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Bioelectrochemistry 55 (2002) 127–130

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Short communication

Adsorption of differently charged forms of horseradish peroxidase on metal electrodes of different nature: effect of surface charges

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Received 1 June 2001; received in revised form 27 June 2001; accepted 17 July 2001

Abstract

The adsorption and bioelectrocatalytical activity in the reaction of H_2O_2 reduction of two forms of horseradish peroxidase (HRP) offering different surface charges at pH 6.0 were studied on gold and silver electrodes. Positively charged HRP was assessed at pH 6.0 for the case of native HRP (isoenzyme C, pI = 8.8), and negatively charged HRP for the case of native HRP exposed to previous oxidation of carbohydrate residues and further introduction of sulfonate groups (pI = 5.0). Under oxidative pretreatment, the gold electrode surface was considered to be negatively charged. Data on the direct immobilisation of HRPs on the bare gold surfaces were estimated with quartz crystal microbalance and data on bioelectrocatalytical activity of peroxidases on gold and silver electrodes were obtained in the course of direct and mediated amperometric detection of H_2O_2. The presented results demonstrate that the surface charges of both the enzyme and the electrode play a dominant role in the immobilisation and, thereby, in the efficiency of the bioelectrocatalytical processes.

Keywords: Bioelectrocatalysis; Horseradish peroxidase; Adsorption; Electrostatic interactions; Silver and gold electrodes; Quartz crystal microbalance

1. Introduction

Direct (mediatorless) electron exchange between the heme group of horseradish peroxidase (HRP, hydrogen peroxide oxidoreductase, EC-1.11.1.7), and the different electrode materials has been extensively studied [1,2 and refs. therein]. Compound 1 (E1), representing oxidized HRP upon addition of H_2O_2 and consisting of oxyferryl iron ($Fe^{4+}=O$), and a porphyrin π cation radical can be directly electro-reduced ($2e^-$, $2H^+$) at the electrode surface with different electron transfer (ET) rate depending on the specific electrode material (e.g., carbon, gold, tin, oxide, etc.). Direct adsorption of HRP could virtually result in efficient direct ET if deposition and orientation of the enzyme can be controlled. Previously, we have characterised the adsorption and bioelectrocatalytical behaviour of different forms of HRP, native and recombinant ones, on gold electrodes [2]. In the case of native HRP it was observed that detectable currents could be only achieved when the enzyme was adsorbed at pH 6.0. However, the enzyme could be deposited at pH 7.4 on preoxidised gold surfaces (23.1 ± 2.1 pmol cm^{-2}) as was demonstrated by microgravimetric measurements [2]. It was thus presumed that if favoured electrostatic interactions can be established by strategic manipulation of the charge of the interacting surfaces (i.e., the enzyme and the electrode), this could, virtually, result in more efficient direct ET.

The aim of this work is a deeper insight into the effect of electrostatic interactions on the adsorption of HRP on gold and silver electrodes, and, therefore, on the efficiency of ET from the electrode to the active site of the heme protein. This is achieved by different pretreatment of metal electrodes which provides different electrode surface charges and by comparison of the deposition and the bioelectrocatalytic efficiency in direct and mediated ET obtained with two differently charged forms of HRP.

2. Experimental

2.1. Instrumentation

The experimental set-up consisted of gold-coated piezoelectric quartz crystals (PQC), with a frequency of 5 MHz

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1567-5394/02/$ - see front matter
PII: S1567-5394(01)00155-4

and a working area of 1.37 cm^2, and quartz crystal microbalance PM-700 Series Plating Monitor (QCM), (both MAXTEK, Torrance, CA, USA). Amperometric measurements with polycrystalline gold and silver disk electrodes (CH-Instruments, Austin, TX, USA, surface area 0.031 cm^2) were performed at room temperature (22 ± 1 °C) in a standard three-electrode wall-jet flow-injection cell connected to a potentiostat AUTOLAB (PGSTAT30, Eco Chemie, Netherlands) equipped with a GPES 4.8 software. An Ag/AgCl/0.1 M KCl electrode and a platinum wire were used as reference and auxiliary electrodes, respectively.

2.2. Materials

Native HRP (nHRP, isoenzyme C, 1300 U mg^{-1} towards ABTS, pI = 8.8) and other reagents of analytical grade were provided by Sigma (St. Louis, USA). Anionic HRP (HRP^-, 330 U mg^{-1}) was obtained from nHRP by previous oxidation of carbohydrate residues and derivatization with sulfonate groups providing a rather anionic isoelectric point (pI = 5.0) [3]. De-ionized Milli-Q water (Millipore, Bedford, MA, USA) was used throughout this work.

2.3. Electrode pretreatment/modification

Prior to use, the gold PQC electrodes were cleaned in a hot Pyranha mixture and 3 M NaOH, stepwise, for 10 min, rinsed with water, dried, and positioned into the QCM holder. One milliliter of a 0.1 mg ml^{-1} HRP solution in 0.01 M phosphate buffer containing 0.15 M NaCl (PBS), pH 6.0, was then added and the frequency change measurement started. A more negative surface charge of the PQC gold electrodes was obtained by chemical pre-oxidation with 500 μl of the hot Pyranha solution for 2 min, followed by quick and thorough rinsing with water and immediate addition of 1 ml of the previously described enzyme solution.

