JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by American Chemical Society

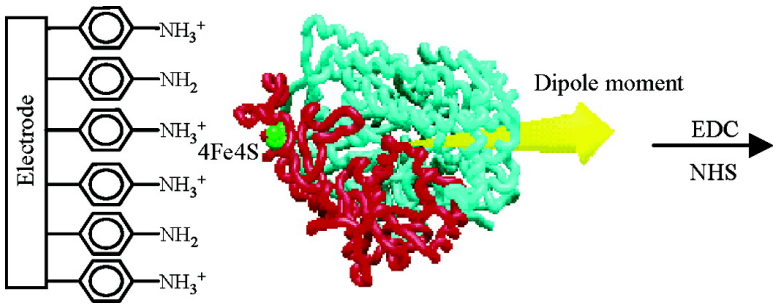
Communication

Oriented Immobilization of *Desulfovibrio gigas* Hydrogenase onto Carbon Electrodes by Covalent Bonds for Nonmediated Oxidation of H

Olaf Rdiger, Jose M. Abad, E. Claude Hatchikian, Victor M. Fernandez, and Antonio L. De Lacey

J. Am. Chem. Soc., **2005**, 127 (46), 16008-16009• DOI: 10.1021/ja0554312 • Publication Date (Web): 28 October 2005

Downloaded from http://pubs.acs.org on March 25, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 13 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 10/28/2005

Oriented Immobilization of *Desulfovibrio gigas* Hydrogenase onto Carbon Electrodes by Covalent Bonds for Nonmediated Oxidation of H_2

Olaf Rüdiger,[†] Jose M. Abad,[†] E. Claude Hatchikian,[‡] Victor M. Fernandez,[†] and Antonio L. De Lacey*[,†]

Instituto de Catalisis, CSIC, C/Marie Curie 2, Cantoblanco, 28049 Madrid, Spain, and Institut de Biologie Structurale et Microbiologie, CNRS, Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France

Received August 9, 2005; E-mail: alopez@icp.csic.es

The controlled and oriented immobilization of redox proteins on electrode surfaces constitutes an attractive goal in bioelectrochemical research for fundamental studies of enzymatic catalysis, biotechnological applications, and mainly for obtaining direct electron transfer (DET). To achieve this, it is necessary to have good structural knowledge of the used redox enzymes and to develop efficient methods of electrode derivatization.[1]

In this work, we report the covalent bonding of *Desulfovibrio gigas* hydrogenase to a functionalized carbon electrode in such a controlled manner that DET between enzyme and electrode can be achieved. Hydrogenases catalyze the oxidation of H_2 in a reversible way, thus their immobilization on electrodes has potential interest for biological fuel cells development.[2] DET has been reported by physical adsorption of different hydrogenases on carbon electrodes.[3] Although very high catalytic currents may be obtained,[4] this immobilization method has the drawbacks of low enzyme stability on the electrode, probably due to its leakage into the bulk solution, and the lack of control on the immobilization process.[3a,b,f,5] More stable or controlled methods of hydrogenase immobilization on electrodes have been reported, but DET was not obtained in those cases.[6]

The rationale of the strategy reported in this work is based on the structural properties of the *D. gigas* hydrogenase. The crystallographic structure indicates that the enzyme has an electron transfer pathway formed by three iron−sulfur clusters that connects the Ni−Fe active site, where heterolytic cleavage of H_2 takes place, to the protein surface, where electrons are exchanged with the redox partner of the hydrogenase. The so-called 4Fe4S distal cluster, which is the farthest from the active site and the nearest from the protein surface, is surrounded by several glutamic residues.[7] Therefore, our aim has been to link covalently the hydrogenase to the electrode by formation of an amide bond between these glutamic amino acids and a carbon electrode functionalized with a monolayer of amine groups to achieve DET. However, as carboxylic acid groups are located in different parts of the enzyme surface, correct orientation of the enzyme molecule with the distal 4Fe4S cluster facing the electrode surface during covalent bond formation is necessary for obtaining efficient DET. Figure 1A shows the surface electrostatic potential distribution obtained from the 3D structure of *D. gigas*, which indicates the existence of a dipole moment in the enzyme molecule. Figure 1B shows that the distal 4Fe4S cluster is located in the negative region of the protein surface; thus during the enzyme immobilization step, it is possible to control the orientation of the enzyme molecules by electrostatic interactions. Such interactions should mimic the electrostatic interactions that most probably control electron transfer between hydrogenase and their in vivo redox partners.[8]

