluster domain (ClpX-ASD, corresponding to residues 71–448), was cloned, purified, and crystallized, as described previously (19). The N-terminal Cys cluster domain was removed to facilitate the crystallization of the remaining ATPase and SSD domains of the ClpX. Briefly, the protein was expressed in \textit{E. coli} and purified to homogeneity (>95% pure by SDS-PAGE) by ion exchange (Q-Sepharose) and gel filtration (Superdex-200) chromatographies. Selenomethionine-substituted protein was expressed in the \textit{E. coli} strain B834(DE3) and prepared in the same way. The Hp ClpX-ASD was crystallized in the hexagonal space group P6\textsubscript{1}, with unit cell dimensions of a = b = 78.52 Å and c = 131.51 Å, by the hanging drop vapor diffusion method at 22 °C, from a reservoir solution containing 100 mM imidazole (pH 8.0), 1.0 mM (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}, and 0.2 mM NaCl. To obtain the crystal of ClpX-ASD complexed with nucleotides, 5 mM ADP or ATP\textsubscript{S} and MgCl\textsubscript{2} were added to the protein solution and incubated before crystallization. One molecule of Hp ClpX-ASD exists in an asymmetric unit.

**The Size Measurement of Hp ClpX-ASD—**The size of the Hp ClpX-ASD was estimated using a TSK-4000 gel filtration column under two different buffer conditions, buffer A (20 mM Tris-HCl at pH 7.0) and buffer B (50 mM imidazole and 0.5 mM ammonium phosphate at pH 8.0). One-hundred microliters of ClpX (1.0 μg/μl) was injected into the pre-equilibrated column. The eluted proteins were monitored by absorbance at 280 nm. Ferritin (440 kDa), catalase (233 kDa), albumin (66 kDa), and carbonic anhydrase (29 kDa) were used as size markers (Amer sham Biosciences and Sigma).

**Data Collection, Structure Determination, and Refinement—**Diffraction data were collected for the native, ADP-bound, and ATP\textsubscript{S}-bound crystals of ClpX-ASD at the beamline 6B of the Pohang Accelerator Laboratory with a Mac-Science 2030b area detector, at a resolution of 2.6, 3.0, and at 2.8 Å, respectively. Data were processed and integrated by DENZO and scaled by SCALEPACK, using the HKL program suite (20). Multiwavelength anomalous diffraction data were collected from a frozen crystal of selenium substituted ClpX-ASD at a beamline 41XU of the Spring-8, with a MAR USA marCCD165 detector. The data collected at three wavelengths (inflection, peak, and remote) were processed using the HKL program suite (Table I). Five selenium sites were found and used for phase calculation in the SOLVE program (21).

The density modification, using RESOLVE (22), resulted in a high quality electron density map, which was suitable for model building. Amino acids were assigned using the O program (23). Several cycles of rigid body refinement, positional refinement, simulated annealing, and B-factor refinement were performed at 3.0 Å resolution, using CNS (24). The refinement was continued at 2.6 Å resolution using the data collected from the Hp ClpX-ASD. Although a nucleotide was not added during the crystallization of Hp ClpX-ASD, the electron density corresponding to ADP was found, and the nucleotide was modeled at this stage. Successive refinement with temperature factors and the addition of solvents resulted in R- and R\textsubscript{free} values of 22.4 and 25.7%, respectively, with bulk solvent correction and overall anisotropic thermal factor refinement. The R\textsubscript{free} value was calculated from 5% of the reflections. The final model includes residues 75–179, 188–219, 234–251, and 267–438, 88 water molecules; and an ADP molecule (Table I).

The crystal structure of Hp ClpX-ASD and ATP\textsubscript{S}, plus magnesium were refined at 3.0 and 2.8 Å, respectively. However, since both were identical with the structure of Hp ClpX-ASD refined at 2.6 Å, and the γ-phosphate of the ATP\textsubscript{S} was not shown, no further studies of these structures were continued. The structural evaluation of the refined model, using PROCHECK (25), revealed that the structure had good geometric parameters, with no residue falling in the disallowed region of the Ramachandran plot. The figures in this article were drawn using the MOLSCRIPT (26) and GRASP (27) programs. The final coordinates and structure factors have been deposited in the Protein Data Bank (Protein Data Bank accession number 1U8M).

**Modeling of Hexameric ClpX-ASD—**To model the ClpX subunit into a hexameric ring, the hexameric structure of \textit{E. coli} HslU (Protein Data Bank accession number 1D00) was used as a template. The Hp ClpX-ASD subunit overlapped onto each subunit of the HslU hexamer, with the resulting hexameric model of ClpX being energy-minimized.

