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Electrocatalytic Photolysis of Water at Photosystem II-Modified Carbon Paste Electrode Containing Dimethylbenzoquinone

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Photosystem II-enriched membranes from spinach chloroplasts were immobilized on the surface of a carbon paste electrode containing 2,6-dimethylbenzoquinone. The PS II membrane-modified electrode showed photocurrent response due to the photolysis of water, in which dimethylbenzoquinone served as an electron acceptor for the PS II reaction and as a mediator of electron transfer between the PS II membrane and the electrode.

The coupling of electron transfer between electrodes and redox proteins has recently attracted increasing interest.[1-3] Enzymic reactions of oxidoreductases such as glucose oxidase could be coupled to the electrode reactions of small molecules serving as electron transfer mediators, thus the electrode could respond specifically to the substrate.[2,3] Mediated amperometric enzyme electrodes rely upon this principle of current response to the substrates.[3,4,] The use of small redox molecules as mediators may also be effective to couple electron transfer between electrodes and multiprotein complexes composing functional units in biological membranes. Here, we show an example of such electron transfer coupling, the coupling of water photolysis taking place in photosystem II (PS II) membranes from chloroplast thylakoids to the electrode reaction of 2,6-dimethyl benzoquinone (DMQ).

PS II-enriched membranes prepared from spinach chloroplasts by the method of Kuwabara and Murata [5] were suspended in 50 mM (1 mM = 10^{-3} mol/dm^3) TRIS-HCl containing 0.4 M sucrose and 10 mM NaCl(pH 7.6). Oxygen evolution by PS II membranes using DMQ as an electron acceptor was assayed with a Clark-type oxygen electrode (Hansatech, England). Chlorophyll was determined by the method of Arnon.[6] Carbon paste electrodes containing DMQ was prepared by the same method described previously.[4] The geometrical surface area of the electrode was 9×10^{-2} cm^2. PS II membranes (5 µg of Chl in 20 µl, activity: 260 µmol O_2 mg Chl^{-1} h^{-1}) was syringed onto the electrode surface and the solvent was allowed to evaporate in the dark. Subsequently the electrode was covered with a 20-µm thick dialysis membrane (Union Carbide Co., No. 20/23) which was fixed with a nylon net covering it. The electrodes prepared in this way are referred to as PS II-DMQ-CPEs in this paper. A PS II-DMQ-CPE was inserted into a cell from the bottom, and the cell was filled with 10 ml of 50 mM TRIS-HCl containing 50 mM NaCl(pH 7.6). A Luggin capillary connected to an Ag/AgCl reference electrode and a platinum coil serving

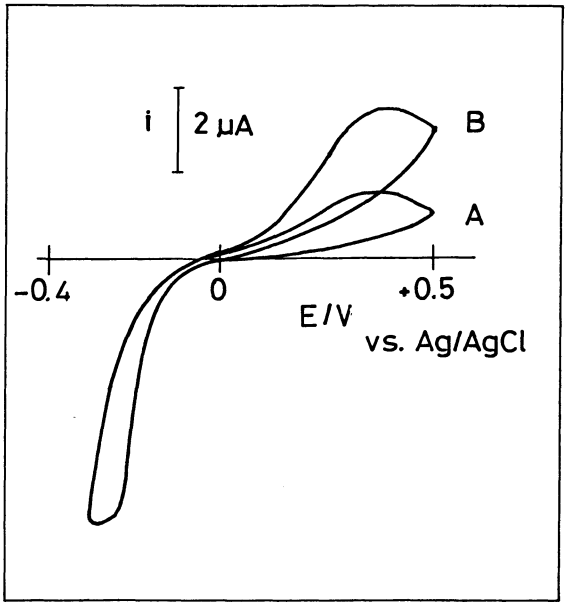

Fig. 1. Cyclic voltammograms of a PS II-DMQ-CPE recorded in the 50 mM Tris containing 50 mM NaCl (pH 7.6) (A) in the dark and (B) under illumination at 100% light intensity (0.76 mW/cm^2). Potential scan rate v = 50 mV/s.

