Technische Universität München

Crystallographic analysis of the ClpP1/2 heterocomplex from *Listeria monocytogenes*

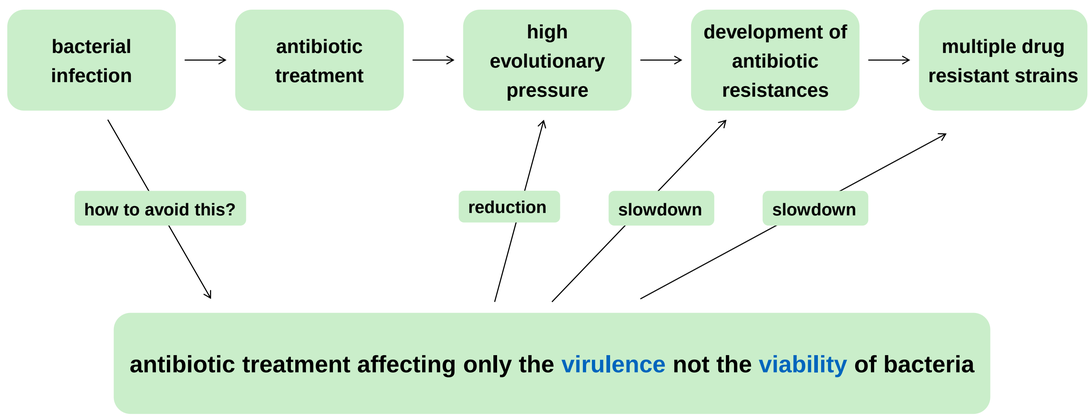
Marie-Theres Vielberg[*1], Maria Dahmen[*2], Stephan A. Sieber[2], Michael Groll[1]

Center for Integrated Protein Science Munich CIPS[M]
[1]Chair of Biochemistry, [2]Chair of Organic Chemistry
Department of Chemistry, Technische Universität München
Lichtenbergstraße 4, 85747 Garching
*equally contributed

