Biochimica et Biophysica Acta, 590 (1980) 339—344

BBA 47836

CORRELATION OF MEMBRANE-POTENTIAL-SENSING CAROTENOID TO PIGMENT-PROTEIN COMPLEX II IN *RHODOPSEUDOMONAS SPHAEROIDES*

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(Received July 23rd, 1979)

Key words: Membrane potential; Carotenoid band shift; Pigment-protein complex; Bacteriochlorophyll; Chromatophore; (Rps. sphaeroides)

Summary

The changes in carotenoid absorbance induced by illumination or by a diffusion potential were larger in chromatophores from cells cultured under low light intensity than those in chromatophores from high-light culture in a photosynthetic bacterium, *Rhodopseudomonas sphaeroides*. The carotenoid molecules which are associated with the pigment-protein complex (with the infrared bacteriochlorophyll peaks at 800 and 850 nm) (complex II) probably respond to the electrical field changes in the chromatophore membrane.

Introduction

The spectral shift of carotenoid has been used as a good indicator of membrane potential in photosynthetic membranes of certain bacteria [1,2]. It is generally accepted that the carotenoid molecules in the membrane respond directly to the changes of the electrical field in which the chromophore molecules are located [3—5]. Therefore, the location of the field-sensing carotenoid is important in analyzing the dynamic processes of energy transduction, including the translocation of electrons and ions. In *Rhodopseudomonas sphaeroides*, de Grooth and Amesz [3] and Symons et al. [4] showed the presence of two different states of carotenoid molecules in the membrane by spectral analysis. The wavelengths of the absorbance maxima differed by approx. 5 nm between the two states, and the carotenoid with the longer-

Abbreviation: Tricine, *N*-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine.

wavelength absorption showed the spectral shift due to light or to a diffusion potential.

Light-harvesting bacteriochlorophylls exist in two types of pigment-protein complexes in *Rps. sphaeroides* and a sibling species, *Rhodopseudomonas capsulata* [6—8]. Pigment-protein complex I contains bacteriochlorophyll with an infrared peak at approx. 870 nm, and pigment-protein complex II contains bacteriochlorophyll with peaks at approx. 800 and 850 nm. The ratio of complex I to complex II varies with growth conditions [6,7,9]. We studied the relationship between the field-sensing carotenoid molecules and the pigment-protein complexes.

Materials and Methods

Cells of *Rps. sphaeroides* were grown under two different conditions of light anaerobically in a nutrient medium [10] at 30°C. The culture bottles (5-cm thick) were illuminated under the white light from incandescent lamps at 15 000 lx and 2000 lx on the surfaces. Cells were harvested in late log phase at a turbidity of 1.2 (660 nm, 1 cm). Generation time was approx. 3.5 h in the high-light culture and approx. 6 h in the low-light culture. Chromatophores were prepared as described previously [10] in 20 mM Tricine-NaOH (pH 7.4), 100 mM NaCl and 5 mM $MgSO_4$. Bacteriochlorophyll concentration was measured in the acetone/methanol extract using the molar absorption coefficient of 76 $mM^{-1} \cdot cm^{-1}$ (at 767 nm) [11]. Concentrations of carotenoids, spheroidene and spheroidenone, were determined by using the method of Cohen-Bazire et al. [9]. Absorption spectra of chromatophores were measured with a Hitachi 124 spectrophotometer. Light- and diffusion-potential-induced absorbance changes were measured by a Hitachi 356 dual-wavelength spectrophotometer as described previously [12].

Results and Discussion

Fig. 1 compares the absorbance spectra of chromatophores from cells cultured under low and high light intensities, with the same bacteriochloro-

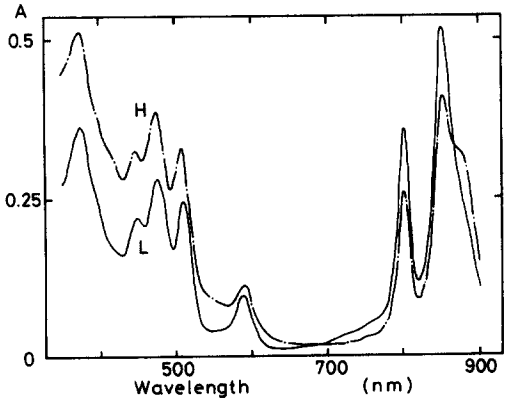

Fig. 1. Absorption spectra of chromatophores from high-light (H) and low-light cultures (L). Bacteriochlorophyll concentration was 5 μM. Culture conditions were as described in the text.

