Electrochemical Control of Hydrogenase Action of *Desulfovibrio vulgaris* (Hildenborough)

Tokuji Ikeda,* Kazuyoshi Takagi, Hirosuke Tatsumi, and Kenji Kano
Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606

(Received August 30, 1996)

A carbon electrode modified with immobilized whole cells of *Desulfovibrio vulgaris* was found to be able to electrochemically drive the reaction: $H_2 \longleftrightarrow 2H^+ + 2e^-$ very effectively in both forward and reverse directions by the use of methyl viologen and vitamin K_3 as a mediator, respectively. Similarly, the reduction of dioxygen was electrochemically driven by the use of *Thiobacillus ferrooxidans* as a biocatalyst and hexacyanoferrate (II) as a mediator.

Hydrogenase is an enzyme catalyzing oxidation of hydrogen and reduction of proton. The enzymatic reaction has been coupled to an electrochemical reaction of a compound functioning as an electron acceptor[1] or donor[2,3] of the enzyme, producing the catalytic current for the oxidation of hydrogen or reduction of proton. The enzyme-catalyzed electrochemical reaction, bioelectrocatalysis, has provided a convenient means of studying kinetics of the enzyme reaction.[1-3] It has also received attention from a practical point of view in terms of a hydrogen enzyme electrode, biofuel cells and specific biological electrosynthesis.[2]

Hydrogenase exists in periplasmic spaces and/or cytoplasmic membranes of a variety of bacterial species.[4] In spite of extensive studies of the kinetics of purified hydrogenase, the physiological function of the periplasmic hydrogenase has not been established yet.[1] In this respect, we have been interested in measuring *in vivo* hydrogenase reaction by the method based on bacterial cell-catalyzed electrochemical reaction. We have recently found[5] that a number of bacterial species work as bags of enzymes to produce large electrocatalytic currents due to the catalytic action of the periplasmic and/or cytoplasmic enzymes.

In this paper we demonstrate that *Desulfovibrio vulgaris* (Hildenborough) works as a biocatalyst as effective as purified hydrogenase in electrocatalytic evolution and consumption of hydrogen. *D. vulgaris* (Hildenborough) contains hydrogenase in the periplasmic space.[4] The bacterial strain supplied by Dr. T. Kakiuchi was grown to early stationary phase according to Van der Westen et al.[6] The cell paste of the bacterium was suspended in 10 mM phosphate buffer solution (pH 7.0); the suspension had an optical density of 54.0 at 610 nm. Five microliter of the suspension was entrapped on the surface of a glassy carbon electrode (BAS Inc., No. 11-2012, 3 mm i.d.) by covering with a dialysis membrane (20 μm in the dry state) by the procedure mentioned previously.[5] The *D. vulgaris*-modified electrode was kept in 10 mM phosphate buffer (pH 7.0) at 4 °C when not in use. Electrochemical measurements were carried out at 30 °C under anaerobic conditions by purging the electrolysis cell with argon unless stated otherwise. Hydrogen in place of argon was used in case of hydrogen consumption experiments. A platinum disk and an Ag/AgCl/saturated KCl electrode were used as the counter electrode and the reference electrode, respectively. All potentials in this paper are referred to the Ag/AgCl/saturated KCl electrode.

We first examined reductive bioelectrocatalysis. Figure 1 shows cyclic voltammograms (CVs) obtained with the *D. vulgaris*-modified electrode in (A) pH 5.0 buffer and in buffer solutions containing 0.10 mM methyl viologen (MV^{2+}) at pH (B) 5.0, (C) 7.0, and (D) 8.0. In the presence of MV^{2+}, all the CVs produce sigmoidal cathodic waves, which are completely different from the peak-shaped cathodic and anodic waves observed at a bare glassy carbon electrode (Figure 1E). The sigmoidal waves start to appear at -0.54 V, and increase to attain limiting current at -0.72 V. The magnitude of the limiting current is dependent on the solution pH; it becomes smaller at higher pH. The results clearly show that hydrogenase in the bacterial cells is functioning as a catalyst to reduce proton by the use of methyl viologen cation radial ($MV^{\bullet 1+}$), which is generated

