

Enzyme Operational Stability in Non Aqueous Solvents

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Background and Objective: During the last 20 years enzymes have been successfully used to catalyze a range of biorelevant reactions in non aqueous solvents. Unfortunately there are still some drawbacks to overcome before their potential can be fully exploited. The most common and detrimental for synthetic applications, especially for lengthy reactions and for the construction of bioreactors, is the low stability and poor reusability of enzymes in both aqueous and organic mediums. The objective of these studies was to elucidate the reasons for the observed decrease in enzyme activity upon prolonged exposure to non-aqueous media.

Methods: The serine protease subtilisin Carlsberg, prepared by lyophilization and covalently modified with polyethylene glycol (5KD), was spin-labeled at its active site with 4-ethoxyfluorophosphinyloxy-TEMPO, and their EPR spectra recorded in different solvents during a 96 hour incubation period.

Results: We observe the presence of two distinct components, a predominant rigid, and a mobile one. The percentage of this rigid component diminishes over time in all solvents. Similar findings were made from a series of fluorescence spectroscopy experiments.

Discussion and Conclusions: The data suggests that prolonged exposure of the enzyme to organic solvents changes the polarity of the microenvironment of the active site, inducing a different, less productive binding of a the substrate.

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