Abstract

Intramembrane proteases from the rhomboid-like superfamily are enzymes widely distributed and conserved in all domains of life. They participate in many important processes such as membrane protein quality control or mitochondrial dynamics. Their activity is also linked with diseases like Parkinson's disease or cancer. This makes them potential therapeutic targets. In this work we tried to elucidate in more detail the mechanism of action of the main model intramembrane protease, GlpG from E. coli. We also focused on the mechanism of eukaryotic rhomboid RHBDL2, one of the four mammalian rhomboids, function of which is poorly understood. To acquire more detailed information about substrate-enzyme interaction, we synthesized a series of novel peptidyl-chloromethylketone inhibitors derived from natural rhomboid substrate TatA from P. stuartii. Crystal structure of the complex of GlpG with these inhibitors revealed four substrate binding subsites (S1 to S4) of the enzyme and explained its observed substrate specificity structurally. This study showed that substrate cleavage rate can be dramatically modified by changing the substrate sequence in positions P1 to P5. This helped us develop fluorogenic transmembrane peptide substrates for rhomboid proteases, which are usable in detergent and liposomes, and compatible with high-throughput screening. Using these substrates we showed that rhomboid proteases require almost the entire transmembrane domain of the substrate for efficient recognition and cleavage, and the enzyme probably interacts with the transmembrane domain of the substrate via a membrane-immersed exosite. Based on this knowledge we have designed novel and potent rhomboid inhibitors based on peptidyl-α-ketoamides. These compounds are active at nanomolar concentrations, and are selective for rhomboids. Crystal structures revealed that peptidyl-α-ketoamides bind the rhomboid covalently by mimicking the tetrahedral intermediate. Finally, by employing advanced fluorescence spectroscopy techniques (FRET and FCCS), we have investigated the behavior of the rhomboid protease RHBDL2 in a natural biomembrane. While it was previously thought that rhomboids are allosterically activated by dimerization, we found no evidence of RHBDL2 dimerization in natural membranes. Importantly, the approaches developed in this work are generally applicable to the assessment of dimerization of transmembrane proteins. In summary, the findings described in this thesis significantly contribute to the understanding of the mechanism of action of rhomboid proteases.