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Implications of the Positive Band at 1712 cm^{-1} in the Light-Induced Infrared Difference Spectrum of the Bacteriochlorophyll-a Special Pair in the Photosynthetic Reaction Center of *Chromatium vinosum*

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Assignments of bands observed in the light-induced infrared difference spectrum of reaction centers from *Chromatium vinosum* are discussed with particular attention to the carbonyl stretching region of the bacteriochlorophyll-a (BChl) special pair radical cation. It is pointed out that the infrared data reflect the localization of an unpaired electron on either one of the two bacteriochlorophyll-a's in the special pair radical cation in the time scale of infrared absorption (10^{-13}s).

The three-dimensional arrangements of the pigments and protein subunits in the photosynthetic reaction center (RC) and their response to the absorption and transfer of light energy have been one of the most important problems in the research on photosynthesis. In recent years, the three dimensional structures of RCs from some bacteria have been solved,1,2) and a better understanding for the above problem has been obtained. Yet, more studies are needed to clarify the dynamic aspect of electron transfer from the BChl special pair (P) to quinones.

In this letter, we show that a detailed analysis of the light-minus-dark infrared difference spectrum of RCs gives information relating to this problem. More specifically, the infrared difference spectrum enables us to discuss the delocalization and localization of an unpaired electron (hole) on the two BChls in the special pair radical cation (P^+), on which some information has been obtained from electron spin resonance,3,4) electron nuclear double resonance,5,6) and electron spin echo7) experiments.

Chromatophores of *Chromatium vinosum* (*Ch. vinosum*) were isolated and purified according to the methods described in the literature.8) For infrared measurements, this sample was washed twice with D_2O and placed between two CaF_2 plates with a spacer of 15 µm thickness. The light-minus-dark difference spectrum was obtained according to the method described previously.9)

Bacteriochlorophyll-a was isolated from *Ch. vinosum*.10) The infrared spectra of BChl and its radical cation generated electrochemically were observed in tetrahydrofuran (THF) and methanol solutions. The concentrations of BChl were adjusted between 0.5 and 2 mM. The solvents were dried with CaH_2 powder, degassed and then purified by repeated distillation (several times) *in vacuo* in the presence of CaH_2 powder. As supporting electrolytes, tetrabutylammonium hexafluorophosphate ($TBAPF_6$) and tetrabutylammonium perchlorate (TBAP) were used. These materials were carefully dried *in vacuo* for two hours at 100 °C prior to use. Only $TBAPF_6$ was recrystallized three times from a mixture of ethyl acetate and n-pentane (1:3) before drying.11)

Generation and spectral measurements of the radical cation were performed by using a home-made cell

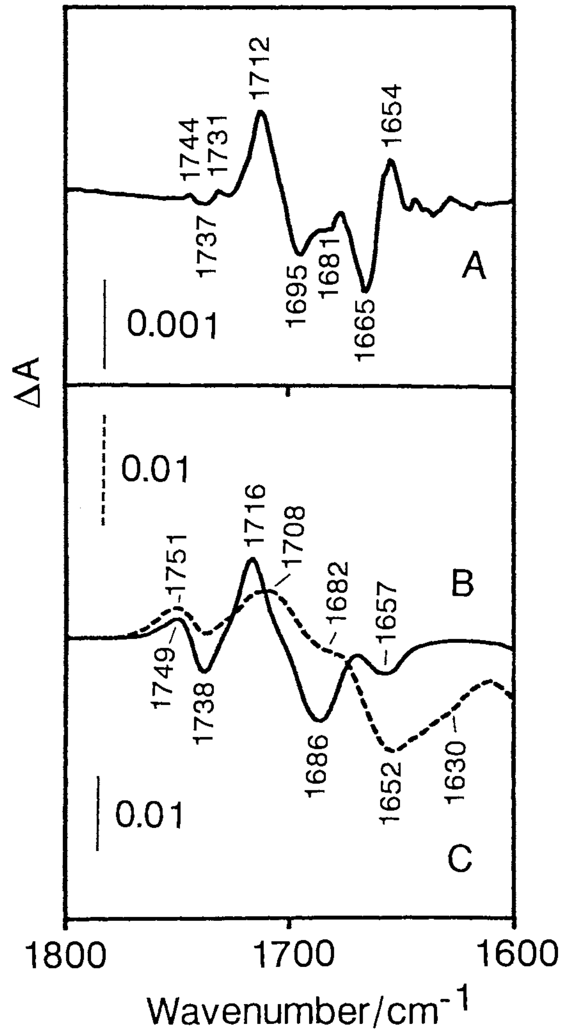

Fig. 1. A, Light-minus-dark infrared difference spectrum of the chromatophores from *Chromatium vinosum* in D_2O. B and C, Infrared difference spectra between electrochemically generated bacteriochlorophyll-a radical cation (positive side) and neutral bacteriochlorophyll-a (negative side) in tetrahydrofuran and methanol solutions, respectively. The ordinate refers to absorbance differences whose scales are indicated on the lefthand side of this figure.

(200 μm path length) containing Pt electrodes and an Ag/AgCl reference electrode. The design of this cell followed that reported by Mäntele *et al.*[12)]

A JEOL JIR-100 FT-IR spectrophotometer equipped with an MCT detector (JUDSON) was used for infrared measurements. Interferograms from 500 scans were averaged to obtain a reliable light-induced difference spectrum for the chromatophores. Only 100 scans were required for obtaining reliable spectra of BChl and its radical cation in organic solvents. Spectral resolution was 4 cm^{-1}.

