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Fabrication of an Electrode-Viologen-Hydrogenase Heterogeneous System and the Electrochemical Hydrogen Evolution

Dong-Jin Qian,[1] Chikashi Nakamura,[1] Kazuyuki Noda,[1] Nickolai A. Zorin,[2] and Jun Miyake*,[1]

[1]National Institute of Advanced Interdisciplinary Research, AIST, 1-1-4 Higashi, Tsukuba, Ibaraki 305, Japan, E-mail: Miyake@nair.go.jp; and [2]Institute of Soil Science and Photosynthesis, Russian Academy of Sciences, Pushchino, 142292 Moscow, Russia

Abstract

An indium tin oxide (ITO) electrode was chemically modified by one layer of viologen (VIO) derivative, which possessed a persistent and reproducible electrochemical response. A monolayer of a thermal stable hydrogenase from *Thiocapsa roseopersicina* was stabilized on a synthesized poly-L-lysine subphase surface and transferred onto the electrode for fabrication of an ITO-VIO-hydrogenase heterogeneous system. Electrochemical properties of both the ITO-VIO monolayer and the heterogeneous ITO-VIO-hydrogenase system have been investigated. Hydrogen evolution could be measured by potentiostating the VIO-hydrogenase-covered ITO electrode to "electroplate" $[(VIO^{+})_n]_{surf}$, and a large increase in hydrogen evolution was observed when using an electrolyte solution containing sodium dithionite. We discuss the possible electron transfer process.

Index Entries: Indium tin oxide-viologen monolayer; electrochemistry; hydrogenase; Langmuir-Blodgett film; hydrogen evolution.

Introduction

Hydrogen, as a powerful energy source as well as a harmless impact on the environment, has attracted much attention in the past several decades. Researchers have tried to obtain abundant hydrogen from several fields; among them biological hydrogen production seems to be a challenging one *(1)*. Microorganisms usually produce hydrogen based on the following mechanism: hydrolysis, reduction of proton, and electron supply *(2)*. One way to reduce of proton is to catalyze the reversible oxidoreduction

*Author to whom all correspondence and reprint requests should be addressed.

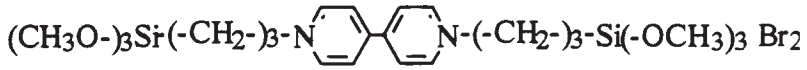
of molecular hydrogen according to the reaction $H_2 \leftrightarrow 2H^+ + 2e^-$ by hydrogenase. As a result, the electric energy could be converted to the chemical energy reserved in hydrogen. To mimic this procedure artificially, many of hydrogenases were isolated and several systems have been designed, such as an electrochemical cell *(3)*, liposomes *(4)*, and membrane *(5)*. Electron donor, mediator, and hydrogenase are three main parts in these systems.

Some reductants and the cathodic electrode could be used as an electron donor. In a biological organism, some proteins play the role of electron carrier (mediator), whereas in an artificial system, researchers sometimes use viologens (VIOs) instead of proteins. The easily synthesized VIOs have three oxidation states with a high reversibility of the first redox reaction VIO^{2+} ´ VIO^+ *(6,7)*, and the VIO^+ can transfer electrons to the hydrogenase *(5)*. VIOs have been extensively investigated as a mediator to hydrogenases in various systems in a solution phase *(3–5)*. However, little work has been done using an immobilized VIO in an electrode as an electron mediator *(8)*, especially in a heterogeneous system composed of an electrode, VIO, and hydrogenase. This system has the advantages that it can overcome the protein deactivation owing to the strong adsorption at a bare electrode, that a controlled arrangement of hydrogenase layers on a monolayer of VIO may enhance the electron transfer efficiency, and that chemically modified electrodes have numerous important applications *(4,9)*. Several methods have been used to prepare the VIO-modified electrodes *(10–13)*. Among them a derivative of the indium tin oxide (ITO)-VIO developed by Bookbinder and Wrighton *(12)* and Palmore et al. *(13)*, in the past 20 yr, was considered to be very effective for immobilization of VIO on ITO electrode. In the present work, a VIO derivative was immobilized on anITO electrode according to these investigators, and the hydrogenase multilayer deposited on this electrode by the Langmuir-Blodgett method was used as an electron acceptor. The hydrogenase used was a thermal stable [NiFe] hydrogenase from *Thiocapsa roseopersicina*, whose structure and properties have been reported previously *(14,15)*. This heterogeneous system was of a persistent and reproducible electrochemical response. The hydrogen evolution was measured by potentiostating the VIO-hydrogenase-covered ITO electrode to "electroplate" $[(VIO^+)_n]_{surf}$. These findings may provide an alternative way for generating electrochemical hydrogen.

