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EDITOR



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Chapter 4

**EFFECT OF ORGANIC SOLVENTS ON THE BINDING OF
COMPETITIVE INHIBITOR PROFLAVIN AND STORAGE
STABILITY OF BOVINE PANCREATIC
 α -CHYMOTRYPSIN**

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ABSTRACT

This review is aimed at analyzing the studies of the competitive inhibitor binding and the storage stability of bovine pancreatic α -chymotrypsin (CT) in organic solvents in order to elucidate what intermolecular processes produce the main effect on the state and functioning of enzymes at high and low water activities in organic media. The binding of competitive inhibitor proflavin and the storage stability of CT in water-organic mixtures were studied in the entire range of thermodynamic water activities (a_w) at 25°C. The moderate-strength hydrogen bond accepting solvents (acetonitrile, dioxane, tetrahydrofuran, and acetone) were used as models due to their ability to vary significantly the size, polarity, denaturation capacity, and hydrophobicity.

The state of water hydrogen bond network in organic solvents was characterized by thermodynamic and spectroscopic data. The absorption spectra of water in organic solvents were measured by FTIR spectroscopy. The state of water in organic solvents was defined in terms of variations in the integral intensity of water and the contour shape of the band of OH stretching vibrations. Excess chemical potentials, partial molar enthalpies, and entropies of water and organic solvents were simultaneously evaluated at 25°C.

The obtained results show:

- 1) The proflavin binding and storage stability curves can be unified in the water activity coordinates. No proflavin binding was observed at low water activity values in the studied solvents.
- 2) At $a_w > 0.35$, the proflavin binding is sharply increased reaching a maximal value at $a_w \sim 0.5-0.6$. This sharp increase in the enzyme activity occurs only above the threshold water activity level, when the self-associated (H-bonded) water molecules appear in the studied organic solvents.