The surface of gold and silver disk electrodes used for amperometry was polished on fine emery paper, then to a mirror luster on alumina slurry (0.1 μm). The electrodes were finally rinsed with water and immersed in a 0.1 mg ml^{-1} HRP solution in PBS, pH 6.0, for 2 h. After thorough washing with PBS, the modified electrodes were mounted in a wall-jet cell and steady state currents were measured at − 50 mV vs. Ag/AgCl. Similar oxidative pretreatment to the described above was also performed. The reproducibility of the data was verified by measurements with at least three equivalently prepared electrodes and was found to be within 15%.

3. Results and discussion

The effect of the electrode/enzyme surface charges on the HRP adsorption and the ET rate was evaluated with two metal electrodes, polycrystalline gold and silver (non-treated and oxidatively pre-treated), and two forms of HRP possessing different surface charges at pH 6.0. Native HRP is considered cationic while chemically derivatized HRP^- a negative one at the working pH. The potential of zero charge (pzc) of polycrystalline gold is close to − 80 mV vs. Ag/AgCl and thus the electrode surface charge can be considered as slightly positive/neutral, whilst a highly positive surface charge corresponds to a silver electrode in accordance with its pzc (− 840 mV) [4]. After the oxidative pretreatment the gold electrode surface is considered negatively charged.

The amount of both HRP forms adsorbed on differently pre-treated gold electrode surfaces was calculated from the PQC frequency measurements and is summarised in Table 1. These results clearly show that a higher level of HRP deposition is achieved if electrostatic interactions are favoured, i.e., when the attraction between the electrode and the enzyme occurs. This is demonstrated by the increasing deposition of cationic nHRP when changing the slightly positive charge of the non-preoxidised electrodes to the negative charge of the preoxidised electrodes. The predicted behaviour for the anionic enzyme is also reflected by the highest immobilisation on the non-preoxidised electrodes. The lowest immobilisation is observed when the cationic and anionic enzymes exposed to the non-oxidised and preoxidised surfaces, respectively, as a consequence of electrostatic repulsion. The intermediate oxidative pre-treatment corresponds to the electrode surface that has been oxidised but the enzyme was not deposited immediately but after an elapsing time of 5 min. It is worth to mention that, in this case, similar amounts of both cationic and anionic enzymes are deposited representing 46% of the maximal observed surface coverage.

The correlation between the enzyme adsorption and the bioelectrocatalytical activity of HRPs was studied amperometrically by the measurement of a steady state current response of the HRP-modified gold and silver electrodes to H_2O_2. These currents were measured in the presence of a diffusing redox mediator (5×10^{-4} M catechol) and with no addition of the redox partner for the comparison of mediated and direct ET, respectively. Data on direct ET enable the estimation of the fraction of HRP molecules that are efficiently connected with the electrode surface and thus are active in direct ET. Mediated ET in the presence of a saturating concentration of a mediator enables the evaluation

Table 1
HRP surface coverage at differently pre-treated gold QCM electrodes, 10^{-12} mol cm^{-2}

HRP form	Surface pre-treatment		
	Non-oxidised	Intermediate	Pre-oxidised
nHRP	3.0 ± 0.8	9.2 ± 1.9	22.0 ± 2.1
HRP^-	19.3 ± 2.7	8.8 ± 2.0	1.5 ± 0.5

of the total amount of the active enzyme at the electrode surface, as all HRP molecules are supposed to be involved in ET [1]. Results are presented in Figs. 1 and 2.

As can be seen from Fig. 1, in accordance with changing surface concentration of HRPs (see Table 1) the amperometric signal increases significantly when occurring from cationic nHRP adsorbed on the non-preoxidised gold surface (curves 1, 1′ in Fig. 1A) to the oxidised one (curves 2, 2′ in Fig. 1A), and, conversely, decreases for HRP^- (Fig. 1B). In the same manner, a twofold increase of the signal due to mediated ET was obtained for anionic HRP^- when compared to cationic nHRP adsorbed on the positively charged surface of a silver electrode, in spite of about four times lower enzymatic activity of HRP^- (see Fig. 2). Thus, data on mediated ET, when all adsorbed HRP molecules are supposed to be active in the redox process, reveal a rigid correlation between the amount of the adsorbed enzyme and the efficiency of bioelectrocatalysis: favoured surface electrostatic interactions assist in the increasing surface concentration of the enzyme, resulting, thereby, in the enhancement of the bioelectrocatalytic response.

