Assembly of Microperoxidase-11 and Co(II)-Protoporphyrin IX Reconstituted Myoglobin Monolayers on Au-Electrodes: Integrated Bioelectrocatalytic Interfaces

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Cytochrome *c*, cyt *c*, acts as an electron transfer mediator for many redox proteins (or enzymes). The formation of organized interprotein complexes is the key process for the cytochrome *c* mediated reactions.[1] Microperoxidase-11 (MP-11) is a heme containing 11 amino acid polypeptide[2] that consists of the active site microenvironment of cyt *c*. We have shown that MP-11 assembled as a monolayer on a Au-electrode[3] mediates the electrocatalyzed reduction of hemoproteins such as hemoglobin or myoglobin.[4] These electrocatalytic reactions were attributed to the formation of MP-11/hemoprotein complexes at the electrode surface. Recently, we developed the concept of generating integrated electrically-contacted enzyme monolayer electrodes via the reconstitution principle. Apoglucose oxidase was reconstituted on a pyrroloquinoline quinone-FAD (PQQ-FAD) monolayer and the resulting enzyme monolayer revealed bioelectrocatalytic activities for the oxidation of glucose.[5] Lactate dehydrogenase generated a sufficiently stable complex with a PQQ-NAD^+ monolayer assembled on a Au-electrode that enabled the cross-linking of the protein monolayer with glutaric dialdehyde. The resulting integrated NAD^+-enzyme monolayer electrode revealed electrical contact without any diffusional cofactor.[6] Here we demonstrate that a Co(II)-protoporphyrin IX reconstituted myoglobin,[7] Co(II)-Mb, is electrically contacted in the presence of a MP-11 monolayer modified electrode. The electrical contact originates from a supramolecular complex between MP-11 and Co(II)-Mb. Cross-linking of the complex monolayer yields an integrated, stable, protein monolayer electrode exhibiting bioelectrocatalytic properties for the hydrogenation of acetylene dicarboxylic acid to maleic acid.

Microperoxidase-11, MP-11, was assembled[8] onto a Au wire electrode as shown in Scheme 1. Figure 1 (curve a) shows the cyclic voltammogram of the resulting MP-11 monolayer electrode. Coulometric analysis of the redox wave indicates a surface coverage for MP-11 corresponding to 1×10^{-10} mole·cm^{-2}. Figure 1 (curve b) shows the cyclic voltammogram of the MP-11 monolayer electrode in the presence of Co(II)-protoporphyrin IX-reconstituted myoglobin, Co(II)-Mb. An electrocatalytic cathodic current at the MP-11 redox-potential is observed implying the electrocatalytic reduction of Co(II)-Mb. Control experiments indeed reveal that no direct electrochemical reduction of Co(II)-Mb occurs. Rotating disc electrode, RDE, experiments, where the MP-11 monolayer was assembled on a Au-disc electrode, revealed that the electrocatalytic one-electron reduction of Co(II)-Mb proceeds in a complex between the MP-11 monolayer and Co(II)-Mb. In the resulting complex $K_M = (k_{-1} + k_{+2})/k_1 = 7.8 \times 10^{-6}$ M, and

