Kinetic and thermodynamic effects of sucrose on chymotrypsin-catalyzed peptide synthesis

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Abstract

Protease-catalyzed peptide synthesis in aqueous media has potential as a green alternative to chemical synthesis of short peptides. These systems can benefit from improved kinetic and thermodynamic stability of the protein[1], as well as the solvent properties, which collectively has the potential to improve enzymatically catalyzed reactions. The addition of a high concentration (up to 3 M) of sugar has the potential to lower the activation energy of peptide bond formation by lowering the dielectric constant of the aqueous media and disfavoring ionisation. In this study, the effects of sucrose on the enzymatic activity of chymotrypsin in peptide synthesis were investigated. The extent to which the folding of the protein was affected by the solvent were measured using Circular Dichroism (CD)[2]. Insignificant alteration in the CD spectra of chymotrypsin in 3 M sucrose and in phosphate buffer indicated that improved activity of the protein did not come from the induced change in shape but was motivated by the solvent-solute interactions. Infrared spectroscopy (IR) upheld the hypothesis that lower dielectric constant in presence of sucrose would change the ionization state of the starting material, which lowered the activation energy of the reaction between various protected amino acids. Subsequently, enzymatic peptide coupling of a model reaction between N-BOC-L-Tyrosine and C-NH_2-L-Glycine in up to 3M sucrose was analyzed using Liquid Chromatography – Mass Spectrometry (LCMS). Monitoring dipeptide coupling through LCMS revealed an improved kinetic and thermodynamic profile of the enzyme in sucrose as opposed to the phosphate buffer. Overall, sugar rich media demonstrates an improved catalytic activity of chymotrypsin.

References

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