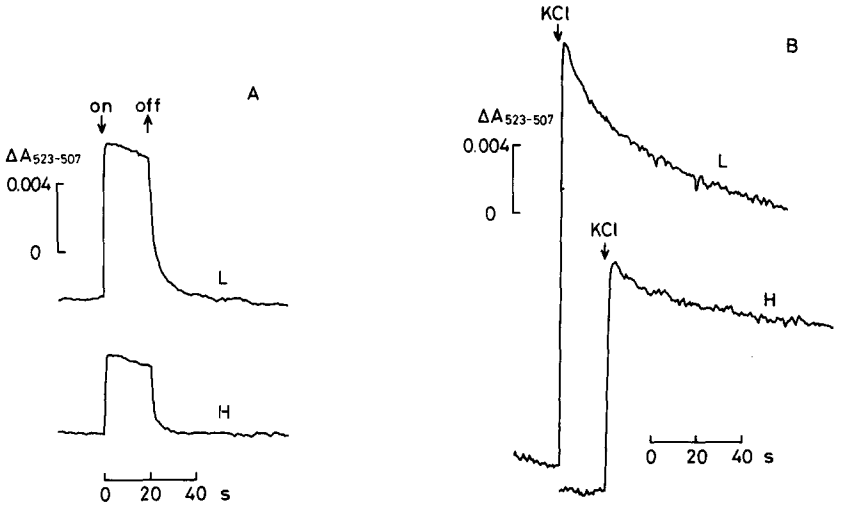

Fig. 2. Absorbance changes of carotenoid induced by light (A) and by diffusion potential of K^+ (B). Chromatophores from high-light (H) and low-light cultures (L) were suspended in 20 mM Tricine-NaOH (pH 7.4), 100 mM NaCl and 5 mM $MgSO_4$. 130 nM valinomycin were also present in the KCl-addition experiments. Bacteriochlorophyll concentrations were 5 μM in A and 15 μM in B. Infrared light was passed through a Wratten 88A filter and a 2-cm water layer (intensity 280 000 erg/cm^2 per s). KCl was added to make the final concentration 10 mM.

phyll concentration. In the near-infrared region, a shoulder at approx. 870 nm was more prominent in the high-light culture and peaks at 800 and 850 nm were higher in the low-light culture. These peaks correspond to the higher content of pigment-protein complex I in the high-light culture than that in the low-light culture. Peak wavelengths of carotenoid were also different in two preparations, 510, 477 and 449 nm in the low-light culture and 508,

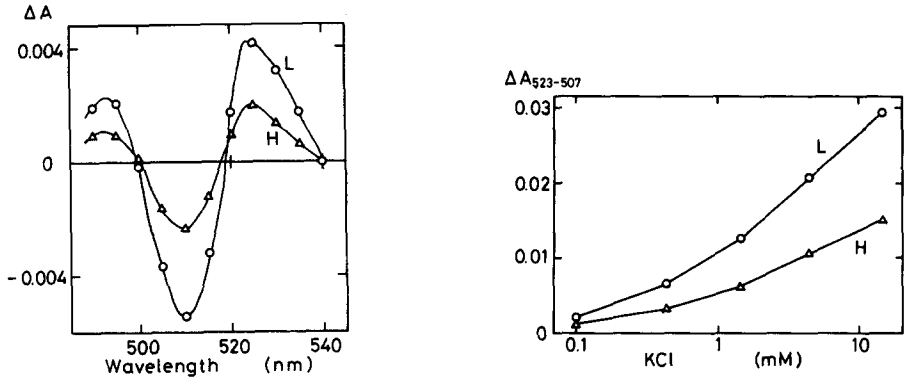

Fig. 3. Absorption spectrum changes caused by light in chromatophores from high-light (△) and low-light cultures (○). Changes 5 s after the start of illumination were measured in a series of experiments similar to those in Fig. 2A. Dual-wavelength measurements with a reference wavelength at 540 nm.

Fig. 4. Dependence of KCl-pulse-induced carotenoid change on final concentration of KCl added. KCl at various concentrations was added in a series of experiments similar to those in Fig. 2B in the presence of 130 nM valinomycin. Changes extrapolated to the time of addition were plotted. Bacteriochlorophyll concentration of chromatophores from high-light (△) and low-light cultures (○) was 15 μM.

TABLE I

EFFECTS OF LIGHT INTENSITY DURING CULTIVATION ON PIGMENT COMPOSITIONS AND CAROTENOID ABSORBANCE CHANGE OF CHROMATOPHORES

BChl represents bacteriochlorophyll. Contents of pigment-protein complex II were estimated from the absorbance at 800 nm in chromatophores (Fig. 1). $\Delta A_{523-507}$/mV was calculated using linear parts in Fig. 4, for optical path-length of 1 cm with the bacteriochlorophyll concentration of 15 μM. Light-induced $\Delta\psi$: illumination by an infrared light passed through a Wratten 88A filter and a 2-cm water layer (intensity 280 000 erg/cm^2 per s). The values 5 s after the start of illumination in the traces of Fig. 2 were used.

