Effect of 1-Methoxy-5-methylphenazinium Methyl Sulfate on Photocurrent Produced by *Rhodospirillum rubrum* Chromatophores

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(Received April 22, 1987)

Synopsis. A cathodic photocurrent produced by chromatophores was measured in the presence of 1-methoxy-5-methylphenazinium methyl sulfate (methoxy-PMS). The magnitude of the photocurrent was a function of the wavelength of the incident light, and the photocurrent action spectrum was in agreement with the absorption spectrum of chromatophores. The photocurrent also depended on the concentration of methoxy-PMS or of chromatophores.

Many studies have demonstrated the possibility of employing photoactive biological components in photoelectrochemical cells, in connection with studies regarding the mechanism of photosynthetic primary process as well as solar energy conversion projects. From this viewpoint, we[1] reported that a cathodic photocurrent was produced, upon illumination, at a platinum electrode immersed in a chromatophore suspension under potentiostatic conditions. This phenomenon was largely enhanced by adding 1-methoxy-5-methylphenazinium methyl sulfate (methoxy-PMS) to the suspension. Although an enhancement of the photocurrent was presumed, that methoxy-PMS functioned as a mediator between the electrode and chromatophore particles, a further discussion was not given because of insufficient data. This paper reports on subsequent measurements of the photocurrent in the presence of methoxy-PMS.

Experimental

The carotenoid-less blue-green mutant strain (G-9) of *Rhodospirillum rubrum* was used throughout the present study. The cells were grown anaerobically at 30 °C for 3 days under continuous illumination from tungsten lamps.[2] Chromatophores were prepared from light-grown cells by sonication,[3] suspended in a 0.12 M (M=mol dm^{-3}) GTA buffer (pH 8.0) which consisted of an equimolar mixture of 3,3-dimethylglutaric acid, tris(hydroxymethyl)aminomethane and 2-amino-2-methyl-1,3-propanediol, and stored at 4 °C in the dark. The concentration of chromatophores was expressed in terms of absorbance of bound bacteriochlorophyll at 873 nm (A_{873}; corresponding to 7.1 μM of bound bacteriochlorophyll[4]).

A schematic diagram of a photoelectrochemical measurement is shown in Fig. 1. The experimental cell, in which the light path length was 0.4 mm, consisted of a platinum plate working electrode (2×3 cm^2 of surface area), a platinum wire counter electrode and a saturated calomel reference electrode. All potentials listed in this paper are corrected against the normal hydrogen electrode. For a potentiostatic measurement, the potential of the electrode was controlled with a Hokuto Model potentiostat HA-101 or HA-501 and the resulting current was recorded. A photocurrent was measured after electrolyzing for 30 minutes in the dark at an arbitrary potential. As the light source, a 60 W tungsten lamp was usually used in combination with a 12 cm thick water filter in order to eliminate the thermal effect; a 500 W

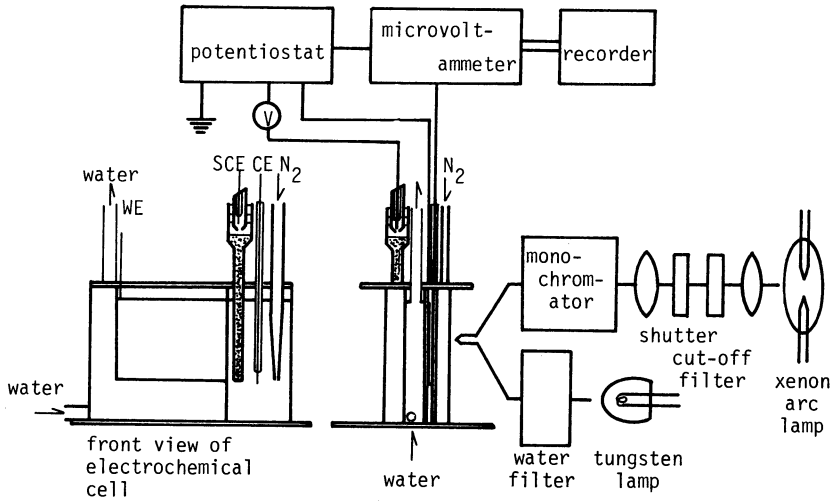

Fig. 1. Electrolytic cell arrangement for measuring photocurrent. The experimental cell was all made of borosilicate glass. A cellulose membrane divides between main compartment (chromatophore suspension) and subcompartment (buffer solution). WE, working electrode; CE, counter electrode; SCE, saturated calomel reference electrode.

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. 10 Units ml^{-1} of glucose oxidase, 2 mM of glucose and 13 units/ml of catalase were added to the suspension in order to remove dissolved oxygen and oxygen-free nitrogen was passed through into the suspension for 30 minutes. The suspension was kept constant at 25°C with a help of a thermostat. Methoxy-PMS was synthesized as described.[5,6]

Results and Discussion

When the cathodic photocurrent by chromatophores was measured in the presence of methoxy-PMS, it was largely enhanced.[1] The photocurrent increased with a greater cathodic shift of the applied potential up to about −200 mV and then gradually decreased. Since methoxy-PMS showed a large enhancing effect of the photocurrent, the behavior of the photocurrent in the presence of methoxy-PMS was examined in some detail. The magnitude of the cathodic photocurrent was a function of the wavelength of the incident light. The resulting action spectrum is shown in Fig. 2. There are three maxima (600, 800, and 875 nm) in agreement with the maxima of the absorption spectrum (588, 804, and 873 nm, ascribed to chromatophore-bound bacteriochlorophyll[7]) of the chromatophore suspension, and not with that of methoxy-PMS solution (508 nm). This suggests that the enhanced photocurrent is independent of the photoexcitation of methoxy-PMS: that is, the photocurrent is not due to a light-induced electron transfer from the electrode to methoxy-PMS, and the photocurrent is actually due to the light absorbed by chromatophores.

