Electrocatalytic oxidation of *L*-lactate by *Saccharomyces cerevisiae* flavocytochrome b_2 on redox mediator-modified glassy carbon electrodes

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L-lactate detection plays a significant role in healthcare and food industries. For that purpose, amperometric biosensors based on lactate oxidizing enzymes are often used. Among them, lactate electrodes based on flavocytochrome b_2 (*L*-lactate: cytochrome *c* oxidoreductase, fcb_2) have attracted some interest. This enzyme consists of four 58 kD subumits which contain protoheme IX and flavinmononucleotide (FMN). Since direct electron exchange between conventional electrodes and fcb_2 is largely impossible, electrodes with surface redox mediators are used for this purpose.

In this work we investigated the electrocatalytic reactions of *Saccharomyces cerevisiae* fcb_2 on glassy carbon (GC) electrodes, modified either by their electrochemical pretreatment or by the products of electrochemical reduction of dinitrobenzenes. After electrochemical pretreatment of GC electrode in the range of 1.8 to -0.8 V (vs. Ag/Agcl) [1], its cyclic voltammogram shows reversible redox peaks centered at 0.08 V (vs. Ag/AgCl, pH 7.0). The electron transfer rate constant (k_s) of the electrogenerated quinone/hydroquinone redox pair, determined by the method of Laviron, is equal to 0.7 s^{-1}. The pH dependence of the redox peaks corresponds to the $2e^-$, $2H^+$ transfer at pH 5.5-9.0. In the presence of 30-100 µM fcb2 entrapped on the electrode surface, *L*-lactate oxidation started at 0.05 V. The linear part of the response was observed up to 0.4 mM *L*-lactate, and the maximum catalytic current obtained at 0.2 V was 4.5 µA/cm^2. Glassy carbon electrodes can also be modified with the products of reduction and reoxidation of nitroaromatic compounds ($ArNO_2$), most likely polymers containing azoxy (Ar-N=N(-O)-Ar) groups [2]. We modified the electrodes by the potential scanning (10 cycles, -0.8 - 0.6 V) in the presence of 1.0 mM *p*- or *o*-dinitrobenzene. This resulted in the formation of stable surface electroactive compounds with redox peaks centered at 0.06 V (*p*-dinitrobenzene) and 0.1 V (*o*-dinitrobenzene) with k_s values of 0.62 and 0.72 s^{-1}, respectively. Like in the case of electrochemically pretreated GC, the pH dependence of the redox peaks was characterized by the slope of -0.06 V/pH unit. In the presence of entrapped fcb2, in both cases *L*-lactate oxidation started at 0.1 V, the linear part of the response reached 0.3-0.4 mM *L*-lactate, and the maximal catalytic current at 0.2 V was 12 to 18 µA/cm^2.

Although these electrodes were good in terms of their catalytic currents and linear part of the response, they were not sufficiently stable. Their activity dropped by 50% after 24 h storage at pH 7.0 and 4 °C. In comparison, previously studied electrodes based on fcb2 from *Hansenula anomala* and carbon black [3], and fcb2 from *Ogataea polymorpha* and gold nanoclusters [4] were at least one order more stable. The reason for the rapid inactivation may be the reactions of Cys200,216,233 with surface quinones and other electrophiles. These cysteines are at 7-10 Å distance from the FMN isoalloxazine ring [5] and are not conserved in fcb2 from *H. anomala*.

References

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