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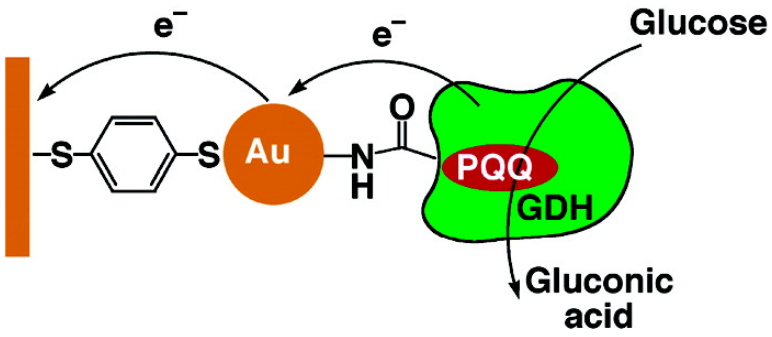
J. Am. Chem. Soc., **2005**, 127 (35), 12400-12406• DOI: 10.1021/ja052841h • Publication Date (Web): 13 August 2005



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Reconstitution of Apo-Glucose Dehydrogenase on Pyrroloquinoline Quinone-Functionalized Au Nanoparticles Yields an Electrically Contacted Biocatalyst

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Abstract: An electrically contacted glucose dehydrogenase (GDH) enzyme electrode is fabricated by the reconstitution of the apo-GDH on pyrroloquinoline quinone (PQQ)-functionalized Au nanoparticles (Au-NPs), 1.4 nm, associated with a Au electrode. The Au-NPs functionalized with a single amine group were attached to the Au surface by 1,4-benzenedithiol bridges, and PQQ was covalently linked to the Au-NPs. The apo-GDH was then reconstituted on the PQQ cofactor sites. The surface coverage of GDH corresponded to 1.4×10^{-12} mol cm^{-2}. The reconstituted enzyme revealed direct electrical contact with the electrode surface, and the bioelectrocatalytic oxidation of glucose occurred with a turnover number of 11 800 s^{-1}. In contrast, a system that included the covalent attachment of GDH to the PQQ-Au-NPs monolayer in a random, nonaligned, configuration revealed lack of electrical communication between the enzyme and the electrode, albeit the enzyme existed in a bioactive structure. The bioelectrocatalytic function of the later system was, however, activated by the diffusional electron mediator 2,6-dichlorophenol-indophenol. The results imply that the alignment of GDH on a Au-NP through the reconstitution process leads to an electrically contacted enzyme-electrode, where the Au-NP acts as a charge-transfer mediator.

Introduction

The electrical contacting of redox enzymes with electrode supports is a fundamental prerequisite to develop biosensors,[1] biofuel cells,[2] or bioelectronic devices.[3] The chemical modification of enzymes with redox relays[4] or the immobilization of the biocatalysts in redox polymers[5] or conductive polymers[6] was employed to electrically communicate the biocatalysts with the electrodes. In all of these systems the electrical contacting efficiency was hampered, however, by the fact that the biocatalysts are randomly modified with the redox relay units linked to nonoptimal positions of the protein and due to the fact that enzyme redox centers are randomly oriented with respect to the electrode. A different approach to electrically contact redox enzymes with conductive supports has involved the orientation of the enzymes on electrodes by a reconstitution process. The precise positioning of relay units between the protein redox center and the conductive support resulted in effective electron transfer between the biocatalytic redox center and the electrode.[7] Recently, nano-objects such as Au nanoparticles (Au-NPs)[8] or carbon nanotubes (CNTs)[9] functionalized with the flavin adenine dinucleotide (FAD) cofactor were employed for the reconstitution of apo-glucose oxidase on surfaces. Unprecedented effective electrical contacting of the enzyme with the electrode was achieved using these nano-objects as electrical connectors. In the present study we extend this concept by demonstrating the electrical contacting of pyrroloquinoline quinone (PQQ)-dependent enzymes by the reconstitution of apo-glucose dehy-

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drogenase (apo-GDH) on PQQ-functionalized Au-NPs assembled on a Au electrode surface. Previous studies have demonstrated the electrical contacting of glucose dehydrogenase (GDH) by the reconstitution of the apo-enzyme on the PQQ cofactor linked to the conductive polyaniline polymer.[7d]

Experimental Section

Chemicals. Apo-glucose dehydrogenase (PQQ-dependent, apo-GDH) from recombinant *Escherichia coli* (EC 1.1.99.17, molecular weight for one subunit ≈ 50 kDa) was purchased from Genzyme (Genzyme Diagnostics, Kent, U.K.). The bridging dithiol 1,4-benzenedithiol was purchased from TCI (Japan). Monoamino-functionalized Au-nanoparticles, containing a single triphenylphosphine ligand functionalized with 1,3-propane diamine,[10] were purchased from Nanoprobes (U.S.A.). Pyrroloquinoline quinone (PQQ), β-D-glucose, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES), 2,6-dichlorophenol-indophenol (DCPIP), and all other chemicals were purchased from Aldrich or Sigma and used as supplied. Ultrapure water from NANOpure Diamond (Barnstead) source was used throughout all the experiments.

