Highly efficient and easy protease-mediated protein purification.

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Abstract

As both research on and application of proteins are rarely focused on the resistance towards nonspecific proteases, this property remained widely unnoticed, in particular in terms of protein purification and related fields. In the present study, diverse aspects of protease-mediated protein purification (PMPP) were explored on the basis of the complementary proteases trypsin and proteinase K as well as the model proteins green fluorescent protein (GFP) from Aequorea victoria, lipase A from Candida antarctica (CAL-A), a transaminase from Aspergillus fumigatus (AspFum), quorum quenching lactonase AiiA from Bacillus sp., and an alanine dehydrogenase from Thermus thermophilus (AlaDH). While GFP and AiiA were already known to be protease resistant, the thermostable enzymes CAL-A, AspFum, and AlaDH were selected due to the documented correlation between thermostability and protease resistance. As proof of principle for PMPP, recombinant GFP remained unaffected whereas most Escherichia coli (E. coli) host proteins were degraded by trypsin. PMPP was highly advantageous compared to the widely used heat-mediated purification of commercial CAL-A. The resistance of AspFum towards trypsin was

improved by rational protein design introducing point mutation R20Q. Trypsin also served as economical and efficient substitute for site-specific endopeptidases for the removal of a His-tag fused to AiiA. Moreover, proteolysis of host enzymes with interfering properties led to a strongly improved sensitivity and accuracy of the NADH assay in E. coli cell lysate for AlaDH activity measurements. Thus, PMPP is an attractive alternative to common protein purification methods and facilitates also enzyme characterization in cell lysate.

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