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Intracellular Voltammetry in a Single Protoplast with an Ultramicroelectrode

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Silver ring ultramicroelectrodes with tip diameter of 1 μm or less were fabricated by electroless plating in a glass capillary. The ultramicroelectrode was inserted in a living protoplast of Bryopsis plumosa and the oxygen concentration inside the cell was estimated from the steady-state current observed in a voltammogram.

Microelectrodes have been receiving growing interest in recent studies since they possess unique and advantageous properties.[1-3] One of the most important characteristics of microelectrodes is their capability to measure the electrochemical behavior in ultrasmall environments, especially in vivo systems. However, at present amperometric measurements using microelectrodes are restricted to a cell aggregation such as central nervous system[4] or giant cells,[5] because it is difficult to prepare the microelectrode which can be inserted into a single cell without any damage.

we describe here an fabrication method of Ag ring ultramicroelectrodes with tip diameter of submicrometers including the insulating sheath, and report the first demonstration of voltammetric measurements in a single cell of ordinary size (ca. 30 μm) using the electrodes.

Recently, Ewing and coworkers[6-8] fabricated carbonring ultramicroelectrodes with tip diameter of ca. 1 μm by pyrolysis of hydrocarbon. We have developed a simple method to prepare metal electrodes of submicrometer size using electroless plating of Ag inside a glass capillary. This method is suitable for preparation of ultramicroelectrodes wiht low tip impedance for intracellular voltammetry, because very thin glass capillaries with tip diameters of submicrometers are easily prepared. The process is as follows: A pyrex glass tube (1 mm O.D.) was cleaned in nitric acid and rinsed with water. The clean tube was then pulled with a capillary puller (NARISHIGE, Model PD-5). The inside of the glass capillary was sensitized in an 0.1% (w/v) $SnCl_2$ solution containing 1% (v/v) HCl, followed by rinsing with water, then activated in an 0.01% (w/v) $PdCl_2$ solution containing 1% (v/v) HCl. This treatment was repeated two or three times. The glass capillary thus obtained was washed in water and dried in an electric hearth at 150 °C for 10 min. Ag was deposited inside the glass capillary by electroless plating, so-called the silver mirror reaction.[9] The plating solution (1:1 mixture of 24% (w/v) $AgNO_3$ 9% ammonia water and

14% (w/v) sodium tartrate solution) was incessantly pushed into the tip of the capillary by using a microinjector. During the plating the tip was immersed in a stirring hot water (60-70 °C) to restrict the plating length inside a capillary and to avoid plating outside the capillary. The tip with an Ag ring thus formed was dried and the inside of the tip was filled with an epoxy resin (Nisshin EM, Spurr Low-Viscosity Embedding Media). Finally the cross section of the tip was polished on a turn table (Narishige EG-3).

A two-electrode configuration without a potentiostat was employed for the electrochemical measurement to avoid system noise. A saturated calomel electrode (SCE) was used as the counter electrode as well as reference electrode. Small currents were measured with a high speed current amplifier (Keithley Model 427). Micromovements of the electrode were accomplished by means of a three-dimensional micro-manipulator system (NARISHIGE MO-188) under a microscope (Nikon Diaphot-TMD). The radius of the Ag ring (r) deposited in a glass capillary was determined from the steady-state current (i_{ss}) for air-saturated oxygen in a voltammogram using the following equation;[6]

$$i_{ss} = 5.8\ nFCDr \qquad (1)$$

where n is the number of electron transferred, F the Faraday constant, C the concentration, and D the diffusion coefficient. Protoplast of Bryopsis plumosa was fabricated by the method reported in the literature.[10]

Figure 1 shows a typical scanning electron micrograph (SEM) of an Ag ring ultramicroelectrode. The tip diameter of this ultramicroelectrode is ca. 1.2 μm. The thickness of the Ag ring formed between the glass capillary and the core epoxy is ca. 100 nm. The Ag ring ultramicroelectrode with tip diameter of submicrometers have also been fabricated by the electroless plating method. However, because of the difficulty in obtaining clear SEM images as pointed out by Ewing and coworkers,[6] the image is not shown here. Figure 2

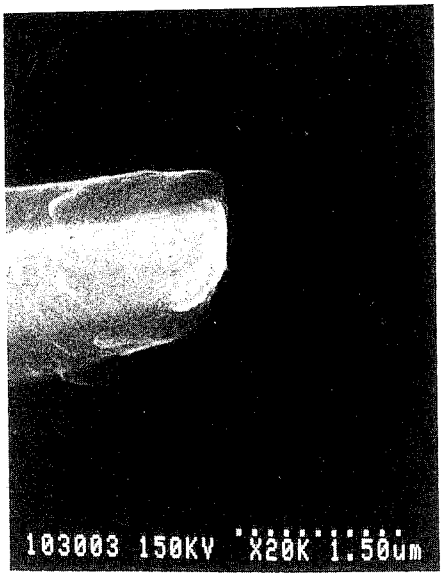

Fig. 1. SEM image of an Ag ultramicroring electrode fabrecated by the metal deposition method.

