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Electrical contacting of glucose dehydrogenase by the reconstitution of a pyrroloquinoline quinone-functionalized polyaniline film associated with an Au-electrode: an *in situ* electrochemical SPR study

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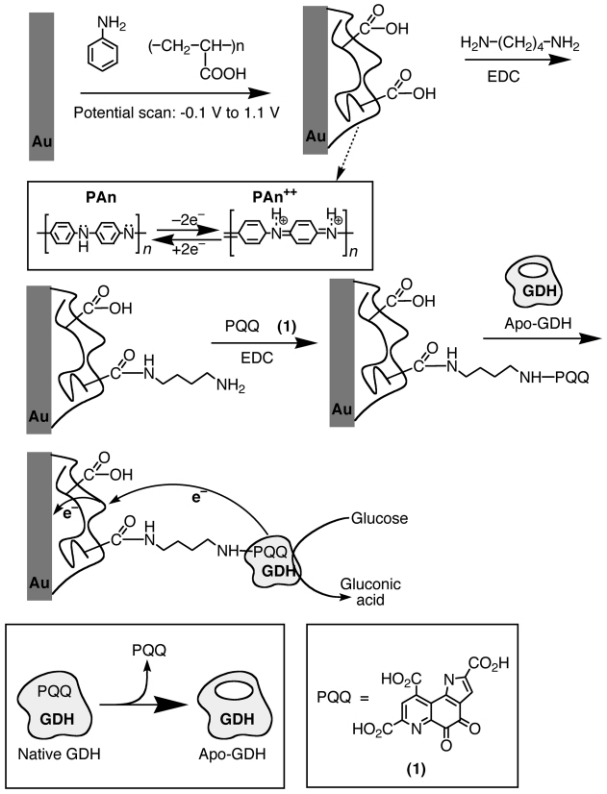
Received (in Cambridge, UK) 6th June 2002, Accepted 16th July 2002
First published as an Advance Article on the web 31st July 2002

A novel method to generate an integrated electrically contacted glucose dehydrogenase electrode by the surface reconstitution of the apo-enzyme on a pyrroloquinoline quinone (PQQ)-modified polyaniline is described. *In situ* electrochemical surface plasmon resonance (SPR) is used to characterize the bioelectrocatalytic functions of the system.

Electrical contacting of redox-proteins with electrodes is a fundamental topic in designing enzyme-electrodes for biosensor applications and biofuel cells design.[1,2] Different methods to electrically contact redox-proteins and electrodes have been reported, including the use of diffusional electron relays,[3] the tethering of redox-active groups to the enzyme,[4] and the immobilization of enzymes in redox polymers.[5] Effective electrical contacting of redox-proteins with electrode supports was accomplished by the alignment of the protein units on surfaces *via* the reconstitution of cofactor monolayer-functionalized electrodes with the respective apo-proteins.[6–8] The reconstitution of apo-glucose oxidase on a relay-FAD monolayer associated with an electrode led to an integrated enzyme electrode exhibiting unprecedented electron transfer turnover properties, and the electrode was used to develop biosensor systems,[6] and biofuel cells.[7] Similarly, horseradish peroxidase was reconstituted on a heme-modified electrode surface to yield a hemoprotein interface exhibiting direct electron contact with the electrode.[8] Pyrroloquinoline quinone (PQQ) cofactor-dependent enzymes represent a broad class of redox-enzymes,[9] however, they were not extensively used in biosensors.[10,11] Here we wish to report on the electrical contacting of the PQQ-dependent glucose dehydrogenase (GDH) by the reconstitution of apo-GDH on a PQQ-functionalized polyaniline (PAn) film associated with an Au-electrode. We characterize the system by *in situ* electrochemical SPR measurements. Previous efforts to generate an electrically-contacted enzyme-electrode by the surface reconstitution of GDH on a PQQ monolayer were unsuccessful, although the enzyme film existed in biologically active form.[12] Therefore, as far as we are aware, the present study represents the first example of the reconstituted GDH directly electrically contacted with the electrode.

