Appendix 1

Candida antarctica lipase B adsorption on the double-brush Langmuir-Blodgett monolayer of an α-helical diblock copolymer

A1.1 Introduction

Ultrathin films of unidirectionally oriented α-helical polypeptides with a large polar order show potential applications in chemical biology, opto-electronics and biosensors. We have studied amphiphilic diblock copolypeptides (PLGA-b-PMLGSLG) of poly(α-L-glutamic acid) (PLGA) and poly(γ-methyl-L-glutamate-ran-γ-stearyl-L-glutamate) with 30 mol% of stearyl substituents (PMLGSLG) for the fabrication of α-helical double-brush Langmuir-Blodgett (LB) monolayers. These films are 4-5 nm thick with the top-brush layer consisting of the PMLGSLG helices unidirectionally aligned. In addition to the special properties of the helix macrodipole, which plays an important role in the structure stabilization and function of proteins and peptides, the liquid-like features of the alkyl side chain mantle of the PMLGSLG block make these films suitable for incorporation of various biomolecules with potential applications as functional materials. In a preliminary study, we investigate the immobilization of Candida antarctica lipase B (Cal-B) onto an LB monolayer of PLGA-b-PMLGSLG via physical adsorption. The immobilization of enzymes onto solid substrate-supported films has received considerable attention due to the effectiveness for multiple use and enzyme property improvement in biocatalytic applications. In particular, Cal-B is an extensively used enzyme which catalyzes a diversity of reactions including the hydrolysis of a wide range of esters and amides as well as many regio- and enantioselective syntheses. In this study, we show that Cal-B can be physically adsorbed onto the monolayer of the PLGA-b-PMLGSLG diblock copolymer.
The enzyme loading and activity were quantified using transmission Fourier transform infrared (FT-IR) spectroscopy and the p-nitrophenyl acetate (pNPA) assay, respectively.\textsuperscript{19}

A1.2 Experimental section

Materials
The diblock copolymer synthesis, silicon wafer cleaning and LB monolayer transfer have been described in detail in Chapters 2 and 3. A monolayer of PLGA-b-PMLGSLG (DP_{PLGA} = 63 and DP_{PMLGSLG} = 39) was transferred onto both sides of a double-sided polished silicon wafer. Candida antarctica lipase B (Cal-B) in the form of a dried powder was purchased from Codexis\textsuperscript{®} (Pasadena, CA, USA). Other chemicals were used as received from Sigma-Aldrich.

Enzyme immobilization
The silicon wafer-supported film of PLGA-b-PMLGSLG was incubated in 0.58 mg/mL Cal-B solution in acetate buffer pH 5.1 for 20 h at room temperature. The sample was rinsed with acetate buffer twice to remove any unbound lipases, rinsed thoroughly with deionized water to remove buffer residues and dried under a gentle nitrogen flow.

Hydrolytic activity
Cal-B immobilized on a silicon wafer-supported film of PLGA-b-PMLGSLG was added to a 1,4-dioxane solution (5 mL) containing p-nitrophenyl acetate (pNPA) (28 mM) and methanol (80 mM). The assay reaction was carried out for 50 min at 35 °C (200 rpm). Subsequently an aliquot (10 μL) was taken, mixed with 4 mL of 1,4-dioxane and immediately recorded by UV/VIS spectroscopy. The concentration of the reaction product p-nitrophenol (pNP) was determined by UV/VIS (PYE UNICAM SP8-200 UV/VIS spectrophotometer) at the λ_{max} (304 nm) of pNP. The same reaction solution was used as the reference to monitor the product of transesterification without catalysis. Enzyme hydrolytic activity is defined herein as the micromoles of pNPA hydrolyzed in 1,4-dioxane per unit of weight of enzyme per time (μmol of pNP·min^{-1}·mg^{-1}).
Fourier transform infrared (FT-IR)

Transmission FT-IR measurements were outlined in the experimental section of Chapter 2. Band areas were calculated with the integration function of the Bruker OPUS software (version 4.2).