Modification of Electrodes. Au electrodes (0.5 mm diameter Au wire, geometrical area ca. 0.13 cm^2, roughness factor ca. 1.4) were used for modifications. The Au electrodes were cleaned by boiling in 2 M KOH for 1 h followed by rinsing with water. The electrodes were stored in concentrated sulfuric acid. Prior to the modification, the electrodes were rinsed with water, soaked for 10 min in concentrated nitric acid, and rinsed again with water. A cyclic voltammogram recorded in 1 M H_2SO_4 was used to determine the purity and roughness of the electrode surface just before modification.[11] The Au electrodes were modified by 1,4-benzenedithiol by the interaction with ethanolic solution of 10 mM overnight, followed by rinsing of the electrodes with ethanol and water. The monoamino-functionalized Au-NPs, 1×10^{-6} M, were adsorbed onto the dithiol-modified Au electrodes in phosphate buffer 0.1 M, pH = 7.3, overnight. The Au-NPs-modified Au-electrodes were incubated for 2.5 h in a solution of PQQ, 3 mM, in 0.05 M HEPES-buffer, pH = 7.3, in the presence of 5 mM EDC to yield covalent coupling of PQQ to the amino groups associated with Au-NPs, and the resulting electrodes were washed with the buffer solution. The PQQ-Au-NPs-functionalized Au-electrodes were reacted with 1 mg mL^{-1} apo-GDH in 0.02 M HEPES-buffer, pH = 7.4, in the presence of 0.03 M $CaCl_2$ for 35 min at room temperature. The modified Au-electrodes were washed with 0.1 M phosphate buffer, pH = 7.4, to yield the GDH-reconstituted electrodes for biocatalytic oxidation of glucose.

A Au electrode with randomly covalently bound GDH was made for a control experiment. Apo-GDH reconstitution with PQQ in solution prior to its immobilization on the electrode was performed by the reported method:[12] Apo-GDH, 1 mg mL^{-1}, was incubated for 1 h at room temperature in a solution containing PQQ, 1 mg mL^{-1}, in 0.02 M HEPES-buffer, pH = 7.4, in the presence of $CaCl_2$, 0.03 M. After the enzyme reconstitution, the excess of PQQ was removed from the solution by gel filtration on a PD10 column (Pharmacia) using 0.05 M HEPES-buffer, pH = 7.4, for equilibration and elution. The biocatalytic activity of the reconstituted GDH was determinated spectrophotometrically by following the reduction of DCPIP at 600 nm, using phenazine methosulfate (PMS) as a primary electron donor.[12] The reconstituted GDH, 1 mg mL^{-1}, was covalently bound to the PQQ-functionalized Au-NPs-modified Au electrode in the presence of 5 mM EDC for 2.5 h, followed by the electrode rinsing with 0.1 M phosphate buffer, pH = 7.4.

The reconstitution of apo-GDH with the PQQ-functionalized Au-NPs was performed in a solution to yield the soluble Au-NP-enzyme hybrid system for the STEM imaging. Monoamino-functionalized Au-NPs, 1×10^{-5} M, were reacted with PQQ, 6×10^{-3} M, in 0.02 M HEPES-buffer, pH = 7.4, in the presence of EDC, 10 mM, for 2.5 h at room temperature. The PQQ-functionalized Au-NP-conjugate was purified from the excess of the nonreacted PQQ and EDC using a centrifugal filter device (Centricon YM-10, Millipore). Then the apo-GDH, 2 mg mL^{-1}, was reconstituted with the PQQ-functionalized Au-NP-conjugate in 0.02 M HEPES-buffer, pH = 7.4, in the presence of 0.03 M $CaCl_2$ for 1 h at room temperature. The GDH reconstituted with the PQQ-functionalized Au-NP-conjugate was purified using a centrifugal filter device (Centricon YM-50, Millipore).

Electrochemical, Microgravimetric, and Spectral Measurements. A conventional three-electrode cell, consisting of the enzyme-modified Au working electrode, a glassy carbon auxiliary electrode isolated by a glass frit, and a saturated calomel reference electrode (SCE) connected to the working volume with a Luggin capillary were used for the electrochemical measurements. All potentials are reported with respect to the SCE. Argon bubbling was used to remove oxygen from the solutions in the electrochemical cell, unless otherwise stated. The cell was placed in a grounded Faraday cage. Cyclic voltammetry, differential pulse voltammetry, and chronoamperometry were performed using an electrochemical analyzer composed of potentiostat/galvanostat (EG&G model 283).

A quartz crystal microbalance (QCM) analyzer (Fluke 164T multifunction counter, 1.3 GHz, TCXO) linked to a computer with a homemade software was used for microgravimetric measurements. Quartz crystals (AT-cut, ca. 9 MHz, EG&G) sandwiched between two Au electrodes (geometrical area 0.2 cm^2, roughness factor ca. 3.5) were used. The Au electrode surfaces were washed with ethanol and modified in the same way as it is described above for the Au wire electrodes. Frequency changes of the quartz crystals were measured in air after each modification step. All the measurements were carried out at ambient temperature (22 ± 2 °C). Absorbance measurements were performed using a spectrophotometer UV-2401PC (Shimadzu).

