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Short communication

Ionophore-sensitive spectral shift of carotenoid induced by ferricyanide in chromatophores from *Rhodopseudomonas sphaeroides*

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In chromatophores from *Rhodopseudomonas sphaeroides*, ferricyanide induced a change in the absorption spectrum of carotenoid. The ionophore-sensitive part of the ferricyanide-induced change was similar to that induced by light or by diffusion potential. The ferricyanide-induced change is explained by the electrochromic shift of the carotenoid spectrum by the inside-positive electrical field change which is probably caused by the electrogenic electron flow from a membrane redox component to ferricyanide in the outer aqueous phase. The ionophore-insensitive part is probably the response of the carotenoid in another pool to the local field change by oxidation of bacteriochlorophyll [Okada, M. and A. Takamiya (1970) *Plant & Cell Physiol.* 11: 713–721].

Key words: Bacterial photosynthesis — Carotenoid band shift — Chromatophores — Electrogenic electron flow — Membrane potential — *Rhodopseudomonas sphaeroides*.

The changes in membrane potential accompanying the redox reactions have been studied to clarify the coupling mechanisms of electron flow and proton transport in photosynthetic membranes of bacteria. The spectral shift of carotenoid can serve as a good indicator of the intramembrane electrical field (*3*). Okada and Takamiya observed a ferricyanide-induced absorbance change of carotenoid, with a simultaneous oxidation of light-harvesting bacteriochlorophyll with a peak at 885 nm, in chromatophores from *Rhodopseudomonas sphaeroides* (*8*). However, the absorbance change was ionophore-insensitive and the peaks of the carotenoid change were 3 to 4 nm shorter than the peaks of the light-induced change. We studied the ferricyanide-induced carotenoid shift further and observed an ionophore-sensitive shift as well as the insensitive one.

R. sphaeroides cells were grown anaerobically under illumination as described previously (*4*). Chromatophores were prepared in 20 mM tricine-NaOH (pH 7.4), 20 mM K_2SO_4 and 5 mM $MgSO_4$ (*4*). Absorbance changes of carotenoid were measured as described previously (*5*, *6*).

Fig. 1 shows the time courses of carotenoid absorbance changes induced by the addition of ferricyanide. Most of the increase of the difference absorbance (523-

Abbreviation: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

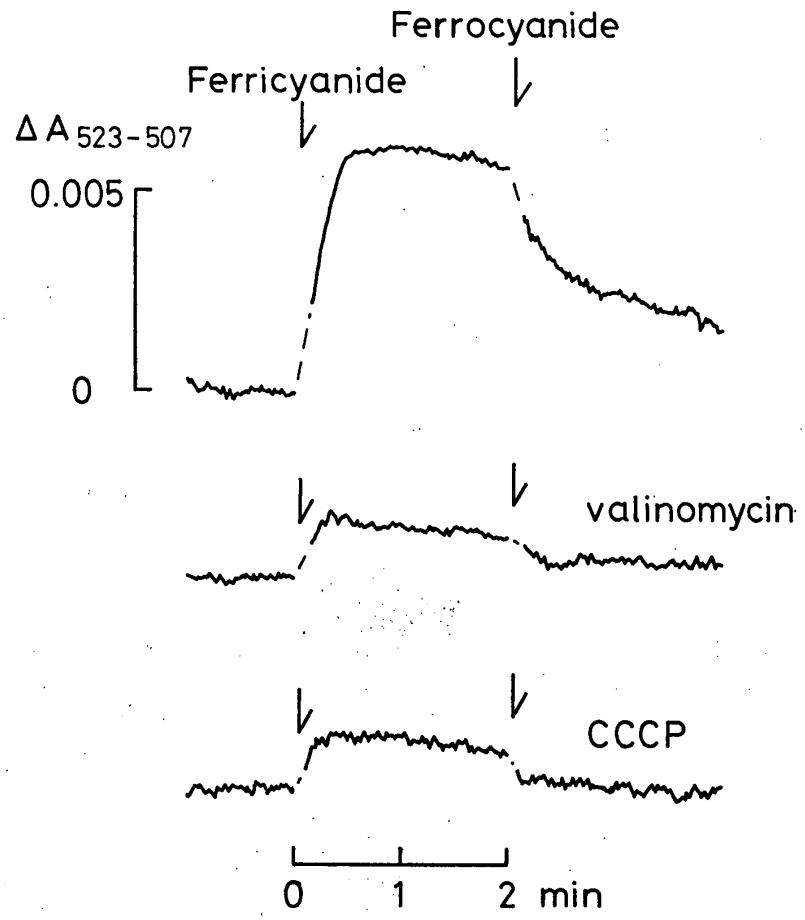

Fig. 1. *Absorbance changes of carotenoid induced by additions of ferricyanide and ferrocyanide.* Chromatophores (20 μM bacteriochlorophyll) were suspended in 20 mM tricine-NaOH (pH 7.4), 20 mM K_2SO_4 and 5 mM $MgSO_4$. The second and third traces were taken in the presence of 130 nM valinomycin and 1 μM CCCP, respectively. 0.1 mM potassium ferricyanide (with 0.33 μM ferrocyanide) and 0.1 mM potassium ferrocyanide were added.