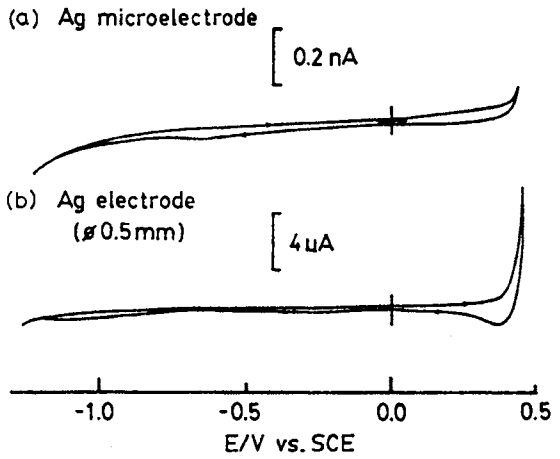

Fig. 2. Cyclic voltammograms at an Ag ultramicroelectrode with ring diameter of 1.5 μm (a) and at a Ag disk electrode with diameter of 0.5 mm (b) in 0.1 M KNO_3. Scan rate : 50 mV s^{-1}.

shows typical background voltammograms observed at an Ag ring ultramicroelectrode in an 0.1 M KNO_3 solution, together with that at an Ag disk electrode of usual size (diameter; 0.5 mm). Similarity of the two voltammograms suggests that the Ag ring ultramicroelectrode can be used for electrochemical measurements. The voltammograms at Ag ring ultramicroelectrodes were stable and no detectable change was observed at least for several hours.

We have used the Ag ring ultramicroelectrode for estimation of oxygen concentration in a single living cell. Dissolved dioxygen plays important roles in many biological reactions such as metabolic and electron transfer processes. Therefore, sensing of oxygen in a single cell is important to clarify the intracellular biological reactions. Oxygen reduction at Ag electrodes is known to proceed via two two-electron steps to form H_2O in neutral solutions.[11] The intermediate is H_2O_2. Therefore, a small amount of Pt, a catalyst for the reduction of oxygen to water, was electrodeposited on the surface of the Ag ring ultramicroelectrode. This procedure eliminates the formation of H_2O_2 which is poisonous to a living cell; in addition, the magnitude of the reduction current increases due to the four-electron process. The Pt deposition was carried out at -0.4 V vs SCE for 30 s in a 1.0 mM $PtCl_6^{4-}$ solution.

The Pt/Ag ultramicroelectrode was inserted into a single cell, a plant protoplast of Bryopsis (Fig. 3). The diameter of this protoplast is ca. 30 μm. Microscopic observation indicated that the diameter of this Pt/Ag ultramicroelectrode including the insulator sheath was ca. 1 μm. The Pt/Ag ring diameter of the electrode is estimated to be 0.72 μm by using Eq. 1. The insertion of the ultramicroelectrode into a protoplast caused no serious damage to the cell, because transformation or destruction of the cell membrane was not observed. Even after the voltammetric measurements, the protoplast was still living, exhibiting continuous growth. Figure 4 shows linear sweep voltammograms for oxygen in the single protoplast, together with that in the air-saturated sea water. The shapes of the two voltammograms are basically the same, indicating that the intracellular concentration of oxygen can be determined directly from the steady-state current observed at the Pt/Ag ultramicroelectrodes. In sea water, the steady-state current increases linearly with the partial pressure of oxygen. The concentration of oxygen in the cell was determined from the steady-state current of the intracellular voltammogram and found to be ca. 0.18 mM, which is similar to that of outer-cell sea water, 0.23 mM.[12] Since oxygen permeates easily the

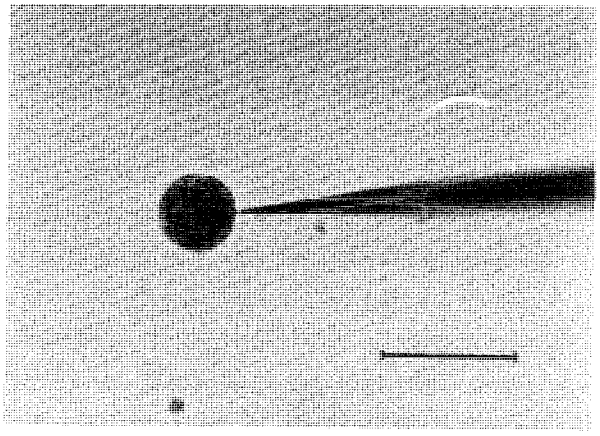

Fig. 3. Photograph of a protoplast of Bryopsis plumosa inserted with a Pt/Ag ultramicroelectrode. The bar indicates 50 μm.

cell membrane, the level of the steady-state current inside the cell is similar to that in the sea water under usual atmosphere. In this estimation, we have assumed that the diffusion coefficient of oxygen inside the cell is equal to that in the sea water, since most of the intracellular fluid consists of sea water.[10]

Further investigation, including the effect of light irradiation to cells and permeation of electroactive chemicals through the cell membrane, is now underway. The voltammetric measurements in a single cell will provide useful information to clarify the mystery of living materials.

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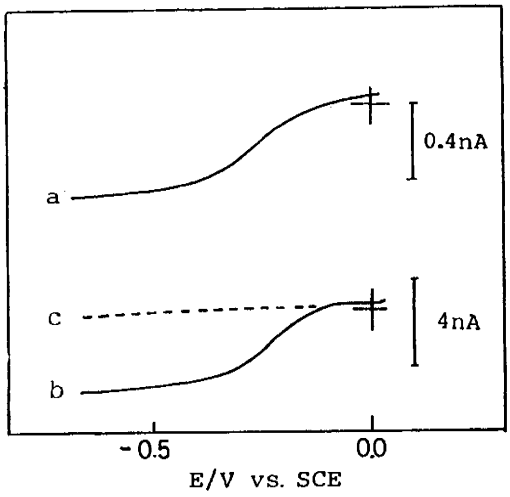

Fig. 4. Voltammograms at Pt/Ag ultramicroelectrodes with ring diameters of 0.72 μm (a) and 5.2 μm (b,c).
a : In a single protoplast placed in an air-saturated sea water.
b : In an air-saturated sea water.
c : In a nitrogen-saturated sea water.
Scan rate : 50 mV s^{-1}.

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