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Effect of dioxane on the structure and hydration–dehydration of α-chymotrypsin as measured by FTIR spectroscopy

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Abstract

A new experimental approach based on FTIR spectroscopic measurements was proposed to study simultaneously the adsorption/desorption of water and organic solvent on solid enzyme and corresponding changes in the enzyme secondary structure in the water activity range from 0 to 1.0 at 25 °C. The effect of dioxane on the hydration/dehydration and structure of bovine pancreatic α-chymotrypsin (CT) was characterized by means of this approach. Dioxane sorption exhibits pronounced hysteresis. No sorbed dioxane was observed at low water activities (a_w<0.5) during hydration. At a_w about 0.5, a sharp increase in the amount of sorbed dioxane was observed. Dioxane sorption isotherm obtained during dehydration resembles a smooth curve. In this case, CT binds about 150 mol dioxane/mol enzyme at the lowest water activities. Three different effects of dioxane on the water binding by the initially dried CT were observed. At a_w<0.5, water adsorption is similar in the presence and absence of dioxane. It was concluded that the presence of dioxane has little effect on the interaction between enzyme and tightly bound water at low a_w. At a_w>0.5, dioxane increases the amount of water bound by CT during hydration. This behavior was interpreted as a dioxane-assisted effect on water binding. Upon dehydration at low water activities, dioxane decreases the water content at a given a_w. This behavior suggests that the suppression in the uptake of water during dehydration may be due to a competition for water-binding sites on chymotrypsin by dioxane. Changes in the secondary structure of CT were determined from infrared spectra by analyzing the structure of amide I band. Dioxane induced a strong band at 1628 cm^{-1} that was assigned to the intermolecular β-sheet aggregation. Changes in the intensity of the 1628 cm^{-1} band agree well with changes in the dioxane sorption by CT. An explanation of the dioxane effect on the CT hydration and structure was provided on the basis of hypothesis on water-assisted disruption of polar contacts in the solid enzyme. The reported results demonstrate that the hydration and structure of α-chymotrypsin depend markedly on how enzyme has been hydrated—whether in the presence or in the absence of organic solvent. A qualitative model was proposed to classify the effect of hydration history on the enzyme activity-a_w profiles.

Keywords: Bovine pancreatic α-chymotrypsin; Thin film; Enzyme hydration; Secondary structure; Organic solvent sorption; Hysteresis phenomenon; Fourier transform infrared spectroscopy

1. Introduction

The hydration of proteins (enzymes) is a phenomenon of considerable fundamental importance and practical interest. It is well known that the interaction of water with proteins plays a key role in determining the structure and functions of proteins [1–3]. Knowledge of processes occurring upon the hydration or dehydration of proteins is also very important in biotechnological and pharmaceutical applications of proteins such as their use as biocatalysts [4–8], biosensors [9,10] and selective adsorbents [11,12] in low water organic solvents. There are many advantages in employing organic solvents for enzymatic processes, including high solubility of organic substrates, synthesis of useful chemicals (for example, chiral drug molecules, emulsifiers, modified fats and oils, flavor esters), suppression of undesirable side reactions caused by water and increased thermostability. However, in general, the enzyme activity in organic solvents is a complex function of the

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