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Layer-by-layer assembly of a redox enzyme displayed on the surface of an elongated bacteria into a hierarchical artificial biofilm based anode

Lin Xia,[a,b] Yehonatan Ravenna[a] and Lital Alfonta*[a]

Experimental Section

Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich (Rehovot, Israel) or Acros (Geel, Belgium) and used without further purification.

Cloning

The details for the construction of plasmid pJM7-ADHII and the surface display of ADHII on *E. coli* JK321 can be found in our previous work.[1] Alcohol dehydrogenase II gene (*adhII*) (1146bp) was amplified from *Zymomonas mobilis* genome by polymerase chain reaction (PCR) using purified genomic DNA as a template, with the following primers:

Forward (pjm-ZADH2-F (containing an underlined XhoI restriction site)):

CTCGCACGTGCTCGAGGCTTCTTCAACTTTTTATATTC

Reverse (pjm-ZADH2-R (containing an underlined KpnI restriction site)):

ACATAAGCGGTACCGAAAGCGCTCAGGAAGAGTTC

PCR products and the autodisplay vector pJM7 (4652 bp), were digested with XhoI and KpnI (all restriction enzymes supplied by Fermentas, Leon-Rot, Germany), and the ADHII gene was ligated into the autodisplay vector, pJM7 which resulted in a new plasmid pJM7-ADHII. pJM7-ADHII was transformed into competent *E. coli* cells strain DH5α by electroporation. The plasmid was purified, sequenced and introduced into competent *E. coli* cells strain JK321. All cloning steps were performed by standard methods.

Surface display of ADHII

E. coli JK321 harboring the plasmid pJM7-ADHII were grown under selective conditions (kanamycin and carbenicillin). The activity of ADHII, displayed on the bacteria surface, was examined both by a colorimetric assay and by an electrochemical assay.

Elongation treatment

Elongation of ADHII surface displayed on JK321 was achieved using a cis-diammineplatinum(II) dichloride (cisplatin) treatment method that was slightly altered from the previously reported method.[2,3] In Brief, bacteria were grown at 37°C in LB medium in the presence of 15μg/mL of cisplatin for 14 h. The cisplatin was added at the beginning of the incubation.

Biochemical measurements

The enzymatic activity of surface displayed ADHII was determined by monitoring the conversion of NAD^+ to NADH, based on an enzymatic alcohol dehydrogenase assay.

Briefly, cells were washed twice with PBS then resuspended in the same buffer, to which NAD^+ was added to a final concentration of 5 mM. Measurements were performed using UV-star 96-well plate (Grenier Bio-one, Germany). To each sample (200 μL final volume), ethanol was added to a final concentration of 20%. Absorbance was measured at 340 nm for more than one hour, in a microplate reader (BioTek instruments, Winoosky, VT).

Cell counting

Cell counting was performed by Luna Automated Cell Counter (Korea). For the sample preparation, different concentrations of bacteria containing growth media (OD=0.2, 0.4, 0.6, 0.8 and 1.0) were mixed with the same volume of trypan blue and incubated for 10min.

Preparation of bioanode

Prior to electrode assembly, multi-walled carbon nanotubes (MWCNTs) were dispersed in DDW with Sodium Dodecyl Benzene Sulphonate (SDBS) by probe-sonication for 1h to obtain a homogeneous solution of negatively charged MWCNTs. The enzyme surface

displayed bacteria was suspended in 50μM methylene blue (MB) solution to adsorb MB molecules to form a positively charged surface, and then centrifuged and washed 3 times in PBS before use. The general self-assembly procedure for preparing multilayer films was as follows; to facilitate the formation of the three-dimensional structure of the bioanode, a hydrophilic carbon paper (CP) was used as the base material. First, carbon cloth was immersed into MWCNTs solution for 1 hour, followed by the spontaneous assembly of MWCNTs on CP via π-π stacking interactions and hydrophobic interactions between carbon materials. Next, the multilayers were formed by alternately dipping the electrode into the positively charged bacteria aqueous solution and the negatively charged MWCNT aqueous solution (0.5 mg mL^{-1}) for 15 min each. Between each dipping, the electrode was immersed into DDW for 2 minutes to thoroughly wash off non-adsorbed reagents, and then electrode was dried under a flow of air.

The elongated bacteria modified anode was prepared by casting 10μL of the elongated bacteria suspension (OD=1) onto the CP electrode and drying it. For the preparation of the natural biofilm modified anode, the CP was dipped into bacterial solution with ADHII-JK321 for 18 hours and gently washed with DDW. Anode performance remained the same during a week of operation and deteriorated to 60% of its initial performance during the next two weeks of operation after which the ~~Mb~~ MB was replenished to gain the original concentrations and original power out puts.

Electrochemical measurements

Electrochemical measurements were performed using a PalmSense potentiostat (Palm Instruments, Houten, the Netherlands). The electrochemical response was measured in a conventional three-electrode based electrochemical cell, the bioanode was used as a working electrode, a graphite rod as an auxiliary electrode and an Ag/AgCl electrode as the reference electrode. All measurements were performed at room temperature (Ca. 23℃).