Materials and Methods

Materials

The derivatizing agent (I) was synthesized by refluxing dry 4,4'-bipyridyl (Wako Pure Chemical, Osaka, Japan) with 1-bromo-3-trimethoxysilylpropane (Gelest, Tullytown, PA) according to Bookbinder and Wrighton *(12)* method.



(I)

Hydrogenase (mol wt = 9×10^4) from *Th. roseopersicina* was prepared as described in the literature *(16)*. The sample was stored at –20°C and reactivated by incubation in the presence of H_2. Poly-L-lysine (PLL) (20 mer), used as a counterion in the subphase, was synthesized by a solid-phase system using a P9050 Plus PepSynthesizer (Millipore, Bedford, MA) and purified by an ODS column (C18; 19 × 150 mm; Millipore).

Electrode Derivatization

An ITO electrode was made by cutting a small (~0.5×3.0 cm^2) rectangular piece, which was first washed with detergent and then treated with H_2CrO_4 solution for 10 s, following a 60-s soak in 10 *M* NaOH solution *(17)*. The electrode was finally washed with ultrapure water and dried for 2 h at 120°C. After pretreatment, the electrode was immersed in a 2 m*M* dimethylformamide/CH_3CN solution of iodine with one drop of 10% HCl at room temperature for 2 d. The derivatized ITO electrode was then washed with CH_3CN, ethanol and water, and dried under N_2.

Preparation of Hydrogenase Langmuir-Blodgett Film

A KSV minitrough (KSV, Finland) was used for the deposition of hydrogenase monolayer by the traditional Langmuir-Blodgett method. The monolayer preparation and Langmuir-Blodgett film deposition have been described previously *(8)*.

Electrochemical Measurement

Cyclic voltammogram experiments were performed with a BAS 100B electrochemical analyzer. A Pt wire and Ag/AgCl electrode were used as the auxiliary and reference electrodes, respectively. An ITO electrode immobilized with a VIO monolayer or with a VIO-hydrogenase multilayer was employed as the working electrode using 0.1 mol/L of $NaClO_4$, or 0.1 mol/L of $NaClO_4$ and a small amount of phosphate (1.2×10^{-5} mol/L of PO_4^{3-}, for pH adjusting) as electrolyte, respectively. An initial potential of –0.20 V was applied for 2 s, and subsequently several cyclic scans to a final potential of –1.20 V were obtained. All experiments were made under Ar atmosphere at room temperature.

Electrochemical Hydrogen Evolution

The activity of hydrogen evolution was measured as described by Zorin et al. *(16)*. The hydrogen evolution of ITO-VIO hydrogenase Langmuir-Blodgett film was measured by a hydrogen electrode in a temperature-controlled cell chamber at 30°C (Biott, Tokyo, Japan). A Pt wire and Ag/AgCl electrode were used as the auxiliary and reference electrodes, respectively. An ITO electrode covered with VIO and hydrogenase was employed as the working electrode using 0.1 mol/L of $NaClO_4$ (pH 7.0, phosphate) as electrolyte. The potential of the ITO electrode was kept at –540 mV vs Ag/AgCl electrode.