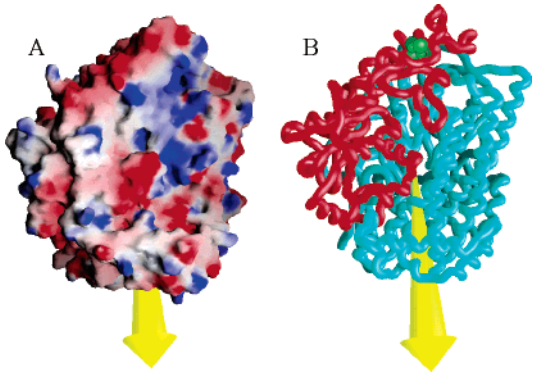


Figure 1. Three-dimensional structure of *D. gigas* hydrogenase obtained from the Brookhaven Protein Data Bank. (A) Surface electrostatic potential distribution: negative regions (red), positive regions (blue), direction of the dipole moment (yellow arrow). (B) Position of the distal 4Fe4S cluster (green), large subunit (cyan), and small subunit (red) relative to the dipole moment. The direction and magnitude (564 D) of the dipole moment of the crystal structure were calculated with GRASP software.

Functionalization of carbon electrodes with a monolayer of amine groups has been carried out in this work using the method developed by Saveant and co-workers of electrochemical reduction of aryl diazonium salts. It has proven to be a versatile, reliable, and robust way for creating functionalized monolayers on carbon electrodes.[9] Despite extensive work done on the characterization and application of these modified surfaces in the last years, the method has seldom been used for redox enzyme coupling to carbon electrodes, and in those few cases, electron transfer was done via diffusing redox mediators.[10] Figure 2A shows cyclic voltammograms obtained after covalent coupling of *D. gigas* hydrogenase at low ionic strength to pyrolytic graphite electrodes modified with a monolayer of 4-aminophenyl groups.[11] In the case of hydrogenase immobilization at pH 7.6, no catalytic current is observed in the presence of H_2 substrate if no redox mediator (methyl viologen, MV) is added in solution, whereas in the case of hydrogenase immobilization at pH 6.0, a clear catalytic current is measured in absence of the redox mediator, indicating DET between the enzyme and the electrode.[12] A pK_a of 6.9 has been measured by capacitance experiments for a monolayer of 4-aminothiophenol groups on gold,[13] so a similar value should be expected for our monolayer. Thus, at pH 6.0, most of the amine groups are protonated, and the electrode surface has a strong positive charge that should favor oriented enzyme immobilization according to its dipole moment, that is, with the 4Fe4S cluster facing the electrode, and allowing DET. At pH 7.6, most of the amine groups of the modified electrode should not be protonated, and hydrogenase immobilization is expected to proceed without electrostatic control, resulting in random orientation and no efficient DET. On the contrary, nonmediated catalytic current for hydrogen oxidation was measured with hydrogenase covalently bound at pH 7.6 to a gold

[†] CSIC, Madrid.
[‡] CNRS, Marseille.