The assembly of the integrated electrically contacted enzyme-electrode is depicted in Scheme 1. A polyaniline/polyacrylic acid composite film (*ca.* 90 nm thickness estimated by theoretical fit of the SPR spectrum), was electropolymerized[13] on an Au-electrode (glass support covered with Au-layer, 1.5 cm^2 active area) from solution composed of 0.2 M aniline, 1.5 mg mL^{-1} polyacrylic acid, PAA, (MW = 450,000), 0.5 M Na_2SO_4 and 0.1 H_2SO_4 (pH 1.2) upon application of one potential cycle from −0.1 to 1.1 V and back, scan rate = 100 mV s^{-1}. The polyacrylic acid component was entrapped into the electrochemicaly generated PAn layer, and it is essential in order to facilitate the redox properties of the polyaniline at neutral pH values,[13,14] as well as to enable the immobilization of PQQ and GDH. The polyaniline film reveals a reversible redox-wave at E^0 = 0.29 V (*vs.* SCE). By coulometric assay of the oxidation wave of PAn the surface coverage of the layer is estimated to be *ca.* 9×10^{-8} g cm^{-2}. 1,4-Diaminobutane, 5 mM, was reacted with the acrylic acid units in the presence of EDC,† 10 mM, as coupling reagent for 15 min in 0.1 M HEPES-buffer,† pH 7.2. It should be noted that the applied concentration of the diamine and the reaction time were optimized in order to provide tethering of the diamine *via* one amino group and to preserve partially non-functionalized acrylic acid residues that retain the PAn electrochemical activity at neutral pH. Then PQQ (**1**), 1 mM, was covalently linked (1 h) to the vacant amine-tethers linked to polyacrylic acid using EDC, 5 mM, in 0.1 M HEPES-buffer, pH 7.2. Apo-glucose dehydrogenase, apo-GDH, (from *Acinetobacter calcoaceticus*, E.C. 1.1.99.17) was prepared according to the published procedure[15] and then it was reconstituted on the PQQ units associated with the polymer film (4 h in solution composed of apo-GDH, 1 mg mL^{-1}, NaCl, 160 mM, $CaCl_2$, 20 mM, 0.1 M MOPS-buffer,† pH 7.3). Parallel microgravimetric quartz-crystal-microbalance (QCM) measurements revealed that *ca.* 1.3×10^{-7} g cm^{-2} of the composite polymer film PAn/PAA, *ca.* 1.5×10^{-11} mol cm^{-2} of 1,4-diaminebutane, *ca.* 1×10^{-11} mol cm^{-2} of PQQ, and *ca.* 2×10^{-12} mol cm^{-2} of GDH are associated with the quartz crystal.

Fig. 1 shows the cyclic voltammograms of the integrated GDH/polymer electrode in the absence, curve (a), and in the presence of variable concentrations, curves (b)–(e), of glucose. Clearly, an electrocatalytic anodic current is observed at the



Scheme 1 Reconstitution of glucose dehydrogenase (GDH) on the PQQ-functionalized polyaniline/polyacrylic acid film associated with an Au electrode.

DOI: 10.1039/b205393d

oxidation potential of PAn. Fig. 1, inset, shows the calibration plot that corresponds to the electrocatalytic currents at different concentrations of glucose. Control experiments reveal that GDH directly linked to the polyaniline/polyacrylic acid lacks bioelectrocatalytic functions, although it is bound to the polymer in a biocatalytically-active configuration. Also, the PAn/PAA/PQQ film is inactive towards the oxidation of glucose. Similarly, GDH that is reconstituted on polyacrylic acid assembled on an Au-electrode without PAn is inactive towards the electrocatalyzed oxidation of glucose. These results clearly indicate that the surface reconstituted GDH is structurally aligned in a configuration that allows the electrical contacting with the electrode by means of PAn that acts as an electron transporting matrix.