Results and Discussion

Electrochemistry of Derivatized ITO Electrode

The VIO-derivatized ITO electrode showed a persistent electrochemical response in aqueous electrolyte solution *(17)*. Rinsing the electrode with pure water ultrasonically for 10 min resulted in nearly identical current-voltage traces, which demonstrated that the VIO was covalently attached to the ITO surface by the formation of an Si-O bond. Figure 1 shows the cyclic voltammogram curves of the derivatized electrode at various scan rates. Well-defined symmetrical surface waves consisting of reversible oxidation and reduction peaks were obtained at ~–540 mV for $VIO^{2+} \rightarrow VIO^{+}$ and ~–500 mV for $VIO^{+} \rightarrow VIO^{2+}$, respectively *(6)*. The redox peak currents of the VIO increased linearly with the potential scan rates (Fig. 2), as expected from the equation that was derived for an adsorbed redox species in a thin-layer cell:

$$i_c = n^2CrF^2\nu/(4RT)$$

in which n, Cr, F, and ν are the number of electrons, surface concentration, Faraday constant, and potential scan rate, respectively *(18)*. Some other dynamic parameters, such as $i_{pc}/\div v$ vs v, i_{pa}/i_{pc} vs v, and $|E_{pa} - E_{pc}|$ vs v, were also calculated and are listed in Table 1. No large difference of the cathodic peak current against the square root of the scan rate was observed (Table 1), which suggested that the charge transfer process in the VIO monolayer was diffusion controlled *(19)*. According to the cyclic voltammogram curve for the VIO-immobilized electrode in the electrolyte solution with a scanning rate of 100 mV/s, the surface coverage (G) of the viologen derivative was about 1.5×10^{-10} mol/cm^2. Tang et al. *(20)* prepared a self-assembled monolayer of a disulfide derivative of VIO on a gold surface and estimated that the saturated surface coverage was about 1.8×10^{-10} mol/cm^2. In addition, we have measured the pressure-area isotherms for monolayers of some VIO derivatives at the air/water interface, indicating that the area of a flat orientation of a VIO group was about 150 – 155 Å^2 *(21)*, corresponding to a saturated surface coverage of a VIO group of about 1.1×10^{-10} mol/cm^2. These results indicate that the VIO-derivatized ITO surface is saturated and covered by one layer of the VIO with an almost flat orientation to the electrode surface.

Several articles have described the splitting of redox peaks, especially for the $VIO^{2+} \rightarrow VIO^{+}$, and attributed it, e.g., the existence of two different conformations and the formation of radical dimers *(10,22)*. We found that the splitting in the ITO-VIO monolayer was very small and hardly distinguished after 10 cycles as shown in Fig. 1. This may be ascribed to the fact that two short alkyl chains ($CH_2 = 3$) with the Si atoms were tightly attached to the ITO electrode surface, resulting in difficulty in forming radical dimers. However, the interactions between VIO parents and reduced species were still rather strong, so the cyclic voltammogram peaks measured were broadened *(10)*.

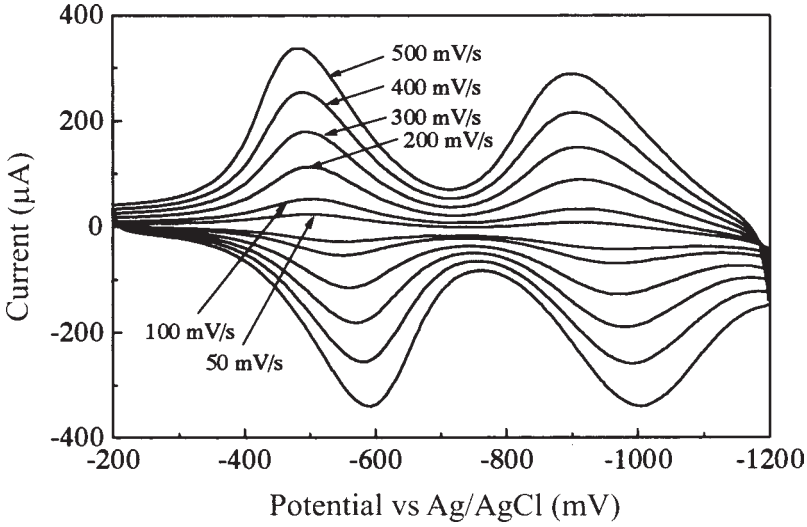


Fig. 1. Cyclic voltammograms of the ITO-VIO monolayer at various potential scan rates.

