Electrochemistry Communications 65 (2016) 1–4

Contents lists available at ScienceDirect

Electrochemistry Communications

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

Short communication

Bioelectrocatalytic oxidation of glucose by hexose oxidase directly wired to graphite

Deby Fapyane [a], Charlotte H. Poulsen [b], Elena E. Ferapontova [a,*]

[a] Interdisciplinary Nanoscience Center (iNANO), Science and Technology, Aarhus University, Gustav Wieds Vej 14, DK-8000 Aarhus C, Denmark
[b] DuPont Industrial Biosciences, DuPont Nutrition Biosciences ApS, Edwin Rahrs Vej 38, 8220 Brabrand, Denmark

ARTICLE INFO

Article history:
Received 19 December 2015
Received in revised form 20 January 2016
Accepted 23 January 2016
Available online 29 January 2016

Keywords:
Hexose oxidase (HOX)
Bioelectrocatalysis
Direct electron transfer
Glucose oxidation

ABSTRACT

Glucose-oxidizing enzymes are widely used in electrochemical biosensors and biofuel cells; in most applications glucose oxidase, an enzyme with non-covalently bound FAD and low capability of direct electronic communications with electrodes, is used. Here, we show that another glucose-oxidizing enzyme with a covalently bound FAD center, hexose oxidase (HOX), adsorbed on graphite, exhibits a pronounced non-catalytic voltammetric response from its FAD, at −307 mV vs. Ag/AgCl, pH 7, characterized by the heterogeneous electron transfer (ET) rate constant of 29.2 ± 4.5 s^{-1}. Direct bioelectrocatalytic oxidation of glucose by HOX proceeded, although, with a 350 mV overpotential relative to FAD signals, which may be connected with a limiting step in biocatalysis under conditions of the replacement of the natural redox partner, O_2, by the electrode; mediated bioelectrocatalysis was consistent with the potentials of a soluble redox mediator used. The results allow development of HOX-based electrochemical biosensors for sugar monitoring and biofuel cells exploiting direct ET of HOX, and, not the least, fundamental studies of ET non-complicated by the loss of FAD from the protein matrix.

1. Introduction

Glucose oxidizing enzymes are highly requested in biosensors for diabetes monitoring [1,2], sugar analysis and biofuel cells [3–5], and, thus, a concomitant research on bioelectrocatalysis of glucose oxidation is strongly promoted. Total amount of publications in this area only for the last decade scales over 9600 (SciFinder Scholar 2015) and in most papers glucose oxidase (GOx) known for its specificity for glucose oxidation is used. The electrochemical biosensors and biofuel cells exploit glucose oxidation by GOx mediated by either redox gels [6,7], polymers [8] or small redox molecules [9–13], and the removal of mediating species might be beneficial both for the price and design of the devices. However, a direct electrical communication between the GOx and the electrode needs quite special conditions, such as GOx reconstitution on electronically wired co-factors [14–16] or the use of de-glycosylated recombinant forms [17]. As a matter of fact, most of hitherto published examples of a so-called direct electron transfer (ET) of GOx show no direct ET-based bioelectrocatalysis of glucose oxidation, but only non-catalytic voltammetric responses from non-covalently bound GOx's FAD [18] that can be ascribed to the FAD lost from the enzyme matrix.

Unsurprisingly, efforts are focused on finding alternative enzymatic systems that can oxidize glucose at moderate potentials and pH in the absence of mediators. Among those are FAD-dependent glucose dehydrogenases (GDHs) [19] and heme- and flavo-containing cellobiose dehydrogenases (CDHs) [20]. The inherent complexity of CDHs requires a quite special electrode environment for their direct bioelectrocatalysis, and the NAD^+-dependence of most GDHs makes them less attractive for practical applications, though recently discovered O_2-independent GDHs quickly gain deserved attention [21,22].

Here, we interrogated ET reactions and bioelectrocatalysis of hexose oxidase (HOX) originating from *Chondrus crispus* and produced recombinantly in the yeast *Hansenula polymorpha* directly immobilized on graphite (Gr) electrodes. HOX is an oxidoreductase enzyme of 126 kDa (pI of 4.7), possessing a single catalytic FAD center covalently bound through His 79 and Cys 138 residues and thus exhibiting improved stability and cofactor integrity, in contrast to GOx; HOX's pH optimum lies between 5.8 and 6.3 [23,24]. HOX catalyzes oxidation of D-glucose with the K_M of 2.7 mM [25], which makes it a good candidate for bioelectrochemical applications. Here we report the first electrochemical study of electron transfer (ET) reactions and bioelectrocatalysis of glucose oxidation by HOX immobilized on Gr electrodes.