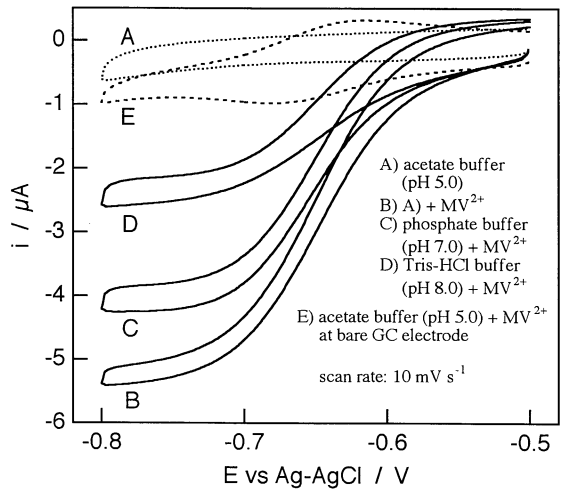

Figure 1. CVs representing bioelectrocatalytic hydrogen evolution at the *D. vulgaris*-modified electrode.

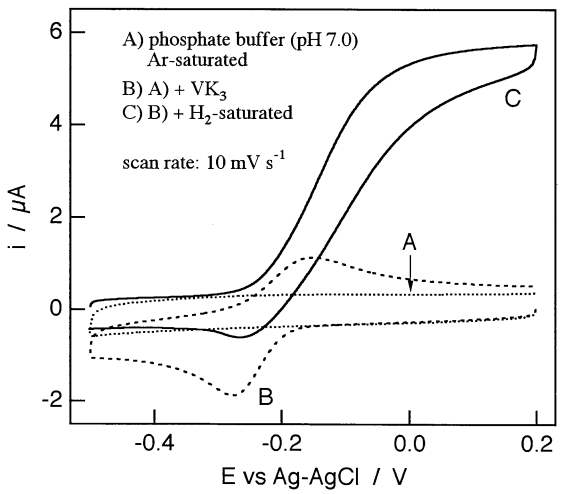

Figure 2. CVs representing bioelectrocatalytic hydrogen consumption at the *D. vulgaris*-modified electrode.

electrochemically at the electrode to produce the catalytic cathodic current as was reported[3] for the purified hydrogenase reaction with methyl viologen as a mediator. MV^{2+} and $MV^{\bullet 1+}$ are considered to be able to permeate rather rapidly through the bacterial outer membrane to reach and react with the periplasmic hydrogenase. Thus, we observe the catalytic current of significant magnitude on the time scale of CV.

Next, we tested oxidative bioelectrocatalysis. Figure 2 shows CVs obtained with the *D. vulgaris*-modified electrode in pH 7.0 buffer containing 0.10 mM vitamin K_3 (VK_3). Under argon-saturated conditions (Figure 2B), VK_3 produces a pair of cathodic and anodic peak-shaped waves. In contrast to this, the CV under hydrogen-saturated conditions (Figure 2C) exhibits a large anodic current starting from -0.25 V to attain a limiting current at 0 V. In the absence of VK_3, such anodic current was not observed under hydrogen-saturated conditions, and the CV was very similar to Figure 2A (data not shown). Accordingly, the anodic current in Figure 2C is attributed to the catalytic action of *D. vulgaris* in the hydrogen consumption reaction using VK_3 as an electron acceptor; it may accept electrons from hydrogenase or from cytochromes c_3, an efficient electron acceptor of the enzyme,[1] in the bacterial membrane. VK_3 may permeates through the outer membrane to reach the periplasmic space.

We have tentatively examined the stability of the *D. vulgaris*-modified electrode, and confirmed that the electrode remained active for at least three days.