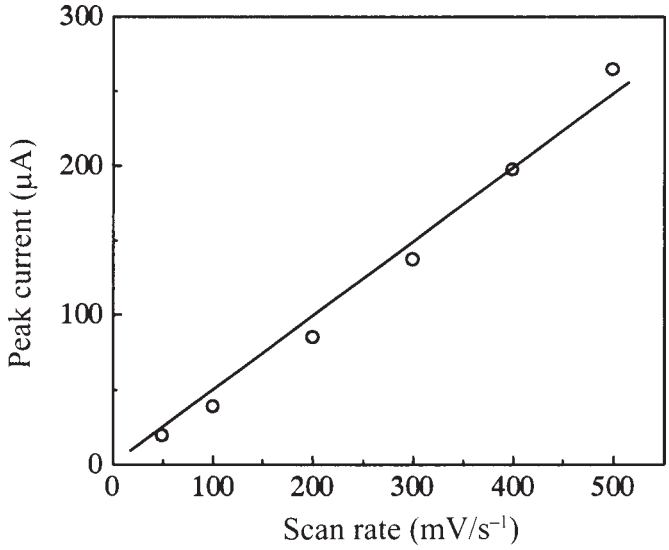


Fig. 2. Dependence of redox peak current on the potential scan rate for ITO-VIO monolayer.

Figure 3 shows the absorption spectra of the ITO-VIO redox systems taken with the electrode potentiostated at –540 mV vs Ag/AgCl in 0.1 mol/L of $NaClO_4$ solution using a "naked" ITO electrode as a reference. Compared with the naked ITO electrode, reduction of the ITO-VIO electrode was accompanied by a color change to purple *(17)*. The reduction reaction was very fast and reached an equilibrium of $VIO^{2+} \leftrightarrow VIO^{+}$ within 20 s. This made the ITO-VIO electrode surface become "electroplated" $[(VIO^{+})_n]_{surf}$.

Table 1
Dynamic Parameters for ITO-VIO Monolayer in 0.1 mol/L of $NaClO_4$ Solution

v (mV/s)	$i_{pc}/v^{1/2}$	i_{pa}/i_{pc}	$\lvert E_{pa}-E_{pc} \rvert$
50	56	1.26	40
100	42	1.18	42
200	40	1.08	56
300	43	1.04	74
400	46	1.02	92
500	47	1.01	106

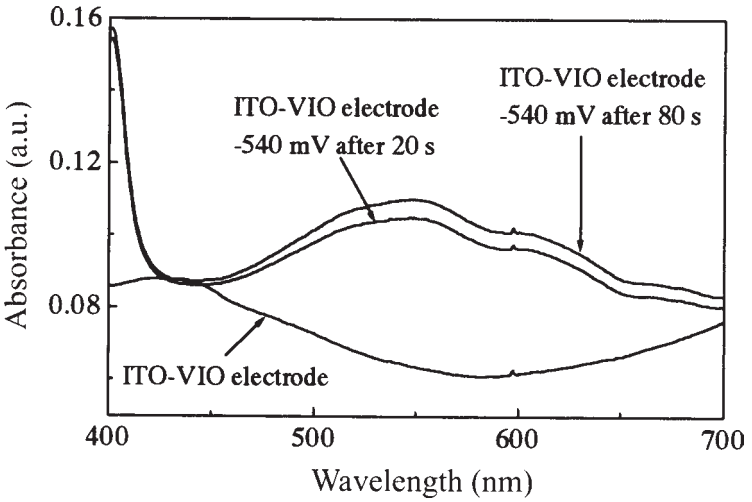


Fig. 3. Absorption spectra of the ITO-VIO monolayer taken with the electrode potentiostated at –540 mV vs Ag/AgCl in 0.1 mol/L of $NaClO_4$ solution.