In order to quantify the amount of enzyme from the integrated area of the amide I band a calibration was performed. KBr pellets with known amounts of enzymes (0.5 to 0.7 mg) were prepared. Enzymes were homogeneously dispersed throughout the pellets. Transmission FT-IR measurements of these pellets were carried out using the same instrument and measurement settings, giving the calibration curve (amount of enzyme versus the integrated area of amide I band). Considering the fact that enzymes are immobilized on both sides of the silicon wafer, the amount of deposited enzyme per area of the wafer was estimated.

A1.3 Results and discussion

Figure A1.1 shows the transmission FT-IR spectra of the LB monolayer of PLGA-b-PMLGSLG before and after Cal-B adsorption. The spectrum of Cal-B immobilized on the silicon wafer-supported film of PLGA-b-PMLGSLG subtracted by that of the PLGA-b-PMLGSLG film reveals absorption bands characteristic of Cal-B (Figure A1.2). This gives strong evidence that a large amount of Cal-B was adsorbed onto the PLGA-b-PMLGSLG film. The spectra of the film before and after enzyme adsorption show the same absorption intensity of the C=O stretching band, indicating the stability of the PLGA-b-PMLGSLG film on the silicon wafer during the enzyme immobilization process.

The integrated area of the amide I band originating from the immobilized enzyme was quantified. From the calibration with known amounts of Cal-B present in KBr pellets, the amount of Cal-B immobilized on each side of the wafer was determined to be 0.074 μg·mm⁻². Assuming that during the immobilization the enzyme molecules maintain their natural conformation (planar dimensions), the amount of enzyme immobilized on the PLGA-b-PMLGSLG film on silicon wafer...
is much higher than the theoretical amount of the maximal possible monolayer load of enzyme molecules on a flat surface, which is 0.0046 μg/mm² calculated on basis of the average spherical diameter (39.15 Å) and the molecular weight (33 kDa) of Cal-B. This indicates that molecules of Cal-B were adsorbed on the surface of the PLGA-b-PMLGSLG film in multilayers. Multilayer adsorption has been observed for some lipases and some other enzymes, since these enzymes have large hydrophobic surfaces surrounding their active centers and thereby easily form clusters.

![Figure A1.1. Transmission FT-IR spectra of the LB monolayer of PLGA-b-PMLGSLG on a double-sided silicon wafer before (dashed line) and after Cal-B immobilization (solid line) and the spectrum of the Cal-B immobilized film subtracted by that of the PLGA-b-PMLGSLG film (dotted line).](image)

The hydrolytic activity of the enzyme adsorbed on the PLGA-b-PMLGSLG monolayer was determined to be 1.929 μmol of pNP·min⁻¹·mg⁻¹, which is lower than the hydrolytic activity of the free Cal-B powder in dioxane, of 2.396 μmol of pNP·min⁻¹·mg⁻¹. Apparently, only the enzyme molecules in the top layer could possibly bind to the substrate (pNPA) in the hydrolytic reaction, expressing catalytic activity. Nevertheless, the activity measured strongly suggests that the enzyme molecules adsorbed on the top surface were not denaturated and their activity might even be enhanced considerably upon immobilization. Assuming that the surface is covered completely with the enzyme and that the hydrolytic activity contributes mainly to the top enzyme monolayer, the hydrolytic activity of the enzyme molecules in the top layer is roughly calculated to be 31.032 μmol of pNP·min⁻¹·mg⁻¹, which is much higher than the activity of the free enzyme.
A1.4 Conclusions

Besides the specific features of the PLGA-\(b\)-PMLGSLG monolayer containing unidirectionally aligned \(\alpha\)-helices, the top brush PMLGSLG layer with the alkyl side chain mantle provides a suitable hydrophobic environment for enzyme adsorption. The experiments presented here have shown the effective adsorption of Cal-B on a PLGA-\(b\)-PMLGSLG film and that the adsorbed enzyme exhibited hydrolytic activity. However, to elucidate the structure as well as the effectiveness of the immobilized enzyme, further characterization, such as atomic force microscopy and X-ray reflectivity, and experiments on enzyme desorption and reusability are required. In addition, the immobilization of Cal-B (with the use of a surfactant) into the PLGA-\(b\)-PMLGSLG monolayer during monolayer stabilization at the air-water interface, which provides the possibility to quantitatively control the incorporated enzyme, is worth studying. In approach, the presence of proper detergents can also improve the activity of lipases.26

A1.5 References