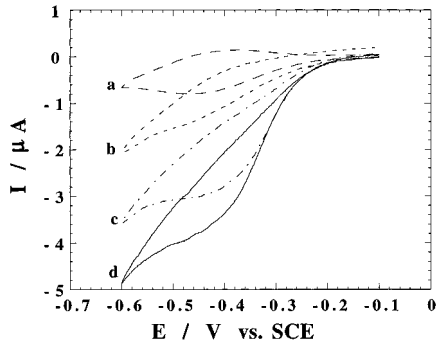
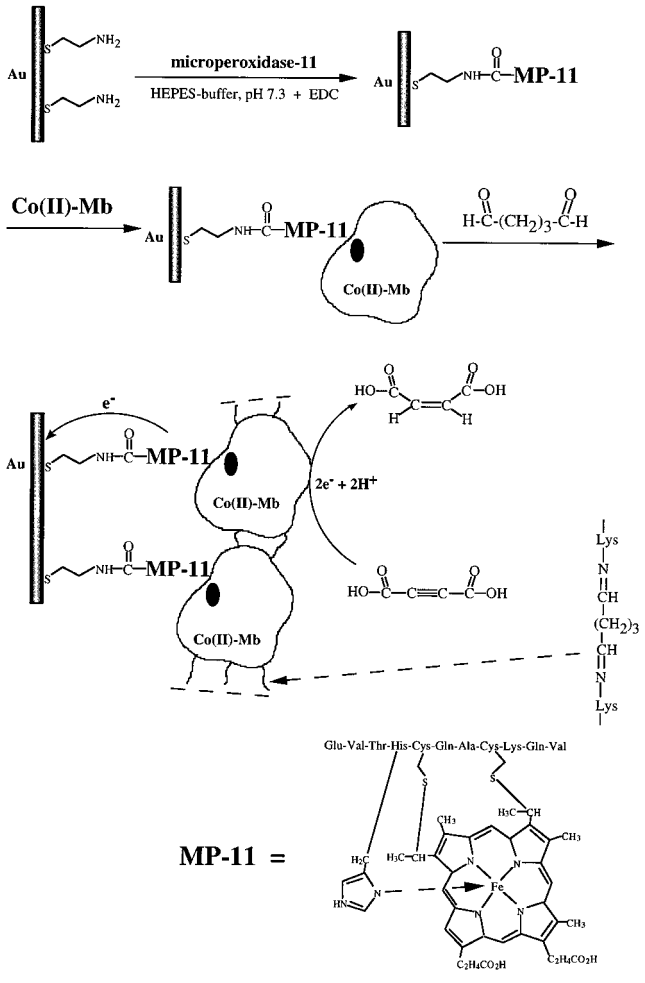


Figure 1. Cyclic voltammograms of the MP-11 monolayer-modified Au-electrode (geometrical area 0.2 cm^2, roughness factor ca. 1.2): (a) vs background solution 0.1 M, phosphate buffer, pH = 7.1, (b) in the presence of 5×10^{-5} M Co(II)-Mb, (c) in the presence of 5×10^{-5} M Co(II)-Mb and 50 mM acetylene dicarboxylic acid, and (d) in the presence of 5×10^{-5} M Co(II)-Mb and 100 mM acetylene dicarboxylic acid. Potential scan rate, 5 mV·s^{-1}.

Scheme 1. Stepwise Organization of the Integrated MP-11/Co(II)-Mb Monolayer-Modified Au-Electrode and Bioelectrocatalyzed Reduction of Acetylene Dicarboxylic Acid



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the electron transfer rate constant,[9] $k_{+2} = 0.13\ s^{-1}$, eqs 1 and 2.

$$|-\text{Fe(II)}-\text{MP}-11 + \text{Co(II)}-\text{Mb} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} |-\text{Fe(II)}-\text{MP}-11\cdots\text{Co(II)}-\text{Mb} \quad (1)$$

$$|-\text{Fe(II)}-\text{MP}-11\cdots\text{Co(II)}-\text{Mb} \xrightarrow{k_{+2}} |-\text{Fe(III)}-\text{MP}-11 + \text{Co(I)}-\text{Mb} \quad (2)$$

Figure 1 (curves c and d) shows the cyclic voltammograms of the MP-11 monolayer electrode in the presence of Co(II)-Mb and added acetylene dicarboxylic acid. A substantial enhancement of the electrocatalytic cathodic current is observed. Control experiments reveal that no electrocatalytic cathodic current is observed upon addition of acetylene dicarboxylic acid to the MP-11 monolayer electrode without Co(II)-Mb and in the absence of MP-11. Previous studies indicated that the reduced Co(I)-Mb is capable of stimulating the hydrogenation of acetylene dicarboxylic acid.[7b] Thus, the enhanced cathodic currents in the presence of the added substrate are consistent with the Co(I)-Mb-mediated hydrogenation of acetylene dicarboxylic acid (*vide infra*).