The light-minus-dark difference spectrum in the carbonyl stretching region obtained from the chromatophores is shown in Fig. 1A. The bands in the positive and negative sides are attributed to P^+ and P, respectively.[9)] The difference spectra between the electrochemically generated radical cation ($BChl^+$) and the neutral state of BChl observed in THF and methanol solutions are shown in Figs. 1B and 1C, respectively. The positive and negative bands in these difference spectra are due, respectively, to the radical cation and neutral species. The difference spectrum in Fig. 1B is very close to the corresponding one of Mäntele *et al.*13)

First, we discuss the assignments of bands in Figs. 1B and 1C. The positive and negative bands at 1749 and 1738 cm^{-1} in Fig. 1B are safely assigned, respectively, to the C_{10} ester carbonyl stretches of $BChl^+$ and BChl, because Leonhard *et al.*14) have shown that these bands are not observed for pyroBChl which lacks the C_{10} ester group. The positive and negative bands at 1716 and 1686 cm^{-1} in Fig. 1B are assigned, respectively, to the C_9 keto carbonyl stretches of $BChl^+$ and BChl, because these bands are observed also for pyroBChl.14) The negative band at 1657 cm^{-1} in Fig. 1B is assignable to the C_2 acetyl carbonyl stretch of BChl, since no corresponding band is observed for chlorophyll-a.14) No positive band which should be paired with the 1657 cm^{-1} negative band is detected.

The most important difference between the spectrum of Fig. 1C and that given by Mäntele *et al.*13) is that a positive band at 1721 cm^{-1} and a positive shoulder at 1710 cm^{-1} are present in the spectrum reported by Mäntele *et al.*,13) whereas only the positive band at 1708 cm^{-1} is observed in Fig. 1C. We observed the same spectrum as in Fig. 1C by using methanol-d_4 as solvent. Only when we used methanol-d_4 without careful purification, we obtained a spectrum similar to that reported by Mäntele *et al.* In Fig. 1C, the positive and negative bands at 1708 and 1652 cm^{-1} are due to the hydrogen-bonded C_9 keto carbonyl stretches of $BChl^+$ and BChl, respectively, and the negative band at 1657 cm^{-1} in Fig. 1B seems to be shifted to about 1630 cm^{-1} in Fig. 1C.

The assignments described above for the bands arising from $BChl^+$ and BChl in organic solvents provide a basis for assigning the bands observed in Fig. 1A for the chromatophores of *Ch. vinosum*. It is noted that the spectrum in Fig. 1A is significantly different from that observed for *Rhodobacter sphaeroides* (*Rb. sphaeroides*).9,13,15,16) Particularly, the regions around 1750 cm^{-1} and of 1720-1700 cm^{-1} are different between these two bacterial species. In the light-minus-dark difference spectrum of RCs from *Rb. sphaeroides*,9,13,15,16) positive bands are strongly observed at 1751, 1714, and 1703 cm^{-1}, whereas, in Fig. 1A, the positive band at 1744 cm^{-1} is very weak, and only a single band is found at 1712 cm^{-1}. This indicates that the structures and/or proteinic environments of P^+ are decisively different between the two bacterial species.

In Fig. 1A, the C_9 keto carbonyl stretching region is most interesting. The positive and negative bands at 1712 and 1695 cm^{-1} are assignable to the C_9 keto carbonyl stretches in P^+ and P, respectively. From the similarity of the positions and shapes between the 1712 cm^{-1} band in Fig. 1A and the 1716 cm^{-1} band in Fig. 1B, it is most likely that the former band arises from the non-hydrogen-bonded C_9 keto carbonyl group in P^+. The following point should be emphasized. The similarity between the 1712 cm^{-1} band of P^+ and the 1716 cm^{-1} band of $BChl^+$ indicates that, in the timescale of infrared absorption ($\sim10^{-13}$s), the state of the special pair in P^+ can be represented as ($BChl_I^{1+}\cdots BChl_{II}^{0}$) and ($BChl_I^{0}\cdots BChl_{II}^{1+}$) where subscripts I and II are used to specify the two BChls in the special pair. In other words, an unpaired electron is localized on either one of BChls in the timescale of 10^{-13}s and is *not* in the delocalized state represented as ($BChl_I^{0.5+}\cdots BChl_{II}^{0.5+}$), which should 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 BChl in Fig. 1B). Thus, the present infrared study leads to a conclusion that the timescale of electron exchange between the two BChls in P^+ is of the order of 10^{-12}s or longer. This is consistent with the conclusion derived from an electron spin echo experiment for RCs of *Rb. sphaeroides*.7)

The absence of a strong positive band at about 1750 cm^{-1} in Fig. 1A may be interpreted in the following two ways. (1) Little change occurs in the electronic state of the C_{10} ester carbonyl group on going from P to P^+.

(2) Due to a possible downshift of the positive band of P^+ to a position closer to the negative band of P, the intensities of these positive and negative bands cancel each other. Both cases can only be realized as a result of strong interactions between the special pair and the proteinic environment. Further studies are needed to clarify such interactions.

The spectral differences observed between *Ch. vinosum* and *Rb. sphaeroides* suggest that measurements of the light-induced infrared difference spectra may provide a clue to the elucidation of structural and dynamical characteristics of the special pair. Comparisons with other bacterial species are now in progress.

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