Light-Induced Electron-Transfer Reaction into *Rs. rubrum* Chromatophores from a Pt Electrode

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A platinum electrode immersed in a chromatophore suspension brought about, upon illumination, a cathodic photocurrent under potentiostatic conditions. The photocurrent action spectrum coincided fairly well with the absorption one of a chromatophore suspension, showing maxima at 600, 800, and 875 nm. The photocurrent increased with an increasing cathodic shift of the applied potential up to about −500 mV; two successive waves were observed on the photocurrent. The mechanism for the generation of the photocurrent is discussed.

Serious environmental problems, including air pollution with NO_x and SO_x and the release of vast amounts of CO_2 into atmosphere, limit the utility of fossil fuels. The development of solar energy technology has therefore been desired, while promising a substantial future contribution to growing energy demands. The primary processes of photosynthesis are the most efficient to convert solar energy to chemical energy and, consequently, could possibly be used as the basis for modified photosynthetic processes applicable in solar energy conversion.[1] Supporting this viewpoint, many studies[2] have demonstrated the possibility of employing photoactive biological components in photoelectrochemical cells, in connection with studies on the mechanism of photosynthetic primary processes as well as solar energy conversion projects. We have been studying photoelectrochemical behavior using a photosynthetic apparatus, chromatophores, of photosynthetic bacterium, *Rhodospirillum rubrum,* at a platinum electrode.[3,4] Still, questions regarding the electron-transfer mechanism between chromatophores and the electrode remain unanswered. The present paper deals with a further detailed photoresponse of chromatophores at a platinum electrode and with a plausible mechanism to account for the photoresponse.

Experimental

A carotenoid-less blue-green mutant strain (G-9) of *Rs. rubrum* was used throughout the present study. The cells were grown anaerobically at 30 °C for 3 days under continuous illumination from tungsten lamps, as described previously.[5] Chromatophores were prepared from light-grown cells by sonication, followed by centrifugation.[6] Chromatophores, thus prepared, were suspended in a 0.12 M (M=mol dm^{-3}) GTA buffer comprising an equimolar mixture of 3,3-dimethylglutaric acid, tris(hydroxymethyl)methanamine, and 2-amino-2-methyl-1,3-propanediol, which was adjusted to various pH values by adding HCl or NaOH. The concentration of chromatophores is expressed in terms of the absorbance of bound bacteriochlorophyll at 873 nm (A_{873}; corresponding to 7.1 μM of bound bacteriochlorophyll[7]). An experimental cell[4] with a light path length of 0.4 mm comprised a platinum plate working electrode (2×3 cm^2 of surface area), a platinum wire counter electrode and a saturated calomel reference electrode. All of the potentials listed in this paper were corrected against a normal hydrogen electrode. For a potentiostatic measurement, the potential of the electrode was controlled with a Hokuto Model potentiostat (HA-104 or HA-501), and the resulting current was recorded. The photocurrent was measured after electrolyzing for 30 min in the dark at a certain potential, while approaching a steady state of electrolysis. The light source was a 300 W projector lamp, usually used in combination with a 12 cm thick water filter in order to eliminate any thermal effects (a 500 W xenon arc lamp (Wacom Model MX-500) was used in combination with a JASCO Model CT-25N grating monochromator equipped with a cut-off filter (UV-39 or VR-69) for measuring the photocurrent action spectrum). The intensity of monochromatic light was measured with a calibrated thermopile (Model 404 of Spectra-Physics). Ten units·ml^{-1} of glucose oxidase, 2 mM of glucose and 13 units·ml^{-1} of catalase were added to the suspension to remove any dissolved oxygen; oxygen-free nitrogen was passed through the suspension for 30 min before electrolysis. The suspension was kept at 25 °C using a thermostat. The electrochemical cell, thus prepared, was placed in a dark box under a nitrogen atmosphere; it was shielded with a common ground against any electromagnetic perturbations.

