Biomolecular Release Triggered by Glucose Input – Bioelectronic Coupling of Sensing and Actuating Systems

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Electronic Supplementary Information

Experimental details

Materials:

Peroxidase from horseradish (HRP) (E.C.1.11.1.7), 2-iminothiolane hydrochloride (Traut's reagent), 3-(*N*-morpholino)propanesulfonic acid (MOPS-buffer), sodium sulfate, sodium salt of alginic acid from brown algae (medium viscosity, \geq 2000 cP), D-(+)-glucose, 3,3',5,5'-tetramethylbenzidine dihydrochloride (TNB), gold (III) chloride trihydrate (HAuCl₄), trisodium citrate, calcium chloride, 2,6dichlorophenolindophenol (DCPIP), phenazine methosulphate (PMS), ferrous sulfate and hydrogen peroxide were obtained from Sigma-Aldrich or J.T. Baker and used without further purification. The bifunctional linker 1-pyrenebutanoic acid succinimidyl ester (PBSE) was purchased from AnaSpec Inc. PQQ-dependent glucose dehydrogenase (PQQ-GDH; E.C. 1.1.5.2, from microorganism – not specified by the company) was purchased from Toyobo Co., Japan, and used as supplied. All experiments were carried out in ultrapure water (18.2 M Ω ·cm; Barnstead NANOpure Diamond). Buckypaper composed of compressed multiwalled carbon nanotubes (CNTs) (Buckeye Composites; NanoTechLabs, Yadkinville, NC) was used as the electrode material support (geometric area exposed to the solution was ca. 1.2 cm²).

Instrumentation:

A Shimadzu UV-2450 UV–Vis spectrophotometer with 1 mL poly(methyl methacrylate) (PMMA) cuvettes, was used for optical spectrum measurements of gold NPs and a BIO RAD Model 680 ELISA microplate reader was used for all optical measurements of the HRP assay. Electrochemical experiments (cyclic voltammetry and application of controlled potential on the alginate-electrode) were performed in a 3-electrode cell: Metrohm Ag|AgCl|KCl, 3M, reference, graphite counter, and buckypaper working electrode. A single compartment cell was used with an electrochemical workstation (ECO Chemie Autolab PASTAT 10) and GPES 4.9 (General Purpose Electrochemical System) software. The Au NPs dimensions and the particle size distribution were investigated using an Atomic Force Microsope (AFM) Veeco Dimension 3100 and scanning electron microscope (SEM) JEOL 6300.

Preparation and characterization of the HRP-NP conjugate:

Gold nanoparticle preparation and characterization: Au NPs were prepared using the standard citrate method.¹ 200 mL of 0.01% (w/v) HAuCl₄ was heated to a boil, and then 7 mL of 1% (w/v) aqueous trisodium citrate solution was added under vigorous stirring. The color changed from grayish-black to

wine-red within a few minutes. The dispersion was allowed to cool. The Au NPs obtained by this method were characterized by SEM and AFM. The diameter of Au NPs was measured by scanning electron microscope (SEM) JEOL 6300. The SEM images were processed with ImageJ (NIH) and MatLAB software to get the diameter distribution. A droplet of Au MPs solution was placed on a cleaned silicon wafer, air dried and imaged by Atomic Force Microsope (AFM) Veeco Dimension 3100.

Conjugation of HRP with Au NPs. 48.8 μ L Traut's reagent stock solution (1 mg Traut's reagent / 0.5 mL water) was added to 1 mL HRP solution (1 mg HRP / 1 mL 100 mM Na₂SO₄ aqueous solution) and allowed to react for one hour at 4 °C. The thiolated HRP mixture containing non-reacted initial reactants was filtered 3 times using 10 kDa Nanosep ultrafiltration tubes at 10,000 rpm for 5 minutes and then rebuffered with MOPS-buffer (20 mM, pH 7.4). The resulting solution was diluted to 800 μ L using MOPS-buffer. Au NP solution (1.17×10⁻⁹ M) was then added to the solution of thiolated HRP in a 1:1 (v/v) ratio and allowed to incubate for 48 hours at 4 °C. Conjugation of the enzyme with the Au NPs was followed by the absorbance maximum ($\lambda = 520$ nm) shift in the spectrum of the NPs.

