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Conformational changes of enzymes adsorbed at liquid/solid interface : relevance to enzymatic activity.

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ABSTRACT

FTIR-ATR spectroscopy has been used to study in-situ adsorption of enzymes at water/solid interfaces so as to better understand how conformational changes may monitor enzymatic activity. As the adsorption process depends on hydrophobic and electrostatic interactions, conformational changes have been studied as a function of the nature of the adsorbing substrates: hydrophobic or hydrophilic character. Adsorption kinetics of two examples of serine enzyme, α -chymotrypsin (α -chym) and Humicola lanuginosa lipase (HLL), have been studied. Both secondary structure and solvation of the adsorbed enzymes have been compared to the dissolved enzymes. While the positively charged α -chym was adsorbed on a negatively charged hydrophilic support with minor structural changes, the negatively charged lipase has no affinity for a similar support. Both enzymes were strongly retained on the hydrophobic support. The secondary and tertiary structures of the α -chym adsorbed on hydrophobic support were strongly altered, in correlation with the inhibition of enzymatic hydrolysis. The specific solvation obtained for the adsorbed HLL is consistent with the existence of the open conformer, in relation with the enhanced enzymatic activity at the water/hydrophobic interface.

Key words: FTIR-ATR, secondary structure, solvation, lipase, protein adsorption.

INTRODUCTION

Several models of protein adsorption include a transition from a reversibly adsorbed state to a more tightly held state. The latter is brought about by a molecular rearrangement of the protein allowing more additional contacts with the solid surface ¹. However, since enzymes retain at least some of their biological activity in the adsorbed state, conformational changes in adsorbed enzymes should be limited in some extent. Hence, studying the interaction between enzymes and solid surfaces in an aqueous environment may contribute to the understanding of the relation between structural flexibility and enzymatic activity. As an illustration, we will compare the adsorption of two serine enzymes, a proteolytic one such as α -chymotrypsin (α -chym) and a lipolytic enzyme such as Humicola lanuginosa lipase (HLL). This esterase has a solvent accessible catalytic site and displays an interfacial activation at the water/lipid interface ².

A strategy to study both adsorption kinetics and protein structural change by FTIR is to use Attenuated Total Reflection mode. The native silica on the ATR crystal was used as a negatively charged hydrophilic surface. It was also chemically modified by grafting a self-assembled monolayer of octadecylsilane so as to present a hydrophobic surface, representing the lipid substrate ³. The ATR measurements should be able to detect the irreversibly and reversibly adsorbed proteins, as well as the protein in solution. However, the contribution to the overall spectrum of the enzyme present in solution was negligible in respect with that of the adsorbed fraction since the bulk concentrations used were in the 10^{-6} M range.

Adsorption kinetics at D₂O /solid interface were monitored by measuring the time dependent Amide I'-band increase in stationary conditions. The secondary structures of the adsorbed enzymes were determined from the analysis of the Amide I' band, and is compared to that of the dissolved enzymes. The kinetics of D₂O diffusion into the core of the proteins probes the

accessibility of the solvent into random or structured internal domains. This diffusion is correlated to the H/D exchange. The measurements of the amounts of residual CONH during the adsorption process provide in situ the change in tertiary structure.

EXPERIMENTAL

The lipase (HLL) was kindly provided by Allan Svendsen of Novo-Nordisk, Denmark. α -Chymotrypsin (C-4129) was obtained from Sigma[®] (St Louis, MO).

The spectra of the enzymes in solution were obtained in transmission mode in a CaF₂ liquid cell (50 μ m) and the spectra of the enzymes adsorbed at the D₂O buffer/solid interface were recorded in a liquid ATR cell described elsewhere ⁴ (Figure 1). The ATR silicon crystal provides 75 useful internal reflections at the liquid/crystal interface. The beginning of the adsorption kinetics was set at the filling of the ATR cell by the enzyme solutions just after the dissolution of the enzymes in D₂O buffers. The protein layer density (Γ) was calculated from the area of the Amide I' using the adsorption density equation ⁵. The amount of reversibly adsorbed protein was checked to be negligible in our case. Curve fitting procedure for the analysis of the Amide I' band has been performed by setting five component bands with the same parameters for the adsorbed state and the solution ^{6,7}. For each component band the integrated area are expressed in % of the overall Amide I' band. The so-called "random" domains (1670-1690 cm⁻¹) correspond to the non-hydrogen bonded CO peptides. The random "self-associated" and "hydrated" domains have absorption bands located near 1610-1620 cm⁻¹ and 1640-1645 cm⁻¹, respectively. The ordered structures are composed of α -helices (1650-1660 cm⁻¹) and β -sheets (1630-1635 cm⁻¹). The CONH contents were determined in % of the overall peptide groups by referring the Amide II/Amide I' area ratios in the adsorbed state, to the ratio measured for the fully CONH enzymes in the solid state.

