Electronic Supporting Information

Electrocatalytic Interconversion of NADH and NAD^+ by *Escherichia coli* Flavohemoglobin

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Materials and Methods

Reagents

Escherichia coli flavohemoglobin (HMP, a 4.1 mg ml^{-1} stock solution) was purified as described elsewhere.[1] β-Nicotinamide adenine dinucleotide, reduced form disodium salt (NADH, purity 98.6%), β-nicotinamide adenine dinucleotide, oxidised form (NAD^+, purity 99%), neomycin sulphate and polymyxin B sulphate (PMB) were from Sigma-Aldrich, St Louis, MO, USA. Glucose dehydrogenase (*Bacillus* sp.) was from Genzyme Diagnostics, UK. Concentrations of NADH were determined spectrophotometrically. A molar absorption coefficient of 6317 M^{-1} cm^{-1} at 340 nm was used,[2] solutions were freshly prepared and used promptly. All components of buffer solutions were from Sigma-Aldrich, Germany. All solutions were prepared with de-ionised Milli-Q water (18.2 MΩ cm, Millipore, Bedford, MA, USA).

Electrodes preparation

Prior protein immobilization, rods of solid spectroscopic graphite (Gr, type RW001, 3.05 mm diameter, SGL Carbon AG, Werk Ringsdorff, Bonn, Germany) were cut; their disk surface was polished on fine emery paper (Tufbak Durite, P600), rinsed with de-ionized water and fitted into Teflon holders. For adsorption of HMP, either 5 μl of a stock solution of HMP in a Tris-HCl buffer solution, pH 7.5, or 5 μl of a 10:1 v/v mixture of a stock solution of HMP and a 2.6 mg ml^{-1} solution of PMB, in the same buffer, were placed on the electrode surface and stored for 4 h at 4°C under the plastic lid. Prior to the experiments the modified electrodes were thoroughly washed with 20 mM Tris-HCl, pH 7.0, and mounted in the cell. NAD^+-dependent glucose dehydrogenase (GDH) was physically adsorbed onto the HMP/PMB-modified Gr electrodes, by adsorption of 5 μL of a 5 mg mL^{-1} protein aqueous solution placed onto the HMP/PMB modified surface for 1-2 h at 4 °C.

In control experiments with PMB, FAD and heme, Gr electrodes were modified by placing 5 µl of either the PMB stock solution or its 1:1 mixture with 1 mg/ml solutions of FAD and heme (in 10% DMSO) onto the Gr surface for 2 h at rt under the plastic lid.

Electrochemical experiments

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments with the HMP-modified Gr disk and rotating disk electrodes were performed at 22±1°C in 20 mM Tris-HCl, pH 7.4, in a standard three-electrode electrochemical cell connected to a three-electrode potentiostat AUTOLAB PGSTAT 300 (Eco Chemie B.V., Utrecht, the Netherlands) equipped with GPES 4.9 software. An $Ag|AgCl|KCl_{sat}$ was the reference (+197 mV vs. NHE) and a Pt plate was the auxiliary electrode. In DPV measurements, the effective scan rate was 10 mV s^{-1} (the modulation amplitude and time were 50 mV and 50 ms, correspondingly, and the interval time was 0.2 s). Prior measurement, the working solutions were purged with nitrogen for 40 min and kept under the gas flow during experiments.

Figures

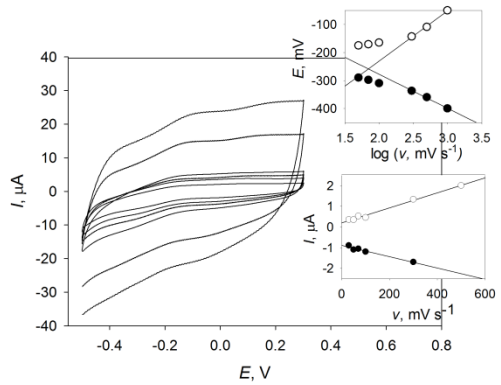

Figure S1. Representative CVs (from 30 to 500 mV s^{-1}) recorded with Gr electrodes modified with HMP in the presence of PMB, 20 mM Tris, pH 7.4. Insets: and peak currents and potential scan rate dependences.

Comment to Figure S1: Electrochemistry of the heme center of HMP was not sufficiently resolved (the cathodic peak) at higher scan rates, which restricted the analysis to scan rate of 300 mV/s. However, the peak potentials could be precisely determined and used in analysis of the rate constant (Main text, Figure 2, inset). The polynomial background analysis of the GPES software was used for the peak position identification.