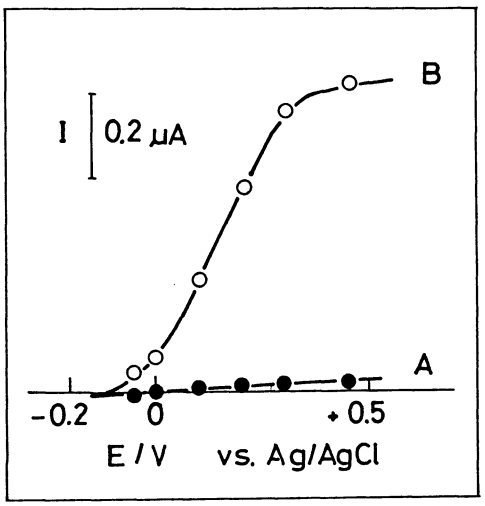

Fig. 2. Dependence of the steady-state currents on the potential applied to the electrode. The currents were measured (A) in the dark and (B) under illumination at 50% light intensity.

as a counter electrode were immersed in the electrolyte solution which was deaerated by passing nitrogen gas. The electrode was illuminated from the top by a visible light conveyed from a 300 W projector through a Toshiba VY-44 filter, sheets of gauze, and a water-filled flask. The light intensity was 0.76 mW/cm^2 at the position of the horizontal plane of the working electrode as measured by a thermocouple (Kip and Zonen, Holland). This light intensity is described as 100%, and when necessary, the light intensity was diminished by neutral density filters. Photoelectrochemical measurements were done at ambient temperature (23 - 25° C).

Figure 1A shows a cyclic voltammogram recorded with a PS II-DMQ-CPE in the dark. The voltammetric waves observed in Fig. 1A are attributable to the redox reaction of DMQ, since such waves were not observed with a bare carbon paste electrode with immobilized PS II membranes (an electrode not containing DMQ). Detailed investigation of the voltammetric behavior showed[7] that the waves in Fig. 1A are due to the redox reaction of DMQ trapped in the PS II-membrane layer between the carbon-paste electrode and the dialysis membrane. It has been shown[2,4] that poorly soluble redox compounds can be effectively entrapped in a layer of proteins behind a dialysis membrane on a carbon-paste electrode when the compounds are mixed with the carbon paste. By the illumination of the electrode the anodic wave was increased (Fig. 1B). A PS II membrane- modified electrode not containing DMQ showed no observable current response to the illumination. Steady-state anodic currents, I, were observed when measured at fixed electrode potentials, E, under illumination (Fig. 2B). I started to appear at -0.05 V and increased with increasing positive potential to approach a limiting current, I_l, at +0.3 V. In the dark, no appreciable steady-state currents were observed (Fig.

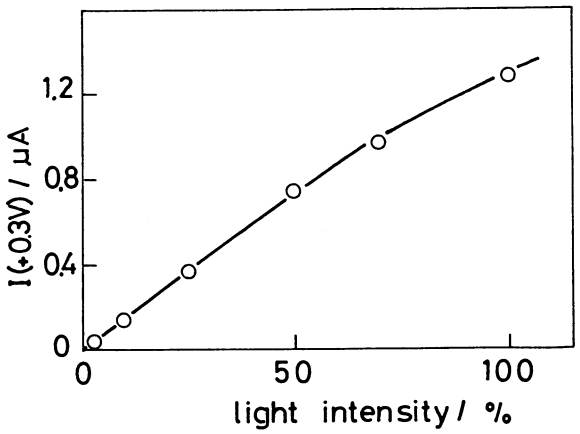

Fig. 3. Dependence of the steady-state current measured at +0.3 V on the light intensity.