Results and Discussion

A platinum electrode immersed in a chromatophore suspension brought about, upon illumination, a cathodic photocurrent (I_p) under potentiostatic conditions, as reported previously.[3,4] Although the magnitude of the cathodic photocurrent depended on the concentration of chromatophores, incident light intensity, and applied potential,[3] no anodic photocurrents could be observed, even under anodic polarization. This may be due to a structural anisotropy in the chromatophore membrane at which the functional positions of such electron-transport components as cytochromes, ubiquinone 10 and the reaction center complex are important in consideration of the transfer of electrons and protons within and across the membrane.

If electrons injected into the electron-transport components bound to the chromatophore membrane equilibrate with the electrode during the primary photoact, the action spectrum of the photocurrent should correspond to the absorption one of the chromatophore suspension. The photocurrent action spectra were mea-

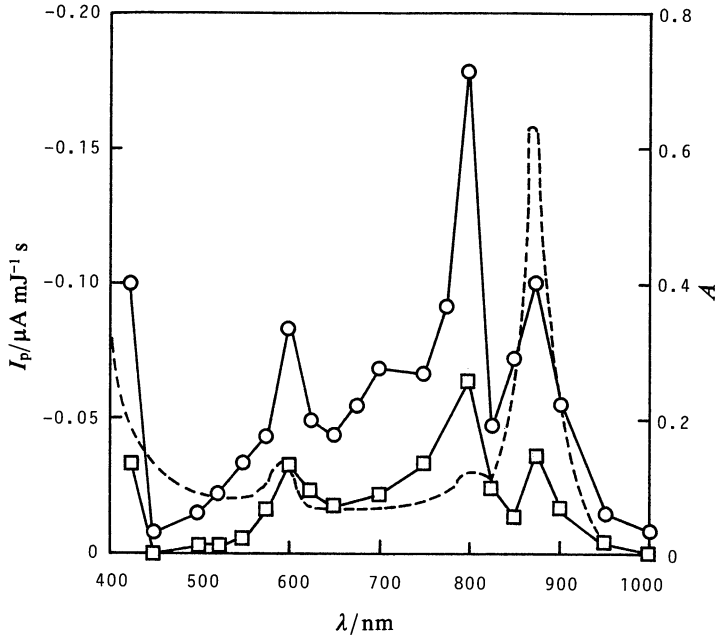

Fig. 1. Photocurrent action spectra (solid lines) and absorption spectrum of chromatophore suspension (dotted line). ○ and □ represent the values of photocurrent obtained at −500 mV and −100 mV of applied potentials, respectively. The concentration of chromatophores was kept constant at A_{873}=75.

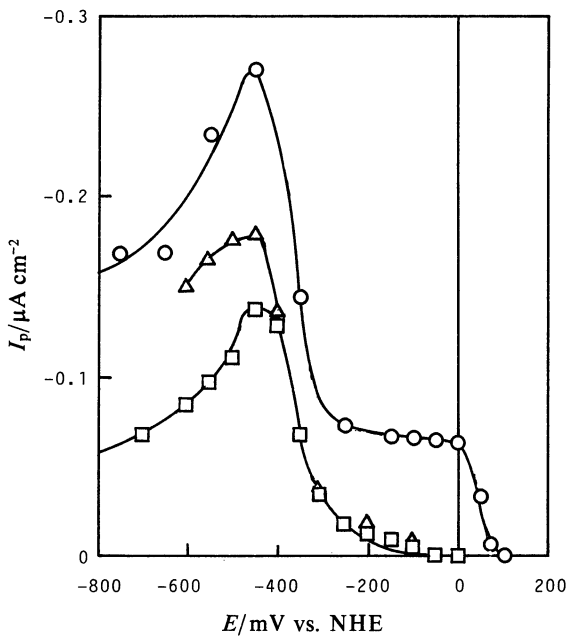

Fig. 2. Dependence of photocurrent on applied potential. ○, □, and △ represent the values obtained by native chromatophores, quinone-extracted ones, and reaction center complexes, respectively. The concentration of chromatophores and reaction centers were at A_{873}=75, and A_{802}=5, respectively. The light intensity was kept at 6000 lx of illuminance.

