

Propeptide-mediated control of protease activity and zymogen maturation in tomato subtilase SBT3.

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Subtilases are ubiquitous extracellular serine proteases, found in all kingdoms. In plants, they are involved in many processes like protein turnover, reaction to biotic and abiotic stresses, plant development, and cell death. They are synthesized as pre-pro-enzymes and targeted to the secretory pathway, where zymogen maturation occurs. We found, that the propeptide of tomato subtilase 3 (SBT3) is a prerequisite for its sorting along the secretory pathway and enzyme maturation.

We observed the intracellular accumulation of an SBT3 mutant lacking its propeptide, while secretion into the apoplast could be restored upon expression of the propeptide *in trans*. The propeptide appears to act as an intramolecular chaperone allowing SBT3 to fold correctly and to pass ER quality control mechanisms as a prerequisite for secretion. We further showed that the propeptide is subsequently cleaved in an autocatalytic process and that the now independent polypeptide acts as a strong inhibitor of the mature protease. This propeptide-subtilase complex is formed with high affinity and temporarily maintains SBT3 in an inactive state. After a pH-dependent intrinsic cleavage of the propeptide, the highly stable complex dissociates, releasing the active subtilase into the apoplastic space.

This highly regulated process allows a compartment-specific activation of the subtilase and prevents premature activity of SBT3 within the secretory pathway.