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THE CAROTENOID BAND SHIFT IN REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES*

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SUMMARY

A specific carotenoid associated with reaction centers purified from *Rhodopseudomonas sphaeroides* shows an optical absorbance change in response to photochemical activity, at temperatures down to 35 K. The change corresponds to a bathochromic shift of 1 nm of each absorption band. The same change is induced by either chemical oxidation or photo-oxidation of reaction center bacteriochlorophyll (*P*-870). Reduction of the electron acceptor of the reaction center, either chemically or photochemically, does not cause a carotenoid absorbance change or modify a change already induced by oxidation of *P*-870. The change of the carotenoid spectrum can therefore be correlated with the appearance of positive charge in the reaction center. In these studies we observed that at 35 K the absorption band of reaction center bacteriochlorophyll near 600 nm exhibits a shoulder at 605 nm. The resolution into two components is more pronounced in the light-dark difference spectrum. This observation is consistent with our earlier finding, that the "special pair" of bacteriochlorophyll molecules that acts as photochemical electron donor has a dimer-like absorption spectrum in the near infrared.

INTRODUCTION

The spectral shift of carotenoid and (bacterio)chlorophyll absorption in certain photosynthetic bacteria and in chloroplasts (the "515 nm effect") has been interpreted as an electrochromic response of the photosynthetic pigments due to a light-induced electric field acting across the membrane. The fact that both bulk (bacterio) chlorophyll and the carotenoid molecules respond in the same manner, coupled with the effects of gramicidin and of valinomycin with K^+ in accelerating the decay of such shifts [1, 2], suggest that this electric field is delocalized over the membrane. However, different lines of study (different behavior between carotenoid and extrinsic dyes [3],

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and observations of carotenoid shifts at low temperature [4–6]) suggest that at least some of the carotenoid molecules may respond to a local field associated with the primary charge separation occurring in the reaction center. The reaction center particle prepared from wild type *Rhodopseudomonas sphaeroides* contains one specific carotenoid molecule [7, 8]. Therefore, the existence of a carotenoid band shift associated with a local field can be demonstrated with such material.

MATERIALS AND METHODS

R. sphaeroides, wild type strain 2.4.1, was grown as described earlier [9]. The method of preparing purified reaction centers was similar to that reported by Jolchine and Reiss-Husson [10]. The purity of the reaction center preparation was assayed by the absorbance ratio $A_{280\ nm}/A_{800\ nm}$, which was in the range 1.3–1.5 for different preparations (material considered to be pure shows a ratio of 1.2).

For measurements at low temperatures, reaction centers were mixed with 80 % glycerol and 20 % water (v/v) containing 0.01 M Tris/Cl, pH 7.5, and 0.1 % lauryl dimethyl amine oxide. Samples were mounted in a quartz cell, 2 mm light path, attached to the cold probe of a cryogenic refrigerator (Cryogenic Technology, Inc., Waltham, Mass.) capable of sustaining any temperature between 300 and approx. 35 K. The optical compartment had windows 2 inches (5 cm) in diameter on all sides, located 2 inches from the sample cell. This device could be placed in any of the absorption spectrometers used in this study.

We found that the addition of glycerol caused the rereduction of the photo-oxidized reaction centers to become very slow. Therefore all manipulations involved in filling and mounting the sample cell were carried out in the dark, and the preparation was kept in darkness until the temperature of the sample was below 250 K. At lower temperatures, photo-oxidation of the reaction centers was rapidly and completely reversible in the dark, even with glycerol. We could then use the same sample at different temperatures. For experiments done at room temperature, the reaction centers were suspended in 0.01 M Tris/Cl, pH 7.5, with 0.1 % lauryl dimethyl amine oxide but without glycerol.

Light-induced difference spectra were measured with a Cary 14R spectrophotometer coupled to a signal averager (Tracor-Northern TN-1500, Middleton, Wisc.) as described earlier [11]. Several spectra were taken alternatively in the dark and in the light (using continuous side-illumination); the averages of "dark" and "light" were then subtracted. Kinetic measurements were made at room temperature with a home-built split beam spectrophotometer [12], and at low temperature (35 K) with a conventional single beam spectrophotometer. The actinic light was provided either by a tungsten-iodine lamp (Sylvania "Sun Gun", 650 W) filtered through 1 inch of water and a Corning CS7-69 filter, or with a Q-switched ruby laser (Korad, Inc., Santa Monica, Calif.). Adequate filters were put in front of the detector to block scattered actinic light.