RESULTS AND DISCUSSION

- Adsorption kinetics

Whereas α -chym was adsorbed onto a negatively charged hydrophilic surface ⁷, HLL did not adsorb on a similar surface even at a bulk concentration of 0.1 mg/ml (Figure 2 and 1f). The saturation of the hydrophobic surface was observed with α -chym and HLL for a bulk concentration of 0.01 mg/ml after 4 hours (Figure 2). Taking into account the molecular size of the native α -chym ⁷, the formation of a densely packed monolayer would give the adsorbed amounts of 1.1-1.4 mg/m², following an end-on or side-on orientation. The experimental Γ value of 1.2 mg/m² suggests the completion of an adsorbed monolayer. Based on the same considerations, the adsorbed amounts for a densely packed monolayer of HLL are calculated to be 3.3, 3.0 or 2.3 mg/m², depending on the protein orientation ⁸. The Γ value of 1.7 mg/m² for HLL would correspond to the formation of a loosely-packed monolayer (Figure 2), in agreement with results reported for the HLL adsorption on a polystyrene support ⁹.

- Secondary structure

As shown in Figure 1, the broadening of the Amide I' band occurs when α -chym is adsorbed on the hydrophobic support, suggesting a significant conformational change (Figure 1-a). The adsorption onto the hydrophobic surface at complete surface coverage causes an unfolding of 10 % of the ordered structure of α -chym (Figure 3A). The newly formed domains in the adsorbed enzyme molecules are either self-associated or hydrated (Figure 3A). For the hydrophilic support, only weak changes were observed on the spectrum of the adsorbed α -

chym (Figure 1b). Previous published results have shown that α -chym loosed only 5 % of β -sheets when adsorbed on a negatively charged polar support such as montmorillonite ⁷.

In contrast, HLL is adsorbed on the hydrophobic surface without significant changes in the Amide I' profile (Figure 1-e). Indeed, weak folding in ordered structures concerned 5 % of the enzyme backbone and is associated to the dehydration of the adsorbed HLL (Figure 3B). Similar dehydration process has been also suggested for some proteins adsorbed onto hydrophobic supports ¹.

- Tertiary structure

The H/D exchange in the CONH bonds of the enzymes in solution has been previously described ^{7,10}. After exposure of 8 min in the D₂O buffer, the residual CONH content in α -chym was 25% ⁷. This content was weaker when α -chym is adsorbed on the hydrophobic surface at 8 min (Figure 4). This decrease in non-exchanged CONH content remained constant with the increase of the protein layer density. All the α -chym molecules adsorbed on the hydrophobic surface undergo a change in solvation, more likely due to the surface interaction than to protein-protein interactions. The larger diffusion of D₂O molecules inside the enzyme core is correlated to the more unfolded state of the adsorbed enzyme.

While HLL in solution contained still 37 % of CONH species after 8 minutes of incubation ¹⁰, the first adsorbed HLL molecules contained only 17 % of CONH. This suggests a change in solvation of the peripheral domains of the first adsorbed HLL molecules. Surprisingly, the CONH content increased with the protein layer density (Figure 4). This could be due to the coexistence of two conformers inside the protein layer. The first adsorbed HLL molecules undergo a change in tertiary structure while the subsequent adsorbed molecules present the same conformation as in solution. The average CONH content of the lipase monolayer increases as far as the proportion of the second conformers increases within the monolayer.