2. Materials and methods

Hexose oxidase (HOX, 883.6 U ml^{-1}) originating from *C. crispus* and produced in yeast *H. polymorpha* as described elsewhere [24] was from

* Corresponding author.
E-mail address: elena.ferapontova@inano.au.dk (E.E. Ferapontova).

http://dx.doi.org/10.1016/j.elecom.2016.01.012
1388-2481/

DuPont Nutrition Biosciences ApS (Brabrand, Denmark). HOX was immobilized on graphite electrodes (3.05 mm diameter, Werk Ringsdorff, Germany type RW001, polished with silicon carbide emery paper, SIC paper #1000HV 30-800, Struers A/S, Ballerup, Denmark) by 3 h adsorption from 3 μl of 2 mg ml^{-1} HOX solution casted on the Gr surface (kept at 4 °C under the plastic lid). Prior to measurements, HOX-modified electrodes were washed with a buffer solution. Cyclic voltammetry was performed in a standard three electrode cell connected to μAUTOLAB potentiostat interfaced with a NOVA 1.8 software. A flow-injection wall-jet system was used for chronoamperometry. If not stated otherwise, Gr rods inserted in the Teflon holder served as the working electrode and a Pt wire and an Ag/AgCl (0.1 M KCl for an electrochemical flow-through wall-jet cell and 3 M KCl for a standard electrochemical cell) as counter and reference electrodes, respectively. The working solution was 0.1 M phosphate buffer solution (PBS).

3. Results and discussion

HOX's ET reactions were studied with HOX-modified Gr electrodes. Hitherto, electrochemistry of another glucose oxidizing enzyme, GOx, showed no direct response from FAD, unless FAD was lost from the protein (the E^0 of FAD lost from the GOx being routinely misinterpreted as the potential of GOx-bound FAD) or GOx was deglycosylated [26]. Cyclic voltammograms (CVs) of HOX adsorbed on Gr demonstrated a couple of redox peaks centered at -307 ± 3.3 mV, pH 7 (Fig. 1(A)) that can be associated with the FAD redox center. The formal potential, E^0, of FAD in HOX estimated as a mean value of the anodic and cathodic peak potentials is less negative than that of free FAD adsorbed directly onto Gr (Fig. 1(B)), thus exhibiting the features of the enzyme-bound FAD (Table 1). The potential of free FAD (-460 ± 25 mV, pH 7) is consistent with the previously reported values for FAD on graphite and gold [27–29].

The slope of the pH dependence of the E^0 of FAD in HOX was -60 mV/pH unit and approached the theoretical slope of -59 mV/pH at 22 °C characteristic for an equal number of electrons and protons involved in the ET reaction [30]. Consistent with previous reports [28], similar data were obtained for free FAD (Fig. 1(B)). Along with that, both FAD in HOX and free FAD showed broadened CV peak widths, exceeding the values expected for a $2e^-$ transfer reaction and correlating, as estimated from the peak widths at their mid-height, $w_{1/2}$ (a quasi-reversible reaction, $w_{1/2}(\text{cathodic}) = 62.5/\alpha n$ and $w_{1/2}(\text{anodic}) = 62.5/(1-\alpha)n$) [31], with a $1e^-$ transfer:

$$\text{FAD} + 1\text{H}^+ + 1\text{e}^- \leftrightarrow \text{FADH(semiquinone)} \quad (1)$$

Table 1
Electrochemical characteristics of FAD in HOX immobilized on Gr.