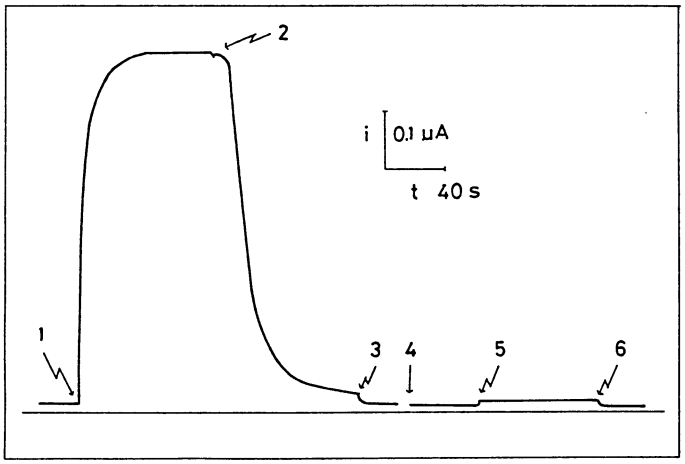

Fig. 4. Effects of DCMU on the photocurrent response. The currents were measured at +0.3 V and the 50% light intensity. At 1, the light was turned on; 2, DCMU added; 3, the light turned off; 4, the solution changed (no DCMU); 5, the light turned on and 6, turned off.

2A). I_1 increased almost linearly with increasing light intensity in a range tested (Fig. 3). The results indicate that PS II membranes immobilized on the surface of the carbon paste electrode remain active and that DMQ trapped in the immobilized PS II membrane layer can mediate the electron transfer between the immobilized PS II membranes and the carbon paste electrode. Dichlorophenyldimethylurea (DCMU) binds irreversibly to Q_B binding site in the PS II complex, causing an inhibition of the electron flow from Q_B site in the PS II complex to an electron acceptor.[8] When DCMU was added to the electrolyte solution to make 20 μM, a photocurrent measured at 0.3 V decreased to almost zero (Fig. 4). The original photocurrent response could not be restored even when the electrolyte solution was exchanged (Fig. 4). The results are consistent with the above indication. The overall reaction for the electron transfer of PS II to DMQ may be written by Scheme 1. When the light is turned on, charge separation in the

$$H_2O + DMQ \xrightarrow[\text{(PS II membranes)}]{h\nu} (1/2)O_2 + DMQH_2 \qquad 1$$

reaction center of PS II occurs producing $p680^+$ and the electrons are donated to DMQ through Q_A and Q_B of the PS II complex. On the other hand, $p680^+$ abstracts the electrons from water, resulting in the oxygen evolution. The reduced form of DMQ ($DMQH_2$) produced by the PS II reaction diffuses to the electrode surface in the immobilized PS II membrane layer and is oxidized at the electrode to regenerate DMQ (Scheme 2), yielding the anodic current. The rather slow current response

$$DMQH_2 \xrightarrow[\text{(electrode)}]{-2e} DMQ + 2H^+ \qquad 2$$

observed with the PS II-DMQ-CPE (Fig. 4) may be attributable to a relatively slow diffusion rate of DMQ and $DMQH_2$ within the immobilized PS II membrane layer. If we assume a homogeneous distribution of the PS II complexes in the immobilized PS II

membrane layer and unit density for the PS II membranes, we may evaluate the thickness of the immobilized PS II membrane layer to be about 3 μm based upon the applied amount of PS II membranes (5 μg Chl/0.09 cm^2) and the contents of Chl (17%, w/w) calculated from the dry weight of the PS II membranes. DMQ and $DMQH_2$ should diffuse in this range of thickness of the immobilized PS II membrane layer to shuttle between the Q_B binding site of the PS II complexes and the carbon paste electrode.

In the PS II-DMQ-CPE, the carbon paste electrode acts as a sink of electrons and does not directly take part in the charge separation process. This is contrasted with photosynthetic components-modified SnO_2 electrodes,[9,10] in which the SnO_2 electrode itself plays a role in the charge separation process. The PS II-DMQ-CPEs produced relatively large photocurrents. This is because a larger amount of PS II complexes compared to that in the monolayer level can take part in the reaction by the electron transfer mediation of DMQ. There have been many papers[11-13] dealing with photocurrent generation at electrodes with thylakoid components and chromatophores immobilized on them or suspended in the solution from the view point of the efficient energy conversion. Our electrodes are applicable to investigations in this direction.

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