10.1021/ja0554312 CCC: $30.25 © 2005 American Chemical Society

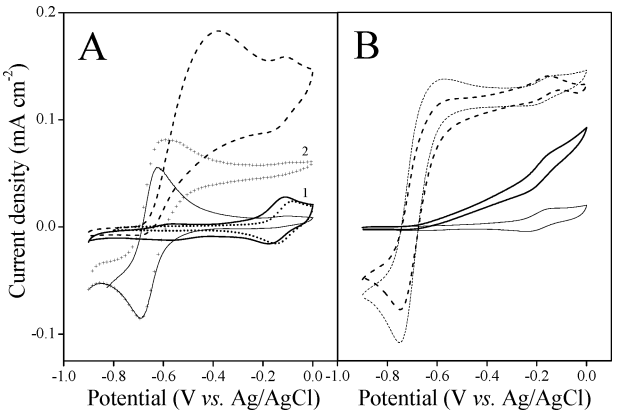


Figure 2. Cyclic voltammograms of H_2 oxidation catalysis with pyrolytic graphite electrodes modified with covalently bound hydrogenase. Measurement conditions were 100 mM phosphate buffer, pH 8.0, 20 mV/s scan rate, room temperature. (A) Electrodes modified with hydrogenase at 0.01 M ionic strength and different pH: pH 6.0 under N_2 (thick solid line) and after 5 h under H_2 (thick dashes) without MV; pH 7.6 after 5 h under H_2 without (dots 1) and with 1.25 mM MV (crosses 2). The thin solid line is a control experiment with 1.25 mM MV and no immobilized hydrogenase. (B) Electrodes modified at pH 6.0 and different ionic strengths: 0.02 M without (thick solid line) and with 1.25 mM MV (thick dashed line); 1.0 M without (thin solid line) and with 1.25 mM MV (thin dashed line). The voltammograms were done after 5 h under 1 atm H_2 in order to obtain full activity of the immobilized hydrogenase.[16]

electrode modified with a cystamine monolayer (Supporting Information). In this later case, the pK_a of the amine groups of the monolayer should be approximately 8.5.[14] However, the operational stability of these hydrogenase electrodes is low due to the desorption of the thiol self-assembled monolayer at negative potentials.[15] Control experiments in which the hydrogenase was just deposited on the modified electrode (carbon or gold) without covalent bond formation gave no direct or mediated catalytic currents, probably due to the low stability of the enzyme adsorbed on the electrode (not shown).

Further experimental evidence in favor of electrostatic interactions modulating the orientation of hydrogenase during immobilization was obtained by cyclic voltammetry of electrodes prepared at different ionic strength (Figure 2B). The electrode prepared at very high ionic strength, at which electrostatic interactions between the electrode surface groups and the enzyme molecules should be screened, hardly gave any catalytic current in absence of redox mediator, despite having very similar mediated catalytic current to the electrode prepared at lower ionic strength, which suggests that in both cases the coverage of active hydrogenase is similar. The ratio between direct and mediated electrocatalysis of covalently bound hydrogenase increased as the ionic strength of the medium during the immobilization step was decreased (Supporting Information).

The operational stability of the hydrogenase-modified carbon electrodes was high; 90% of the initial catalytic current was maintained after 1 week in continuous measurement under 1 atm H_2 bubbling at room temperature.

In summary, we have developed a robust and controlled immobilization method of hydrogenase onto carbon electrodes based on electrostatic interactions directing the correct orientation of the enzyme for DET. This method could be applied to other redox enzymes.

Acknowledgment. This research was financially supported by the MCYT, Grant BQU 2003-04221. We thank Dr. Juan Hermoso for his help with Figure 1. O.R. thanks MEC for a fellowship.

Supporting Information Available: Experimental details of electrode preparation, ratio of directed/mediated catalytic currents with electrodes prepared at different ionic strength (Figure S1 and Table S1), and cyclic voltammograms of hydrogenase−cystamine electrode (Figure S2). This material is available free of charge via Internet at http://pubs.acs.org.

References

(1) (a) Willner, I.; Katz, E. *Angew. Chem., Int. Ed.* **2000**, *39*, 1180−1218. (b) Hess, C. R.; Juda, G. A.; Dooley, D. M.; Amii, R. N.; Hill, M. G.; Winkler, J. R.; Gray, H. B. *J. Am. Chem. Soc.* **2003**, *125*, 7156−7157. (c) Fantuzzi, A.; Fairhead, M.; Gilardi, G. *J. Am. Chem. Soc.* **2004**, *126*, 5040−5041. (d) Shleev, S.; Tkac, J.; Christenson, A.; Ruzgas, T.; Yaropolov, A. I.; Whittaker, J. W.; Gorton, L. *Biosens. Bioelectron.* **2005**, *20*, 2517−2554. (e) Armstrong, F. A. *Curr. Opin. Chem. Biol.* **2005**, *9*, 110−117.

(2) *Hydrogen as a Fuel: Learning from Nature*; Cammack, R., Frey, M., Robson, R., Eds.; Taylor & Francis: London, 2001.