pH	E^0 (V)	Γ (pmol cm^{-2})	k_s (s^{-1})
5	-197.2 ± 1.7	131 ± 61	27.9 ± 3.2
6	-247.3 ± 1.1	135 ± 52	27.1 ± 2.9
7	-307.0 ± 3.3	121 ± 41	29.2 ± 4.5

consistent with a $1e^-/1H^+$ transfer reaction earlier demonstrated for free FAD [28]. The redox transformation of FAD during biocatalysis involves a $2e^-/2H^+$ transfer reaction [32], which is essential for enzymatic activities of FAD-containing enzymes and FAD itself in such reactions as electrocatalytic oxidation of NADH [28,33]. Free FAD seems to be electrocatalytically inactive unless it is stabilized within polymers [28,33] or by nanoparticles [34]e.g. entrapment of FAD into the cationic polyethyleneimine matrix provides a proton rich local environment that favors a $2e^-$ transfer mechanism, exhibited through the narrower voltammetric peak width and electrocatalysis of NADH oxidation [28]. In non-catalytic voltammetry of FAD-containing enzymes, a $2e^-$ transfer reaction might be also expected, since enzymatic activity requires a concomitant $2e^-$ oxidation of such substrates as glucose and NADH. However, non-catalytic voltammetry of GOx routinely shows $1e^-/H^+$ transfers [14,29,35,36] and similar data are obtained here for FAD in HOX.

The HOX's FAD peak currents depended linearly on the potential scan rate (Fig. 1(A), inset) designating a surface-confined ET process [30], with a heterogeneous ET constant, k_s, calculated according to the Laviron formalism [31] approaching 30 s^{-1} (Table 1). The k_s values did not significantly change with pH (Table 1), suggesting that proton transfer may not be the limiting step contrary to some heme proteins, for whose $1e^-/1H^+$ reaction the k_s can increase up to 30-fold upon solution acidification facilitating the protonation step [37–39]. The HOX surface coverage, Γ, evaluated from the voltammetric peak areas, Q ($\Gamma = Q/nFA$, number of electrons $n = 1$, referred to the geometric surface area, A) was consistent with a compact protein layer, one HOX molecule occupying ca. 3.8×3.8 nm^2 if the Gr surface roughness of 10 is taken into account [40,41] (Table 1).

Glucose oxidation by HOX follows the same biocatalytic pathway as GOx's one: glucose is oxidized to gluconolactone at the expense of oxygen, producing H_2O_2 [12]:

$$\text{HOX(FAD)} + \beta - \text{d} - \text{glucose} + \text{O}_2 \rightarrow \text{HOX(FADH}_2) + \text{d} - \text{glucono} - 1,5 - \text{lactone} + \text{H}_2\text{O}_2 \quad (2)$$

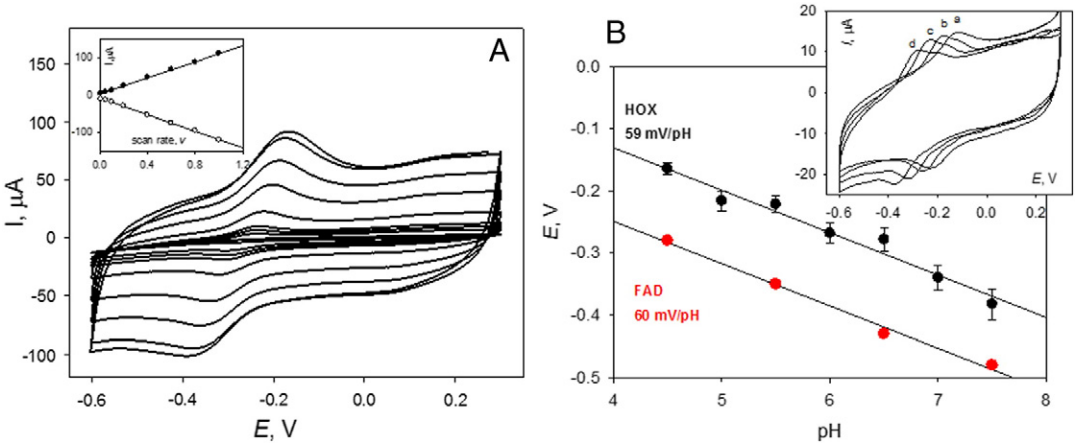

Fig. 1. (A) Representative CVs of HOX recorded in 0.1 M PBS, pH 7, at potential scan rates from 0.01 to 1 V s^{-1} and (B) pH dependences of the formal potentials, E^0, of free FAD and FAD in HOX, both immobilized on Gr. Insets: (A) Dependencies of the cathodic and anodic peak currents for FAD in HOX on the potential scan rate; (B) representative CVs of HOX on Gr recorded in 0.1 M PBS at pH 4.5 (a), 5.5 (b), 6.5 (c) and 7.5 (d), potential scan rate 0.1 V s^{-1}. All CV data are obtained in the absence of O_2.