Results and Discussions

The Au-NPs electrically contacted PQQ-dependent glucose dehydrogenase (GDH) enzyme electrode was constructed as depicted in Scheme 1. A 1,4-benzenedithiol monolayer was assembled on a Au wire electrode, and Au-NPs (1.4 nm) functionalized with a single amine unit were linked to the thiolated interface. PQQ (a redox cofactor of GDH) was covalently bound to the amino groups associated with the Au-NPs, and subsequently apo-GDH was reconstituted on the PQQ sites. The surface coverages of the different components associated with the Au electrode were determined by analyzing the similar buildup of the system on a piezoelectric Au-quartz crystal. The surface coverage of the Au-NPs corresponded to 3×10^{-11} mol cm^{-2} (the same value was obtained for the surface coverage of PQQ by the electrochemical measurements, vide infra). The surface coverage of reconstituted enzyme was estimated by QCM measurements to be 1.4×10^{-12} mol cm^{-2}. Knowing the dimensions of GDH,[13] its footprint was calculated to be 1.2×10^{-12} cm^2. Thus, the observed surface coverage of GDH translates to ca. 60% coverage of a randomly densely

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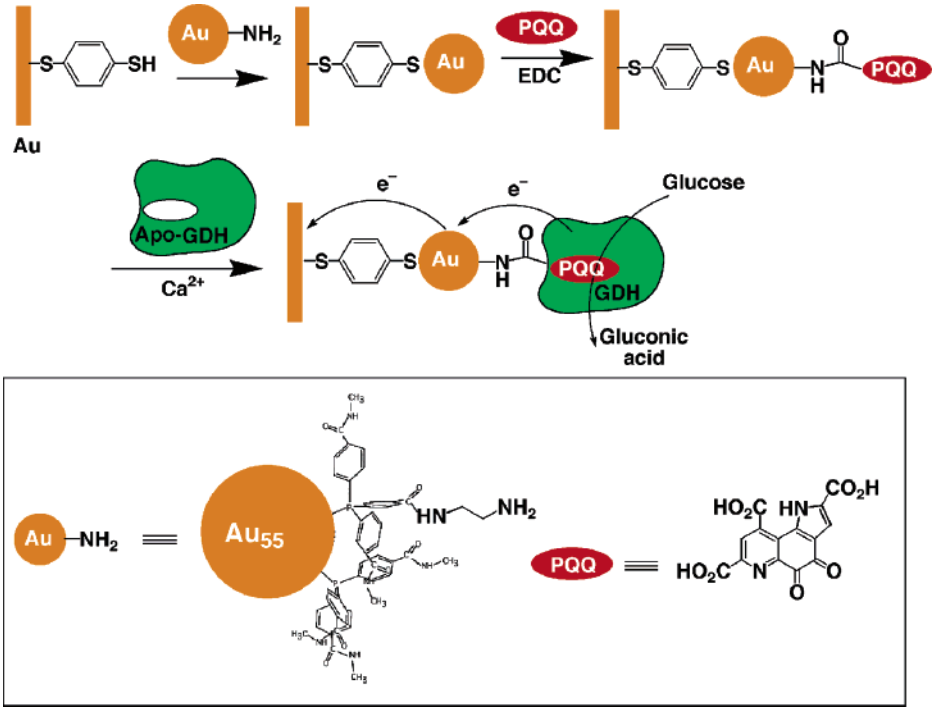
Scheme 1. Assembly of Glucose Dehydrogenase (GDH) Reconstituted on the PQQ-Functionalized Au Nanoparticles Associated with a Au Electrode and the Nonmediated Electrical Contacting of the Enzyme



packed GDH monolayer on the electrode surface (assuming ca. 70% of the ordered packing).[14]

Figure 1A, curve a, shows the cyclic voltammogram of the PQQ units linked to a Au-NP-functionalized electrode. A quasi-reversible redox-wave at $E° = -0.14$ V (pH = 7.3) is observed as it is expected for the immobilized PQQ.[15] Coulometric analysis of the reduction (or oxidation) wave of PQQ indicates a surface coverage that corresponds to 3×10^{-11} mol cm^{-2} (taking into account a two-electron redox process).[15] This value is identical to the surface coverage of the Au-NPs, derived from the QCM measurements, indicating that ca. 100% of the Au-NPs were functionalized with the PQQ cofactor units. To follow possible minor changes of the redox potential of the immobilized PQQ upon the reconstitution process, we recorded a differential pulse voltammogram of the PQQ-functionalized Au-NPs on the Au electrode surface that showed a single peak at $E° = -0.14$ V, pH = 7.3, Figure 1A, inset. Figure 1B shows the chrono-amperometric transient corresponding to the reduction of the PQQ units upon the application of a potential step on the electrode from 0.1 V to −0.3 V. The electron-transfer rate constant to the PQQ units is given by eq 1, where k_{et} is the interfacial electron-transfer rate constant and Q_{PQQ} is the charge associated with the reduction of the PQQ units.[16] Figure 1B, inset, shows the kinetic analysis of the transient according to eq 1. The transient current decay follows a single-exponential process, and the value $k_{et} = 3.3 \times 10^3$ s^{-1} is derived. From the pre-exponential value of the transient decay curve, using the derived electron-transfer rate constant and assuming that the reduction of PQQ involves two electrons, the surface coverage of PQQ is estimated to be 3×10^{-11} mol cm^{-2}. This value is in excellent agreement with the value derived from the analysis of the cyclic voltammogram.

