Chapter 2
Objective

Structural information on the yCP alone and in complex with ligands strongly supported the development of the currently known potent CP inhibitors bortezomib and carfilzomib. Both compounds do not discriminate between the three mammalian CP types and, thereby, exert anti-cancer activity. However, recent studies demonstrate that iCP-specific inhibitors might qualify as therapeutics in autoimmune diseases. So far, only a few iCP-specific inhibitors were identified, mostly because structural data on the iCP were lacking.

The aim of this thesis was to elucidate the atomic structures of the murine iCP and cCP by X-ray crystallography. The crystal structures of both CP types from one organism enable the direct comparison of the structural features of the iCP and the cCP from *Mus musculus*, the cCP from *B. taurus* and the CP of *S. cerevisiae*. The structural data were expected to explain observed differences in the substrate specificities of the iCP and cCP. Moreover the structural characterization aimed at providing insights into the generation of the distinct MHC I peptide patterns by the cCP and iCP and into the pivotal role of the iCP in the adaptive immune system of vertebrates. Besides the examination of the cleavage preferences, it was intended to elucidate the molecular basis for the (non-) selectivity of known CP inhibitors. In particular, cCP and iCP structures in complex with the iCP-specific epoxyketone inhibitor ONX 0914 were supposed to unravel the reason for its β5i selectivity.

Amino acid substitutions in the substrate binding channel are known to affect cleavage and inhibitor specificities. To evaluate the impact of amino acid differences between β5 subunits on the proteolytic activity mutagenesis experiments with the model organism *S. cerevisiae* were envisioned. These aimed at imitating the active site surroundings of subunit β5c, β5i and β5t. Structural data on these mutant yCPs and analysis of their affinity towards ONX 0914 and bortezomib were expected to reveal the effect of the amino acid substitutions on substrate and inhibitor specificities.

In summary, a multidisciplinary approach combining X-ray crystallography, yeast mutagenesis and inhibition assays was intended to provide detailed information on the architecture of all active sites of murine CP types for the development of potential lead structures for diverse medicinal applications.

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