Rhomboid-Catalized Intramembrane Proteolysis Requires Hydrophobic Matching with the Surrounding Lipid Bilayer

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Membrane thinning of the rhomboid GlpG has been proposed to reduce the hydrophobic mismatch between the enzyme and its surrounding lipid environment. Here, we directly show that the membrane environment of the rhomboid influences the velocity of substrate cleavage. We first measure the impact of GlpG on the hydrophobic thickness in phosphatidyl-choline membranes of varying thickness, where the rhomboid only marginally alters the surrounding membrane. However, in an *E. coli* relevant lipid mix of phosphatidylethanolamine and phosphatidylglycerol, a decrease in hydrophobic thickness of -1.1 Å per leaflet is observed. The cleavage velocity of GlpG is highest in DMPC followed by POPC, POPE/POPG and DLPC, while in the thickest membranes (DPPC/cholesterol) enzyme function is abolished. This suggests that an optimal window of membrane thickness (between ~24 – 26 Å) exists while headgroup specificity does not seem to be decisive for protein function. We infer from these results that the lipid environment can fine-tune GlpG function. By adjusting membrane thickness, for instance through dynamic domain formation, the cell can regulate membrane protein function.

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