RESULTS AND DISCUSSION

Absorbance (A), first derivative of absorbance ($dA/d\lambda$), and light-induced difference spectra (ΔA) were recorded on the same sample with the Cary 14R between

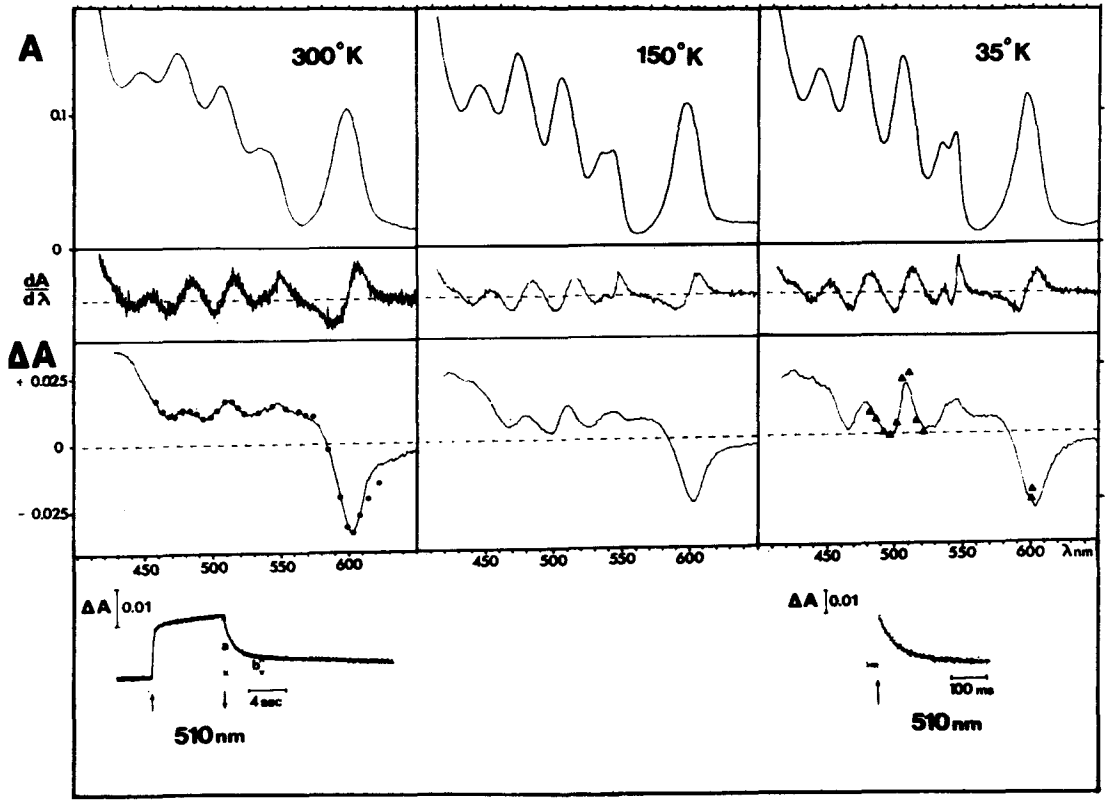

Fig. 1. Absorption (A), first derivative ($dA/d\lambda$) and light-induced difference spectra (ΔA) measured at 300, 150 and 35 K. Reaction centers (1 μM) in 0.01 M Tris/Cl, pH 7.5+0.1 % lauryl dimethyl amine oxide in measurements at 300 K; in the same with 80 % glycerol/20 % water (v/v) at 150 and 35 K. These curves were obtained with a Cary 14R spectrophotometer. Absorbance changes obtained at 300 and 35 K in kinetic measurements (bottom traces) using a home-built spectrometer are replotted (closed circles and triangles) on the corresponding light-induced difference spectra obtained with the Cary 14R. At 300 K closed circles correspond, respectively, to the absorbance changes indexed as a and b on the kinetic trace, normalized to the difference spectrum at 600 nm.

250 and 35 K, approximately every 50 K. Fig. 1 shows these data for three different temperatures. Similar light-induced difference spectra can be obtained if one plots absorbance changes measured from kinetic data (bottom traces and full symbols) obtained at successive wavelengths. This is true at low temperature (triangles) and at room temperature (circles), whether the actinic light was a flash or was continuous. At room temperature, light-induced difference spectra measured just after the cessation of continuous illumination (indexed as a) or 4 s after the cessation of the illumination (indexed as b) are similar when normalized at 600 nm. These difference spectra are indicated in Fig. 1 by the closed circles.