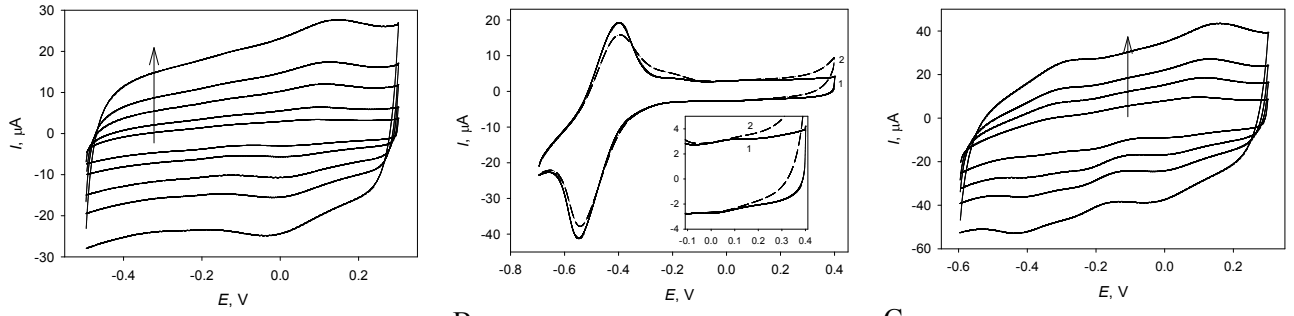

Figure S2. Representative CVs recorded with (A) bare Gr, (B) FAD-PMB-modified, and (C) heme-PMB-modified Gr electrodes, scan rates changing from (A) 50 to 500 mV s^{-1} and (C) 100 to 500 mV s^{-1}. In (B) scan rate is 50 mV s^{-1}, (1) CV in the absence and (2) in the presence of 1 mM NADH/1 mM NAD^+, inset: zoomed NADH oxidation region, oxidation is catalysed by the Gr surface qiunoid functionalities.

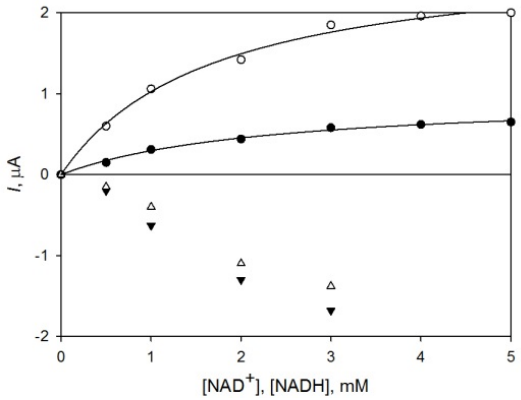

Figure S3. Dependences of the catalytic currents of (circles) NADH oxidation and (triangles) NAD^+ reduction on the concentration of the substrates; derived from CVs, at (●) -200, (○) 70, (Δ) -200 and (▼) -251 mV. Solid lines are Sigma-Plot fitting of the data to the Michaelis-Menten equation.

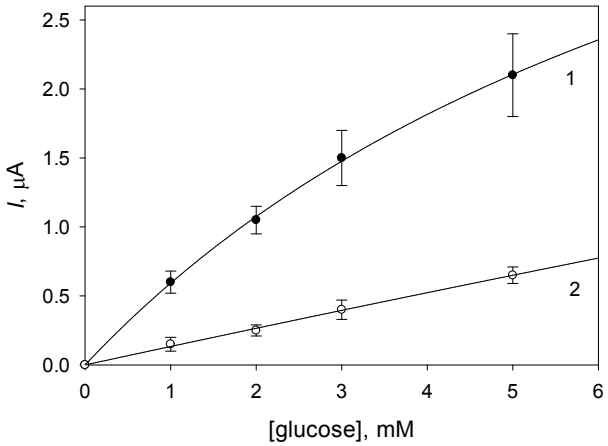

Figure S4. Dependences of the catalytic currents of glucose oxidation on the glucose concentration at the GDH/HMP-PMB-modified electrodes in the presence of 5 mM NAD^+ and different concentrations of glucose in 20 mM Tris, pH 7.4, chronoamperometry at -200 and +70 mV.

REFERENCES

1. Bonamore, A.; Chiancone, E.; Boffi, A., *Biochimica et Biophysica Acta* **2001,** *1549* (2), 174-178.
2. McComb , R. B.; Bond, L. W.; Burnett, R. W.; Keech, R. C.; Bowers Jr, G. N., *Clin. Chem.* **1976,** *22*, 141-150.