$$I = k_{et} \cdot Q_{PQQ} \exp(-k_{et} \cdot t) \qquad (1)$$

The cyclic voltammogram, Figure 1A, curve b, and differential pulse voltammogram of the surface with the reconstituted GDH overlap with those of the PQQ-functionalized Au-NPs prior to the enzyme reconstitution. This suggests that there is no observable redox potential change for the reconstituted PQQ and that the redox process of the PQQ cofactor is not screened (or blocked) upon its interaction with the apo-GDH. This result is surprising compared to previous studies, where apo-flavo-enzymes were reconstituted on the FAD cofactor resulting in the blocking of the redox wave of the FAD cofactor due to its steric insulation by the protein shell.[7] Inspection of the 3D structure of GDH,[17] Figure 2A, indicates, however, that the PQQ cofactor units are located close to the protein surface. Thus, the alignment of the protein on the PQQ-functionalized Au-NPs results in an intimate contact between the reconstituted PQQ units/Au-NPs/bulk Au electrode giving rise to the visible cyclic voltammogram of the reconstituted PQQ units.

Further support to the reconstitution of apo-GDH on the PQQ-functionalized Au-NPs is obtained from scanning transmission electron microscopy (STEM) imaging of the reconstituted enzyme[10] and complementary absorbance spectroscopy. The STEM images reveal that ca. 80−85% of the protein units include reconstituted Au-NPs, and most of them include two implanted Au-NPs. Figure 2B shows the STEM image of the reconstituted GDH on the PQQ-modified Au-NPs in an aqueous solution. Two Au-NPs are associated with each protein structure.

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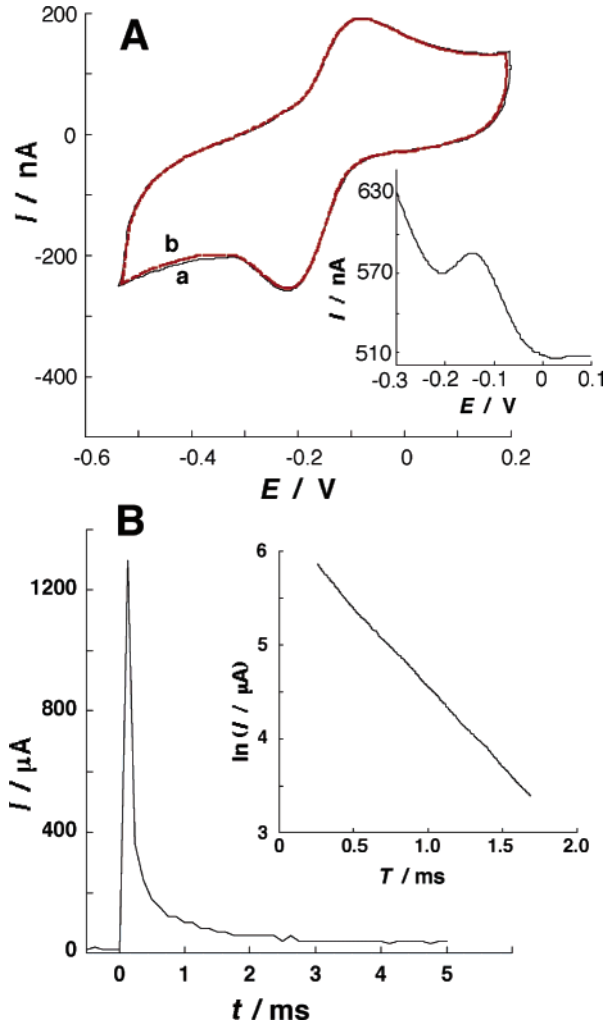

Figure 1. (A) Cyclic voltammogram of the following: (a) the Au electrode modified with the PQQ-functionalized Au nanoparticles (black curve); (b) the Au-NPs/PQQ-functionalized electrode after the reconstitution with apo-GDH (red curve). Potential scan rate, 20 mV s^{-1}. Inset: differential pulse voltammogram of the PQQ-functionalized Au nanoparticles. Potential scan rate, 20 mV s^{-1}. (B) Chronoamperometric transient measured upon the application of a potential step from 0.1 V to −0.3 V. Inset: Kinetic analysis of the current transient. The data were recorded in 0.1 M phosphate buffer, pH = 7.3, under Ar.

