Protein Templates & Spectroscopic Rulers for Silica Surfaces

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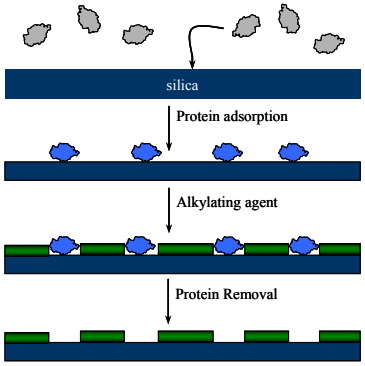
Introduction

Designating well defined regions for constructing catalytic active sites has the potential to develop methods for preparing more consistent model catalysts. To address this issue, we developed a method for using proteins to template isolated regions on a commercial silica. The basic scheme for this work is shown in Figure 1. A cationic protein is deposited onto an oxide surface, the remaining surface is functionalized, and the protein is removed leaving behind a footprint. Additionally, the use of a protein template offers opportunities for employing biochemical characterization methods for evaluating the distances between proteins on the surface.

Figure 1. Protein Templating Scheme



Materials and Methods

Lysozyme (Lys) solutions (nominally 0.192g Lys in 1.5L buffer) were prepared in pH 4.0 and pH 7.2 buffers. Davicat SI-1403 silica powder (245m^2/g, 40-60 & 60-80 mesh) was supplied by Grace Grace-Davison and calcined overnight at 500 °C prior to use. Lys adsorption capacity and kinetics were monitored as a function of Lys concentration (9-900 µM) using a solution Bradford assay. The free silica hydroxyl groups were alkylated using isobutyltrimethoxysilane; Lys was removed using 0.5 M morpholine and monitored with ATR-IR spectroscopy. Surface interactions between the Lys molecules were evaluated using modified Fluorescence Resonance Energy Transfer (FRET) experiments. In these experiments, half the proteins were labeled with a fluorescent donor (TX Red) and half were labeled with an appropriate acceptor (CNF).

Results and Discussion

Lysozyme adsorption was monitored by following the solution protein concentration with a Bradford assay for 2 hours. The total Lys capacity (0.305 ± 0.009 µmol/m^2, strongly bound) was determined from assays on subsequent washings. The adsorption was evaluated in terms of successive two-step Langmuir adsorption models, which describe the kinetics data well. Linear portions of the adsorption kinetics plots had consistent non-zero intercepts (0.158 ± 0.005 µmol/m^2), which were interpreted as the end of the first adsorption event. In these models, the first adsorption event involves Lys adsorbing along its long axis until it saturates the surface. Possibilities for the second adsorption event include a strongly bound bilayer model or a shift to end-on protein adsorption.

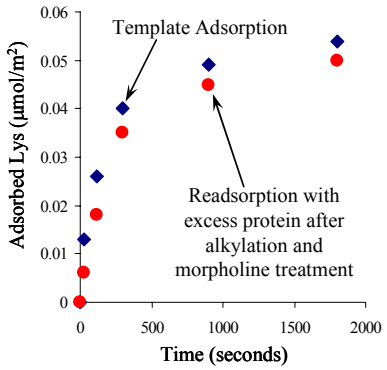


Figure 2. Lys Readsorption

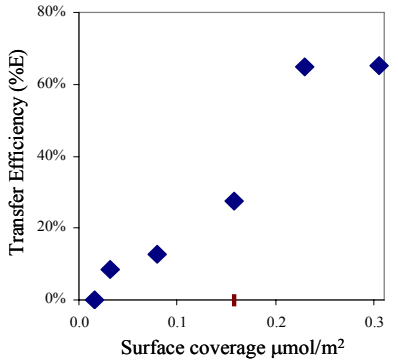


Figure 3. FRET Data

With the Lys adsorption capacities in hand, sub monolayer adsorption regimes were examined. ATR-IR spectroscopy was used to confirm changes in protein surface coverage by monitoring the amide bond stretching frequencies. Functionalization of the uncovered silanol groups was achieved with isobutyltrimethoxysilane or trimethylpropoxysilane in ethanol. IR spectra showed enhanced C-H stretching frequencies after the alkylation, yet the amide bands remained unaffected. The protein could be quantitatively desorbed (via ATR-IR) with morpholine. To test the retention of the template footprint, readsorption experiments were performed with excess Lys. As Figure 2 shows, there the same quantity of Lys adsorbs in the readsorption experiment, indicating that the template footprint is retained.

Modified FRET experiments were used to characterize the protein-protein interactions on the silica surface. In these experiments, samples of Lys were partially labeled with Texas Red, a fluorescence donor, or CNF, a fluorescence acceptor. The labeled proteins were adsorbed on silica and fluorescence intensity was compared against controls with donor labeled and unlabeled Lys. As Figure 3 shows, the fluorescence transfer or quenching efficiency increases substantially with surface coverage. There is no quenching of the fluorophore at low coverages, indicating that the proteins do not cluster on the surface. Because quenching efficiency is distance dependant, this serves as a crude molecular ruler for the adsorbed proteins, indicating that they are on the order of 10+ nm apart at 5% of a monolayer. These sites are subsequently used to anchor catalysts such as dendrimer encapsulated nanoparticles.

Significance

We show that it is possible to use proteins to template isolated footprints on oxide surfaces. To the best of our knowledge, this is the first attempt to employ FRET techniques in catalyst preparation and characterization, thus providing an additional tool for studying catalyst preparation and properties.