Conference track «2.2 Bioinformatics»

In silico analysis of structural lability of the phytaspase-1 active site: mechanisms of conformational inactivation

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Phytaspase-1 is a serine aspartate-specific protease which is known to participate in programmed cell death during oxidative stress (e. g. induced by antimycin A or methyl viologen) and to increase its gene expression and activity during wounding stress in *Nicotiana benthamiana* [1, 2, 4]. Under normal conditions, the enzyme is localized in the apoplast, but upon stress induction, it undergoes relocalization into the intracellular environment [3]. During protein purification, preparations of phytaspase-1 exhibit considerable instability and undergo progressive inactivation over time. To mitigate this, methodological protocols commonly recommend the addition of sodium chloride to final concentrations of 300 mM or even 500 mM, which exerts both a stabilizing and a partial activating effect on the enzyme [2, 4]. The present study aimed to elucidate the molecular basis of this phenomenon through an in silico analysis of the structural lability of the enzyme's catalytic triad. We employed an AlphaFold-predicted model of *N. benthamiana* phytaspase-1, which was used to construct a physiologically relevant simulation system mimicking leaf apoplastic conditions, followed by energy minimization, heating, NVT and NPT equilibration according to the Langevin dynamics protocol in OpenMM, as well as a short trial simulation. Subsequently, based on the molecular dynamics trajectory obtained under apoplastic conditions, representative starting frames were selected for subsequent molecular dynamics simulations under varying ionic conditions, including pure water and high-salt conditions (500 mM NaCl). The analysis focused on the positional stability of the catalytic triad residues (Asp-His-Ser) and the oxyanion hole; in catalytically favorable conformations, alignment of the heavy backbone atoms of the triad was performed. In silico molecular dynamics modeling revealed that Na^+ ions stabilize the spatial positioning of the catalytic aspartate and serine residues in the active site of phytaspase-1. This conformational ordering likely underlies the observed increase in enzyme stabilization upon addition of NaCl during commonly used methodological protocols. These results highlight ion-dependent subtle structural adjustments within the phytaspase-1 active site and outline a potential role for saline interactions in modulating its function under stress conditions. The study was funded by the Shenzhen Municipal Government and Shenzhen MSU-BIT University.

Источники и литература

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