- Relation to enzymatic activity

α -Chym is known to retain enzymatic activity when adsorbed on a hydrophilic surface ⁷, but is fully inhibited on a hydrophobic support ¹¹. Relatively large changes in both secondary and tertiary structures could explain the inhibition of the enzyme on hydrophobic surfaces. It has been assumed that the weaker unfolding observed for the adsorption on hydrophilic surfaces does not alter drastically the catalytic site ⁷.

The catalytic activity of HLL has been shown to be enhanced by addition of hydrophobic polystyrene beads to the solution ⁹. The lipase in solution adopts a closed conformation and has no enzymatic activity ^{2,9}. The present results for the HLL adsorption are consistent with the existence of an open form of the enzyme at the water/hydrophobic interface. Only the open form enables the accessibility of the solvent to the catalytic triad of the adsorbed enzyme. The flexibility of the adsorbed open form is nevertheless associated to the maintenance of the native secondary structure. More structural insights on the adsorption of open/closed forms, depending on the concentration of the enzyme-containing solution, are discussed in a forthcoming publication ¹⁰.

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FIGURES and FIGURE LEGENDS

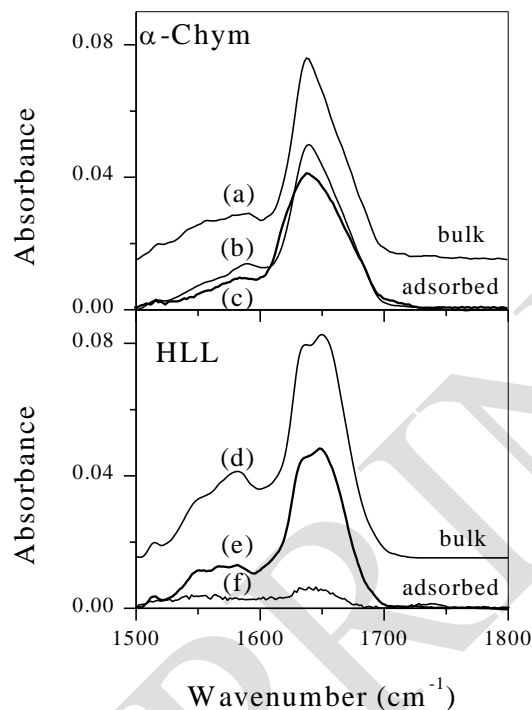


Figure 1 : FTIR transmission spectra for α -chym at 5 mg/ml in solution (a) and adsorbed on montmorillonite particles (3 μm diameter) in suspension (b) (see reference 6), compared to the ATR spectrum of α -chym adsorbed on the hydrophobic support with bulk concentration of 0.01 mg/ml (c). FTIR transmission spectrum of HLL at 5 mg/ml in solution (d) compared to the ATR spectra of HLL adsorbed at 0.01 mg/ml on the hydrophobic support (e) and at 0.1 mg/ml on the hydrophilic support (f). All spectra were recorded in D_2O phosphate buffer (0.055 M Na_2DPO_4) at pD 7.5, after 2h of exposure to D_2O and/or in presence of the solid surfaces.

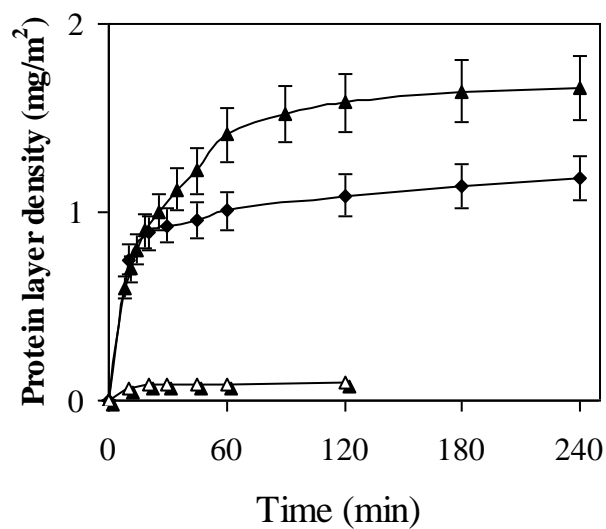


Figure 2 : Adsorption kinetics of α -chym (∇) and HLL (\square) on the hydrophobic surface at a bulk concentration of 0.01 mg/ml. Adsorption kinetics of HLL (\triangle) on the hydrophilic surface at a bulk concentration of 0.1 mg/ml.

