Electrically Contacted Enzyme-Electrodes for Biosensors and Biofuel Cells

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The active sites in redox-proteins usually lack direct electrical communication with electrodes due to the spatial separation of the redox centers from the conductive surface by means of the protein shells. The development of artificial means for the electrical contacting of redox-proteins and electrodes has been a subject of extensive research efforts.[1,2] The tethering of electroactive redox groups to the proteins[3] or the immobilization of the redox-proteins in conductive polymers[4] or electroactive polymers were reported as general means to establish electrical communication between the redox-sites in proteins and electrode supports. Nonetheless, in all of these electrically contacted enzyme-electrodes the exchange rates of electrons between the redox-site and the electrode is relatively inefficient, due to the lack of appropriate structural alignment of the redox proteins relative to the electrode.

We have developed a general strategy for the electrical contacting of redox-proteins and electrodes by the reconstitution of the apo-enzymes (e.g., apo-glucose oxidase) on relay units associated with electrodes functionalized with a semi-synthetic flavin adenine dinucleotide (FAD) cofactor, Fig. 1. This is exemplified with the preparation of electrically contacted glucose oxidase (GOx)-functionalized electrodes where the electrically contacting relay units consist of the molecular electron transfer mediator, pyrroloquinoline quinone (PQQ) (**1**),[5] the Au_{55}-nanoparticle, (**2**),[6] or the carbon tubes of variable lengths, (**3**).[7] The resulting glucose-oxidase-functionalized electrodes reveal unprecedented electrical communication, and electron-transfer exchange rates that correspond to 700 s^{-1} to 5000 s^{-1} were detected in the different systems, far above the electron transfer exchange rates of the enzyme with its native electron acceptor (O_2). The high turnover rates of the electrically contacted enzyme-electrodes have important consequences on the biocatalytic functions of the electrodes, and besides high sensitivities, the enzyme-electrodes reveal high specificities and are not interfered by oxidizable substrates or molecular oxygen.

A different approach to electrically contact redox-proteins and electrodes involves the generation of affinity complexes between the redox-protein and its cofactor. This is exemplified in Fig. 2 with the assembly of a cytochrome oxidase (COx)-Cytochrome c electrode for the biocatalytic reduction of O_2 to water. The assembly of the O_2-insensitivie glucose oxidizing electrode and of the biocatalytic O_2-reducing electrode has enabled the construction of an integrated biofuel cell[8] that uses the two electrodes as anode and cathode and glucose and oxygen as the fuel and oxidizer, respectively, Fig. 3. This biofuel cell does not include a separating membrane between the anolyte and catholyte solutions. The performance of the system as a biofuel cell[8] and as a biofuel cell-based glucose sensor[9] was investigated

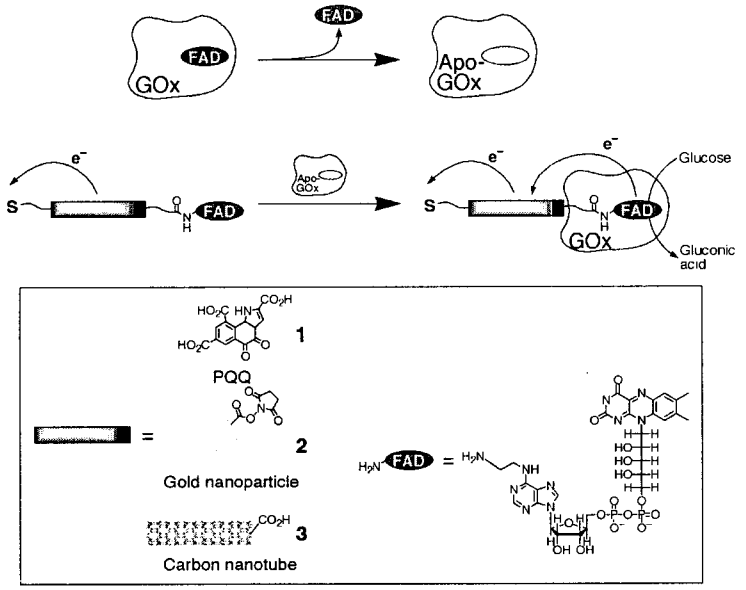
FAD
GOx
Apo-GOx
e⁻
S
FAD
Glucose
Gluconic acid
1
PQQ
2
Gold nanoparticle
3
Carbon nanotube
FAD

Fig. 1.

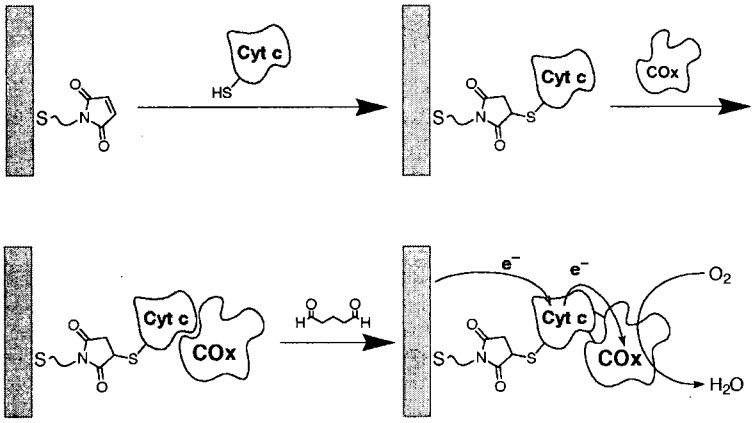
Cyt c
HS
COx
S
e⁻
O₂
H₂O

Fig. 2.

