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## **Analysis of the organic solvent effect on the structure of dehydrated proteins by isothermal calorimetry, differential scanning calorimetry and FTIR spectroscopy**

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### **Abstract**

This review describes the basic principles of a novel method for studying the structure of the dehydrated proteins in the presence of organic solvents. This method, based on combined calorimetric and FTIR spectroscopic measurements, allows the simultaneous monitoring of the thermochemical parameters (interaction enthalpies, DSC thermograms) of the dried proteins and the corresponding changes in the protein structure in anhydrous organic solvents. This review aims to analyse the effect of organic solvents on dehydrated protein systems in order to understand what intra- and intermolecular processes produce the main effect on the structure and functioning of proteins in low water organic media. Two unrelated proteins with a high  $\alpha$ -helix content (human serum albumin, HSA) and with a high  $\beta$ -sheet content (bovine pancreatic  $\alpha$ -chymotrypsin, CT) were used as models. Two groups of model organic solvents were used. The first group included hydrogen bond accepting solvents. The second group included hydrogen bond donating liquids. The results obtained showed that: 5) The enthalpy and integral structural changes accompanying the interaction of dried proteins with anhydrous organic solvents depend cooperatively on the solvent hydrophilicity. The solvent hydrophilicity was characterized by an excess molar Gibbs energy of water in organic solvent at infinite dilution and 25°C. Based on this solvent hydrophilicity parameter, the solvents were divided into two groups. The first group included hydrophilic solvents such as methanol, ethanol, and dimethylsulphoxide (DMSO). Considerable structural rearrangements were observed in this group of solvents. The interaction enthalpies of the dried proteins with hydrophilic liquids were strongly exothermic. The second group included the hydrophobic and medium hydrophilic liquids such as benzene, dioxane, butanol-1, and propanol-1. The enthalpy and structural changes in the second group of solvents were close to zero. 6) The FTIR spectroscopic results can be attributed to the formation of different unfolded states of CT and HSA obtained upon dehydration-, alcohol- and DMSO-induced denaturation. The denatured state obtained in DMSO has a maximal degree of unfolding compared with that observed in alcohols or in the presence of dry air. 7) The effect of the organic solvent on the protein structure is "protein selective". On the other hand, the organic solvent-induced integral structural changes versus solvent hydrophilicity profiles do not depend on the predominant form of secondary structure in the protein. 8) Heat-induced exothermic peaks were observed on the DSC thermograms of the dried proteins in anhydrous organic solvents in the temperature range 60-105 °C. This means that dehydrated proteins in anhydrous solvents is the non-equilibrium state at room temperature. These results give strong support to the idea that the non-equilibrium status of the dehydrated proteins results from the protein-organic solvent interactions being "frozen" at near room temperature. The thermodynamic and structural data were analysed to give a unified picture of

the state of the dried proteins in anhydrous organic solvents. According to this model, the dehydration-induced protein-protein contacts and the potential of the organic solvent to form the hydrogen bonds are key factors in determining the structure of the dehydrated proteins in the liquids under study. © 2011 by Nova Science Publishers, Inc. All rights reserved.

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