The kinetic analysis of the electron transfer from MP-11 to Co(II)-Mb, eqs 1 and 2, indicates the formation of a peptide−protein complex. This affinity complex between Co(II)-Mb and the MP-11 monolayer functionalized electrode allowed us to construct an integrated MP-11/Co(II)-Mb electrode (using a rough Au-electrode,[10] roughness factor ca. 15, to increase the amount of the immobilized biocatalyst) for the electrocatalyzed hydrogenation of acetylene dicarboxylic acid. The MP-11 monolayer electrode was treated with Co(II)-Mb, rinsed with water (1 s) and treated with glutaric dialdehyde (15 min, 10% v/v), Scheme 1. Figure 2 shows the cyclic voltammograms of the resulting electrode in the absence and in the presence of added acetylene dicarboxylic acid. The resulting electrocatalytic cathodic current indicates the electrocatalyzed reduction of the substrate. A control electrode was prepared, where the MP-11 monolayer electrode was similarly treated with Co(II)-Mb but incubated in water (15 min) but not with the cross-linking agent, glutaric dialdehyde. The resulting electrode was found to be catalytically inactive. Thus, the associative affinity interactions between MP-11 and Co(II)-Mb resulted in a complex of temporary stability that enables its latheral cross-linking on the electrode surface. The cross-linked Co(II)-Mb layer exhibits high stability and does not dissociate from the electrode surface. The MP-11/Co(II)-Mb cross-linked electrode was employed for steady-state electrolysis of acetylene dicarboxylic acid at constant potential (−0.5 V vs SCE) in order to analyze the electrochemical reaction product(s). Electrolysis was conducted for 2 h under an Ar atmosphere, and electrolyte samples were analyzed at time intervals of electrolysis.[11] Maleic acid (**1**) was formed with a current yield corresponding to ca. 80%. No

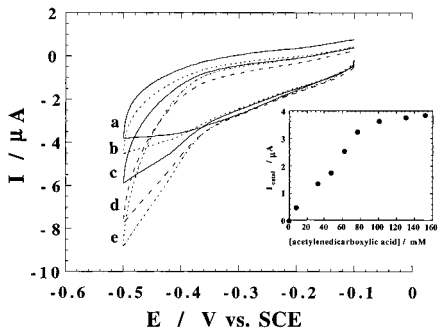
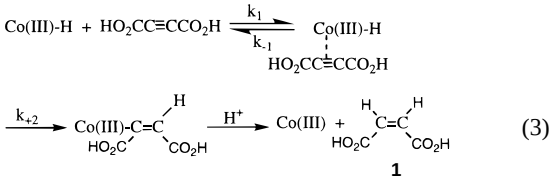


Figure 2. Cyclic voltammograms of the integrated MP-11/Co(II)-Mb monolayer-modified Au-electrode (geometrical area 0.2 cm^2, roughness factor ca. 15): (a) vs background solution 0.1 M phosphate buffer, pH 7.1, (b), (c), (d), and (e) in the presence of 8.2, 32, 62, and 76 mM acetylene dicarboxylic acid, respectively. Potential scan rate, 5 mV·s^{-1}. Inset: Concentration dependence of the electrocatalytic current registered at $E = -0.5$ V vs SCE.

fumaric acid (<2% of total maleic acid) could be detected, suggesting stereoselective Co(I)-mediated hydrogenation of acetylene dicarboxylic acid. A tentative pathway for the hydrogenation of the substrate by the electrocatalytically reduced Co(I)-Mb was previously formulated[7b] and involves the formation of a Co-hydride species.[12] Insertion of the acetylene substrate, followed by protonation, yields maleic acid, eq 3. The



(3)

formation of a complex between the bioelectrocatalyst and the subsrate is supported by the fact that the electrocatalytic cathodic current is enhanced upon increase of the concentration of acetylene dicarboxylic acid, Figure 2 (curves (b)−(e)), but it levels off at a substrate concentration corresponding to 0.1 M, Figure 2, inset. This is consistent with the saturation of the catalyst sites by the substrates. Indeed, the electrocatalytic cathodic currents, I_c, reflecting the rate of hydrogenation of acetylene dicarboxylic acid as a function of the substrate concentration, can be analyzed in terms of the Michaelis−Menten model. This leads to the values $I_{max} = 5.8$ mA and $K_M = 90$ mM.

In conclusion, we have demonstrated a novel method to assemble an integrated bioelectrocatalytic electrode consisting of Co(II)-reconstituted myoglobin acting as a semisynthetic biocatalyst. The enzyme electrode is generated by cross-linking of an affinity-complex formed between MP-11 and Co(II)-Mb on the electrode surface. The base layer of MP-11 acts as an electrical contacting element between Co(II)-Mb and the electrode. The bioelectrocatalytic electrode stimulates the stereoselective hydrogenation of acetylene dicarboxylic acid to maleic acid with a high current yield. The generation of integrated electrically-contacted hemopeptide-protein electrode represents a new element in the tailoring of bioelectrocatalytic interfaces via affinity associative interactions.

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