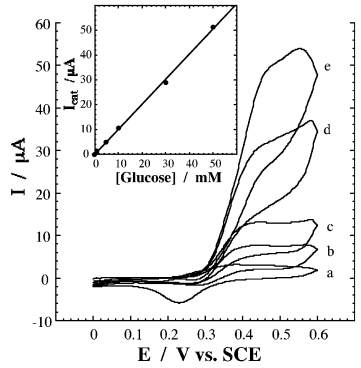


Fig. 1 Cyclic voltammograms of the Au electrode modified with the PQQ-functionalized polyaniline/polyacrylic acid film and the reconstituted GDH in the presence of various concentrations of glucose: (a) 0 mM, (b) 5 mM, (c) 10 mM, (d) 20 mM, (e) 50 mM. The data were recorded in 0.1 M phosphate buffer, pH 7.0 under air, potential scan rate 5 mV s^{-1}. Inset shows the calibration plot of the electrocatalytic current *vs.* glucose concentration.

The bioelectrocatalyzed oxidation of glucose by the PQQ-GDH reconstituted system associated with the PAn/PAA film described so far, was also recorded using an SPR instrument (Biosuplar-2, Analytical-μSystem, Germany), thus allowing us to follow the process by *in situ* electrochemical-SPR measurements. Fig. 2 shows the SPR spectra of the reduced PAn-GDH film maintained at the potential of $E = -0.3$ V before and after the addition of glucose, 2×10^{-2} M, curves (a) and (b), respectively. Addition of glucose results in a slight increase in the minimum reflectivity angle. Application of a potential corresponding to $E = 0.3$ V on the electrode in the absence of glucose results in the SPR spectrum shown in curve (c). The minimum reflectivity angle is shifted to a higher value. Since at the applied potential the polymer exists in its oxidized state, PAn^{2+}, the shift in the spectrum is attributed mainly to changes in the refractive index of the film. Addition of glucose to the system, 2×10^{-2} M, while a potential of $E = 0.3$ V is applied on the electrode, yields the spectrum shown in curve (d). The minimum reflectivity angle is significantly shifted to a lower value. This is consistent with the fact that in the presence of glucose the biocatalytic reaction provided by GDH results in partial reduction of the polyaniline film, thus producing steady-state populations of the reduced and oxidized polymer film, PAn/PAn^{2+}. Upon increasing the glucose concentration, the rate of the biocatalytic reaction is enhanced and the steady-state population of the reduced polyaniline, PAn, is increased. Thus, the difference between the minimum reflectivity angle, $\Delta\theta_{min}$, measured upon biasing the electrode at −0.3 and 0.3 V should decrease when the glucose concentration is increased. Fig. 2, inset, shows the differences in the minimum reflectivity angles,

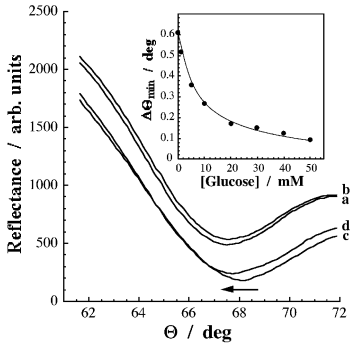


Fig. 2 SPR spectra of the Au electrode modified with the PQQ-functionalized polyaniline/polyacrylic acid film and the reconstituted GDH: (a) in the absence of glucose, $E = -0.3$ V, (b) in the presence of glucose, 20 mM, $E = -0.3$ V, (c) in the absence of glucose, $E = 0.3$ V, (d) in the presence of glucose, 20 mM, $E = 0.3$ V. The data were recorded in 0.1 M phosphate buffer, pH 7.0 under air. Arrow shows the direction of the SPR spectrum shift upon the addition of glucose, $E = 0.3$ V. Inset shows the difference, $\Delta\theta_{min}$, between the values of the minimum reflectivity angle measured at the applied potentials –0.3 and 0.3 V *vs.* glucose concentration.

$\Delta\theta_{min}$, of the integrated PAn-GDH electrode derived from the SPR spectra measured at −0.3 and 0.3 V when the polyaniline layer is electrochemically reduced and oxidized, respectively, at different concentrations of glucose. As expected, the $\Delta\theta_{min}$ value decreases as the concentrations of glucose increase.

In conclusion, the studied system based on the electrically contacted GDH allows simultaneous electrochemical and optical transduction of the biocatalytic oxidation of glucose.

This study is supported by the German–Israeli Program (DIP).

Notes and references

† EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HEPES = 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; MOPS = 4-morpholinepropanesulfonic acid.

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