**RESULTS AND DISCUSSION**

**Size Determination of Hp ClpX-ASD in Solution—**The ClpX-ASD was crystallized in the P6\textsubscript{1} space group, with only one ClpX-ASD molecule in an asymmetric unit, instead of the hexameric ring found in an electron microscopy study (10). To investigate the oligomerization state of Hp ClpX-ASD in solution, the molecular size of Hp ClpX-ASD was measured by gel filtration under different buffer conditions. The Hp ClpX-ASD was eluted as a hexamer in buffer A (20 mM Tris-HCl at pH 7.0), but it was eluted as a monomer in the initial crystallization solution (50 mM imidazole and 0.5 mM ammonium phosphate at pH 8.0). These results suggest that Hp ClpX-ASD exists as a hexamer in low salt concentrations, while but under the crystallization conditions, it dissociates into a monomer.

**Overall Structure of Hp ClpX-ASD—**The crystal structure of Hp ClpX-ASD has been solved by selenium-multiple anomalous dispersion and refined at resolutions of 3.0 and 2.6 Å, respectively (Table I and Fig. 1). The experimental electron density map, calculated from multiple anomalous dispersion phases and improved by solvent flattening, has sufficient quality to find the correct position of most of the main chains and of the
FIG. 1. The structures of Hp ClpX-ASD, *E. coli* HslU, and *E. coli* ClpA D2. A, the overall structure of Hp ClpX-ASD is presented as a ribbon diagram. The ATPase core domain, SSD domain, and LGF tripeptide are colored magenta, green, and red, respectively. The ATP molecule is shown in orange as a ball-and-stick model. Each secondary structure and the N and C termini are labeled. The protease interface and substrate interface...
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some side chains. Interestingly, a clear electron density was observed near the nucleotide binding region in the experimental map, and modeled into ADP, although ADP was not added during the protein preparation and crystallization. Hp ClpX-ASD shows the general structural architecture found in the AAA- superfamily (5), with two structural domains: the ATPase core domain at the N terminus and the SSD domain at the C terminus (Fig. 1A). The ATPase core domain is composed of a β-sheet of five parallel strands (β1, β2, β3, β6, and β7) surrounded by α-helices (α1–α7 and α10–α11). A conserved tripeptide (LGF), which is responsible for ClpP recognition, is located in the center of the loop (ClpP binding loop) between the α8 and α9 helices of the ATPase domain (Fig. 1, A and D). The SSD domain contains a helical bundle, with three parallel helices (α12, α1’, α13, and α14) and a β-sheet (β8–β10) at the C terminus (Fig. 1A). Four residues at the N terminus and eight residues at the C terminus are disordered. The ATPase and SSD domains are connected by a box VII motif (according to the motif designation in Ref. 12) as a hinge, with relatively loose connections (binding surface area of 488 Å²). The average temperature factor of the residues in the ClpP binding loop is relatively higher than that of the entire molecule, suggesting the flexibility of this loop. There are three disordered regions (residues 180–187, 220–233, and 252–266), mostly in the loops near the center of the hexameric ClpX.

**Structural Comparison with Homologous Proteins**—The structural comparison, using the DALI server (29), reveals that the Hp ClpX has a similar topology to the ATPases of the AAA+ family, such as HslU (18, 30), ClpA (17), NSF (31), and PtsH (32, 33). Of these, the *E. coli* HslU (30) could be superimposed on the Hp ClpX-ASD, with the lowest r.m.s.d., 1.8 Å, for 263 Ca atoms (Fig. 1B). For the crystal structure of ClpA, which contains two AAA+ domains, the second AAA+ domain (D2) is more similar to ClpX, and can be superposed on Hp ClpX-ASD, with a r.m.s.d. of 2.8 Å for 250 Ca atoms (Fig. 1C). A structure-based sequence alignment reveals that the Hp ClpX-ASD and *E. coli* HslU share a 32.4% sequence identity (Fig. 1D). Therefore, ClpX is most homologous to HslU in its amino acid sequence, as well as in the three-dimensional structure. On the contrary, despite the structural similarity, Hp ClpX-ASD has only a 19.5% sequence identity with ClpA D2.