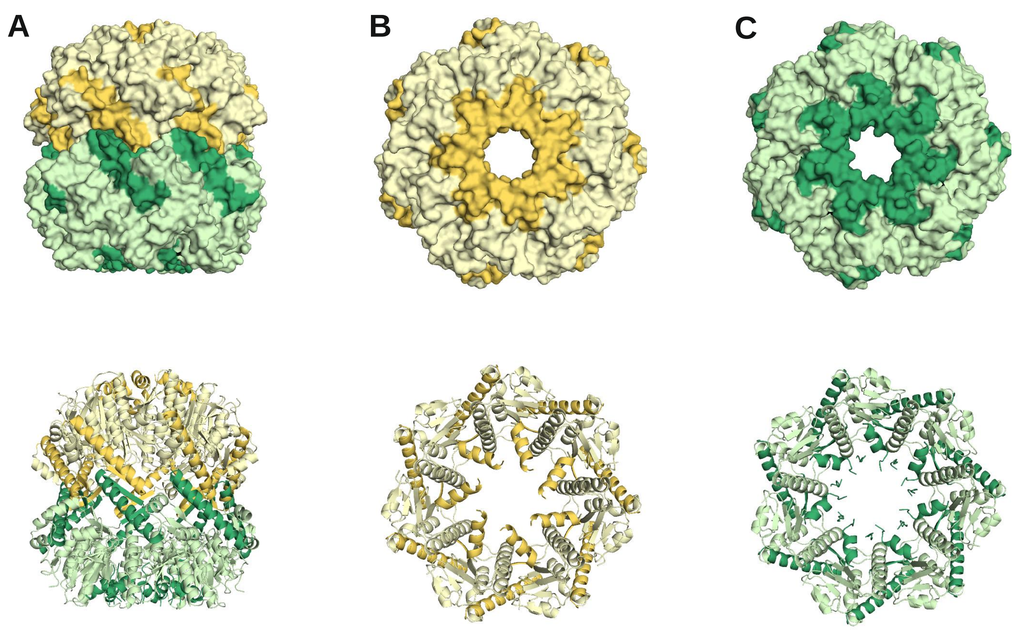
Abstract

The virulence of many obligate as well as facultative pathogens is mediated by the caseinolytic protease P (ClpP). This multimeric serine protease was shown to degrade small peptides independently, whereas digestion of proteins requires the interaction with an AAA$^+$-chaperone, such as ClpX or ClpA. *Listeria monocytogenes* encodes not only one, but two isoforms of ClpP. Labeling studies with different inhibitors proved that both isoforms interact to build up a functional degradation machinery *in vivo*[1]. Recently, we could determine the crystal structure of the LmClpP1/2 heterocomplex at 2.8 Å resolution (PDB: 4RYF)[2]. It preserves all characteristic features of active ClpP proteins which is particularly reflected in the correct alignment of all catalytic centers. The substitution of Asp172 with an asparagine in LmClpP1 leads to replacement of the highly conserved triad by a functional dyad. The reactivity of this assembly is strengthened by the polarizing properties of the mutated residue. Further differences between LmClpP1 and LmClpP2 were found in both the N-terminal region and the S1-substrate pocket. The latter may be useful for isoform-specific drug design in the future.

Biological background



Crystal structure of the heterocomplex



A: Side view of the LmClpP1/2 heterocomplex; B, C: Top views of A, LmClpP1- resp. LmClpP2-interface

The LmClpP1/2 heterocomplex has the typical form of a hollow cylinder. Both LmClpP1 and LmClpP2 (shown in yellow and green respectively) assemble as two distinct heptameric rings. These interact *via* the expanded E-helices of each subunit (highlighted in **A**), a hallmark of active ClpP proteins. The top views of the complex illustrate how the general fold of the enzyme, with exception of the N-terminal residues highlighted in **B** and **C**, is preserved in both isoforms. This observation is reflected in the primary structure: in addition to replacement of the catalytic Asp172 with an asparagine, major variations are found in the N-terminal regions. Overall, LmClpP1 and LmClpP2 share a sequence identity of 44%.

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LmClpP1    8 M-------AENTKNENITNILTQKLIDTRTVLIYGEINQELAEDVSKQLLLLESISN-DPITIFINSQ  60
LmClpP2    1 MNLIPTVIEQTSRGERAYDIYS-RLLKDRIIMLGSAIDDNVANSIVSQLLFLDAQDPEKDIFLYINSP  60

LmClpP1   61 GGHVEAGDTIHDMIKFIKPTVKVVGTGWVASAGITIYLAAEKENRFSLPNTRYMIHQPAGGVQGQSTE 135
LmClpP2   61 GGSISAGMAIYDTMNFVKADVQTIGMGMAASMGSFLLTAGANGKRFALPNAEIMIHQPLGGAQGQATE 135

LmClpP1  136 IEIEAKEIIRMRERINRLIAEATGQSYEQISKDTDRNFWLSVNEAKDYGIVNEIIENRDGL-K     197
LmClpP2  136 IEIAARHILKIKERMNTIMAEKTGQPYEVIARDTDRDNFMTAQEAKDYGLIDDIIINKSGLKG     198
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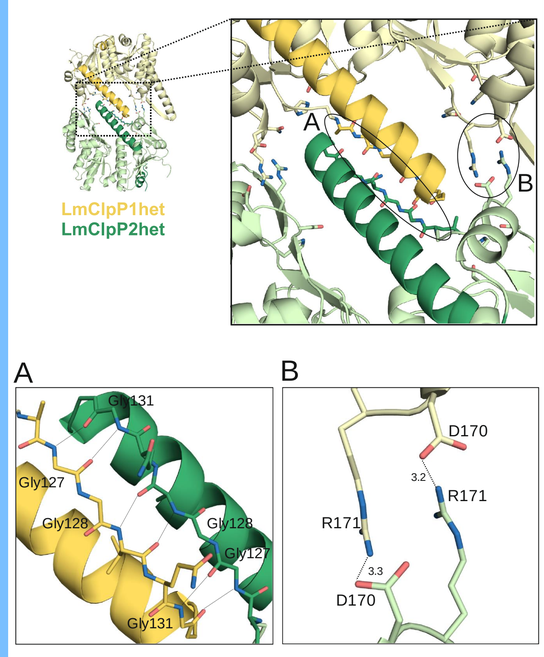
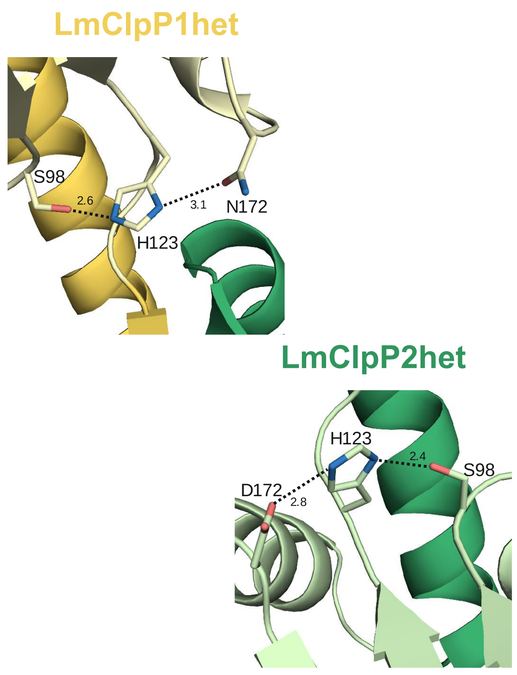
Characteristic features



