Imaging of Photosynthetic and Respiratory Activities of a Single Algal Protoplast by Scanning Electrochemical Microscopy

Tomoyuki Yasukawa, Takatoshi Kaya, and Tomokazu Matsue*
Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Sendai 980-8579

(Received June 8, 1999; CL-990485)

Scanning electrochemical microscopy (SECM) based on the reduction current for oxygen was used for imaging of respiratory and photosynthetic activities of single, living protoplasts. In the dark, the image of the protoplast appeared as a spot with lower oxygen concentration due to consumption of oxygen by respiration. Under light irradiation, the protoplast appeared as the opposite image because it generated oxygen by photosynthesis. SECM images clearly displayed a decrease in photosynthetic activity of the protoplast injected with 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU), a photosynthetic inhibitor.

Electron and ion transfers are deeply related with energy production and signal transmission in a living cell. Among various methods localized electrochemical measurements are particularly suitable for understanding these biological processes. Recently, new electrochemical methods have been developed for studying localized chemical and biological reactions with the progress of microtechnology. One of the new tools is scanning electrochemical microscopy (SECM)[1-5] which detects electrochemical processes occurring in localized areas. SECM affords the imaging information not only on topography, but also on electroactive species and reactions proceeding in localized spaces. SECM has been applied to investigate a single cellular activity.[6-8] Tsionsky and coworkers measured the topography of guard cells in a plant leaf and obtained images of the photosynthetic oxygen evolution from stomata with SECM.[7] Our group reported the SECM imaging of respiration activity of single cancer cells and the influence of cyanide on cellular activity.[8]

In this paper, we report the SECM imaging of the respiratory and photosynthetic activities of single protoplasts based on reduction current for oxygen. We have measured quantitatively the concentration of oxygen near the single protoplast under light irradiation with different intensity.[9, 10] Since oxygen is a key species in respiration and photosynthesis, SECM imaging based on oxygen reduction around single cells gives information an cellular activity at the single cell level. We also describe here the influence of 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU), a photosynthetic inhibitor, on the photosynthetic activity of single protoplasts.[11]

Tips for the SECM measurements were fabricated by the method reported previously.[10, 11] All measurements were done using a self-made SECM system. Details of the SECM setup and equipment used in this study have been described before.[12] A Pt microdisk electrode (Pt radius, 1.2 μm; tip radius, 3.0 μm) was used as a probe for the SECM measurements. The potential of the probe was set at -0.50 V vs. Ag/AgCl to detect localized oxygen reduction current. The reduction current for oxygen was converted into the concentration using a calibration line. For SECM imaging, the microdisk electrode was scanned two dimensionally above a protoplast sitting on a substrate at a speed of 9.8 μm/s in a constant height mode (height from the substrate, protoplast diameter +5-10 μm). The distance was determined the approach curves of the reduction current for oxygen using a motor-driven XYZ stage (LM-641-2AE1, Chuo Seiki) with a piezo actuator (17PAZ-3310, Melles Griot) and also by the optical microscopic measurements. The time required to obtain a image was about 15 min. The SECM measurement of respiratory activity was started about 20 min after the protoplast was prepared in the dark. The SECM measurement of photosynthetic activity of the same protoplast was carried out about 20 min after the SECM measurement of respiratory activity was finished. The photosynthetic activity was imaged under light irradiation (15 kLx). An aliquot (100 pL) of 5.0 μM DCMU solution was injected into a single protoplast using a microinjector (IM-26-2, Narishige). The amount of the injected DCMU is sufficient to inhibit the photosynthesis of the protoplast. The negative decadic logarithms of the concentrations needed for 50% inhibition (pI_{50}) value of DCMU for the protoplast is 6.9.[11] The measurement was carried out in a two-electrode system with a Ag/AgCl (saturated KCl) as a counter/reference electrode. Light irradiation for photosynthesis was provided by a halogen lamp attached to the microscopy. Light intensity was maintained at 15 kLx for all the photosynthetic measurement. All the measurements were performed at 25 °C in a shield box. DCMU were purchased from Wako Pure Chemicals (Osaka, Japan) and used without further purification. All the solutions were prepared from distilled and deionized water by AQUARIUS GS-200 (Advantec) and Milli-Q Jr. (Millipore). Protoplasts were made from marine alga *Bryopsis plumosa* by the method reported previously[13] in a synthetic artificial sea water containing 480.2 mM NaCl, 2.3 mM $NaHCO_3$, 11.1 mM $CaCl_2$ and 83.3 mM $MgCl_2$.

SECM images were obtained based on the oxygen reduction current at the microdisk electrode. Figure 1 shows SECM images of a single protoplast in the dark (a) and under light irradiation (b). The dark area with low reduction current in the dark coincided with the location of the protoplast in an optical microscopic image. The oxygen concentration around the protoplast decreases because the cellular respiration consumes oxygen. Thus, the

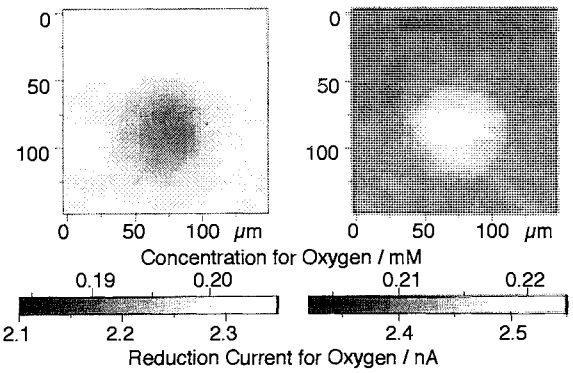

Figure 1. SECM images of the respiration (a) and the photosynthesis (b) of the same single protoplast. Imaging is based on the reduction current for oxygen. Tip radius, 1.2 μm; Protoplast radius, 25 μm; Pixel size, 5 x 5 μm.