(3) (a) Butt, J. N.; Filipiak, M.; Hagen, W. R. *Eur. J. Biochem.* **1997**, *245*, 116−122. (b) Leger, C.; Jones, A. K.; Roseboom, W.; Albracht, S. P. J.; Armstrong, F. A. *Biochemistry* **2002**, *41*, 15736−15746. (c) Morozov, S. V.; Karyakina, E. E.; Zorin, N. A.; Varfolomeyev, S. D.; Cosnier, S.; Karyakin, A. A. *Bioelectrochemistry* **2002**, *55*, 169−171. (d) Leger, C.; Dementin, S.; Bertrand, P.; Rousset, M.; Guigliarelli, B. *J. Am. Chem. Soc.* **2004**, *126*, 12162−12172. (e) Lojou, E.; Giudici-Orticoni, M. T.; Bianco, P. *J. Electroanal. Chem.* **2005**, *577*, 79−86. (f) Johnston, W.; Cooney, M. J.; Liaw, B. Y.; Sapra, R.; Adams, M. W. W. *Enzyme Microb. Technol.* **2005**, *36*, 540−549.

(4) Jones, A. K.; Sillery, E.; Albracht, S. P. J.; Armstrong, F. A. *Chem. Commun.* **2002**, 866−867.

(5) Leger, C.; Jones, A. K.; Albracht, S. P. J.; Armstrong, F. A. *J. Phys. Chem. B* **2002**, *106*, 13058−13063.

(6) (a) Mege, R. M.; Bourdillon, C. *J. Biol. Chem.* **1985**, *260*, 14701−14706. (b) Eng, L. H.; Elmgren, M.; Komlos, P.; Nordling, M.; Lindquist, S. E.; Neujahr, H. Y. *J. Phys. Chem.* **1994**, *98*, 7068−7072. (c) De Lacey, A. L.; Detcheverry, M.; Moiroux, J.; Bourdillon, C. *Biotechnol. Bioeng.* **2000**, *68*, 1−10.

(7) Volbeda, A.; Charon, M. H.; Piras, C.; Hatchikian, E. C.; Frey, M.; Fontecilla-Camps, J. C. *Nature* **1995**, *373*, 580−587.

(8) (a) Pereira, I. A. C.; Romao, C. V.; Xavier, A. V.; LeGall, J.; Teixeira, M. *J. Biol. Inorg. Chem.* **1999**, *3*, 494−498. (b) Morelli, X.; Czjzek, M.; Hatchikian, E. C.; Bornet, O.; Fontecilla-Camps, J. C.; Palma, N. P.; Moura, J. J. G.; Guerlesquin, F. *J. Biol. Chem.* **2000**, *275*, 23204−23210.

(9) (a) Delamar, M.; Hitmi, R.; Pinson, J.; Saveant, J. M. *J. Am. Chem. Soc.* **1992**, *114*, 5883−5884. (b) Allongue, P.; Delamar, M.; Desbat, B.; Fagebaume, O.; Hitmi, R.; Pinson, J.; Saveant, J. M. *J. Am. Chem. Soc.* **1997**, *119*, 201−207.

(10) (a) Bourdillon, C.; Delamar, M.; Demaille, C.; Hitmi, R.; Moiroux, J.; Pinson, J. *J. Electroanal. Chem.* **1992**, *336*, 113−123. (b) Blankespoor, R.; Limoges, B.; Schöllhorn, B.; Syssa-Magalé, J. L.; Yazidi, D. *Langmuir* **2005**, *21*, 3362−3375.

(11) See Supporting Information for experimental details.

(12) The redox process at −150 mV, also observed in the control experiments, is due to hydroxylamine groups present in the electrode monolayer as reported by: Brooksby, P. A.; Downard, A. J. *Langmuir* **2004**, *20*, 5038−5045. H_2 production catalyzed by the immobilized hydrogenases is hardly observed in the cathodic direction because, at pH 8.0 and 1 atm H_2, this activity is very low as reported in ref 3b and by: De Lacey, A. L.; Moiroux, J.; Bourdillon, C. *Eur. J. Biochem.* **2000**, *267*, 6560−6570.

(13) Bryant, M. A.; Crooks, R. M. *Langmuir* **1993**, *9*, 385−387.

(14) Katz, E.; Lion-Dagan, M.; Willner, I. *J. Electroanal. Chem.* **1996**, *408*, 107−112.

(15) Zhong, C. J.; Zak, J.; Porter, M. D. *J. Electroanal. Chem.* **1997**, *421*, 9−13.

(16) Fernandez, V. M.; Hatchikian, E. C.; Cammack, R. *Biochim. Biophys. Acta* **1985**, *832*, 69−79.

JA0554312