STABILITY OF SOLID-SUPPORTED ENZYME CATALYSTS 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. To achieve this goal, a thorough understanding is needed 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. Furthermore, the enzyme surface can induce adsorption of other species which hinder activity of the surface-confined catalyst.

Commercially available catalysts (Novozyme 435, N435) have been previously 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. In situ adsorption and desorption at the solid support/enzyme interface was monitored while deconvoluting background events, such as polymer swelling or non-specific binding. Enzyme adsorption, then PCL binding to the CAL B modified surface was monitored under flow conditions.

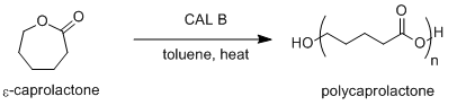

Figure 1. Enzyme catalyzed ring-opening polymerization of ε-caprolactone to polycaprolactone.

Experimental

Materials. Toluene was purchased from Sigma Aldrich[§] and distilled over CaH_2 prior to use. Poly(methyl methacrylate), M_n = 172,000 g/mol, M_w/M_n = 1.28, and polycaprolactone, M_n = 10,000 g/mol, M_w/M_n = 1.25 were 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 dual chamber syringe pump with a 100 µL/min refill rate. Buffer was drawn through the cells for at least 1 hour to establish a stable equilibrium with the sensor surface. Enzyme solution of 0.1 mg/mL CAL B in buffer was added to one of the modules 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.

Monitoring of CAL B Stability through Quartz Crystal Microbalance with Dissipation (QCM-D). Two sensors were run in parallel modules for enzyme/PMMA and PMMA control samples. Dry toluene (water mass fraction of 19 mg/kg) at 45°C was flowed over the modules containing the QCM sensors to establish a baseline. Toluene and PCL (1mg/mL) solutions with increasing water content, measured by Karl Fischer coulometric titration, were recorded at (19, 220, 466 and 515) mg/kg, respectively. Each PCL solution flowed over the crystals for approximately 20 minutes, followed by rinsing with toluene. Changes in frequency with time were recorded for each sensor and the net surface mass change due to enzyme was calculated.

Results and Discussion

Experimental parameters, such as temperature, flow rate, solvent composition, and monomer concentration can be systematically varied within the QCM to elucidate the effects of reaction parameters on catalyst activity and stability. 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). The mass surface coverage is calculated 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 Sauerbrey relationship uses three principal assumptions to correlate frequency change to mass adsorbed. The first is that adsorbed enzyme mass is small compared to mass of the crystal. Secondly, the mass of the polymer and the enzyme are rigidly adsorbed with no slip or deformation imposed by the oscillating surface. The final assumption is that the added enzyme mass is evenly distributed across the sensor.

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, as shown in Figure 2. 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.

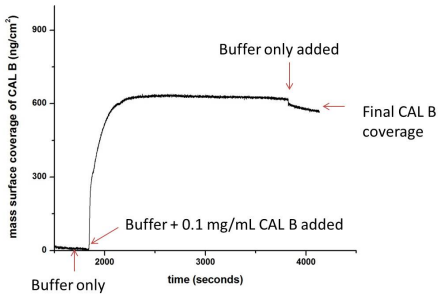

Figure 2. Mass surface coverage of CAL B enzyme binding to PMMA-modified sensor under flow rate of 100 µL/min.

Previous studies have determined that water content within the polymerization controls the equilibrium between active enzyme-polymer chains that propagate, and free enzyme, which can degrade polyester sites along the polymer backbone.[5,6] Following enzyme deposition, the CAL B modified surface and PMMA controls were exposed to polymerization

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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.

conditions with increasing water content. Dry toluene was first introduced to equilibrate the surfaces in organic media. PCL (1 mg/mL in toluene) was then added to the modules. This cycle of PCL addition and toluene rinsing were repeated with increasing water concentrations in each step. The net response of the enzyme at the surface (Figure 3) was calculated from subtracting the mass surface coverage of the PMMA control from the enzyme-functionalized PMMA to account for frequency changes due to bulk water content of the introduced solutions and to deconvolute swelling and non-specific binding of PCL to PMMA. For three PCL/rinsing cycles, the overall increase in surface mass coverage was 100 ng/cm^2, with minimal mass losses of less than 20 ng/cm^2 due to rinsing. The increasing surface coverage indicates that enzyme leaching did not occur under these reaction conditions or was outweighed by the adsorption of PCL onto the surface, which was facilitated by surface adsorbed enzymes.

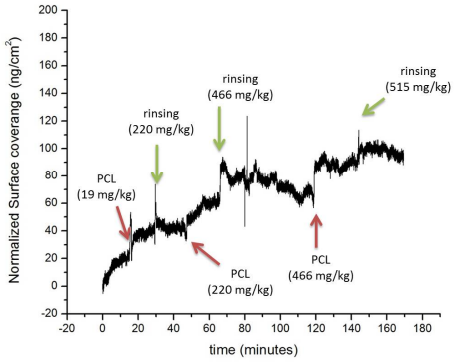

Figure 3. Increasing mass surface coverage due to PCL adsorption on the surface through three PCL/rinsing cycles of increasing water content.

Ongoing work includes using frequency and dissipation changes from multiple frequency overtones to generate a viscoelastic model for the PMMA and enzyme functionalized surfaces. An accurate model for the enzyme modified surfaces will allow for the quantitative study of the enzyme layer under stress by measuring changes in surface viscosity, shear modulus, and film thickness. Additionally, 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. Preliminary studies using the Sauerbrey model indicate moderate polycaprolactone-enzyme complexation at the surface (100 ng/cm^2) with increasing water concentration under reaction conditions. Enzyme loss during rinsing of the sensor surface is minimal for the reaction conditions studied.

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