ENZYME ASSEMBLY AND CATALYTIC ACTIVITY IN A REUSABLE BIOMEMS PLATFORM FOR METABOLIC ENGINEERING

Xiaolong Luo, Angela T. Lewandowski, Gregory F. Payne, Reza Ghodssi, William E. Bentley and Gary W. Rubloff

University of Maryland, USA

ABSTRACT

We report a reversible, chitosan-mediated biofunctionalization strategy for assembling a biocatalytically-active enzyme in reusable bioMEMS devices as a first step toward an experimental platform for metabolic engineering applications, e.g. drug discovery. We demonstrate (a) the assembly of a Pfs enzyme at a specific electrode address and (b) the efficient catalytic activity of this enzyme in the bioMEMS. Enzymatic activity is robust, remaining over days. In addition, biofunctionalization can be reversed, making the bioMEMS reusable for repeated assembly and catalytic activity.

Keywords: Enzyme assembly, catalytic activity, bioMEMS, chitosan

1. INTRODUCTION

Robust and reproducible enzyme assembly within microfluidic devices is challenging. We report a reversible, chitosan-mediated biofunctionalization strategy for assembling a biocatalytically-active enzyme in reusable bioMEMS devices. We exploit an integrated device and package design [1] which supports programmable bio-component assembly at selected sites [2]. The aminopolysaccharide chitosan is utilized as the interfacial biofunctionalization material for (1) the chemical signal-guided conjugation of chitosan to a Pfs enzyme through its pro-tag, which is genetically engineered at the C-terminal of Pfs and is activated by tyrosinase for the conjugation, and (2) the electric signal-guided electrodeposition of the Pfs-chitosan conjugate to a selective electrode under negative bias in the microfluidic channel.

2. THEORY

Fig. 1 illustrates our concept that the programmable and reversible assembly of biologically-active components (i.e., enzymes) enables prefabricated MEMS to be repeatedly biofunctionalized for multiple uses. First, the bioMEMS device in Fig. 2 was prefabricated for multiple uses [1]. Second, Pfs is conjugated to the aminopolysaccharide chitosan through a C-terminal pro-tag genetically-engineered into the Pfs enzyme. As illustrated in Fig. 1b, tyrosinase mediates the conversion of tyrosyl residues of Pfs into active o-quinones that conjugate to chitosan and confers stimuli-responsive properties. Third, the Pfs-chitosan conjugate in a slightly acidic solution (pH<6.3) is introduced into a microchannel and electrodeposited onto a selected electrode under negative bias as illustrated in Fig. 1c. With biofunctionalization complete, Fig. 1d shows the Pfs-mediated reaction is performed by introducing the substrate S-adenosylhomocysteine (SAH) into the microchannel for conversion into the products S-ribosylhomocysteine (SRH) and adenine. Downstream reaction solutions are collected and analyzed with high-performance-liquid-chromatography (HPLC).
Figure 1. Schematic flow of reversible enzyme assembly and catalytic activity in reusable bioMEMS device. (a) Device prefabrication, (b) enzyme-chitosan conjugation, (c) electrically programmed assembly of Pfs-chitosan conjugate, (d) small-molecule reaction by enzyme catalysis, (e) mild acid wash to remove biofunctionalization and reuse bioMEMS device.

3. EXPERIMENTAL METHODS

The experimental sequence of operations to demonstrate catalytic activity and reversibility of the enzyme and reuse of the bioMEMS device is shown in Fig. 2(c), with tests of catalytic conversion color-coded (steps 6, 9, 15, 17) for comparison with the experimental results in Fig. 3. After demonstrating efficient enzyme activity (Day 2, step 6), the acid wash (Day 2) removed catalytic activity (step 9). Subsequent biofunctionalization with Pfs-chitosan (Day 3) re-established enzymatic activity (step 15) comparable to the original (Day 2, step 6), illustrating enzyme reversibility and bioMEMS.

Figure 2. Experimental setup and processdure. (a) Prefabricated bioMEMS device with electrical connections and fluidic inputs/outputs. (b) one microfluidic channel with colored dye flowing and enlarged view of one electrode. (c) Experimental sequence of operations, including enzyme assembly and catalytic activity measurement (steps 1-6), removal of biofunctionalization and measurement (steps 7-9), enzyme re-assembly and measurement (steps 10-15), storage (step 16) and measurement (step 17).
reuse. Storage in PBS buffer at room temperature for 4 days degraded the conversion efficiency slightly (to 35%), demonstrating robustness of the assembled enzyme.

4. RESULTS AND DISCUSSION

This work demonstrates the efficacy of a biological agent – here, the enzyme Pfs – in carrying out a biochemical function it serves in living cells: Pfs converts SAH to SRH and adenine in one step of a multi-step cell-signaling process (autoinducer-2 production), a quorum sensing phenomenon that determines pathogenicity of bacteria. These results are very encouraging for use of bioMEMS as a metabolic engineering platform, e.g. for drug discovery through an efficient screening of potential enzyme inhibitors as antimicrobial drug candidates. They also illustrate the flexibility of the bioMEMS platform in enabling reversible biofunctionalization and device reuse.

Figure 3: Results of experimental sequence (Table 1) to demonstrate enzyme catalytic activity, its reproducibility after enzyme removal, and its robustness over time. The background colors in each step correspond to the colors in experimental process in Table 1. (a) Schematic flow of enzymatic reaction, (b) % conversion vs. various flow rates.

5. CONCLUSIONS

This work demonstrates a chitosan-mediated biofunctionalization strategy for the assembly of catalytically active enzymes within completely packaged bioMEMS devices. The HPLC results show that assembled enzymes are catalytically active, robust and stable with time, and that our strategy is reversible for multiple uses of our bioMEMS devices.

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REFERENCES