Light intensity during growth	BChl/protein (nmol/mg)	Carotenoid/BChl (molar ratio)	Spheroidene/BChl (molar ratio)	Complex II/BChl relative	$\Delta A_{523-507}$/mV	$\Delta\psi$ mV
High (15 000 lx)	25	0.57	0.44	1	0.000 15	92
Low (2000 lx)	64	0.54	0.45	1.4	0.000 28	95

475 and 447 nm in the high-light culture. The slope of the baseline due to light-scattering, which was larger in chromatophores from high-light culture, cannot explain the difference of 2 nm. A difference in the ratio of two carotenoid pools [3,4] probably explains the difference in the peak wavelengths.

The extent of carotenoid absorbance changes induced by illumination and by KCl addition in the presence of valinomycin were different between two preparations when the bacteriochlorophyll concentrations were the same (Fig. 2). Either illumination or the KCl addition increased the difference in absorbance (523 minus 507 nm), which corresponds to a red shift of the carotenoid spectrum. The change in the difference in absorbance was about two times larger in chromatophores from low-light culture than in those from high-light culture. The spectra of the absorbance changes were similar in both preparations (Fig. 3). The decay curves after the KCl addition were also similar. Fig. 4 shows calibration of the difference in absorbance (523 minus 507 nm) induced by a diffusion potential with KCl addition in the presence of valinomycin [1,2]. The absorbance changes were linear with respect to the logarithm of KCl concentration above 1 mM in both preparations. The slope was steeper in low-light culture than in high-light culture.

The differences between chromatophores from high-light and low-light cultures are summarized in Table I. The bacteriochlorophyll/protein ratio was higher in low-light culture, but the carotenoid/bacteriochlorophyll ratio was similar in both preparations. The ratio of spheroidene, which is the major carotenoid in anaerobically grown *Rps. sphaeroides* [9,12,13] and is considered to respond to membrane potential change [3,14], to bacteriochlorophyll was also similar. The relative electrochromic response, $\Delta A_{523-507}$/mV, was 1.9 times larger in the low-light culture. The difference in $\Delta A_{523-507}$/mV, when bacteriochlorophyll concentration was the same, is explained by the difference in the proportions of the two carotenoid pools [3,4]. The high proportion of the longer-wavelength carotenoid as suggested in Fig. 1 is consistent with the large $\Delta A_{523-507}$/mV in the low-light culture. The correlation of the content of complex II to $\Delta A_{523-507}$/mV also suggests the association of field-sensing carotenoid to the pigment-protein complex II. Feick and Drews [8] reported the presence of carotenoid in the complex II preparation isolated from a mutant of *Rps. capsulata*. Okada and Takamiya [14] showed the correlation of the shorter-wavelength carotenoid to the light-harvesting bacteriochlorophyll with a peak at approx. 870 nm. Sewe and Reich [5] proposed that these two pigments may form a complex with proteins, the pigment-protein complex I. The observation and the interpretation are compatible with the present data which indicate that the longer-wavelength membrane-potential-sensing carotenoid is associated with complex II.

In Fig. 5, light-induced carotenoid changes are plotted against the absorbance at 800 nm from the data of different sets of experiments. In this figure, the carotenoid changes are compared at the same bacteriochlorophyll concentration (but with different values of absorbance at 800 nm). The extent of the carotenoid change was linear, with some scattering of data, with respect to the absorbance at 800 nm which reflects the amount of pigment-protein complex II. The light intensity during culture changed not only the ratio of complex I to complex II, but also the membrane organization as indicated

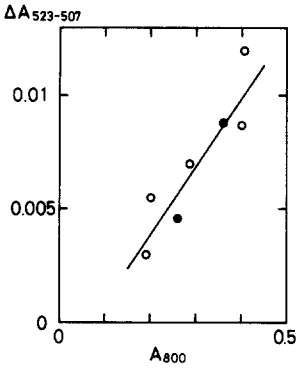

Fig. 5. Relationship between carotenoid absorbance change induced by light and the absorbance at 800 nm of various chromatophore preparations with the same bacteriochlorophyll content. Cells grown under low or high light intensity were harvested at various stages of culture. The carotenoid changes 5 s after the start of illumination as in the traces of Fig. 2 were plotted against the absorbance at 800 nm of the chromatophore suspension (5 μM bacteriochlorophyll). Closed circles are from results in Fig. 1 and Fig.2.

by the chlorophyll/total protein ratio (Table I) and the degree of light-scattering (Fig. 1) (see also Ref. 15). The membrane organization may also influence the extent of the carotenoid change. However, in a study with mutants lacking the pigment-protein complex I or II in *Rps. capsulata*, Zannoni et al. (Zannoni, D., Scolnik, P. and Marrs, B., personal communication) observed that a mutant without complex II lacked the carotenoid shift induced by light or by a K^+ diffusion potential. Their observation supports the conclusion that carotenoid molecules bound to the pigment-protein complex II have longer absorption peaks, and show a spectral shift responding to membrane potential.

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