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Temperature Dependence of the Light-Induced Infrared Difference Spectra of Chromatophores and Reaction Centers from *Rhodobacter sphaeroides*

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The light-induced infrared difference spectra have been measured for chromatophores (intracytoplasmic membranes containing reaction centers) and isolated reaction centers from *Rhodobacter sphaeroides* at 300 and 80 K. Analysis of the C_9 keto carbonyl stretching region suggests that the radical cation of the bacteriochlorophyll-a special pair formed after photoexcitation might have multiple structures which differ in the degree of delocalization of an unpaired electron.

The photosynthetic reaction center (RC) is a pigment-protein complex which plays a central role in converting light energy to chemical energy. Photophysical and photochemical responses of RCs to the absorbed light energy and to the energy transferred from surrounding light-harvesting pigment-protein complexes have been one of the most important problems to be elucidated in photosynthesis research.[1-3] In recent years, the three-dimensional structures of RCs from two species of photosynthetic bacteria were determined.[4,5] The relationships between the structures and kinetic behavior of the RCs can now be discussed on this basis. However, some interesting problems such as the structures and dynamic aspects of the bacteriochlorophyll-a (BChl) special pair radical cation (P^+) and its interaction with the proteinic environment still remain to be clarified.

In a previous paper[6] dealing with the RCs from *Chromatium vinosum*, we have shown that, in P^+ of this bacterial species, the positive charge is localized on either one of the two BChls in the special pair in the timescale of infrared absorption (ca. 10^{-13}s). In the present paper, we report the light-induced infrared difference spectra obtained for chromatophores and RCs from *Rhodobacter sphaeroides* under various conditions, and discuss implications in the observed results.

Chromatophores and RCs were isolated from *Rb. sphaeroides* (wild type) and purified according to the procedure described in the literature.[7] Chromatophores were collected as paste-like material by an ultracentrifuge. An aqueous solution containing RCs was dialyzed against a sodium phosphate buffer (pH 7.9, without any detergent), and RCs were also collected as paste-like material by a high-speed centrifuge. Collected chromatophores and RCs were washed twice with D_2O

Infrared measurements were made for the following two states. One was D_2O suspensions of the paste-like material, which were placed with a 15 μm thick spacer between two CaF_2 plates. The other was dry films obtained by casting D_2O suspensions of the paste-like material on a CaF_2 plate under Ar atmosphere. These samples were set in a cryostat (OXFORD DN704). A JEOL JIR-100 FT-IR spectrophotometer equipped with an MCT detector (JUDSON) was used for infrared measurements. The light-minus-dark difference spectra were

obtained in a manner similar to that described previously.[8] Interferograms from 1000 scans were averaged to obtain reliable difference spectra with 2 cm^{-1} resolution.

The light-induced infrared difference spectra in the carbonyl stretching region observed for chromatophores (film), chromatophores (suspension), and RCs (suspension) at 300 and 80 K are shown in Fig. 1. The bands in the positive and negative sides arise, respectively, from P^+ and P (neutral state of the special pair). It is clearly seen that the spectra of the three samples differ from one another and that the spectra of each sample, particularly those of chromatophores (suspension) and RCs (suspension) in Figs. 1C-F, show significant differences between 300 and 80 K. We have not succeeded in observing the difference spectra from RCs in dry film form, whereas the difference spectra from chromatophores (film) have been obtained as shown in Figs. 1A and B.

The experimental results outlined above indicate that both P^+ and P are sensitive to the environment where the RC is located. This is rather surprising because, according to the three-dimensional structure of the RC from *Rb. sphaeroides*,[5] P is surrounded by the protein chains and is not close to the surface of the RC. We will discuss below the spectral differences among the three samples and the spectral changes for each sample on going from 300 to 80 K.