This is consistent with the fact that the GDH consists of two subunits that each include the PQQ cofactor.[18] The dimensions of Au-NPs, which look as bright spots, and the dimensions of the protein matrixes, appearing as shadows around Au-NPs, nicely correspond to their real sizes. Thus, two PQQ-functionalized Au-NPs may be incorporated into the apo-enzyme, Figure 2A. The absorbance spectra of the apo-GDH prior to the reconstitution of the PQQ-functionalized Au-NPs and after the reconstitution with the PQQ-functionalized Au-NPs are depicted in Figure 2C, curves a and b, respectively. The absorption spectrum of the apo-GDH, Figure 2C, curve a, having a maximum at 278 nm, exhibits the characteristics expected for a cofactorless enzyme. The characteristic absorbance of the PQQ cofactor units at $\lambda = 338$ nm is observed,[12] indicating that the cofactor is indeed integrated into the protein. Indeed the PQQ-reconstituted GDH reveals biocatalytic functions confirmed by the standard colorimetric method (see Experimental Section).

Figure 3 depicts the cyclic voltammograms of the GDH-reconstituted electrode in the presence of different concentrations of glucose. A bioelectrocatalytic anodic current is observed, and it increases as the concentration of glucose is elevated. We observe a catalytic anodic current already at the thermodynamic redox potential of the PQQ units ($E° = -0.14$ V, pH = 7.3), Figure 3A, inset. A sharp increase in the electrocatalytic anodic current is, however, observed at $E > 0.2$ V. Figure 3B shows the derived calibration curve. The bioelectrocatalytic current levels off at a concentration of ca. 100 mM to a maximum value of $I_{cat}^{sat} = 270$ μA. Knowing the surface coverage of the reconstituted GDH, $\Gamma_{GDH} = 1.4 \times 10^{-12}$ mol cm^{-2} and the maximum current density extracted from the system, i_{cat}^{sat}, at the potential of 0.7 V, the number of electrons obtained upon oxidation of one glucose molecule, $n = 2$ (F is the Faraday number), and realizing that two subunits are active in the bioelectrocatalyzed oxidation of glucose, we estimate, using eq 2, the maximum turnover rate of the reconstituted enzyme to be $TR_{max} = 11\,800$ s^{-1} (electrons generated by one GDH per second). This value is similar to the turnover rate of GDH in the presence of its native PQQ cofactor.[19] The high turnover rate of the reconstituted enzyme may originate from the relatively high overpotential used to oxidize glucose and from the possible distortion of the enzyme structure upon the incorporation of PQQ cofactor linked to the Au-NP.

$$TR_{max} = i_{cat}^{sat}/(F \cdot n \cdot G_{GDH}) \qquad (2)$$

The rate constant of the bioelectrocatalytic process was calculated to be ca. 6.7×10^4 M^{-1} s^{-1}.[20] This rate constant reflects the bimolecular interaction between the surface-immobilized enzyme and the soluble substrate. The electrochemical rate constant, which reflects the rate of the reaction between the soluble substrate and the catalytic interface, was calculated to be 9×10^{-5} cm s^{-1}, taking into account the coverage of the enzyme on the electrode surface. The effective electrical contacting of the GDH reconstituted on the PQQ-functionalized Au-NPs toward the oxidation of glucose is attributed to the structural alignment of the biocatalyst on the Au-NP that acts as a nanoelectrode for mediating electron transfer between the PQQ unit and the electrode. It should be noted that the cyclic voltammograms depicted in Figure 3A show a sharp increase of the bioelectrocatalytic currents at ca. 0.2 V that corresponds to an overpotential of ca. 350 mV as compared to the quasi-reversible redox wave of the PQQ cofactor. One should note, however, that the current scales are different for the bioelectrocatalytic currents and the amperometric response of the PQQ cofactor (by a factor of ca. 4×10^3-fold, taking into account the different scan rates).

Furthermore, it should be noted that, in control experiments where the Au-NPs-functionalized electrode or the Au-NPs/PQQ-modified electrode were treated with glucose, no electrocatalytic current was detected. Also, the reconstitution of apo-GDH on a PQQ monolayer directly linked to the Au support, in the