Characterization of the HRP-NP conjugate. The loading of the NP with HRP was measured using the optical assay² of HRP with TMB from a portion of HRP-NP conjugate, centrifuged and rebuffered with 100 kDa Nanosep ultrafiltration tubes and MOPS-buffer (20 mM, pH 7.4) to discard the free unbound HRP. The units of HRP were calculated from the absorbance of TMB_{ox}. Then the units were translated to the number of HRP molecules assuming that the enzyme specific activity remained unchanged after its immobilization. This was related to the known concentration of NPs to give the number of HRP molecules per NP.

Electrode modification, characterization and use:

Deposition of alginate on a buckypaper support. Sodium alginate (1.5% w/w) was dissolved in 100 mM Na₂SO₄ (pH 6.0) and stirred for 30 minutes at 45 °C. The solution was cooled to room temperature and FeSO₄ (35 mM) and HRP-NP (9.3×10^{-11} M) were added and mixed well. The HRP-NP conjugate was not purified from the unbound HRP and the mixture of the free HRP and HRP-NP conjugate was entrapped into the Fe⁺³-alginate upon its electrochemical deposition. The mixture was deposited on a buckypaper electrode upon oxidation by a potentiostat (+0.8 V, 15 min) of Fe²⁺ cations, yielding Fe³⁺ and resulting in an alginate crosslinked film on a buckypaper surface containing entrapped HRP-NP and HRP. The resulting electrode was washed sequentially in Na₂SO₄ solution (0.1 M) four times for 20 seconds, once for 30 minutes, and once more for 20 seconds to wash out free HRP. The thickness of the alginate film deposited on the buckypaper electrode was roughly estimated to be ca. 1.3 µm extrapolating the data described in our previous work to the present deposition time (15 min).³

Alginate electrode characterization. Cyclic voltammetry measurements on the Fe⁺³-crosslinked alginatemodified electrode were performed in a 3-electrode system containing Ag|AgCl|KCl 3M (Metrohm) reference electrode and graphite rod (diam. 3 mm, low density, 99.99 % trace metals basis; Sigma-Aldrich) counter electrode in addition to the modified working electrode. The background aqueous electrolyte solution was composed of MOPS-buffer (50 mM, pH 7.0, containing 100 mM Na₂SO₄ and 1 mM CaCl₂).

Functionalization of buckypaper with PQQ-GDH. Four 7 mm × 20 mm pieces of buckypaper were cut and washed in 600 μ L isopropyl alcohol for 15 minutes, followed by washing in 500 μ L of MOPS-buffer (50 mM, pH 7.0, containing 100 mM Na₂SO₄ and 1 mM CaCl₂) while shaking. The electrodes were then incubated in 500 μ L PBSE (3.6 mg / mL EtOH) for 1 hour while shaking. Finally, the electrodes were washed again in the same manner as the first washing and incubated in PQQ-GDH solution (1.2 mg / 500 μ L) in MOPS buffer (50 mM, pH 7.0, containing 100 mM Na₂SO₄ and 1 mM CaCl₂) for 1 hr with shaking. The electrodes were stored at 4 °C.

PQQ-GDH electrode characterization. The activity of PQQ-GDH immobilized on the electrode was determined according to the protocol published elsewhere by measuring the decrease in absorbance of 2,6-dichlorophenolindophenol (DCIP) upon its biocatalytic reduction with glucose.⁴ The PQQ-GDH-modified electrode was immersed in a MOPS-buffer solution (10 mM, pH 7.0) containing 10 mM glucose, 120 μ M PMS, 200 μ M DCPIP, 1 mM CaCl₂ and the absorbance change ($\lambda = 600$ nm; $\varepsilon_{DCPIP} = 21$ mM⁻¹·cm⁻¹) in time was measured. The PQQ-GDH activity was measured as 250 mU per electrode. Cyclic voltammetry measurements were performed in a 3-electrode system composed of the buckypaper functionalized with PQQ-GDH working electrode, Ag|AgCl|KCl 3M (Metrohm) reference electrode and graphite rod (diam. 3 mm, low density, 99.99 % trace metals basis; Sigma-Aldrich) counter electrode. The background acupates electrode acupates accepted of MOPS buffer (50 mM, pH 7.0) containing 100

background aqueous electrolyte solution was composed of MOPS buffer (50 mM, pH 7.0, containing 100 mM Na_2SO_4 and 1 mM $CaCl_2$). Cyclic voltammograms of the PQQ-GDH electrode were obtained in the absence and presence of glucose (25 mM).