The main differences between Hp ClpX-ASD and *E. coli* HslU are the presence of a ClpP-binding loop in the ClpX and the structural features in the C-terminal β-sheet. *E. coli* HslU has two β-strands and an α-helix at the C terminus, whereas there are three β-strands (β8, β9, β10) at the C terminus of Hp ClpX-ASD (Fig. 1A). The last β-strand (β10) of the Hp ClpX-ASD forms a β-sheet with β8 and β9, which composes the outside wall of the ClpX hexamer (Fig. 2), while the last helix of HslU points toward the protease interface (Fig. 1B). Considering that 8 amino acids in the C terminus of Hp ClpX are invisible in the current structure, the C-terminal end could be extending from the core of SSD domain toward the substrate interface. Unlike HslU, the Clp family is expected to have a ClpP-binding loop, with additional surrounding helices (α8, α9, and α10), which are absent in *E. coli* HslU (Fig. 1). In the crystal structure of Hp ClpX-ASD, the LGF peptide is located on the tip of ClpP binding loop, which protrudes from the protease interface surface. The LGF peptide composes a rather straight loop, with no side chain interaction. Interestingly, the loop and surrounding helices (α8–α10) are completely disordered and missing in the ClpA-D2 (Fig. 1C), suggesting flexible movement of this region. As the amino acid sequence of the tripeptide is relatively well preserved among the ClpP binding loops and neighboring helices located between the two conserved motifs, sensor I and box VII (12), the LGF peptide might be critical for the initial recognition of ClpP, and the other loops and helices (α8–α10) could act as linkers or be involved in nonspecific binding to the ClpP. These structural interpretations are in agreement with proteolytic (10) and mutation experiments (13), which proposed that the tripeptide is open to the protease interface surface, and involved in ClpP recognition. The C-terminal residues of HslU, which are missing in ClpX, are thought to take part in protease recognition. Therefore, it can be concluded that HslU and ClpX employ different structural motives for protease recognition and complex formation with the protease core, although they show similar structural architectures. The structural differences described above may partially explain how HslU and ClpX recognize and bind the specific protease cores, HsiV and ClpP, respectively.

The other structural difference between ClpX and HslU is the presence of an I-domain in the HslU, which might account for their differential substrate recognition (34). In HslU, an I-domain emerges from the ATPase domain to the substrate interface (Fig. 1B). Instead of an I-domain in the HslU, there is a N-terminal ClpA-D2 domain on the ClpX, although it is missing in this model. Considering the position of the N-terminal residue (Leu⁷⁸⁹) in the crystal structure of Hp ClpX-ASD, it is possible that a N-terminal Cys cluster domain might be located near the substrate interface surface and involved in substrate recognition (Fig. 1, A and B), as suggested in the proteolysis experiment of ClpX (10).

Overall, the structure of Hp ClpX-ASD is also similar to *E. coli* ClpA-D2. While, α12 is connected to α1’ by a large bulge at residues, Gin¹³⁵–Leu¹³⁹, in the Hp ClpX-ASD, the corresponding helix is straight in ClpA (Fig. 1, A and C). This bulge in ClpX is involved in additional subdomain contact between the SSD and ATPase domains. Pro⁵⁵⁵ in the bulge forms hydrogen bonds with Thr⁴⁸⁴ and Ile⁴⁵¹, which is packed against the adenine ring of the ADP. Lys³⁶⁶ and Asn³⁵⁷ of the bulge in the SSD domain form hydrogen bonds with Asn⁴⁸⁴ and the carbonyl group of Tyr⁴⁵⁵ via the water molecules. Interestingly, these features, the bulge between the helices and the inter-residue interaction, are also found in HslU, but not ClpA, suggesting that the subdomain interaction of ClpX is only conserved in HslU. Hp ClpX also contains relatively long helices, α2 and α3, compared with those in HslU and ClpA, which extend toward the substrate interface surface and near the N terminus.

**Hexameric Assembly of Hp ClpX-ASD**—Since *E. coli* ClpX and *E. coli* ClpX-ASD were observed as six-membered ring structures, by electron microscopy (7, 35), and the Hp ClpX-ASD also confirmed to form a hexameric structure in solution, it is appropriate to construct a hexameric model of Hp ClpX-ASD to explain its biological implication.

