

Electrocatalytic activity of hydrogenases at carbon electrodes: hydrogen production via [Fe]-hydrogenase from *Desulfovibrio vulgaris* Hildenborough

M. Guiral-Brugna, M.-T. Guidici-Orticoni, M. Bruschi, P. Bianco
BIP-CNRS
31 chemin Joseph Aiguier
13402 Marseille Cedex 20
France

2. K. Draoui, P. Bianco, J. Haladjian, F. Guerlesquin, and M. Bruschi, *J. Electroanal. Chem.*, **313**, 201 (1991).
3. P. Bianco, J. Haladjian, M. Bruschi, and F. Guerlesquin, *Biochem. Biophys. Res. Comm.*, **189**, 633 (1992).
4. P. Bianco and J. Haladjian, *J. Electrochem. Soc.*, **139**, 2428 (1992).
5. H.R. Pershad, J.L.C. Duff, H.A. Heering, E.C. Duin, S.P.J. Albracht, and F.A. Armstrong, *Biochemistry*, **38**, 8992 (1999).

Hydrogenases are a class of enzymes that catalyze the reversible oxidation of hydrogen / reduction of protons in the presence of appropriate electron acceptors or donors. Some of them are involved in microorganisms such as sulfate-reducing bacteria. Different hydrogenases have been isolated, and it is remarkable that three types of hydrogenases varying in active center structure, namely [Fe], [NiFe] and [NiFeSe], can co-exist in the same *Desulfovibrio vulgaris* Hildenborough (DvH) bacterium.

This communication is concerned essentially in [Fe] hydrogenase. This redox protein is an heterodimer composed of one large (46 KDa) and one small (10 KDa) subunit. The large subunit contains two [4Fe-4S] clusters and a more peculiar [Fe-S] cluster presumably involved in the activation of molecular hydrogen.

Electrochemistry offers attractive possibilities for investigating the electron exchanges between either hydrogenase and a physiological partner (cytochrome c3) or hydrogenase alone and an electrode. The former process has been well investigated in previous papers [e.g.1-3], but very few electrochemical approaches are known to the latter one [4,5]. Because of its size (MW of about 55 KDa) and its relatively low isoelectric point (pI of about 5-6), it can be expected that hydrogenase is not, or only sluggishly reactive at conventional working electrodes. Nevertheless, it is demonstrated that positively charged species (e.g. polylysine) are able to improve the electron exchange and in consequence the electrode response.

Results presented in this communication provide evidence for the catalytic electroreduction of protons by DvH hydrogenase from cyclic voltammetry at pH 6, using a carbon (pyrolytic graphite) working electrode. It is shown that the amplitude of catalytic currents depends upon several parameters, especially pH, hydrogenase concentration, the presence of strong electrolytes or other proteins. Polishing of the electrode surface and repetitive cycling have prominent effects on the quality and stability of the electrode response. The stabilization of hydrogenase molecules on the electrode surface seems to be a determinant factor for a productive electrocatalytic process to be observed. In particular, hydrogenase adsorption onto the graphite surface appears as a major (and probably mandatory) step for productive electrocatalysis.

1. J. Haladjian, P. Bianco, F. Guerlesquin, and M. Bruschi, *Biochem. Biophys. Res. Comm.*, **147**, 1289 (1987).