Deposition of Hydrogenase onto ITO-VIO Substrate

Stable monolayer of hydrogenase could form on a PLL subphase surface in about 1 h after spreading *(8)*. The barriers were reopened after one layer of hydrogenase deposited, but then required another hour for continuous deposition. The transfer ratio for the first down deposition was nearly 0, and the second up about 1. This result means that the deposited hydrogenase Langmuir-Blodgett multilayer was of a Z-type structure *(23)*. Although it is very difficult to describe the structure of the hydrogenase Langmuir-Blodgett film, a simple schematic model is suggested in Fig. 4. In this case, two layers of hydrogenase were deposited with the hydrophilic part toward the substrate.

Electrochemistry of Hydrogenase Langmuir-Blodgett Film

The electrochemical property of the ITO-VIO hydrogenase Langmuir-Blodgett film was measured under the same condition as that for the ITO-

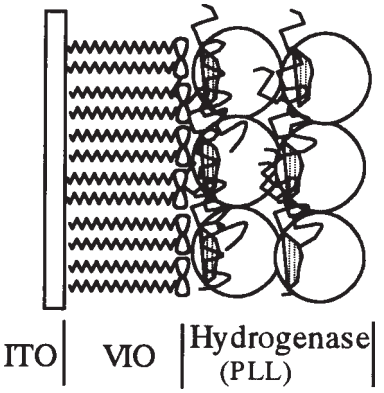


Fig. 4. Possible arrangement of hydrogenase Langmuir-Blodgett film on ITO-VIO substrate.

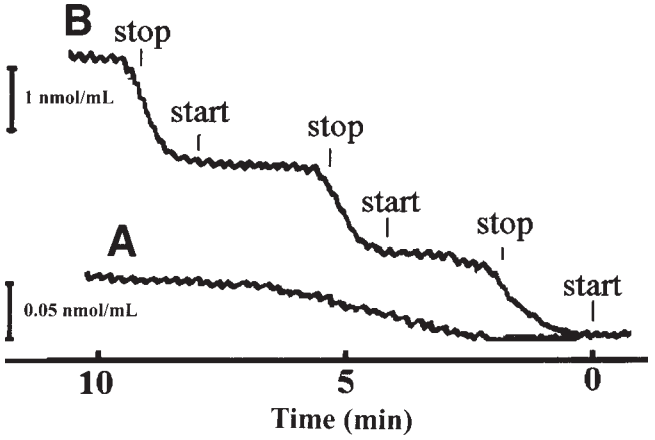


Fig. 5. Electrochemical hydrogen evolution for an ITO-VIO hydrogenase heterogeneous multilayer potentiostated with a bias of –540 mV vs Ag/AgCl in 0.1 mol/L of $NaClO_4$ solution (a) and containing $Na_2S_2O_4$ (b).

VIO monolayer. It was difficult to distinguish the redox characters of hydrogenase from the cyclic voltammogram curve because the redox current of VIO was very strong. However, the cathodic peak of VIO was observed to shift to a low position after the hydrogenase deposition. This may be attributed to the fact that the hydrogenase layer hinders the access of the supporting electrolyte to VIO.

Electrochemical Hydrogen Evolution

An ITO-VIO electrode covered with two layers of hydrogenase was used for the measurement of the electrochemical hydrogen evolution. As a contrast, we also measured a naked ITO electrode with the hydrogenase in electrolyte solution and the electrodes without hydrogenase Langmuir-Blodgett films. The results are shown in Fig. 5. For a naked ITO electrode

in hydrogenase solution with bias potentials from –500 to –650 mV vs Ag/AgCl, hydrogen evolution was not detected. It was also difficult to detect the hydrogen evolution using an ITO-VIO electrode with hydrogenase in electrolyte solution. Only when using a hydrogenase-covered ITO-VIO electrode could, the hydrogen evolution be measured (*see* Fig. 5a). The hydrogen concentration was about 0.05 nmol/mL under a bias of –540 mV for 10 min. That the rate levels off after 5 min may be owing to the hydrogen reaching its equilibrium pressure. In this case, if we added a little sodium dithionite as a sensitizer, the hydrogen evolution accelerated, and if the concentration of the sensitizer was further increased (about 0.01 mol/L), large amount of hydrogen evolution could be measured (*see* Fig. 5b). Furthermore, the hydrogen evolution was found to depend closely on the bias applied, i.e., with a measurable bias hydrogen evolution. Otherwise, a very weak signal was detected.