In the wavelength range studied, three main features emerge in the absorption spectrum as the temperature is lowered: (1) The 600 nm band, almost symmetrical at room temperature, becomes asymmetrical. (2) The Q_x bands of bacteriopheophytin absorption split into two components (maxima near 535 and 546 nm), as already reported for reaction centers prepared from the carotenoidless mutant strain R-26 [13]. (3) The absorption bands of the reaction center carotenoid (sphaeroidene) sharpen and shift by a few nanometers to longer wavelengths, in a way similar to that reported by Ke et al. [14] for carotenoids in vitro.

It is apparent, when comparing the shapes of the first derivative spectra and the corresponding light-induced difference spectra, that at each temperature there is a good but not perfect fit between both spectra in the region of carotenoid absorption. The exact positions of minima and maxima in the light-induced difference spectra are located 4–5 nm to shorter wavelengths compared to those of the first derivative spectra. This can be explained if one supposes that the light-induced absorbance changes are composed of a small red shift plus an increase of the absorption band width of the carotenoid molecule. Comparing absolute absorption spectra recorded in the light and in the dark and assuming that each reaction center contains only one molecule of carotenoid, one can estimate that the band shift is about 1.0 nm.

The carotenoid absorption band shift followed the same intensity dependence as the 600 nm bleaching at each temperature studied (Fig. 2). These results suggest that the absorption bands of the carotenoid molecule are affected by the appearance of positive and/or negative charges in the reaction center. One can ask whether these changes are induced by the presence of both positive and negative charges, or by only one of these. To check these possibilities we took difference spectra between an untreated sample and either an oxidized or a reduced sample of reaction centers. We used H_2O_2 for oxidation, and illumination in the presence of the electron donor phenazonium methosulfate for reduction. No carotenoid shift was induced by reduction of the reaction centers. Light-induced absorbance changes measured in the presence of phenazonium methosulfate and ascorbate as electron donors, which should reflect only the photochemical reduction of the primary acceptor, gave a difference spectrum similar to those reported for reaction centers prepared from carotenoidless strain R-26 [15], with no indication of a carotenoid band shift. On the other hand chemical oxidation of reaction centers did induce a carotenoid band shift as shown in Fig. 3. Finally, in the presence of excess ubiquinone as secondary acceptor one can expect to obtain, under strong illumination, oxidized bacteriochlorophyll ($P\text{-}870^+$) and fully reduced ubiquinone by dismutation of the anion radical $UQ^- \cdot$ in the medium. If one plots the absorbance changes occurring 4 s after the cessation of the light, one obtains a difference spectrum showing a carotenoid shift (Fig. 1, closed circles).

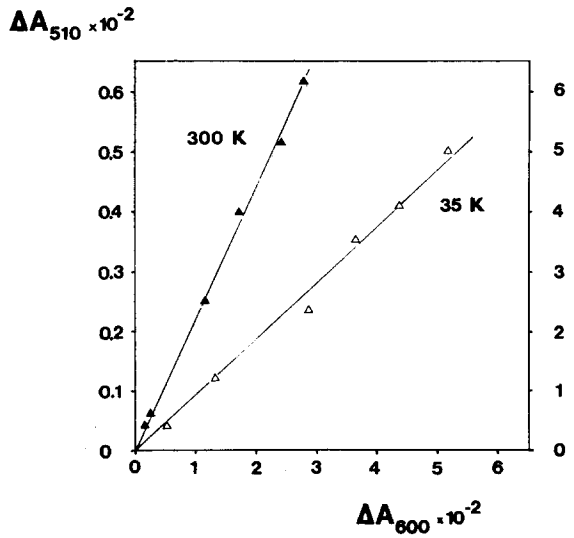

Fig. 2. Plot of the absorbance changes occurring at 600 nm ($\Delta A_{600\,nm}$) against the absorbance changes occurring at 510 nm ($\Delta A_{510\,nm}$) for different intensities and at two temperatures, 300 and 35 K. The left and right scales correspond to the absorbance changes occurring at 510 nm at 300 and 35 K, respectively.

It seems, therefore, that the carotenoid absorption band changes are only induced by the presence of a positive charge on the bacteriochlorophyll.