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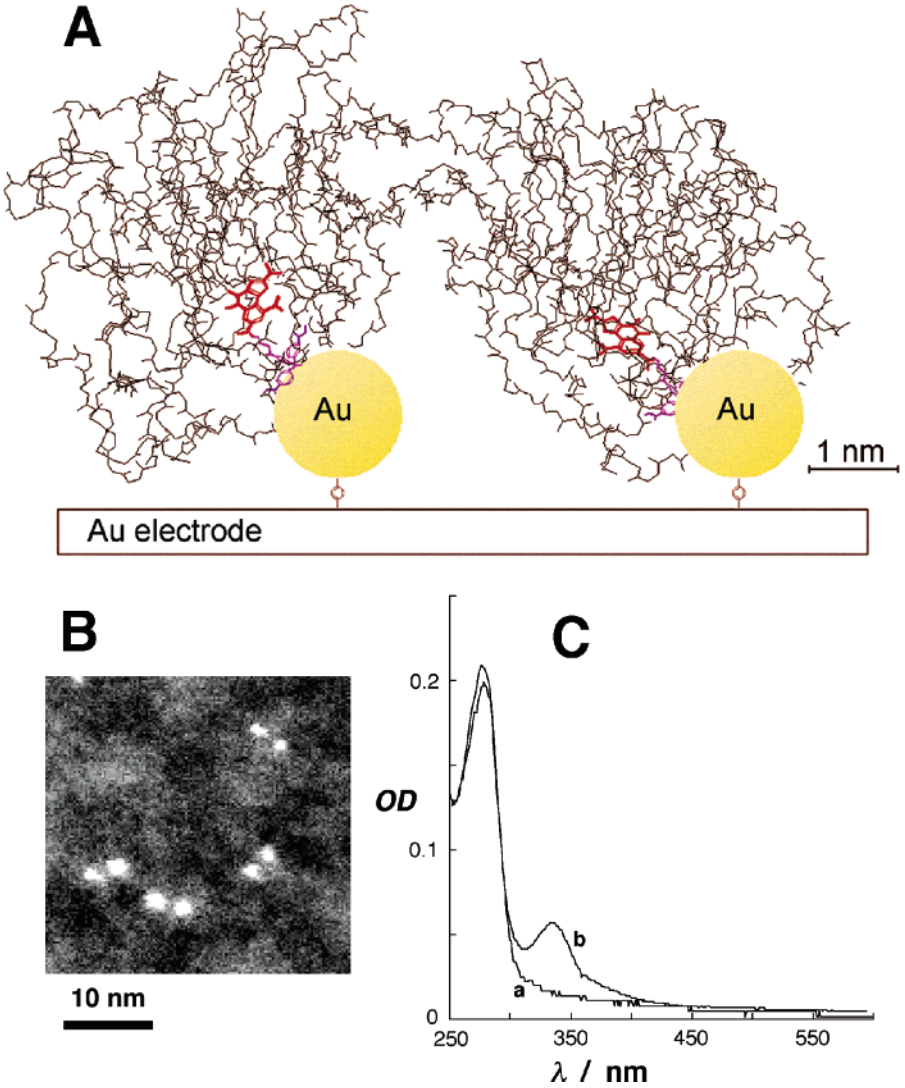

Figure 2. (A) Scheme of the GDH reconstituted by the PQQ-functionalized Au-NPs on the Au electrode surface. The red contours correspond to the PQQ cofactors associated with the enzyme subunits. The enzyme subunits, PQQ-cofactors, and Au-NPs are drawn in the real scale. (B) STEM image of GDH reconstituted with the PQQ-functionalized Au-NPs in solution. The gray "clouds" represent the enzyme molecules, and the bright spots are Au-NPs. (C) Absorption spectra of the apo-GDH: (a) prior to the reconstitution, (b) after the reconstitution with the PQQ-functionalized Au-NPs. The spectra were recorded in 0.05 M HEPES-buffer, pH = 7.4, containing 0.03 M $CaCl_2$.

absence of the Au-NPs, did not lead to the bioelectrocatalyzed oxidation of glucose. These results clearly indicate that the electrocatalytic oxidation of glucose originates from the biocatalyst reconstituted on the Au-NPs/PQQ assembly. It should be noted that the sharp increase in the electrocatalytic anodic current as a result of glucose oxidation in the present system occurs at an overpotential that is similar to that observed for the bioelectrocatalyzed oxidation of glucose by the analogous system where apo-glucose oxidase was reconstituted on the Au-NPs/FAD units. This is consistent with the fact that the rate-limiting step in both systems is identical, namely, the transfer of electrons from the Au-NPs through the dithiol bridge to the bulk Au electrode.

The oxidation of glucose by the PQQ-dependent GDH is known not to be affected by oxygen,[21] thus providing an advantage for the glucose biosensing as compared to the FAD-dependent GOx. In addition, the efficient electrical wiring of GDH with the high turnover rate between the enzyme and the electrode suggests that nonspecific oxidizable interfering compounds for biosensing of glucose will not significantly contribute to the resulting anodic current. Indeed, this kinetically stimulated specificity for detecting glucose is observed, Figure 4. We find that the cyclic voltammograms of the GDH-reconstituted electrode are similar in the absence or presence of O_2, Figure 4, curve a and b, respectively, or when ascorbic acid, 1 mM, is added to the system, Figure 4, curve c, in the presence of glucose, 10 mM. The lack of oxidation of the ascorbate interfering substrate is attributed to the high turnover rate of the biocatalyst and to the possible contribution of blocking the interfacial electron transfer at the electrode by the monolayer structure.

One might argue, however, that GDH associated with the electrode is electrically contacted by PQQ units associated with the monolayer that were not reconstituted with apo-GDH. These PQQ units could then mediate the bioelectrocatalytic activation of the enzyme. To exclude this possibility, the enzyme electrode depicted in Scheme 2 was assembled. In this configuration, the PQQ-functionalized Au-NPs were covalently linked in a random orientation to lysine residues of GDH reconstituted by PQQ