In relation to above observation, we have also examined the electrocatalytic reduction of oxygen by using *Thiobacillus ferrooxidans,* which is known to use ferrous ion for the reduction of oxygen to water.[4] *T. ferrooxidans* supplied by Dr. T. Sugio was grown to early stationary phase according to Silverman and D. G. Lundgren,[7] and a *T. ferrooxidans*-modified electrode was constructed by the same way as the *D. vulgaris*-modified electrode. Figure 3 shows the CVs recorded at pH 2.0 buffer containing 0.48 mM $Fe(CN)_6^{3-}$. Under air-saturated conditions (Figure 3C), a large cathodic wave appears from the potential at which the reduction of $Fe(CN)_6^{3-}$ begins to start (Figure 3B). This indicates that oxygen is reduced by the catalytic action of *T. ferrooxidans*[4] using $Fe(CN)_6^{4-}$ as an electron donor. Similar electrocatalytic effect of *T. ferrooxidans* has been reported[8] in the absence of $Fe(CN)_6^{3-}$ at pH 1.5, in which peak current appears at more negative by 0.25 V than that in Figure 3C.

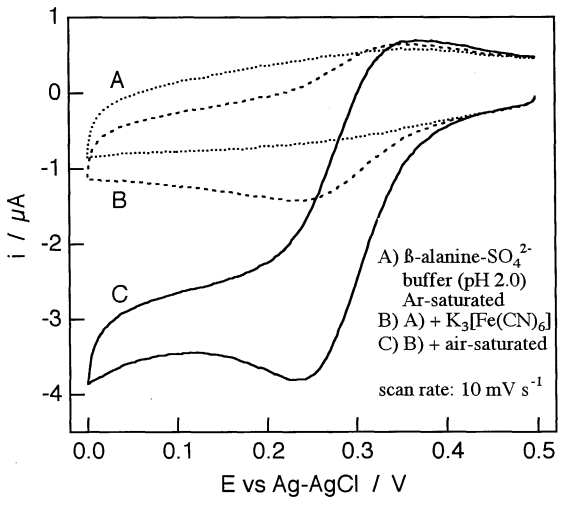

Figure 3. CVs representing bioelectrocatalytic oxygen reduction at the *T. ferrooxidans*-modified electrode.

To our knowledge, this is the first report demonstrating that intact bacterial cells work as effective biocatalysts for both electrochemical hydrogen evolution and consumption. This may provide a convenient means of monitoring *in vivo* hydrogenase reactions. The electrochemical hydrogenase reaction may open a route for developing hydrogenase/oxydase-based electrochemical energy conversion system in combination with the oxygen reduction reaction with *T. ferrooxidans*, and for achieving photo-assisted electrochemical hydrogen production by combining with a system of photo-bioelectrochemical oxidation of water such as that reported previously.[9] Further study is in progress.

We thank Dr. Takashi Kakiuchi, Yokohama National University, and Dr. Takeshi Sugio, Okayama University for kind supplying the bacterial strains. This work was supported by Grant-in-Aids for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

1 M. F. J. M. Verhagen, R. B. G. Wolbert, and W. R. Hagen, *Eur. J. Biochem.*, **221**, 821 (1994), and references cited therein.
2 S. D. Varofolomeyev and S. O. Bachurin, *J. Mol. Catal.*, **27**, 305 (1984); S. D. Varofolomeyev and S. O. Bachurin, *J. Mol. Catal.*, **27**, 315 (1984).
3 J. C. Hoogvliet, L. C. Lievense, C. van Dijk, and C. Veeger, *Eur. J. Biochem.*, **174**, 273 and 281 (1988).
4 K. Yamanaka, "Biseibutsu no Enerugi Taisha (in Japanese)," Gakkai Shuppan Senta, Tokyo (1986), pp. 28-62, 147-151.
5 T. Ikeda, T. Kurosaki, K. Takayama, and K. Kano, *Anal. Chem.*, **68**, 192 (1996), and references cited therein.
6 H. M. van der Westen, S. G. Meyhew, and G. Veeger, *FEBS Lett*, **86**, 122 (1978).
7 M. P. Silverman and D. G. Lundgren, *J. Bacteriol.*, **77**, 642 (1959).
8 S. Nakasono, N. Matsumoto, and H. Saiki, Abstract of the 63rd Electrochemical Society Meeting, April 3-5, Koganei, Japan, 1996, p. 316.
9 K. Amako, H. Yanai, T. Ikeda, T. Shiraishi, M. Takahashi, and K. Asada, *J. Electroanl. Chem.*, **362**, 71 (1993).