Electrochemistry Communications 70 (2016) 39–42

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Enhanced electron transfer between gold nanoparticles and horseradish peroxidase reconstituted onto alkanethiol-modified hemin

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ARTICLE INFO

Article history:
Received 7 May 2016
Received in revised form 6 June 2016
Accepted 24 June 2016
Available online 29 June 2016

Keywords:
Gold nanoparticles
Hemin
Peroxidase
Electron transfer
Reconstitution

ABSTRACT

Robust molecular bioelectronic devices require a programmable and efficient electronic communication between biological molecules and electrodes. With proteins it is often compromised by their uncontrollable assembly on electrodes that does not provide neither uniform nor efficient electron flow between proteins and electrodes. Here, horseradish peroxidase reconstituted onto C_{11}-alkanethiol-conjugated hemin and self-assembled onto the gold nanoparticle (NP)-modified electrodes via the exposed alkanethiol tail exhibits enhanced electron transfer (ET), proceeding via the gold NP relay with the ET rate constant approaching 115 s^{-1} vs. 14 s^{-1} shown on bare gold, by this offering an advanced controllable design of interfaces for bioelectronic devices based on heme-containing enzymes with a non-covalently bound heme.

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1. Introduction

Programmable and efficient electronic communication between biological molecules and electrodes forms the basis of molecular bioelectronics and allows the development of electronically addressable biosensing and actuating systems, information storage and processing devices, and nanoscale-regulated nanomachines and nanorobotic systems [1]. In these applications, ability of biological components to self-assemble is an important feature biomolecular electronic devices benefit from. Advantages of programmable DNA and RNA self-assembly onto electrodes and their electronic functioning have been numerously demonstrated [1]. In contrast, biomolecular interactions of proteins are less predictable, and their use for protein integration with electrodes often results in uncontrollable and non-uniform protein assemblies exhibiting dispersions in orientations and resulting electronic properties such as electron transfer (ET) rates [2,3].

Several strategies can be used to control orientation of proteins at electrodes: its electrostatic modulation via oppositely charged electrode and protein domains [4]; promoter-assisted orientation [5]; chemical coupling of protein surface groups to functional groups on the electrode surface [6,7]; immobilization through affinity tags genetically introduced in specific sites of the protein surface [8]; and wiring of protein redox cofactors directly to the electrode via synthetic linkers [9,10]. With the last two strategies, a specific and anisotropic protein orientation onto electrodes, forming the basis of programmable bioelectronics, can be achieved.

Since genetic engineering of proteins can be quite complicated and time consuming, direct wiring of protein cofactors to electrodes and further protein reconstitution onto them represents a simpler and nevertheless efficient methodology [11]. Non-covalently bound cofactors such as FAD in glucose oxidase (GOx) have been electrically connected to electrodes using electron-mediating relays; most intriguing, such relays as gold nanoparticles induced enhancement of ET between FAD and electrodes [12]. If this strategy were valid for other biotechnologically significant systems it might be used as a general platform for controllable enhancement of the bioelectronic signal transduction.

Heme proteins are most challenging for such applications: being one of the most functionally and structurally versatile groups, they are involved in a large variety of biological processes related to electron transport, oxygen activation, transformation of peroxides, nitric oxide production, and some other [13]. In many of them, such as sulphite oxidase [14,15] or flavohemoglobins [16] heme is non-covalently bound and comprises an ET relay shuttling electrons between the external redox partner of a protein (which may be an electrode) [17] and the protein catalytic center, often with internal ET rates exceeding those of external ET [2,15,18]. For such systems, enzyme reconstitution on the linker-conjugated heme cofactor and further wiring of the reconstituted enzyme to the electrode via this linker may allow production of highly-efficient bioelectronic devices exploiting the controllable anisotropic "bottom-up" assembly of electrically addressable enzyme molecules on electrodes. In horseradish peroxidase (HRP), the heme *b* cofactor is not covalently bound and can be easily removed from the

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holoenzyme/built-in back by protein reconstitution, thus making HRP a perfect model for these studies.

Here, a directional ET in HRP reconstituted onto the alkanethiol-modified hemin and self-assembled onto Au nanoparticles (AuNPs)-modified electrodes via the alkanethiol tail facing out of the protein surface was interrogated (Fig. 1).

2. Experimental

Hemin (referred to as heme in HRP) and ethanedithiol (EDT) were from Sigma-Aldrich (Denmark). Apo-HRP4C, produced by removal of heme from native HRP, and AuNPs (20 nm in diameter, suspended in H_2O, 7×10^{11} AuNPs per ml) were from the BBI solutions (UK). 11-amino-1-undecanethiol ($HS\text{-}C_{11}NH_2 \cdot HCl$) was from Dojindo (Germany). Hemin's propionic acid residues were coupled to $HS\text{-}C_{11}NH_2$ by succinimide ester chemistry [19], under conditions favoring formation of a mono-adduct (a four-fold excess of hemin). Apo-HRP4C reconstitution on either hemin (rHRP) or purified by reverse phase HPLC $HS\text{-}C_{11}$-hemin **1** (r**1**HRP) was performed in 0.1 M phosphate buffer solution (PBS), pH 6, with a 10-fold molarity excess of the cofactor over apo-HRP4C, with unreacted cofactor removed by dialysis. The reconstituted proteins were self-assembled either directly onto Au electrodes (kept in 0.5 mg ml^{-1} r**1**HRP (or rHRP) overnight at 4 °C, further treated with 1 mM mercaptoethanol in PBS for 15 min) or on AuNPs coupled to Au electrodes via EDT (electrodes were first 1 h kept in a 1 mM EDT solution in ethanol, then allowed to react with 5 μl of 2.87×10^{-4} M AuNPs overnight at 4 °C, rinsed with PBS and modified with reconstituted HRPs).