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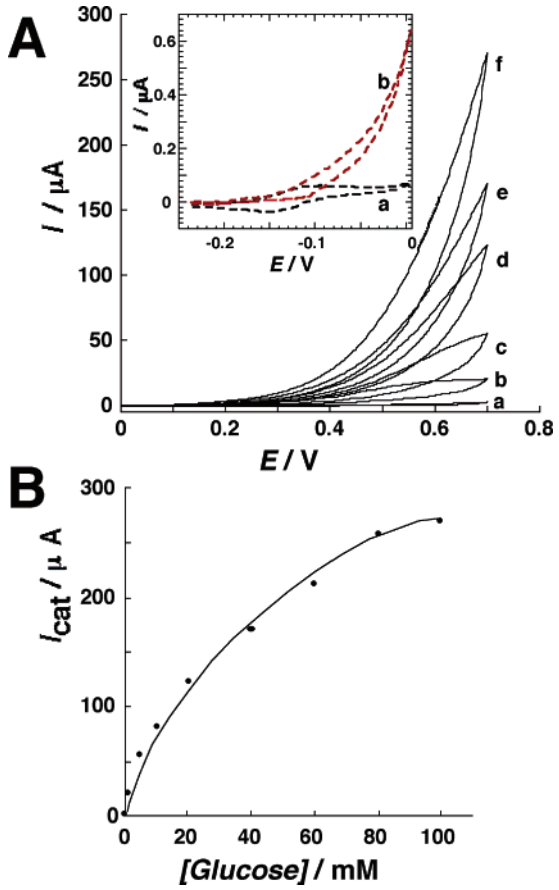

Figure 3. (A) Cyclic voltammograms corresponding to the bioelectrocatalyzed oxidation of glucose by the GDH reconstituted on the PQQ-functionalized Au-NPs associated with a Au electrode in the presence of different glucose concentrations: (a) 0 mM, (b) 1 mM, (c) 5 mM, (d) 20 mM, (e) 40 mM, and (f) 100 mM. Inset: Cyclic voltammograms of the GDH-reconstituted Au-NPs/PQQ-functionalized electrode in the absence of glucose (a) and in the presence of glucose, 100 mM (b), recorded in a selected potential region. The data were recorded in 0.1 M phosphate buffer, pH = 7.3, under Ar, potential scan rate 5 mV s^{-1}. (B) Calibration plot derived from the cyclic voltammograms at $E = 0.7$ V.

cofactor in solution prior to the covalent binding to the electrode surface. One would expect that the bioelectrocatalyst would be activated toward the bioelectrocatalytic oxidation of glucose, provided that the protein-adjacent PQQ units mediate the electrical contacting of the protein. Figure 5, curve a, shows the cyclic voltammogram of the electrode modified with the randomly oriented PQQ-bridged GDH in the presence of glucose. Only the reversible redox process of PQQ is observed at $E^\circ = -0.14$ V. Evidently, no electrocatalytic current is observed up to $E = 0.7$ V, implying that the protein is not electrically contacted with the electrode support. The linked GDH units exist, however, in a bioactive structure, and addition of the diffusional electron mediator 2,6-dichlorophenol-indophenol (DCPIP) activates the enzyme toward the bioelectrocatalytic oxidation of glucose.[22] A cyclic voltammogram recorded on the enzyme-modified electrode in the presence of the diffusional DCPIP, 1×10^{-4} M, and in the absence of glucose shows the reversible redox process of the mediator at $E^\circ = 0.05$ V, Figure 5, curve b. Upon addition of glucose, 10 mM, the bioelectrocatalytic oxidation of glucose mediated by the diffusional

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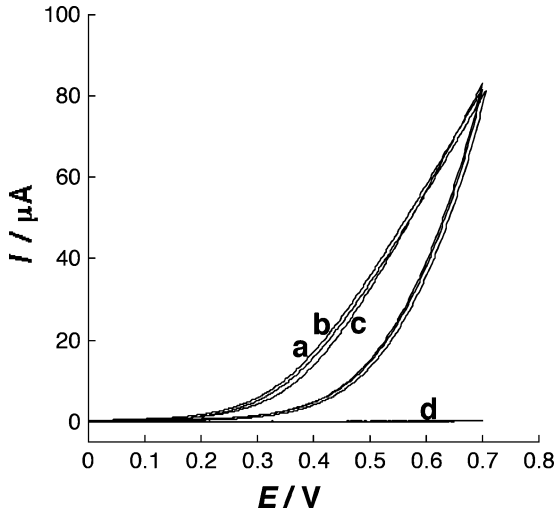


Figure 4. Cyclic voltammograms corresponding to the bioelectrocatalyzed oxidation of glucose by the GDH reconstituted on the PQQ-functionalized Au-NPs associated with a Au electrode: (a) 10 mM glucose under Ar, (b) 10 mM glucose under air, (c) 10 mM glucose and 1 mM ascorbic acid, (d) the background current in the absence of glucose. The data were recorded in 0.1 M phosphate buffer, pH = 7.3, potential scan rate 5 mV s^{-1}.