The hexameric structure of *E. coli* HslU (Protein Data Bank accession number 1DOO) was used as a template for the modeling of the ClpX hexamer, because ClpX shows high sequence and structural homology with *E. coli* HslU (Fig. 1). The model are indicated in the figure to display the relative orientation of Hp ClpX. *E. coli* HslU (B) and *E. coli* ClpA D2 (C), positioned in the same orientation with Hp ClpX-ASD, are displayed as ribbon diagrams, with the same color codes. D, the sequences of Hp ClpX, *E. coli* ClpX, and *E. coli* HslU were aligned by the CLUSTALW program (28), following the manual adjustment based on a structural comparison. The secondary structures of Hp ClpX-ASD are indicated by a *cylinder* for the α-helix and an *arrow* for the β-strand. The amino acids in the LGF peptide are boxed in *blue*. In the alignment, identical residues are boxed in *red*, with homologous residues boxed in *yellow*. 
Figure 2. The hexamer model of Hp ClpX-ASD and E. coli ClpP heptamer. The ribbon diagrams of the hexamer model of Hp ClpX-ASD viewed along the 6-fold axis from the protease interface (A) and from the side (B) are shown. The same color schemes as described in the legend to Fig. 1A are used. However, each subunit is colored with a different brightness. The N and C termini of one subunit are labeled. The surface charge distribution of Hp ClpX-ASD (C) and E. coli ClpP (D) is shown. The protease interface of Hp ClpX and the ATPase interface of ClpP are drawn to show the possible ClpX-ClpP interface. The red and blue areas represent the negatively and positively charged surfaces, respectively. The white region represents the hydrophobic surface. The LGF peptide of ClpX (residues 297, 298, and 299), and the conserved hydrophobic cleft of ClpP (residues 60, 62, 82, 90, 92, 112, 114, and 189), are colored in yellow.

was easily constructed by overlapping the ClpX monomer into each subunit of the HslU hexamer, followed by energy minimization. The overall shape and size of the hexameric model of Hp ClpX-ASD (140 Å wide and 70 Å thick) are similar to those of the E. coli ClpX lacking the N-terminal Cys cluster domain, as shown by electron microscopy (150 Å wide and 75 Å thick; compare Fig. 2 in this manuscript and Fig. 5 in Ref. 10), which supports the validation of our resulting model. Viewed along the 6-fold axis, the Hp ClpX ASD is shaped like a toothed wheel, with an empty space inside of the hexamer located between the subunits (Fig. 2A). Because of the disordered loops (180–187 and 220–233) near the center, the hole in the center of the hexameric model of ClpX appears to be a little wider than that observed in HslU (18, 30) or ClpA (17).

A large area of the surface of ClpX is involved in hexamerization: on one face, α4, α5, α12, α14, and β2, and on the other face, the N-terminal region of α3, α7, α11, β1, and β7 (Figs. 1 and 2). The amino acids in these regions are well conserved in the ClpX family and HslU (Fig. 1D), implying that the hexameric interactions are conserved and supporting the validity of a hexameric model. The SSD domain is mostly involved in a hexameric interaction outside of the ring, while the ATPase domain composes the inside of the ring and contacts the SSD domain of a neighboring subunit (Fig. 2, A and B). The nucleotides near the subunit interface, as well as the subdomain interface, seem to facilitate a hexameric assembly (Fig. 2A). Considering the relative orientation of the ATPase domain and the position of the N-terminal residues, the Cys cluster domain could be located near the apical part of the hexamer, where the I-domain of the HslU is located (Fig. 2, A and B; Ref. 30), suggesting the Cys cluster might have a functional similarity to the I-domain of HslU in recognizing specific substrates. However, in ClpX, an additional regulating factor, such as SspB, is thought to contribute to the substrate binding with the Cys cluster domain (36, 37).