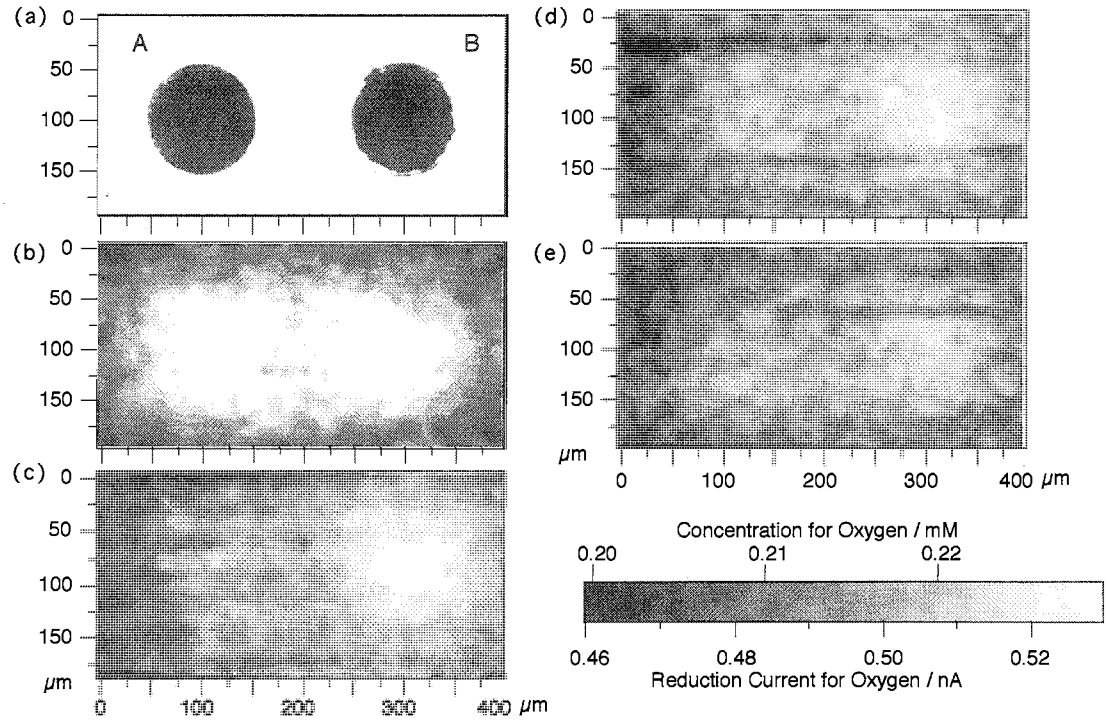

Figure 2. (a) Optical microscopic and SECM images of the photosynthesis of the two protoplasts (A and B) (b) before and (c) 20, (d) 40 and (e) 60 min after a 100 pL of 5.0 μM DCMU was injected into the protoplast A. Tip radius, 1.2 μm; Protoplasts radii, 50 μm; Pixel size, 10 x 10 μm.

image in the dark indicates the respiratory activity of the protoplast. When the light was irradiated the protoplast appeared as a area with large reduction current in the SECM image (Figure 1b) due to the photosynthetic evolution of oxygen from the illuminated protoplast. The above results suggest that the images of the respiratory and photosynthetic activities of the same single cell can be obtained from the SECM image based on the oxygen reduction current in the dark and under light irradiation.

Measurements of living cells by SECM are effective to investigate the influence of chemical stimulation at individual cells. Figure 2 shows SECM images of the photosynthetic activity of two protoplasts (A and B) before and after (20, 40 and 60 min) a 100 pL of 5.0 μM DCMU, a photosynthetic electron-transfer inhibitor, was injected into the protoplast A. Before the injection the two protoplasts (radius, 50 μm) equivalently emit an oxygen by photosynthesis (Figure 2a). After the injection, the SECM image of the protoplast A becomes gloomy and deformed (Figure 2b, 2c and 2d). DCMU inhibits the photosynthetic electron transport and, as a consequence, lowers the generation of oxygen from the protoplast A in the time scale of the measurements. The SECM images of the protoplast B also becomes gloomy and finally disappears although DCMU was not injected into the protoplast B. This fact suggests that DCMU in protoplast A permeates the membrane and diffuses into protoplast B to reduce the photosynthetic activity of protoplast B.

In conclusion, SECM imaging affords the information on respiratory and photosynthetic activities of single, living plant cells, although it is not suitable for monitoring rapid responses. The present procedure can be widely applied for detailed investigation of cellular responses to chemical and physical stimulations and also to the screening cells in cellular engineering.

This work was partly supported by Grants-in-Aid for Scientific Research (10145104 and 11450323) from the Ministry of Education, Science and Culture, Japan. T. Y. acknowledges a research fellowship from the Japan Society for the Promotion of Science.

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