minus-507 nm) by ferricyanide was reversed by the addition of ferrocyanide of the same concentration. The presence of valinomycin or CCCP decreased the extent of the ferricyanide-induced change. The extent of the remaining part was independent of the species of the ionophore. The ratio of the ionophore-sensitive change to the insensitive one, as well as their extents, varied with the concentration of ferricyanide added (data not shown). At lower ferricyanide concentrations, the ionophore-sensitive change was dominant. The concentrations used for the time courses shown, 0.1 mM ferricyanide with 0.33 μM ferrocyanide, gave a large ratio of the ionophore-sensitive fraction. The concentration dependence is probably caused, at least in part, by the partial reduction of ferricyanide with the endogenous reductants as indicated by the data of Fig. 3 in which higher concentrations of ferricyanide and ferrocyanide were used.

The spectrum of the ionophore-sensitive change was different from that of the insensitive one (Fig. 2). The ionophore-sensitive component had peaks at 523 and 509 nm. On the other hand, the ionophore-insensitive component had peaks at

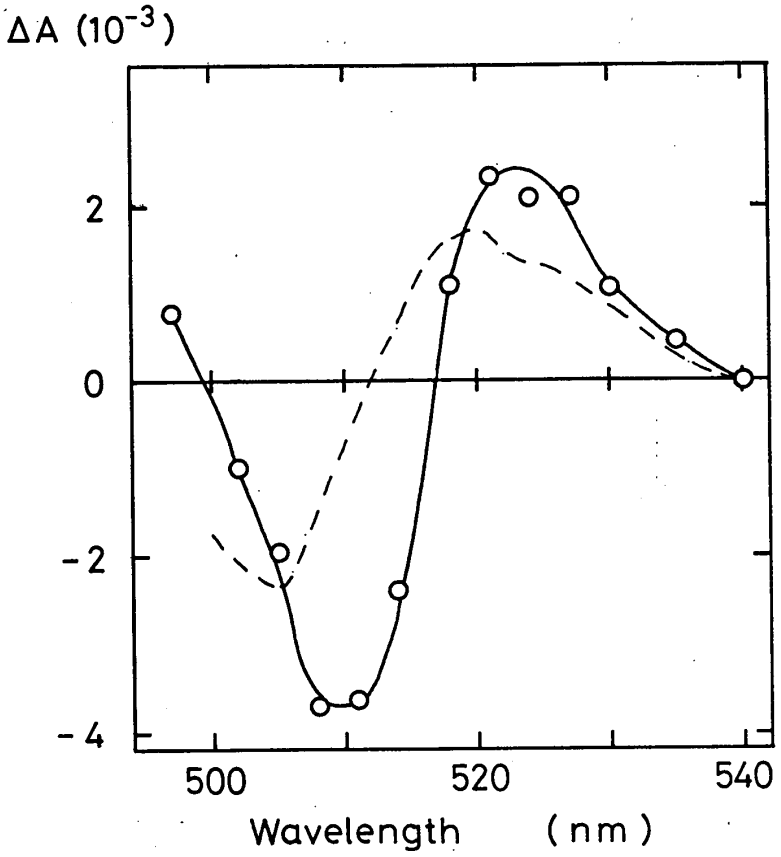

Fig. 2. *Spectra of ionophore-sensitive and -insensitive components of ferricyanide-induced change.* -O-: Difference between the ferricyanide-induced spectra in the presence and absence of valinomycin (no valinomycin *minus* 130 nM valinomycin). 0.1 mM ferricyanide (with 0.33 μM ferrocyanide) was added. ----: Change in the presence of 130 nM valinomycin, 0.5 mM ferricyanide was added. Other conditions were as those in Fig. 1.

520 and 505 nm, as observed by Okada and Takamiya (*8*). The difference can be explained by the presence of two types of carotenoid molecules in different states with different spectra. De Grooth and Amesz (*1*) and Symons et al. (*11*) analyzed the carotenoid absorption spectrum and showed the presence of two different states of carotenoid with a difference of about 5 nm in their absorption maxima. The carotenoid with shorter absorption maxima did not show the spectral shift by light or diffusion potential (*1*, *11*).

