DIRECT ELECTROCHEMISTRY OF **DESULFOVIBRIO GIGAS** [NiFe]-HYDROGENASE COVALENTLY BOUND AND ORIENTED ON CARBON NANOTUBE ELECTRODES.

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The use of hydrogen technology for energetic purposes, as an alternative to fossil fuels, is one of the solutions to obtain a clean energy free of CO\textsubscript{2} emissions. In nature the oxidation or evolution of H\textsubscript{2} is catalyzed by hydrogenases. The stable immobilization of such enzymes on electrodes is an interesting target for their application on biological fuel cells. In order to study the mechanism of catalytic hydrogen evolution or consumption by *Desulfovibrio Gigan* [NiFe]-Hydrogenase, through direct electrochemistry, several methods of covalent oriented immobilization were studied.

In the *Desulfovibrio Gigan* [NiFe]-Hydrogenase the distal 4Fe4S cluster is used by the protein to exchange electrons with his redox partner, the Cytochrome c\textsubscript{3}. The coupling between both proteins has a strong electrostatic component[1,2]. The target of this work was to emulate this interaction and force the enzyme to face the electrode with the aim of having direct electrochemistry. Near the distal cluster, the [NiFe]*Deslifovibrio Gigan* hydrogenase has several glutamate residues resposable of a negative potential, compared to the other side of the protein. This phenomenon, used to orientate the protein on an electrode, was studied by modifying the pH of incubation and the ionic strength[4]. The MWCNT were grown on the electrodes by chemical vapor deposition (CVD) of acetylene with a floating iron catalyst[5]. These deposited carbonaceous materials were analyzed by scanning electron microscopy (SEM, Hitachi S-3000N) and transmission electron microscopy (TEM, JEOL JEM 2000FX) measurements. The supporting carbon nanotube electrode was modified with a 4-aminophenyl monolayer and the hydrogenase was covalently bound to the amine groups through the glutamic residues. The electrode showed a very stable non-mediated catalytic H\textsubscript{2} oxidation current[3]. The catalytic current was also much higher than the one measured on polished HOPG carbon electrodes “edge” modified in the same way[4]. The increase of the catalytic current was also in good agreement with the electroactive area of the electrodes measured by chronocoulometry. This result confirms that only the electroactive surface sites of the MWCNT electrode are modified by the 4-aminophenyl rings. The hydrogenase-modified
MWCNT were imaged with an atomic force microscope (Nanotec Electronica, Madrid, Spain). The height and roughness difference shown in Figure 1 can be attributed to the presence of a 5 nm thick protein layer covering the modified nanotube, which is in agreement with the three dimensional structure of *D. gigas* hydrogenase[6]. All those results have been just published on Nano Letters.

**References:**


**Figures:**

*Figure 2.* AFM characterization of the height and roughness of control MWCNT (panels C, D, and dashed line in panels E and F) and hydrogenase-coated MWCNT (panels A, B and solid line in panels E and F) deposited on highly oriented pyrolytic graphite.