As a byproduct of this study we found that at very low temperatures (35 K) a shoulder is apparent at 605 nm in the absorption spectrum of the reaction center (Fig. 4, upper part). We also found that the 600-nm bleaching was sometimes resolved into two components, one of which corresponded to the shoulder of the absolute absorp-

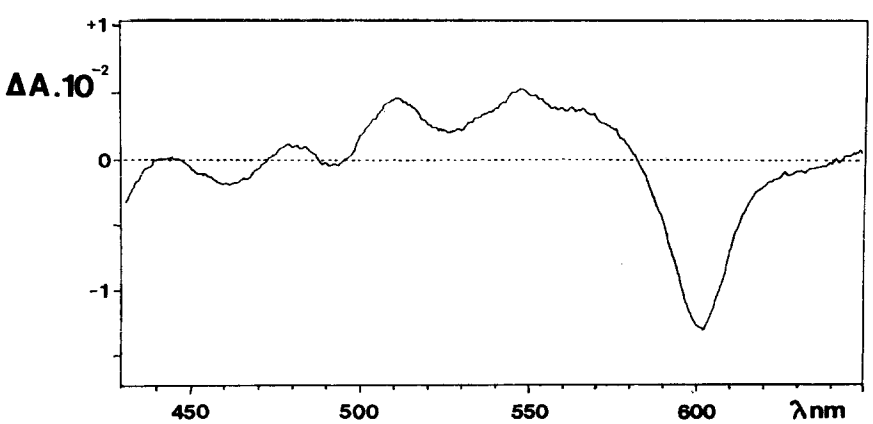

Fig. 3. Difference spectrum induced by chemical oxidation of reaction centers. The oxidant was a drop of 30 % H_2O_2.

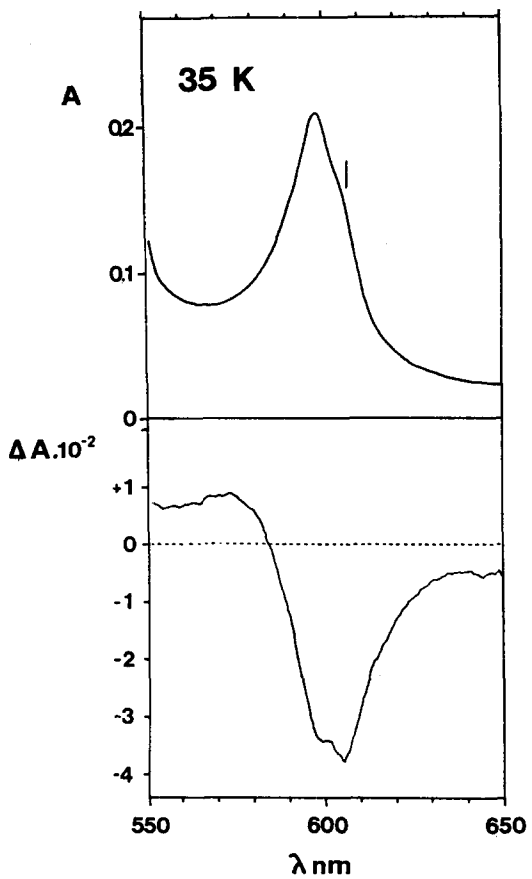

Fig. 4. Upper trace: absolute absorption spectrum of reaction centers (2 μM) at 35 K. The bar indicates a shoulder in the absorption near 607 nm. Lower trace: light-dark difference spectrum recorded at 35 K with the Cary 14R spectrophotometer using continuous actinic illumination.

tion spectrum (Fig. 4, lower part)*. This observation is particularly relevant to a new interpretation of the light-induced absorption changes for the bacteriochlorophyll reaction center dimer *P*-870 [11]. It is supposed that due to molecular excitonic interaction the reaction center bacteriochlorophyll dimer $(Bchl)_2$ exhibits two new absorption bands in place of one for each monomer. These two new transition moments correspond to the two photobleachable absorption bands centered, respectively, at 810 and 865 nm. The increase at 790 nm reflects the appearance of the cation radical $(Bchl)_2^+ \cdot$, with some spectral properties similar to those of monomeric bacteriochlorophyll. If this interpretation is correct, one can expect a splitting of the Q_x transition moment centered near 600 nm. We therefore interpret the shape of the 600 nm absorbance change as revealing the splitting of the two Q_x transition moments of the dimer.

* This result was not reproducible; compare for example Fig. 1 (35 K) and Fig. 4 (35 K); it seemed to depend on how the reaction centers were brought to low temperature.

In summary, a light-induced bathochromic carotenoid band shift of about 1.0 nm has been observed in reaction centers prepared from wild type *R. sphaeroides*. Chemical oxidation and photochemical reduction of reaction centers suggest that the carotenoid shift is induced only by the presence of oxidized bacteriochlorophyll dimer $(Bchl)_2^+ \cdot$; the negatively charged electron acceptor has no such effect. These results indicate that the absorption spectra of carotenoids in vivo can be affected by a local field at the reaction center (Stark effect?) as well as by a delocalized field in the membrane. Finally, we interpret the structure of the 600 nm bleaching at low temperature as due to the splitting of two Q_x transition moments of the reaction center bacteriochlorophyll dimer, *P*-870.

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