We focus our attention on the spectral region from 1720 to 1700 cm^{-1}. Bands observed in this region give information on the structure and dynamic aspect of P^+. In the spectrum of chromatophores (film) at 300 K in Fig. 1A, a shoulder at 1712 cm^{-1} and a band at 1706 cm^{-1} are observed. At 80 K (Fig. 1B), the former stays almost unchanged at 1713 cm^{-1}, whereas the latter consists of a band at 1708 cm^{-1} and a new shoulder at 1703 cm^{-1}. These spectral changes with decreasing temperature are more clearly observed for chromatophores (suspension) in Figs. 1C and D; the shoulder at 1712 cm^{-1} observed at 300 K stays unchanged at 80 K, and the 1705 cm^{-1} band at 300 K is split into two components at 1709 and 1704 cm^{-1} of equal intensities at 80 K. A very similar trend is observed for RCs (suspension) in Figs. 1E and F; in this case the bands at 1714 cm^{-1} (300 K) and the one upshifted to 1716 cm^{-1} (80 K) are observed separately from the lower-wavenumber bands.

The next problem is what we can learn from the experimental findings described above. There is no doubt about the assignment of the bands observed in the region of 1720-1700 cm^{-1} to the C_9 keto carbonyl stretch in P^+.[6,9] Some other points to be taken into account in interpreting the experimental results are summarized as follows. (1) The electrochemically generated $BChl^+$ (radical cation of BChl-a) shows a band at 1716 cm^{-1} in tetrahydrofuran solution, which is shifted to 1708 cm^{-1} in methanol solution.[6] This downshift is due to the formation of hydrogen bonding between the C_9 keto carbonyl group of $BChl^+$ and the hydroxyl group of methanol. (2) In the light-induced infrared difference spectrum of chromatophores (suspension, 300 K) from *Chromatium vinosum*, a single band is observed at 1712 cm^{-1} for P^+.[6] The presence of this band has been interpreted as implying that the C_9 keto carbonyl group is free from hydrogen bonding, and that an unpaired electron is localized on either one of BChls in the special pair in the timescale of infrared absorption (10^{-13} s).

The shoulder at 1713-1712 cm^{-1} in Figs. 1A-D would result from a band whose peak position is located at a slightly higher wavenumber. In fact, it seems most reasonable to consider that this shoulder and the band at 1714 cm^{-1} in Fig. 1E (upshifted to 1716 cm^{-1} in Fig. 1F) arise from the same origin. This band position ($\approx$1714 cm^{-1}) corresponds very well to the 1716 cm^{-1} band of $BChl^+$ in tetrahydrofuran solution as well as to the 1712 cm^{-1} band of P^+ in chromatophores from *Ch. vinosum*. Therefore, the shoulders and the bands at 1716-1712 cm^{-1} in Fig. 1 can be assigned in the same way as described above for the 1712 cm^{-1} band of P^+ in *Ch. vinosum*.[6]