The effect of the concentration change of methoxy-PMS or of chromatophores on the photocurrent is shown in Fig. 3. In this figure, a linear relationship can be observed between the photocurrent and the concentration of methoxy-PMS or of chromatophores. If

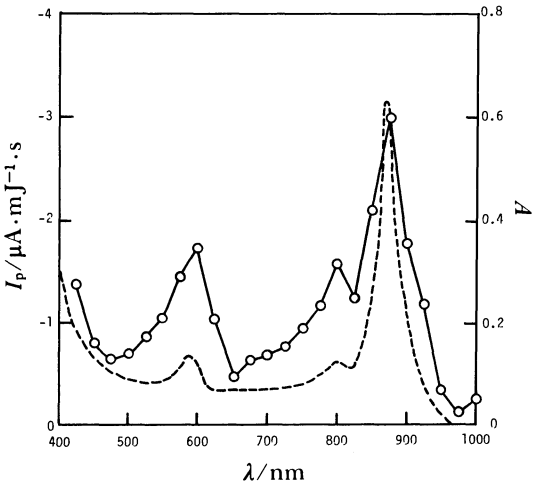

Fig. 2. Comparison of photocurrent action spectrum with absorption spectrum. The concentrations of chromatophores and methoxy-PMS were kept constant at A_{873}=75 and 100 μM, respectively. The photocurrent was measured at −200 mV of applied potential. The dotted line shows the absorption spectrum of chromatophore suspension.

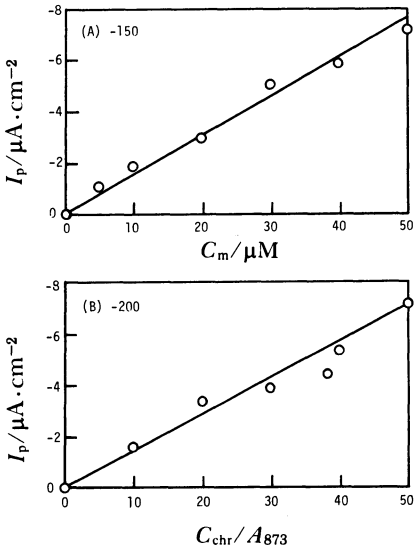

Fig. 3. Dependence of photocurrent on concentration of methoxy-PMS (A) or of chromatophores (B). Numbers in the figure represent the applied potential by mV. The concentration of chromatophores (A) and that of methoxy-PMS (B) were kept constant at A_{873}=75 and at 100 μM, respectively. The illumination was carried out at 6000 lx of illuminance.

the enhancing effect of the photocurrent is based on the ability of methoxy-PMS as the electron mediator,[1] the concept of a catalytic current[8] can be applied to an analysis of the reaction. According to the concept of a reaction layer as a maximal approximation for the treatment of the catalytic current, the enhanced photocurrent is

$$I_p = nF\mu k_f C_{chr} C_m,$$

where I_p, C_{chr}, and C_m are the enhanced photocurrent, bulk concentration of chromatophores and that of methoxy-PMS, respectively. Thus, I_p comes to be proportional to C_{chr} or C_m. As shown in Fig. 3, the result that linear relationship was observed between the photocurrent and the concentration of chromatophores or methoxy-PMS suggests that the methoxy-PMS function as the mediator between the platinum electrode and chromatophore particle, as expected. Moreover, the photocurrent increased with an increasing concentration of the reduced form of methoxy-PMS.[1] This suggests that the main reactant with chromatophores in the production of photocurrent is the reduced form of methoxy-PMS. This suggestion is supported by a study[9] on flash photolysis and electron paramagnetic resonance, in which an electron transfer from PMS in reduced form to reaction center bacteriochlorophyll (BChl) cation was observed.

In conclusion, it has become clear that the photocurrent produced by chromatophores at a platinum electrode under potentiostatic conditions is greatly enhanced in the presence of methoxy-PMS, and that the enhancing effect is based on the ability of methoxy-PMS as an electron mediator. A possible mechanism for the production of photocurrent is shown below.

$$\text{BChl}\cdot\text{I}\cdot\text{Q}\cdot\text{Fe} \xrightarrow{h\nu} \text{BChl}^{*}\cdot\text{I}\cdot\text{Q}\cdot\text{Fe} \longrightarrow \text{BChl}^{+}\cdot\text{I}\cdot\text{Q}^{-}\cdot\text{Fe}\ (\text{in chromatophores}^{10})$$

$$\text{BChl}^{+}\cdot\text{I}\cdot\text{Q}^{-}\cdot\text{Fe} + \text{methoxy-PMS (reduced)} \longrightarrow \text{BChl}\cdot\text{I}\cdot\text{Q}^{-}\cdot\text{Fe} + \text{methoxy-PMS (oxidized)}$$

$$\text{methoxy-PMS (oxidized)} + \text{e} \longrightarrow \text{methoxy-PMS (reduced) (production of photocurrent)}$$

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