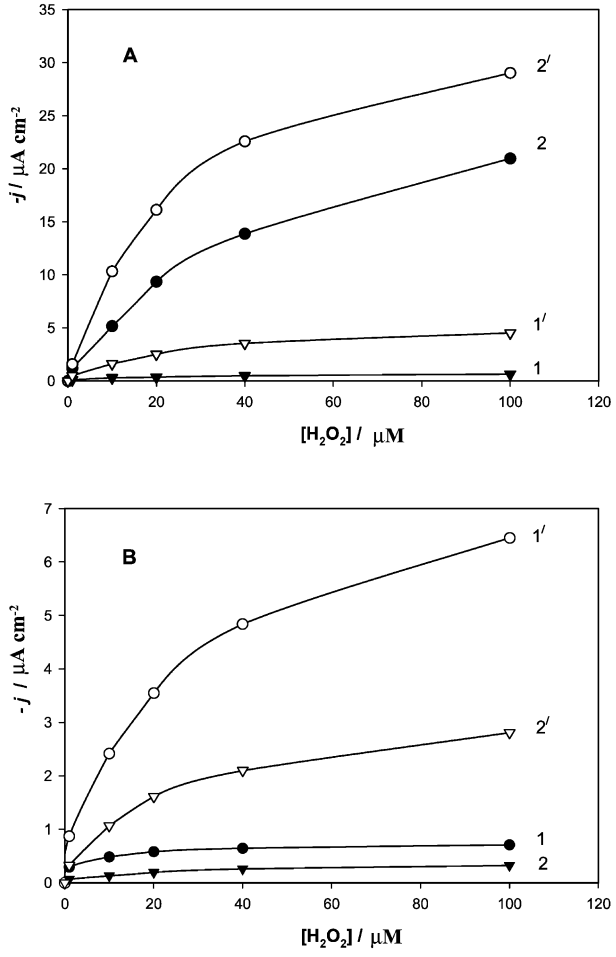

Fig. 1. Dependence of the steady-state current density on the H_2O_2 concentration determined with (1, 1′) polished and (2, 2′) pre-oxidised gold disk electrodes modified with (A) nHRP and (B) HRP^-. In the case of (1, 2) direct and (1′, 2′) mediated ET in the presence of 0.5 mM catechol. Flow rate of the carrier (PBS, pH 6.0) 0.9 ml min^{-1}, potential applied − 50 mV vs. Ag/AgCl.

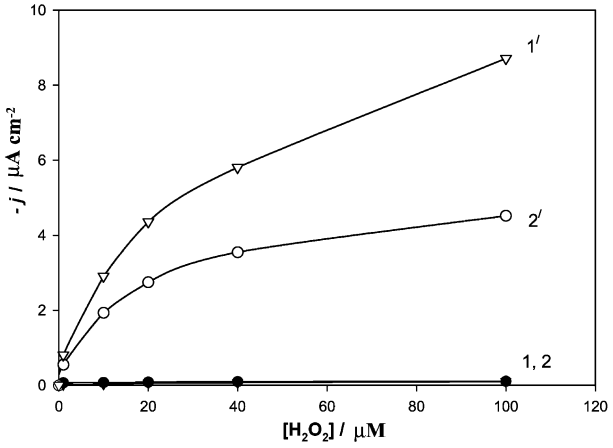

Fig. 2. Dependence of the steady-state current density on the H_2O_2 concentration determined with polished silver disk electrodes modified with (1, 1′) HRP^- and (2, 2′) nHRP. In the case of (1, 2) direct and (1′, 2′) mediated ET in the presence of 0.5 mM catechol. Flow rate of the carrier (PBS, pH 6.0) 0.9 ml min^{-1}, potential applied − 50 mV vs. Ag/AgCl.

Extremely low efficiency of direct ET when compared to mediated one for HRP^- adsorbed both on gold (curves 1, 2 in Fig. 1B) and silver surfaces (curve 1 in Fig. 2), as well as for nHRP on silver (curve 2 in Fig. 2) demonstrates that only a small proportion of HRP molecules is responsible for direct ET orientation on the electrode surface. Comparatively, very different currents (curve 2 in Fig. 1A, preoxidised electrodes, and curve 1 in Fig. 1B, non-pretreated electrodes) are observed for similar HRP depositions (22.0 and 19.3 pmol cm^{-2} for nHRP and HRP^-, respectively). This different efficiency of direct ET cannot be attributed to the different enzymatic activity of immobilised HRPs, contrary to the difference in the efficiency of mediated ET (curve 2′ in Fig. 1A and curve 1′ in Fig. 1B). Further investigation is necessary to elucidate the reasons of these distinctions. The fact that the gold surface upon oxide formation changes its hydrophobic properties towards more hydrophilic ones opens up the possibility of ascribing the higher electronic coupling to a favoured specific orientation through the hydrophobic/hydrophilic surface clusters of the adsorbed peroxidase molecules.

4. Conclusion

The results obtained demonstrated that the surface charges of both the enzyme and the electrode play a dominant role in the adsorption process. Favoured surface charges of the electrode and the enzyme molecule, providing the enhancement of the electrostatic interactions, resulted in the increasing amount of the adsorbed enzyme and, thereby, in the higher current response to H_2O_2. This presents a possibility to control the adsorption and, consequently, the bioelectrocatalysis at the electrode/solution

interface both through the right selection and pretreatment of the electrode surface.

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