The ionophore-insensitive component of the ferricyanide-induced change must be caused by the shorter-wavelength carotenoid molecules. As discussed by Sewe and Reich (*10*), the ionophore-insensitive part probably reflected a change of the carotenoid-bacteriochlorophyll complex [in light-harvesting pigment-protein complex I (*4*)] by a local electrical field change accompanying the oxidation of the bacteriochlorophyll with a peak at 885 nm.

The ionophore-sensitive component, which reflected the delocalized field change, corresponded to the longer-wavelength carotenoid molecules, which are associated with the pigment protein complex II (*4*), as did the light-induced or diffusion-potential-induced carotenoid change. The ferricyanide-induced iono-

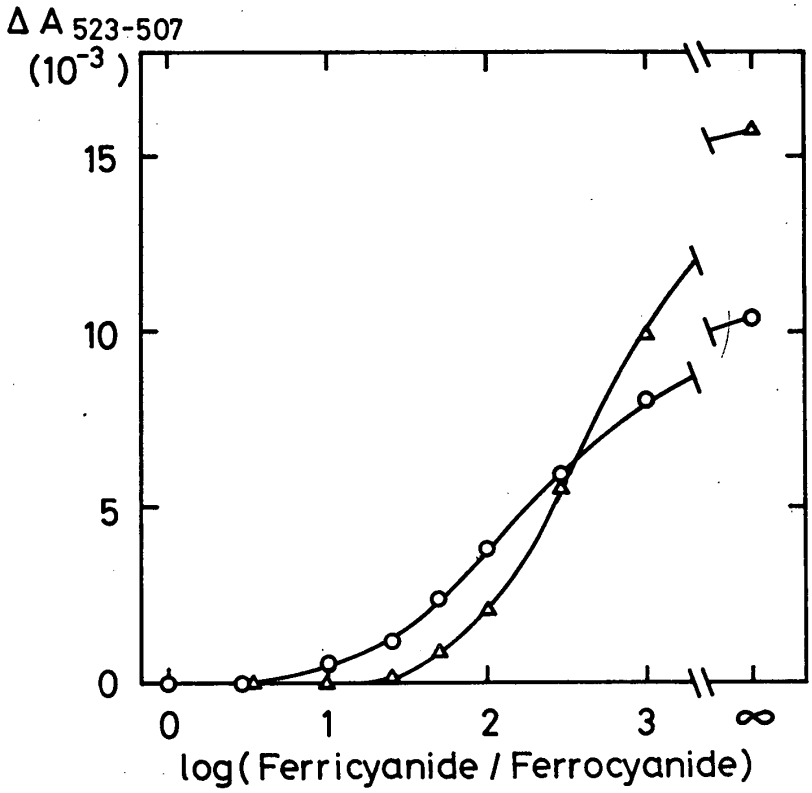

Fig. 3. *Dependence of the carotenoid absorbance change on ferricyanide-ferrocyanide ratio.* The carotenoid changes induced by adding 7 mM potassium ferricyanide/ferrocyanide were plotted. ○: Valinomycin-sensitive change. △: Valinomycin-insensitive change. Other conditions were as those in Fig. 1.

phore-sensitive red shift which corresponded to the inside-positive potential change (*5*), was calibrated to be about 60 mV of membrane-potential change at the maximum. The inside-positive field change was probably caused by the electrogenic electron flow from redox components in the membrane to ferricyanide in the outer aqueous phase. Such reactions can proceed quite rapidly (*7*).

Dependence on the ferricyanide-ferrocyanide ratio of the ionophore-sensitive and -insensitive components of the carotenoid change gave two distinctive curves (Fig. 3). Using 420 mV as the standard oxidation-reduction potential of ferri-ferrocyanide at neutral pH (*9*), the apparent midpoint potential was calculated to be about 550 mV for the ionophore-sensitive component. The value might have been somewhat underestimated because of the uncertainty of the full oxidation level. The midpoint potential for the ionophore-insensitive part was more positive. The midpoint potential of the ionophore-sensitive part was too high for a redox component of cyclic electron transfer. The midpoint potential of bacteriochlorophyll dimer in the reaction center, which is the highest among the components of the cyclic electron transfer, was reported to be 450 mV (*2*). Actually, an equimolar mixture of ferricyanide and ferrocyanide, which did not induce detectable carotenoid changes (Fig. 3), oxidized the reaction-center bacteriochlorophyll (data not shown). It is not clear whether the redox component responsible for the ferricyanide-induced ionophore-sensitive carotenoid change is a physiological electron carrier or not.

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