The C-terminal 14 residues of HslU are known to form a short and well ordered loop and helix for the formation of a HslUV complex and they bind between the subunits of the HslV to induce conformational changes (18). However, ClpX is considered to have a different mechanism for ClpP recognition. Instead of the C-terminal residues in HslU, the ClpP binding loop of the ClpX, containing the LGF peptide, is responsible for the binding and activation of the ClpP proteolytic subunit (13). The ClpP binding loop is exposed on the protease interface surface of the hexamer, with the LGF peptide located on top of the loop, possibly for binding with the ClpP (Fig. 2, A–C). The LGF peptide is located 36 Å from the center of hexamer and 25 Å from the bottom of the protease interface surface. The non-perfect symmetry of the heptameric ClpP structure (8), and the presence of hydrophobic clefts on its surface, suggest that the complex formation of ClpXP is possible by the docking of the ClpX to the ClpP through the hydrophobic interaction of the LGF peptide with a hydrophobic cleft on the ClpP (Fig. 2D). High sequence conservations in both the LGF of the ClpX (13) and the hydrophobic residues in the cleft of ClpP (8) strongly demonstrate that a hydrophobic interaction is important for their interaction. This hypothesis is also strengthened by the fact the hydrophobic cleft on the ClpP is located ~34 Å (8) from the center, while the LGF peptide is located at 36 Å (Fig. 2). The flexible nature and rather extended shape of the ClpP binding loop, which is ~25 Å long from the protease interface surface, suggests that this loop spreads over and easily fits into any hydrophobic clefts of the ClpP heptamer rather nonspecifically, making asymmetric binding between the ClpX and ClpP possible. A broad striation shown between the ClpP and ClpX by the electron microscopic image of E. coli ClpXP (10) also indicates that the ClpX and ClpP are connected with a long loop. This type of asymmetric docking was also proposed by a mutation experiment of E. coli ClpX (13).
Nucleotide Binding Site—The nucleotide binding in Clp protease is associated with a multimeric assembly and with hydrolysis inducing conformational change. In ClpA, nucleotide binding at the D1 and D2 domains plays different roles: hexameric assembly in D1 and ATP hydrolysis in D2 (15). However, ClpX contains only one nucleotide binding site, which could play both roles. In the crystal structure and hexameric model it is clear that nucleotide binding promotes the oligomeric assembly, as several residues (Glu139, Glu246, Glu329, and Arg333) of the neighboring symmetry-related subunit are close enough to directly interact with the nucleotide or through water hydrogen bonding (Fig. 3). The residues involved in the interaction with the nucleotide bound to the neighboring subunit are well conserved (Fig. 1D), and the oligomeric assembly facilitated by nucleotide binding seems to be a common feature of ClpX and HslU. In addition to the nucleotide-mediated interaction, many residues are directly involved in the interaction between subunits, suggesting that ClpX should make a hexamer, even in the absence of a nucleotide, as shown in biochemical experiments (10). The enhancing effect of ATP on multimerization can be explained by the extra interaction between the subunits, which is mediated by the nucleotide binding. Conversely, no such interaction is found in ClpA D2. The molecular surface areas of ADP exposed to the surface of the subunit, which could be proportional to the possible interaction with neighboring subunit, are 210.7, 96.0, and 188.9 Å² for ClpA D1, ClpA D2, and ClpX, respectively, indicating that the contribution of the ADP to the subunit interaction is comparable in ClpA D1 and ClpX.

The modulation of ATP hydrolysis in ClpX by substrate or ClpP binding can also be explained by the crystal structure of Hp ClpX-ASD. Arg396 at the sensor-2 motif of the SSD domain, which is proposed to be a substrate binding domain (14), is close to the nucleotide in ATPase domain (Fig. 3). As a result, it is predicted that substrate binding to the SSD domain causes the conformational change of the sensor-2 motif, affecting the ATPase activity through Arg396. This is also true for HslU and ClpA D2, but not for ClpA D1 (17, 18, 30). A recent experiment also supports that substrate binding to the SSD domain could modulate the ATP hydrolysis and unfolding activity by demonstrating that the mutations in the SSD domain uncouple the substrate binding from ATP hydrolysis required for unfolding (38). The crystal structure of Hp ClpX-ASD also reveals that the sensor I (β6; Fig. 1, A and D) motif, which senses nucleotide binding or hydrolysis (12), is located near the nucleotide and directly connected to the ClpP binding loop (Figs. 1A and 3). As a result, it is possible that conformational change of the ClpP binding loop, caused by the binding of ClpP protease, modulates the ATPase activity of ClpX through the sensor I motif.

CONCLUSION

ClpX and HslU show similarities in their sequences, structures, and hexameric assemblies, although their binding partner and binding mode, to the protease or substrate, are considerably different. Conversely, ClpX shows several structural differences from ClpA, which could be primarily due to the presence of two AAA+ domains in the ClpA. A structural difference between the ClpA and ClpX is also manifested in that human mitochondrial ClpP can only bind to E. coli ClpX, but not to ClpA (4). However, ClpA and ClpX have common ClpP binding loops and an additional N-terminal domain, which might be involved in substrate recognition. The detailed mechanism of ClpXP docking, and the role of ATP hydrolysis, in the conformational change have not been fully elucidated in this report. However, the crystal structure of ClpP-ASD was first described, and the importance of the ClpP binding loop and conserved tripeptide in ClpP recognition was identified, which will contribute to the understanding of the role of ClpX as a modulator of ClpP and their asymmetric interaction.

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