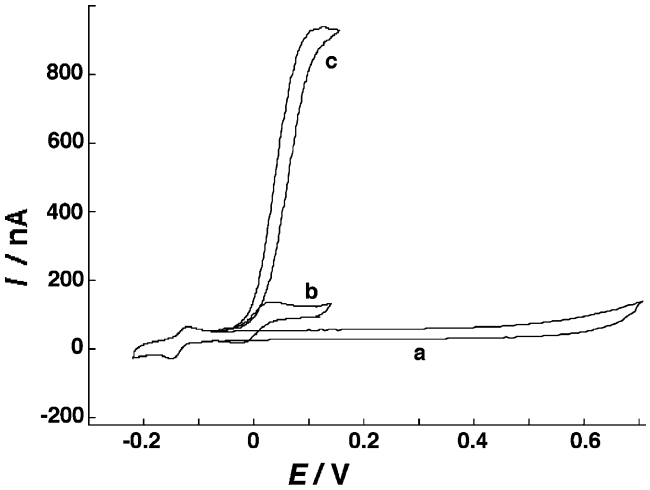
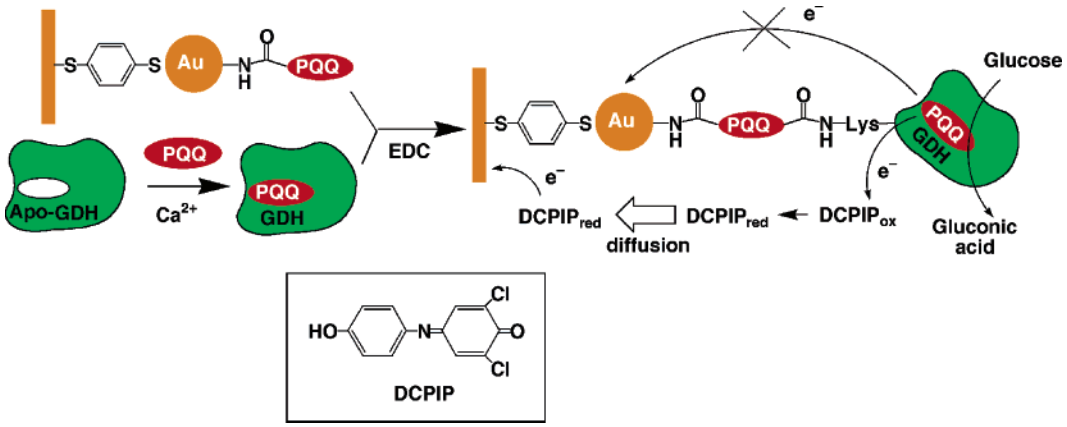


Figure 5. Cyclic voltammograms recorded on a Au electrode modified by GDH randomly covalently bound to the PQQ-functionalized Au nanoparticles in the presence of the following: (a) 10 mM glucose, (b) 1×10^{-4} M DCPIP without glucose, (c) 1×10^{-4} M DCPIP and 10 mM glucose. The data were recorded in 0.1 M phosphate buffer, pH = 7.3, under Ar, potential scan rate 5 mV s^{-1}.

mediator is observed at $E > 0$ V, Figure 5, curve c. It should be noted, however, that the bioelectrocatalytic current generated by the nonaligned enzyme in the presence of the diffusional mediator is ca. 50-fold smaller compared to the current generated by the aligned GDH electrically contacted by the Au-NPs. These results reveal that the free surface-confined PQQ units cannot electrically contact the biocatalyst and that the effective electrical contacting of the GDH reconstituted on the PQQ-Au-NPs originates from the Au-NPs-mediated transport of electrons from the aligned enzyme redox center to the conductive support.

In conclusion, the present study has demonstrated the electrical contacting of the PQQ-dependent GDH by the reconstitution on PQQ-functionalized Au-NPs. It should be noted that the resulting electrode revealed impressive stability and did not show any degradation in the activity upon continuous operation for 8 h, after storage (4 °C) for two weeks, and for an additional operation time interval of 8 h. The

Scheme 2. Assembly of Glucose Dehydrogenase (GDH) Randomly Covalently Bound to the PQQ-Functionalized Au Nanoparticles Associated with a Au Electrode and the Electrical Contacting of the Enzyme by a Diffusional Electron Transfer Mediator.



importance of the structural alignment of the redox enzyme on the electrode surface was demonstrated. Highly efficient electrical contacting of the enzyme by means of the reconstitution of the apo-enzyme on Au-NPs was achieved, and this led to the specific glucose detection. The electrical contacting of GDH in the present system is ca. 25-folds improved as compared to a system where polyaniline (instead of the Au-NPs) acts as the matrix for the electrical contacting of the reconstituted enzyme and the electrode support.[23] The effective electrical contacting of the enzyme could find potential applications in different biosensor and bioelectronic systems. At this point, the system suffers from the limitation that the bioelectrocatalyzed oxidation of glucose proceeds with a high overpotential and, thus, limits the applicability of the electrode for certain bioelectronic systems, e.g., biofuel cells. The understanding of the fundamental barriers of this bioelectrocatalytic process suggests, however, that by appropriate tailoring of the molecular wires linking the Au-NPs to the electrode, the overpotential for the oxidation of glucose could be reduced.

Acknowledgment. This research was supported by the German-Israeli Program (DIP). M.Z. acknowledges the Levi Eshkol fellowship, the Israeli Ministry of Science. We thank Dr. James F. Hainfeld, Brookhaven National Laboratory, Biology Department, Bldg. 463, Upton, NY 11873, U.S.A., for providing us with the STEM images of the Au-NPs-reconstituted enzyme.

JA052841H

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