Electrochemical experiments were performed in 10 mM PBS, pH 6, at 20 ± 1 °C, in a three-electrode cell with an Ag|AgCl (KCl_{sat}) reference electrode and a Pt wire auxiliary electrode. Square wave voltammograms (SWV) were recorded within the 300-1 Hz frequency range, with pulse amplitude of 25 mV and step potential of 1 mV. Standard ET rate constants, k_s, were estimated by plotting the SWV peak currents I_p measured at different frequencies in the I_p/f - log f coordinates (here, f is the specific frequency at which the I_p was measured) and processing those data within the Komorsky-Lovrić – Lovrić formalism [20]. The I_p/f - log f bell-shaped dependences exhibited a maximum at a frequency f_{max} that directly relates to the k_s [20]:

$$k_s = \kappa_{max} \times f_{max} \quad (1)$$

where κ_{max} is a critical kinetic parameter equal to 1.19 in our case ($1e^-$ transfer) [20]. To facilitate comparative analysis, the dependences of I_p/f on log f were normalized to unity by dividing by $(I_p/f)^{max}$, $(I_p/f)^{max}$ relating to the critical frequency f_{max}. Electrochemical impedance spectroscopy (EIS) was performed at 0.2 V, within the 100 kHz–0.1 Hz frequency range; the charge transfer resistance (R_{ct}) was evaluated by data fitting to the Randels circuit generated by the NOVA 1.8.17 software.

3. Results and discussion

A 16–18 Å length of the heme-conjugated C_{11}-alkanethiol linker was estimated to expose 2–4 CH_2-segments with thiol out of the reconstituted HRP [19]. Glycosylated apo-HRP4C was reconstituted onto $HS\text{-}C_{11}$-hemin **1** (producing r**1**HRP) with a 88% efficiency relative rHRP reconstitution on unmodified hemin, as estimated from the Soret maximum in the absorption spectra of HRPs. Enzymatic activity of r**1**HRP decreased 94% compared to that of rHRP, from 2900 ± 50 U mg^{-1} to 98 ± 8 U mg^{-1}, consistent with the reports on heme propionate modifications [21]. Loss of enzymatic activity was unimportant for current research aimed at the enhancement of protein ET wiring to electrodes (a non-catalytic process).

Reconstituted r**1**HRP and rHRP were immobilized onto gold electrodes and on AuNPs-modified electrodes, with r**1**HRP coupled to gold via the thiol of the heme-conjugated linker (Fig. 1) and rHRP physically adsorbed [8,22,23]. EIS spectra recorded for each modification step, demonstrated the increased R_{ct} for both HRPs immobilizations (Fig. 2, data for rHRP are similar and not shown). In agreement with reports on "electron antennae" properties of AuNPs facilitating ET of soluble species on AuNP-terminated SAMs of alkanethiols [24], the ET reaction of ferricyanide was improved (a lower R_{ct}) at the AuNPs/EDT-modified electrodes compared to the EDT-electrodes, with the R_{ct} approaching that observed with a naked Au electrode (Fig. 2).

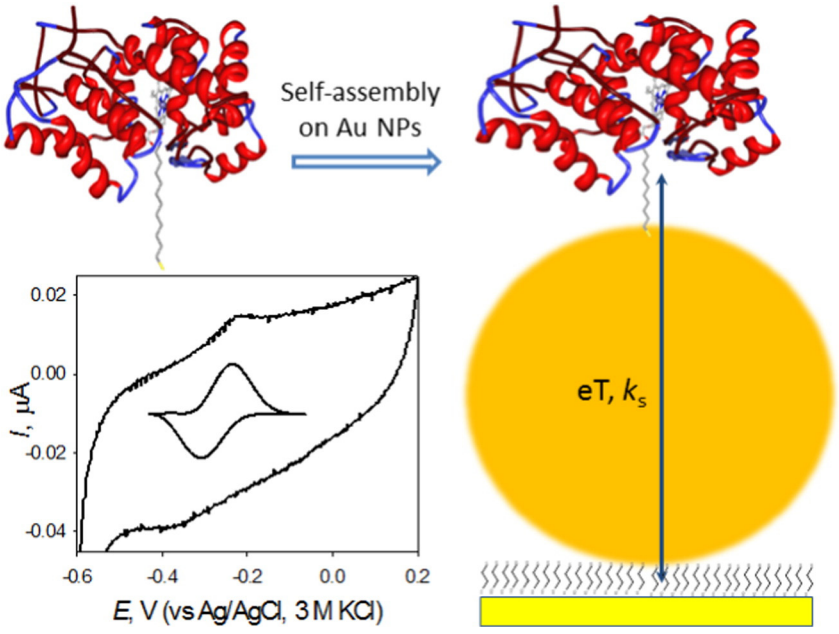

Fig. 1. Schematic representation of self-assembly of r**1**HRP reconstituted onto alkanethiol-modified hemin **1** onto the Au electrode, and representative CV of the r**1**HRP/AuNPs-modified Au electrode recorded in deaerated PBS, scan rate 5 mV s^{-1}, and background corrected voltammetric peaks of the heme $Fe^{2+/3+}$ couple of r**1**HRP.

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