SOLID-SUPPORTED ENZYME CATALYST MODELS FOR RING-OPENING POLYMERIZATION[†]

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Introduction

Biodegradable polyesters have been previously synthesized by the ring-opening polymerization (ROP) of lactones using enzymatic and organic catalysts as an alternative to metal-catalyzed reactions.[1,2] Commercialization of these green polymerization processes relies on catalyst recycling to maintain low production costs, requiring a thorough understanding of catalyst activity, surface interactions and stability under varied reaction parameters.

The enzyme *Candida antarctica* Lipase B (CAL B), immobilized on a solid support, catalyzes the ring-opening polymerization of ε-caprolactone to make biodegradable polycaprolactone (PCL) (Figure 1). CAL B immobilization occurs through physisorption at the surface of a crosslinked poly(methyl methacrylate) (PMMA) resin or bead. Weak hydrophobic interactions between the enzyme and the surface can permit catalyst desorption, which decreases the concentration of remaining enzymes for polymerization and contaminates the PCL product.

Commercially available catalysts (Novozyme 435, N435) have been utilized in reusable packed microfluidic columns for controlled synthesis of PCL under flow conditions.[3] Determination of optimal reaction conditions at the polymer/enzyme interface can afford better control of polymerization from solid-supported catalysts and improve catalyst retention throughout reactions over multiple re-use cycles. In this work, a model of the crosslinked PMMA/CAL B interface was fabricated to mimic the surface of N435 within a quartz crystal microbalance (QCM). QCM is a real time, label free technique that can quantitatively measure surface adsorption processes under flow conditions.

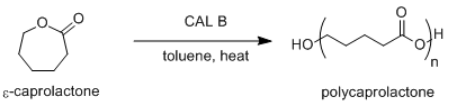

Figure 1. Enzyme catalyzed ring-opening polymerization of ε-caprolactone to polycaprolactone.

Experimental

Materials. Poly(methyl methacrylate), M_n = 172,000 g/mol, M_w/M_n = 1.28, was obtained from Polymer Source.[§] CAL B was obtained from Novozymes. QCM sensors with 50 nm SiO_2 coating (QSX303) were purchased from Q-sense. All other reagents were purchased from Sigma Aldrich and used as received.

Monitoring of CAL B Adsorption through Quartz Crystal Microbalance with Dissipation (QCM-D). QCM-D was performed using a Q-Sense E4 module controlled by Q-Soft integrated software. A quartz crystal sensor was modified with crosslinked PMMA (50 nm) and sealed within the module. Ammonium bicarbonate buffer (pH 7.7) was drawn through each module using a peristaltic pump with a 100 µL/min flow rate. Enzyme solution of 0.1 mg/mL CAL B in buffer was added to one module and enzyme adsorption was monitored. After the baseline stabilized indicating no further enzyme adsorption, buffer was flowed through the cell to remove any loosely bound enzymes and to establish the final enzyme concentration at the PMMA surface.

Atomic Force Microscopy (AFM) of PMMA Surface Morphology. Height images of the CAL B and unmodified PMMA surfaces from QCM-D experiments were taken using a Bruker Dimension Icon Atomic Force Microscope with ScanAsyst. The cantilever was a TESPA tip (spring constant, k = 42 N/m) with reflective coating.

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[§] Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

Results and Discussion

The immobilization of CAL B on PMMA is governed by hydrophobic interactions at the surface; therefore using a PMMA mimic on the QCM sensor with similar hydrophobicity to native PMMA (static water contact angle of 80°) yields an analogous platform for in situ studies at the catalyst/solid support interface.

Enzyme depositions were monitored under flow by measuring the decrease in frequency from the resonant frequency of the oscillating crystal (f_o, 5 MHz) using QCM-D. The amount of enzyme adsorbed, or mass surface coverage (Δm), is calculated proportionally to the frequency change (Δf) using the Sauerbrey equation,[4] where n is the overtone number, and C is the mass sensitivity of the crystal (17.7 ng cm^{-2} Hz^{-1} for a 5 MHz quartz crystal).

$$\Delta m = -C\frac{1}{n}\Delta f$$

The mass surface coverage of CAL B on the PMMA surfaces increases upon addition of enzyme and remains consistent for over 30 minutes under enzyme flow. The CAL B functionalized surfaces are rinsed with buffer to remove any unbound enzyme, yielding a final mass surface coverage of 540 ng/cm^2.

AFM images of PMMA surfaces before and after CAL B immobilization are shown in Figure 2. The morphology of the CAL B modified surface is smooth and homogenous relative to unmodified PMMA, with a slight decrease in RMS roughness from 7.7Å to 6.7Å. This observation is consistent with enzyme distribution in N435, where protein-protein interactions and the strong affinity of CAL B to the matrix restrict the enzyme to the outer shell of the porous bead.[5]

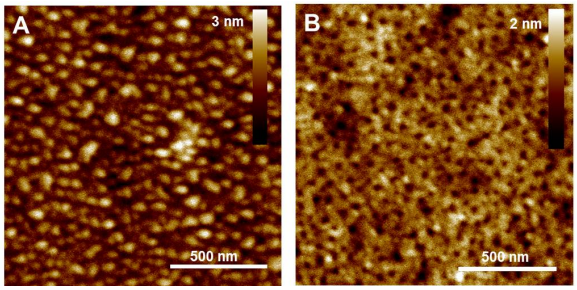

Figure 2. AFM height images of crosslinked PMMA surface (A) and crosslinked PMMA surface with immobilized CAL B (B).

Ongoing work focuses on enzyme activity and stability at the PMMA interface under varied reaction parameters. Frequency and dissipation changes from multiple overtones will be used to generate a viscoelastic model for the CAL B surface. Changes in surface viscosity, shear modulus, and film thickness from the model will determine the stability of the enzyme under stress. Enzyme activity within the QCM model system is being studied using small molecule probes to correlate CAL B activity of the PMMA surface mimic to N435 beads.

Conclusions

PMMA model thin films, immobilized on a QCM sensor, are stable to enzyme adsorption in situ, yielding a final enzyme surface coverage of 540 ng/cm^2. Surface morphology becomes smoother upon addition of CAL B to the PMMA surface, indicating that the enzyme is primarily localized at the outer PMMA interface, consistent with N435 beads.

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