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## Calorimetric and Fourier transform infrared spectroscopic study of solid proteins immersed in low water organic solvents

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### Abstract

Calorimetric heat effects and structural rearrangements assessed by means of Fourier transform infrared (FTIR) amide I spectra were followed by immersing dry human serum albumin and bovine pancreatic  $\alpha$ -chymotrypsin in low water organic solvents and in pure water at 298 K. Enthalpy changes upon immersion of the proteins in different media are in a good linear correlation with the corresponding IR absorbance changes. Based on calorimetric and FTIR data the solvents were divided into two groups. The first group includes carbon tetrachloride, benzene, nitromethane, acetonitrile, 1,4-dioxane, *n*-butanol, *n*-propanol and pyridine where no significant heat evolution and structural changes were found during protein immersion. Due to kinetic reasons no significant protein–solvent interactions are expected in such systems. The second group of solvents includes dimethyl sulfoxide, methanol, ethanol, and water. Immersion of proteins in these media results in protein swelling and involves significant exothermic heat evolution and structural changes in the protein. Dividing of different media in the two groups is in a qualitative correlation with the *solvent hydrophilicity* defined as partial excess molar Gibbs free energy of water at infinite dilution in a given solvent. The first group includes the solvents with hydrophilicity exceeding 2.7 kJ/mol. More hydrophilic second group solvents have this energy values less than 2.3 kJ/mol. The hydrogen bond donating ability of the solvents also assists in protein swelling. Hydrogen bonding between protein and solvent is assumed to be a main factor controlling the swelling of dry solid proteins in the studied solvents. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Fourier transform infrared; Immersion calorimetry; Organic solvent; Solvent hydrophilicity, swelling; Human serum albumin; Bovine pancreatic  $\alpha$ -chymotrypsin

### 1. Introduction

Enzymes suspended in organic solvents with low water contents may catalyze reactions not feasible in aqueous medium [1,2], and demonstrate greatly en-

hanced thermostability [3,4] and ‘molecular memory’ [5,6]. This biotechnological potential of enzymes in organic medium depends strongly on the nature of the organic solvent. For example, solvents may activate or suppress enzymatic activity [7,8], affect the enantioselectivity of the suspended enzymes [9,10], result in exothermic peaks on differential scanning calorimetry (DSC) curves of the protein suspended in organic solvents [11,12], and influence ligand binding to the imprinted protein [5].

Abbreviations: FTIR, Fourier transform infrared; HSA, human serum albumin; CT, bovine pancreatic  $\alpha$ -chymotrypsin

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