Release of HRP-NP conjugate in the presence of glucose. Electrochemical release of the conjugate from the crosslinked alginate matrix was first facilitated by an applied potential from a potentiostat (+10 mV and -150 mV mimicking the absence and presence of glucose, respectively). In subsequent experiments, the potential was produced from a wired PQQ-GDH electrode in the same electrochemical cell, with and without applied glucose (25 mM) in the presence of MOPS buffer (50 mM, pH 7.0, containing 100 mM Na₂SO₄ and 1 mM CaCl₂).

Enzymatic assay of released HRP-NP. A solution composed of 50 μ L H₂O₂ (0.035 % v/v), 30 μ L TMB (4 mM in DMSO) and MOPS buffer (50 mM, pH 7.0, containing 100 mM Na₂SO₄ and 1 mM CaCl₂ was prepared for the analysis of the released HRP-NP conjugate. This solution was mixed with 40 μ L of buffer the HRP-NP was released into and an increase in absorbance at λ = 655 nm was measured using a microplate reader (BIO RAD 680). The HRP biocatalytic activity was derived from the absorbance changes using a standard assay protocol.²



Additional figures illustrating the experiments

Figure SI1. Photographs represent formation of the alginate gel with Fe^{3+} cations from a solution composed of alginate (1.5% w/w FeCl₃ (35 mM), and 100 mM Na₂SO₄ (right), while alginate solution with Fe^{2+} cations composed of alginate (1.5% w/w), FeSO₄ (35 mM), and 100 mM Na₂SO₄ remains a viscous liquid (left). (This illustrating photo was originally published in ref. 3.)



Figure SI2. SEM image of the buckypaper support used for both electrodes, the anode biocatalytically oxidizing glucose through immobilized PQQ-GDH, and the cathode coated with the an electrochemically deposited iron-alginate film.



Figure SI3. (A) SEM image of Au NPs. (B) Diameter distribution for Au NPs derived from the SEM image.



Figure SI4. Enzymatic assay of the leakage of HRP / HRP-NP from the alginate film during the washing steps in 100 mM Na_2SO_4 after deposition. Washing was performed until there was no leakage of HRP observed. The electrode was washed four times for 20 seconds (D, B, C, E), followed by a 30 minute wash (A), and finally washed once more for 20 seconds (F).



Figure SI5. Enzymatic assay of the HRP-NP conjugate released from the alginate film: (A) upon application of the potential mimicking absence (+10 mV) and presence of 25 mM glucose (-150 mV), curves b and a, respectively; note that the experiment was performed in a 3-electrode cell configuration and the potential was applied from a potentiostat; (B) in the absence and presence of 25 mM glucose, curves b and a, respectively; note that the releasing alginate-modified electrode was directly connected to the PQQ-GDH-modified electrode as shown in Figure SI5.



Figure SI6. Electrochemical setups for glucose-triggered release of HRP-NP conjugate from Fe^{+3} -crosslinked alginate polymer film. Two electrodes, one for glucose oxidation and another for HRP-NP release, are connected together.

Electrode stability and use for other bioactive species:

The alginate-modified electrode was able to respond to the electrochemical signal after at least 4 hours incubation in a working solution,⁵ while the PQQ-GDH-modified electrode was operating for at least several weeks in a biological environment.⁶ Other bioactive species / drugs were tested with the alginate modified electrode, for example lysozyme loaded into the polymer film was released by the electrochemical signals.⁵

References

- (1) *Metal Nanoparticles: Synthesis, Characterization, and Application*, D. L. Feldheim, C. A. Foss Jr., (Eds.), Marcel Dekker, New York, 2002.
- (2) P. D. Josephy, T. Eling and R. P. Mason, J. Biol. Chem., 1982, 257, 3669–3675.
- (3) Z. Jin, G. Güven, V. Bocharova, J. Halámek, I. Tokarev, S. Minko, A. Melman, D. Mandler and E. Katz, *ACS Appl. Mater. Interfaces*, 2012, **4**, 466–475.
- (4) A. R. Dewanti and J. A. Duine, *Biochemistry*, 1998, **37**, 6810–6818.
- (5) Z. Jin, A. M. Harvey, S. Mailloux, J. Halámek, V. Bocharova, M. R. Twiss and E. Katz, *J. Mater. Chem.*, 2012, **22**, 19523–19528.
- (6) L. Halámková, J. Halámek, V. Bocharova, A. Szczupak, L. Alfonta and E. Katz, J. Am. Chem. Soc., 2012, **134**, 5040–5043.