Thermal Denaturation of Dubtilisin C. in Scetonitrile/H_2O Mixtures

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Background and Objectives: It has been suggested that in organic solvents, the unfolded state of an enzyme is thermodynamically favorable. However, due to the high-energy barrier needed for them to unfold most enzymes are thought to be kinetically stable. The melting temperature () of subtilisin C. was studied in acetonitrile/H_2O mixtures by Differential Scanning Calorimetry (DSC) to determine its melting temperature in pure organic solvents directly, and to determine if the enzyme is kinetically or thermodynamically stable in organic solvents.
Methods: DSC was used to measure the enzyme's T_m in organic/water solutions of different concentrations of acetonitrile. Enzyme activity and structure were determined for each solution by UV/VIS and FTIR.
Results: Initially T_m decreases linearly as the percentage of acetonitrile increases up to 50%. However, a linear increase in T_m is observed as the concentration of acetonitrile increases further (up to 90%), suggesting a change in the enzyme stability thermodynamics. Extrapolating to 100% acetonitrile yields a T_m of about 90°C.
Conclusion: These results, which represent a direct measure of an enzyme's T_m in a pure organic solvent, show that enzymes are thermally stable in organic solvents, and suggest that they are thermodynamically rather than kinetically stable as first though.

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