A: Glycine-rich beta sheet, B: Asp170-Arg171 sensor

There are several indications that the crystal structure of LmClpP1/2 shows a snapshot of the enzyme in its active state. The so called E-helices in their extended conformations (shown in bold) cause alignment of the adjacent Gly-rich beta-sheet that links the two rings (**A**). The Asp170-Arg171 sensor leads to further stability of the tetradecamer by another inter-ring contact (**B**). Notably, no interactions were found that favour formation of the hetero- over the homocomplex.

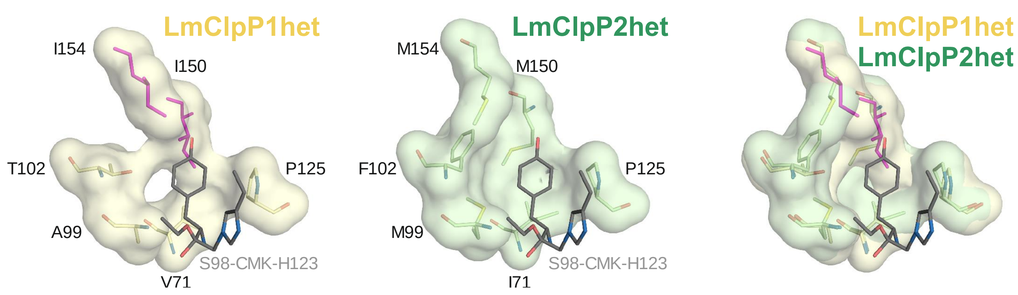
Active dyad versus triad



Catalytic centers of LmClpP1 and L mClpP2

The functionality of the LmClpP1/2 heterocomplex is shown by the correct alignment of all active sites. In LmClpP1 the catalytic aspartate is replaced by an asparagine. The three residues are orientated in the same way and in similar distances as in LmClpP2. Although the proton transfer is limited to two residues, the polarization of the catalytic dyad by asparagine is sufficient to assure the functionality of LmClpP1 during the reaction cycle.

Modeling of the S1-pockets



S1-pockets in the heterocomplex

The similarity between a CMK-inhibitor bound structure of ClpP from *Escherichia coli*[3] and LmClpP2 allowed the assignment of the S1-pockets in the unliganded heterocomplex structure. For LmClpP2 all critical residues are identical, whereas, apart from Pro125, they differ in LmClpP1. Particularly, Ile150 and Ile154 reduce the size of the pocket and provide it with a more hydrophobic character. This potentially restricts the binding of large and polar residues such as P1-Tyr.

Outlook

The crystallization of the LmClpP1/2 heterocomplex showed how two ClpP isoforms in one organism act together to form a functional protease. The assignment of the S1-substrate pockets offers the possibility to design specific inhibitors for LmClpP1 and LmClpP2. This would in turn be helpful to identify the role of LmClpP1/2 during the life and infection cycle of *Listeria monocytogenes.*

References

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[3] Agnieszka Szyk, Michael R. Maurizi: Crystal structure at 1.9 Å of *E. coli* ClpP with a peptide covalently bound at the active site, *J. Struct. Biol. 2006 156 (1) 165-174*

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