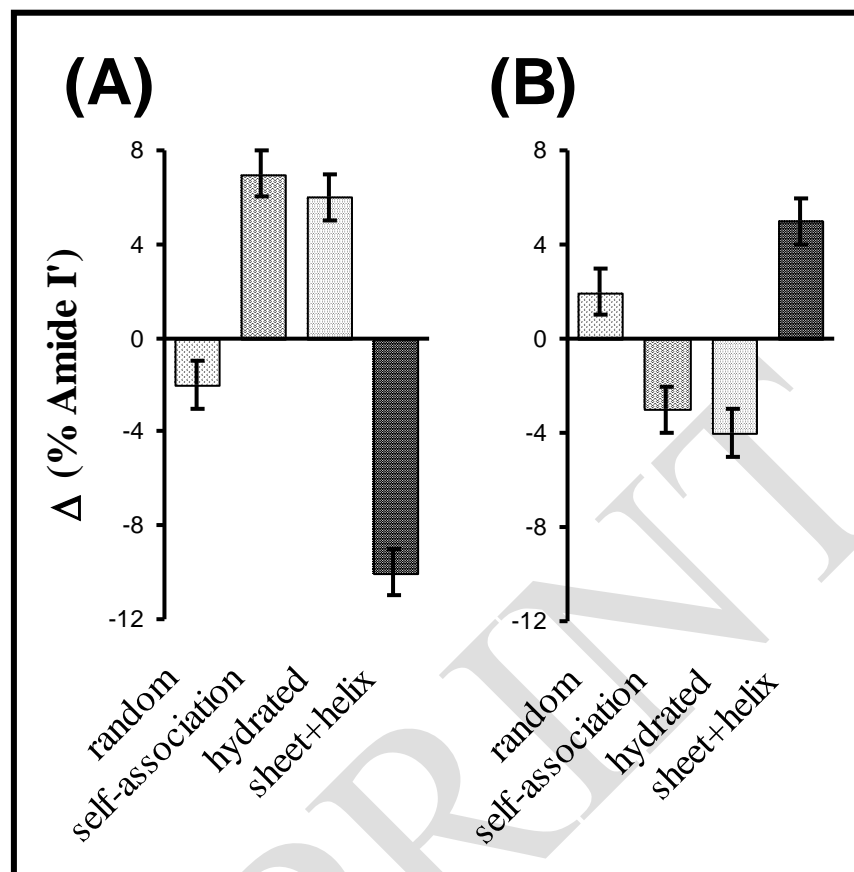


Figure 3 : The differences of the integrated area between the adsorbed state on the hydrophobic support and the state in solution $\Delta(\% \text{ Amide I}')$ were obtained for α -chym (A) and HLL (B) at 2 hours.

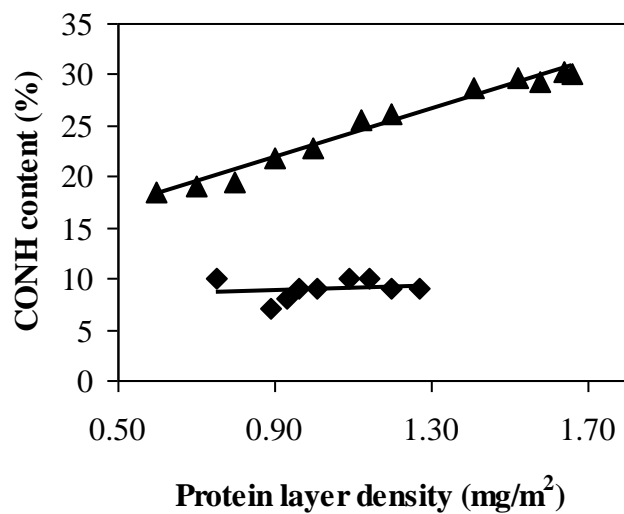


Figure 4 : The time-dependent residual CONH contents of α -chym (\blacktriangledown) and HLL (\blacklozenge) are plotted as a function of the adsorbed amount of enzymes. The lowest and highest protein layer densities correspond respectively to 8 min and 4 h of adsorption at the D₂O buffer/hydrophobic interface.