Oriented Immobilization of a Membrane-Bound Hydrogenase onto an Electrode for Direct Electron Transfer

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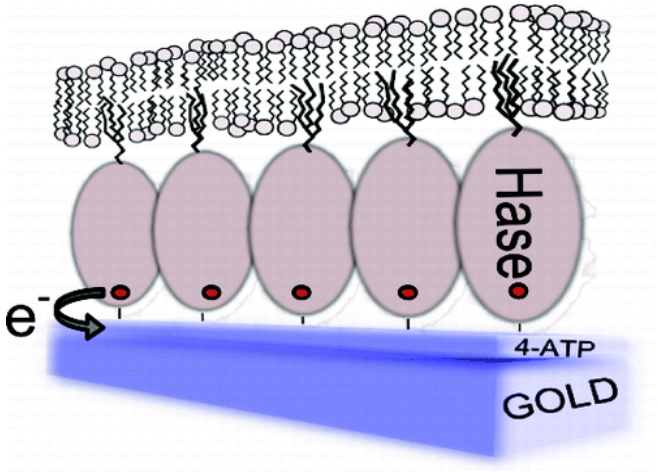

Figure 1. Schematic representation of the insertion of hydrogenase molecules in the phospholipidic bilayer. The red circles represent the distal 4Fe4S clusters.

The interaction of redox enzymes with electrodes is of great interest for studying the catalytic mechanisms of redox enzymes and for bioelectronic applications. Efficient electron transport between the biocatalysts and the electrodes has achieved more success with soluble enzymes than with membrane enzymes because of the higher structural complexity and instability of the latter proteins. In this work, we report a strategy for immobilizing a membrane-bound enzyme onto gold electrodes with a controlled orientation in its fully active conformation. The immobilized redox enzyme is the Ni–Fe–Se hydrogenase from *Desulfovibrio vulgaris* Hildenborough, which catalyzes H_2-oxidation reversibly and is associated with the cytoplasmic membrane by a lipidic tail. Gold surfaces modified with this enzyme and phospholipids have been studied by atomic force microscopy (AFM) and electrochemical methods. The combined study indicates that by a two-step immobilization procedure the hydrogenase can be inserted via its lipidic tail onto a phospholipidic bilayer formed over the gold surface, allowing only mediated electron transfer between the enzyme and electrode. However, a one-step immobilization procedure favors the formation of a hydrogenase monolayer over the gold surface with its lipidic tail inserted into a phospholipid bilayer formed on top of the hydrogenase molecules (Fig. 1). This latter method has allowed for the first time efficient electron transfer between a membrane-bound enzyme in its native conformation and an electrode.