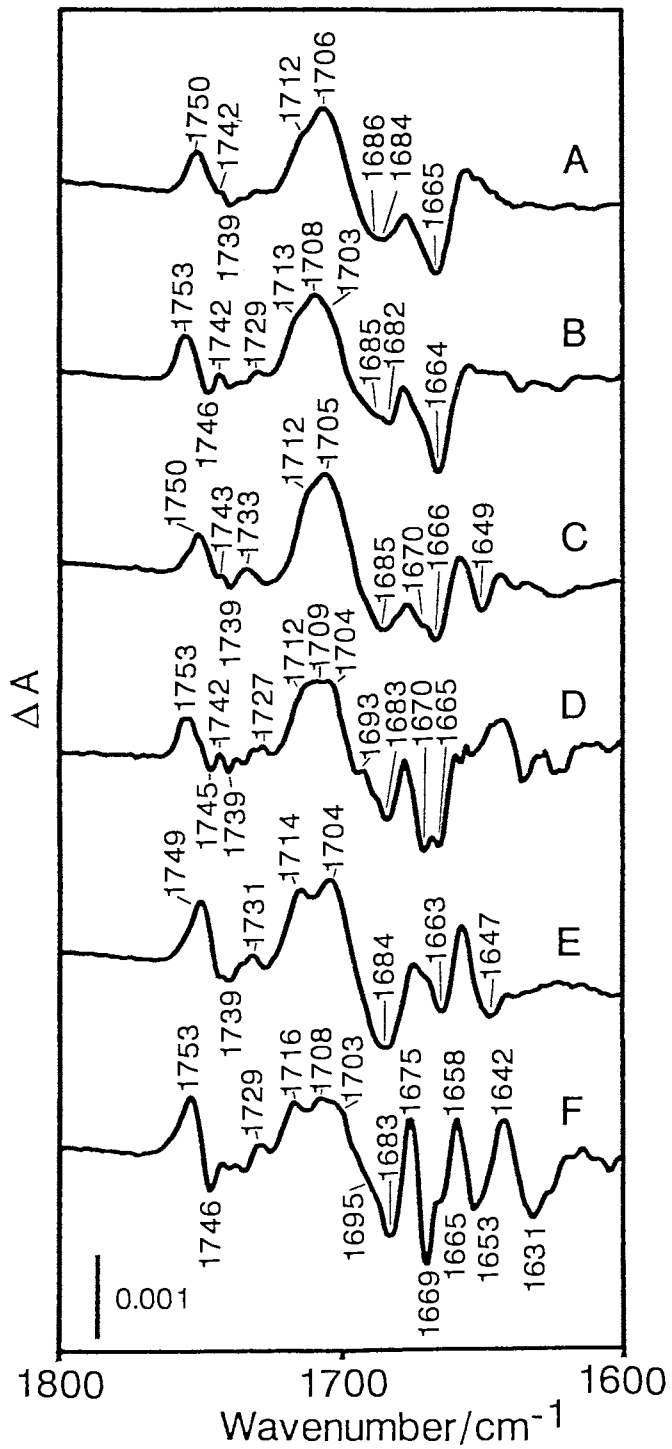

Fig. 1. Light-minus-dark infrared difference spectra of chromatophores and reaction centers from *Rhodobacter sphaeroides*. A, Chromatophores (film) at 300 K; B, chromatophores (film) at 80 K; C, chromatophores (suspension) at 300 K; D, chromatophores (suspension) at 80 K; E, reaction centers (suspension) at 300 K; F, reaction centers (suspension) at 80 K. The absorbance scale in the ordinate is indicated with a bar at the lefthand bottom.

A simple assignment of the bands observed at 1709-1703 cm^{-1} is to correlate them to the 1708 cm^{-1} band of $BChl^+$ in methanol solution; in other words, the bands at 1709-1703 cm^{-1} arise from $BChl^+$ in which the C_9 keto carbonyl group is hydrogen bonded. However, this assignment is not consistent with the X-ray analysis of the RC of *Rb. sphaeroides*,[5] which claims that no hydrogen bond exists between the C_9 keto carbonyl group and a proton donor in the main and side chains of the protein.

Then, it is likely that the bands at 1709-1703 cm^{-1} reflect the delocalization of an unpaired electron over the two BChls in the special pair. As discussed in our previous paper,[6] the completely delocalized P^+ state represented as ($BChl_I^{0.5+}\cdots BChl_{II}^{0.5+}$) would give rise to a positive band at about 1700 cm^{-1} (halfway between the 1716 cm^{-1} band of $BChl^+$ and the 1686 cm^{-1} band of neutral BChl in tetrahydrofuran solution). If the delocalization is not complete, the special pair in P^+ may be represented as ($BChl_I^{(1-\delta)+}\cdots BChl_{II}^{\delta+}$) where $0<\delta<1$. It is expected that such partially delocalized states would give rise to positive bands in the region of 1709-1703 cm^{-1}. At present it is difficult to correlate the band position with the δ value. However, a plausible explanation for the temperature dependences of the bands at 1709-1703 cm^{-1} may be as follows. The strongest band at 1706-1704 cm^{-1} in the spectra at 300 K corresponds to a state where the delocalization is nearly complete, although this band position is 6-4 cm^{-1} higher than 1700 cm^{-1} suggested above. The degree of delocalization seems to change with decreasing temperature, and the band at 1706-1704 cm^{-1} (300 K) is split into two bands at 1709-1708 and 1704-1703 cm^{-1} (80 K), which are considered to correspond to $BChl^{(1-\delta)+}$ and $BChl^{\delta+}$, respectively.

The above temperature dependences of the bands at 1706-1704 cm^{-1} (300 K) and the fact that the band at 1714-1712 cm^{-1} (300 K) is relatively insensitive to temperature change suggests that the special pair radical cation might have multiple structures depending on the interaction with the proteinic environment, which differ in the degree of delocalization of an unpaired electron.

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