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Effect of hydrogen bond accepting organic solvents on the binding of competitive inhibitor and storage stability of α-chymotrypsin

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Abstract

This review aims to analyse 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 (aw) 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 results obtained showed that:4) The proflavin binding and storage stability curves can be unified in the water activity coordinates. At the highest water activities (aw>0.95), the water hydrogen bond network is bond -percolated. In this composition region, the storage stability values are close to 100%.5) At the lowest water activities, the water molecules exist predominantly as single molecules complexed with organic solvent molecules. No proflavin binding was observed at low water activity values in the studied solvents. At aw>0.3, the proflavin binding is sharply increased reaching a maximal value at aw~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.6) In the intermediate composition region, the solution consists of two kinds of clusters, each rich in each component. There is a sharp transition from the water-rich region to the intermediate one. This transition is associated with an anomaly in the thermodynamic, structural, and enzyme activity properties. This transition may involve loss of the bond percolated nature of the hydrogen bond network of liquid water. The residual catalytic activity of CT changes from 100 to 0% in the transition region. A minimum on the competitive inhibitor binding and storage stability curves was observed at aw of 0.8-0.9.The thermodynamic, structural, and enzyme activity data were analysed to give a unified picture of the state of enzymes in low water organic solvents. According to this model, the dehydration-induced protein-protein contacts and the state of water hydrogen bond network play a key role in determining the enzyme activity - water activity profiles in organic liquids. © 2011 by Nova Science Publishers, Inc. All rights reserved.