sured at low light intensity (1—10 J m^{-2}s^{-1}) and at different electrode potentials. The results are shown in Fig. 1, together with the absorption spectrum of the chromatophore suspension. It is evident that both the photocurrent action spectra and that for absorption coincide fairly well, showing maxima at 600, 800, and 875 nm assigned to chromatophore-bound bacteriochlorophyll absorption (588, 804, and 873 nm).[8] This result suggests that the bound bacteriochlorophyll, especially the reaction center bacteriochlorophyll called P870, assumes some roles in the generation of the cathodic photocurrent.

In order to specify the electrochemically active species bound to chromatophores the behavior of the photocurrent was examined in some detail against the applied potential under potentiostatic conditions at various pH values. A typical result at pH 8.0 is shown in Fig. 2. In this figure the cathodic photocurrent increases with the more cathodic shift of the applied potential up to about −500 mV, and then decreases. The decrease in the photocurrent in a more cathodic region than about −500 mV may be due to competitive hydrogen evolution resulting from the electrolysis of water, as well as an irreversible reduction of the chromatophore membrane.[9] In addition, two successive waves were observed on the cathodic photocurrent and two potential onsets of the waves were +100 and −275 mV, respectively.

Earlier, Higuti et al.[10] found that 90—95% of ubiquinone 10 and rhodoquinone are easily extractable from lyophilized chromatophores with 2,2,4-trimethylpentane; the various activities, thus depressed, are restored by adding extracts or ubiquinone 10 to the extracted chromatophores. The photocurrent generated by quinone-extracted chromatophores was lowered to about 45% or less than the original level, as also shown in Fig. 2. The effect of extracting ubiquinone 10 was especially larger in the more positive potential region, and the photocurrent became hardly observable. In addition, the voltammetric behavior of ubiquinone 10 bound to chromatophores has been reported,[9] and the redox potential at pH 8.0 was estimated to be +20 mV. These facts indicate a possibility that the first photocurrent-generating component is ubiquinone 10 bound to the chromatophores.

The chromatophore membrane can be easily disintegrated with *N*,*N*-dimethyldodecylamine *N*-oxide; a reaction center complex was purified by repeating molecular-sieve and ion-exchange chromatographies for the disintegrated membrane.[11] When the photocurrent was measured for the reaction center complex, thus prepared, the potential dependence of the cathodic photocurrent was similar to the more negative wave of the photocurrent generated by chromatophores. This result is also shown in Fig. 2. It therefore seems reasonable that the second photocurrent-generating component is one of the photooxidized constituents localized on the outside of the reaction center complex. It is generally accepted that in chromatophores the majority of bacte-

riochlorophyll molecules are harvesters of light quanta and the remainder are functional in driving the electron-transport system (reaction center bacteriochlorophyll, P870). Earlier, Okayama et al.[12] distinguished the reaction center bacteriochlorophyll into two different kinds regarding functions, Liac-860 (E_{m7}=450 mV) and Liac-890 (E_{m7}<−100 mV). In addition, we[9] have reported that chromatophores bound two reactable components on a voltammetric electrode; one is ubiquinone 10 and the other was a component named POC_{-170}, corresponding to Liac-890. It therefore seems presumable that the photooxidized constituent may correspond to a reaction center bacteriochlorophyll, Liac-890. However, it has been indicated by immunological experiments[13] that the reaction center bacteriochlorophyll should be situated at or near the periplasmic aspect of the chromatophore membrane (inside of chromatophore vesicle). It may thus be difficult for the P870 to be directly reduced on the electrode, due to the insulation of the redox center from the electrode by the structure protein. It is now being attempted to specify which of the extrinsic constituents takes role in the generation of a second cathodic photocurrent; more details regarding the photoelectrochemical behavior of reaction center complexes will be reported elsewhere.

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