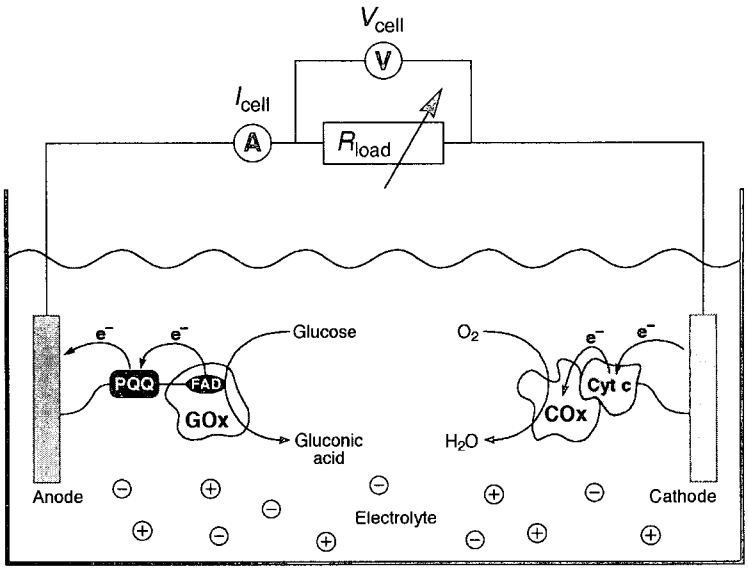

Fig. 3.

An important advance in the development of biofuel cells has involved the organization of electroswitchable and electrotunable biofuel-cells,[10] Fig. 4. This is exemplified with the organization of an electroswitchable and tunable biocatalytic glucose oxidizing electrode. A polyacrylic acid film associated with an electrode acts as an active interface for the complexation of Cu^{2+}-ions, and for the secondary deposition of a polyethyleneimine (PEI) layer. The glucose oxidase (GOx) electrode is generated by the reconstitution of apo-GOx onto pyrroloquinoline quinone (PQQ)-FAD units covalently-linked to the PEI interface. In the Cu^{2+}-carboxylate state of the interface the GOx-biocatalyst lacks electrical contact with the electrode and its bioelectrocatalytic functions are switched-off. Electroreduction of the Cu^{2+}-ions to Cu -nano-clusters, E = -0.5 volt, transforms the polymer interface into a conductive film and this activates the biocatalyst towards the electrocatalyzed oxidation of glucose. The re-oxidation of the Cu -clusters to Cu^{2+}-ions, E = +0.5 volt, switches-off the bioelectrocatalytic function. By the reversible electrochemical reduction and oxidation of the film to the Cu and Cu^{2+}-states the bioelectrocatalyzed oxidation of glucose is switched between "ON" and "OFF" states, respectively. An identical Cu^{2+}/Cu -electroswitchable COx/ Cytochromec O_2-reduction bioelectrocatalytic electrode was assembled. The respective Cu^{2+}/Cu -electroswitchable glucose-oxidizing and O_2-reducing electrodes were integrated into an electroswitchable biofuel cell. The biofuel cell operation can be electroswitched between "ON" and "OFF" states, and its power output is tuned by the degree of transforming of the Cu^{2+}-ions into Cu -nanocluster.[10]

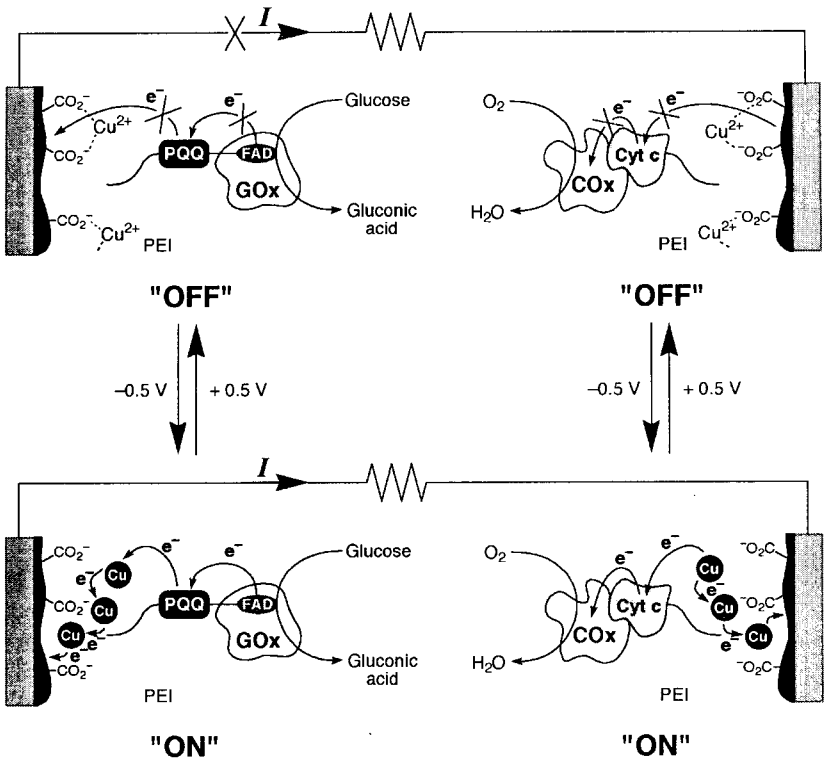

Fig. 4.

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