In comparison with our previous result for hydrogenase monolayer directly deposited on the electrode, the concentration of hydrogen produced was similar *(8)*. This similarity indicates that the hydrogenase covered on the VIO monolayer or on the electrode dominates the yield of hydrogen. A low efficiency of the access of hydrogenase to the reduced VIO surface ($[(VIO^{+})_n]_{surf}$) was considered for the hydrogenase in the solution, which led to the yield of hydrogen evolution being too low to be measured. This means that only the hydrogenase directly contacted or interacted with $[(VIO^{+})_n]_{surf}$ can accept electron and catalyze hydrogen generation. Kovacs and Der *(4)* have reported on the hydrogen evolution in the systems of surfactant, lipids, and amphiphilic VIO mediators with dithionite and hydrogenase, and suggested that the amphiphilic VIO was the most efficient electron mediator in their investigation. We found that a higher efficiency for hydrogen evolution could be measured by potentiostating the VIO-hydrogenase-covered ITO electrode to electroplate $[(VIO^{+})_n]_{surf}$ as well as by using an electrolyte solution containing 0.01 mol/L of sodium dithionite. In addition to the previously mentioned advantages of chemically modified electrodes, there are two more advantages of using immobilized VIO monolayer as the electron mediator. The first is that a reduced VIO enhances the electron transfer rate between electrode/donor (dithionite) and hydrogenase; this may be considered to provide an optimum condition for electrons transferring from both electrode and sodium dithionite to hydrogenase. The second advantaged is that a VIO in the electrode could overcome several limits of those in buffer solution, in which electrode and VIO are easily affected by some ions *(3)*. Thus, a modified ITO-VIO hydrogenase heterogeneous system could play an important role in the generation of electrochemical hydrogen.

It is considered that the main steps for electrons transferring from the electrode to hydrogenase are as follows: electrode (or donor) to oxidation VIO VIO^{2+}, $VIO^{2+} \rightarrow VIO^{+}$, and reduced VIO to hydrogenase. In the present study, we found that the amount of hydrogen evolution was determined by the hydrogenase attached to the reduced VIOs. Thus, the last step may be

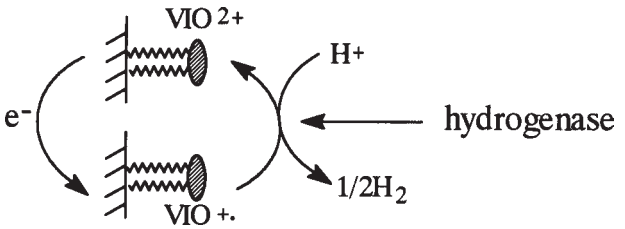


Fig. 6. Reduction mechanism for an ITO-VIO hydrogenase heterogeneous system.

a controlled one because an affinity for mediator to hydrogenase is important for hydrogen evolution *(24)*. A schematic model for the electron transfer process is suggested in Fig. 6. When a sensitizer (electron donor) was added in electrolyte solution, the electron transfer efficiency was assumed to be largely enhanced, a phenomenon that deserves further investigation.

Conclusion

The monolayer of a hydrogenase could be stabilized on a PLL (mer 20) subphase surface and transferred onto a VIO-derivatized ITO substrate by Langmuir-Blodgett method to form a heterogeneous multilayer. The well-reversible redox reaction of VIO-derivatized ITO electrode makes it possible for VIO to be an effective mediator for electrons transferring from electrode/donor to hydrogenase. The assembly of an ITO-VIO hydrogenase heterogeneous system may be a useful route for the electrochemical hydrogen evolution.

Acknowledgment

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