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Impact of substrates and deep eutectic solvents on thermal stability and biocatalytic properties of beta-galactosidase during transglycosylation

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Aspergillus oryzae β-galactosidase is a glycosyl hydrolase with transglycosylation activity, enabling glycosylation of bioactive compounds such as tyrosol, a phenylethanoid with known antioxidant properties. This study explores how substrates and deep eutectic solvents (DESs) affect enzyme stability and activity during the synthesis of tyrosol β-D-galactoside (TG).

Enzyme stability was assessed using both nano differential scanning fluorimetry (nanoDSF) and circular dichroism (CD) spectroscopy to provide complementary structural and thermal insights. NanoDSF revealed that lactose stabilized the enzyme in a concentration-dependent manner, increasing the melting temperature (Tm) from 69.5°C (in buffer) to 76.5°C at 0.83 M. To correlate these thermal transitions with structural changes, CD spectra were recorded at 42°C, 60°C, and 70°C. At 42°C, β-galactosidase retained its native secondary structure. Minor changes were observed at 60°C, while spectra at 70°C indicated partial unfolding, primarily involving helical structures, without complete denaturation confirming and complementing the nanoDSF data.

Among DESs tested, betaine-based solvents provided the greatest stabilizing effects. However, several DESs altered enzyme specificity, often favoring hydrolysis over transglycosylation. Polyols present in DESs could act as alternative glycosyl acceptors, impacting product profiles. Both glycerol and tyrosol inhibited enzyme activity and TG synthesis, though glycerol also enhanced thermal stability.

While CD spectroscopy could not assess structural changes in the presence of substrates or DESs due to signal interference, the combined data from CD and nanoDSF offer a comprehensive view of the enzyme's conformational behavior under different conditions. These findings highlight the importance of reaction environment in tuning β-galactosidase performance for sustainable synthesis of glycosylated bioactives.

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