Fuel cell construction and performance characterization

The assembled electrodes served as the anode in a single compartment semi-biofuel cell (10 mL); consisting of 0.1 M PBS buffer (pH 7.4), NAD^+ (5 mM) and ethanol (2%). The cathode was potentiostatically controlled, using a three-electrode configuration: gold disk (3 mm in

diameter) as a working electrode, platinum wire as a counter electrode and Ag/AgCl as a reference electrode (ALS, Tokyo, Japan). The cathode was biased to a potential of +700 mV against Ag/AgCl. The voltage generated from the biofuel cell was measured by a hand held multimeter (DM-97, Sinometer, China). Various external resistances were applied between the anode and cathode by a resistance decade box (RBOX 408, Lutron Electronic Enterprise, Taipei, Taiwan). The generated voltage at each resistance was measured after reaching equilibrium. Measurements were carried out at ambient temperature.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) images were obtained using JSM-7400F (JEOL) ultrahigh resolution cold FEG-SEM (Japan) operating at 6 kV. Samples for SEM characterization were prepared in a similar manner as mentioned for the bioanode preparation, after which they were washed with DDW and dried at room temperature overnight.

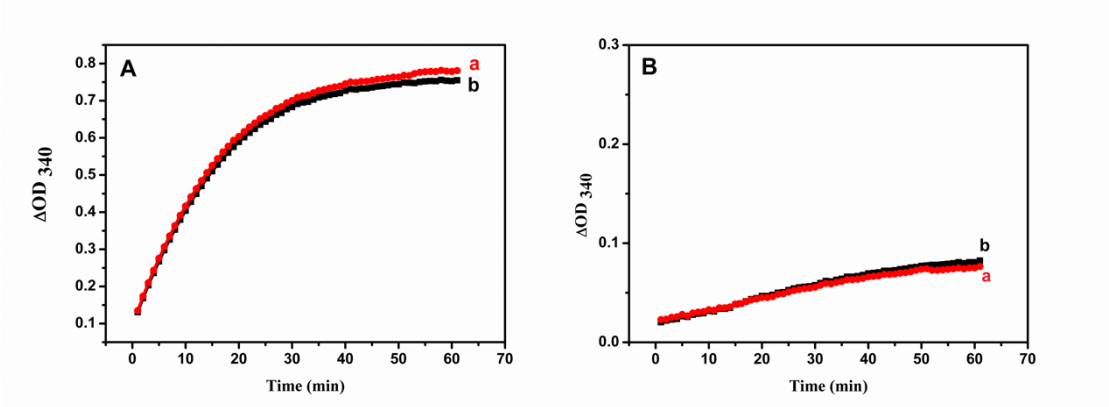

Figure S1. Determination of ADHII activity for （A）a commercial ADHII enzyme and (B) WT JK321 *E. coli* with (curve a) and without (curve b) 15μg/mL cisplatin treatment.

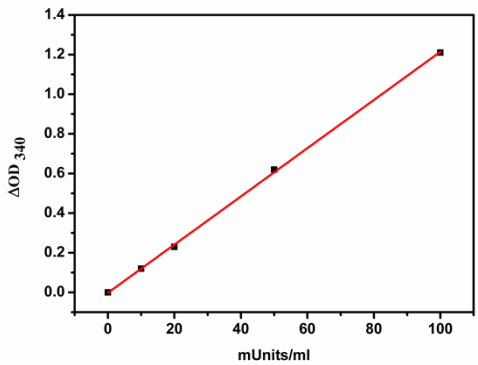

Figure S2. Calibration curve of ADHII activity, based on commercial ADHII activity. Correlation coefficient R^2 = 0.9953.

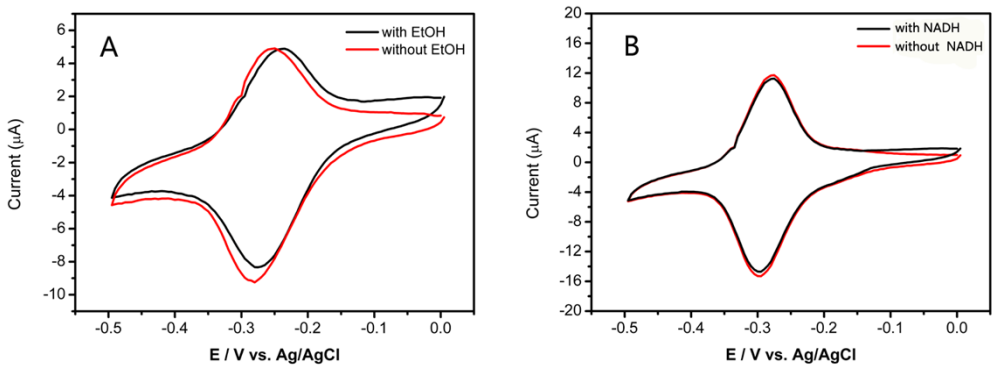

Figure S3. Cyclic voltammograms of electrodes prepared using MB adsorbed on MWCNTs followed by assembly of ADHII-JK321, with (black) and without (red) 1.5% (wt) ethanol, in the presence of 5mM NAD^+, (B) electrodes prepared by MB adsorbed on MWCNTs without further modification with bacteria, with (black) and without (red) 2mM NADH. Scan rate 5 mV/s in PBS buffer (pH 7.4).

References:

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