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Plant defense protease oligomeric structure: is phytaspase from Nicotiana benthamiana monomer or dimer?

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Phytaspases are plant aspartate-specific proteases belonging to the family of plant subtilisin-like proteases. Phytaspases participate in stress responses to some abiotic and biotic factors, including salt, drought, insect invasion, viral infection and so on, helping the plant survive unfavourable effects. Mature phytaspase is exported into intercellular space, called apoplast, and stored there as an active enzyme (Vartapetian A. B., 2011).

The quaternary structure of phytaspases was determined to be monomeric for *Nicotiana tabacum* phytaspase, while for *Arabidopsis thaliana* phytaspase dimerization was reported (Chichkova N. V., 2018).

Nicotiana benthamiana is a promising plant both for biotechnology and fundamental research. This plant grows quickly, produces endogenous proteins well, and easily undergoes various treatments required for research. However, as mature phytaspase is secreted into the apoplast, it can accidentally hydrolyze the exported proteins, thus compromising the fidelity of many targeted recombinant proteins used for therapy (Puchol Tarazona A. A., 2021). Thus, the study of *N. benthamiana* phytaspase simultaneously aims to solve fundamental problems about the molecular aspects of plant defense mechanisms, as well as an improved methodological approach for targeted protein production.

Here, we characterize the oligomeric structure of *N. benthamiana* phytaspase. First, with the method of size exclusion chromatography (SEC) we surprisingly found that active phytaspase had a molecular weight between a monomer and a dimer. To solve this mystery, we use denaturing and non-denaturing PAGE techniques.

There are no other studies on this protein structure before. The results obtained provide a new information about the structure of *N. benthamiana* phytaspase and improve our knowledge about plant defense proteases in general.

References

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