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Light-induced Changes in the Infrared Spectra of Reaction Centers from *Rhodopseudomonas sphaeroides* in H_2O and D_2O Solutions

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Light-induced changes were observed in the infrared spectra of the reaction centers from *Rhodopseudomonas sphaeroides* in aqueous solutions. These changes were suppressed in the presence of ferricyanide, demonstrating that the light-induced changes were due to the primary photochemical reactions in photosynthesis.

In a reaction center (RC) of photosynthetic bacteria, light energy absorbed by bacteriochlorophylls (Bchls) is converted into electrochemical energy, generating the charge separation between the electron donor and acceptor. The isolated RC is a pigment-protein complex which usually consists of three protein subunits, four Bchls, two bacteriopheophytins (Bphs), two ubiquinones, and one Fe atom.[1] A recent X-ray analysis of the RC has succeeded in determining the arrangements of these pigments and protein subunits in the complex.[2] Kinetics of intermediate species produced by the photochemical reactions has been studied extensively with a variety of spectroscopic techniques.[3]

Generally speaking, vibrational spectroscopy is considered to be a useful source of information on the molecular structures and interactions of biological systems. In fact, resonance Raman spectroscopy has already been applied to the study of pigments in photosynthetic systems,[4] since the Raman spectra of pigments can be selectively observed due to the resonance effects. On the other hand, infrared spectroscopy has been regarded as a less useful technique, because no selective enhancement of infrared absorptions is expected for any component of photosynthetic systems. However, recent developments of Fourier-transform infrared spectrophotometers have made it possible to determine surprisingly small differences in the spectra of complex systems. Mäntele et al. succeeded in observing light-induced changes in the infrared spectra of RCs and chromatophores of some photosynthetic bacteria.[5] In their measurements, air-dried films of the RCs (reconstituted in lipid vesicles) and chromatophores on CaF_2 or Ge plates were used.

In the present communication, we wish to show that the light-induced changes in the infrared spectra of the RCs can be observed even in aqueous

solution which imposes much more severe conditions on the infrared measurements but is easier to prepare as compared with the RCs in lipid vesicles. Aqueous solutions are more convenient for studying the effects of adding redox mediators or inhibitors than are the air-dried films. In fact, we have succeeded in observing the effects caused by ferricyanide in aqueous solution.

Reaction centers were isolated from a carotenoidless mutant of *Rhodopseudomonas sphaeroides* with lauryl dimethylamine N-oxide, and purified by repeated column chromatography using DEAE-cellulose. For infrared measurements, 10 μl of a highly concentrated (1 – 3 mM) RC solution (10 mM Tris acetate buffer, pH 8.4) was put between two CaF_2 plates with a 15 μm spacer. To observe the effects of deuterium substitution, D_2O (20 volumes) was added to the H_2O solution (1 volume), and the whole solution was concentrated to the original volume of the H_2O solution. Infrared spectra were measured at room temperature with a JEOL JIR-100 Fourier-transform infrared spectrophotometer equipped with a HgCdTe detector. Normally 500 interferograms were accumulated and averaged to obtain one spectrum. To observe light-induced spectral changes, the sample was irradiated with actinic light from a 50 W lamp (an ordinary microscope accessory for sample illumination). A glass bottle of 5 cm thickness containing a $CuSO_4$ solution was placed between the sample and the lamp as a filter to cut the heating effects by near-infrared and infrared lights. A Ge filter was put in front of the HgCdTe detector to protect it from possible damage caused by a part of the actinic light.

The infrared spectrum (2000 – 1000 cm^{-1}) of the RCs in H_2O solution is shown in Fig. 1a. This spectrum is dominated by the very intense H_2O band at about 1650 cm^{-1}, which completely overlaps with the amide I band of protein subunits. The amide II band is seen at about 1551 cm^{-1} as a shoulder of the H_2O band. This spectrum was observed without irradiation ('dark' spectrum), but it was so similar to the 'light' spectrum observed under irradiation that at first glance it was impossible to discern the 'light' from the 'dark'. It seemed unlikely that any useful information on the light-induced changes could be extracted from the comparison of the two spectra. However, by taking the difference between the two, the 'light-minus-dark' difference spectrum shown in Fig. 1b was obtained. The positive and negative peaks in Fig. 1b correspond, respectively, to the intensity increases and decreases under irradiation. Although these intensity changes are of the order of 0.001 absorbance unit, they are definitely reproducible except for the 1680 – 1620 cm^{-1} region where the intense H_2O band exists.

Comparing the spectrum in Fig. 1b with the spectrum obtained by Mäntele et al.[5] for an air-dried film of the RCs in lipid vesicles, we find an impressive similarity between the two, although there is a slight shift of zero level. This agreement between the results of the two independent measurements (different bacterial strains, different sampling conditions, and

different spectrophotometers) leads us to the conclusion that the observed difference spectra (both theirs and ours) are real and due to the light-induced changes.

Utilizing the advantage of treating aqueous solutions, we next observed the effects of adding ferricyanide to the sample solution. As is well known, ferricyanide oxidizes the primary electron donor (P870) and, as a result, no primary photo-reaction takes place in the RC. Therefore, it was expected that the 'light-minus-dark' difference spectrum would be flat in this case. The difference spectrum shown in Fig. 1c was obtained by adding ferricyanide to the aqueous RC solution. As expected, this difference spectrum is almost flat except for the region of the intense H_2O band and has nothing in common with the difference spectrum in Fig. 1b, although the origin of the two small positive peaks at 1720 and 1480 cm^{-1} is not clear.

The 'light-minus-dark' difference spectrum obtained from ca. 95% D_2O solution of the RCs is shown in Fig. 1d. In this case the difference spectrum in the 1680 – 1620 cm^{-1} region was reproducible, although on the other hand the 1260 – 1240 cm^{-1} region became less certain due to the disturbance caused by the D_2O band. Deuteration shifts can be expected only for bands due to

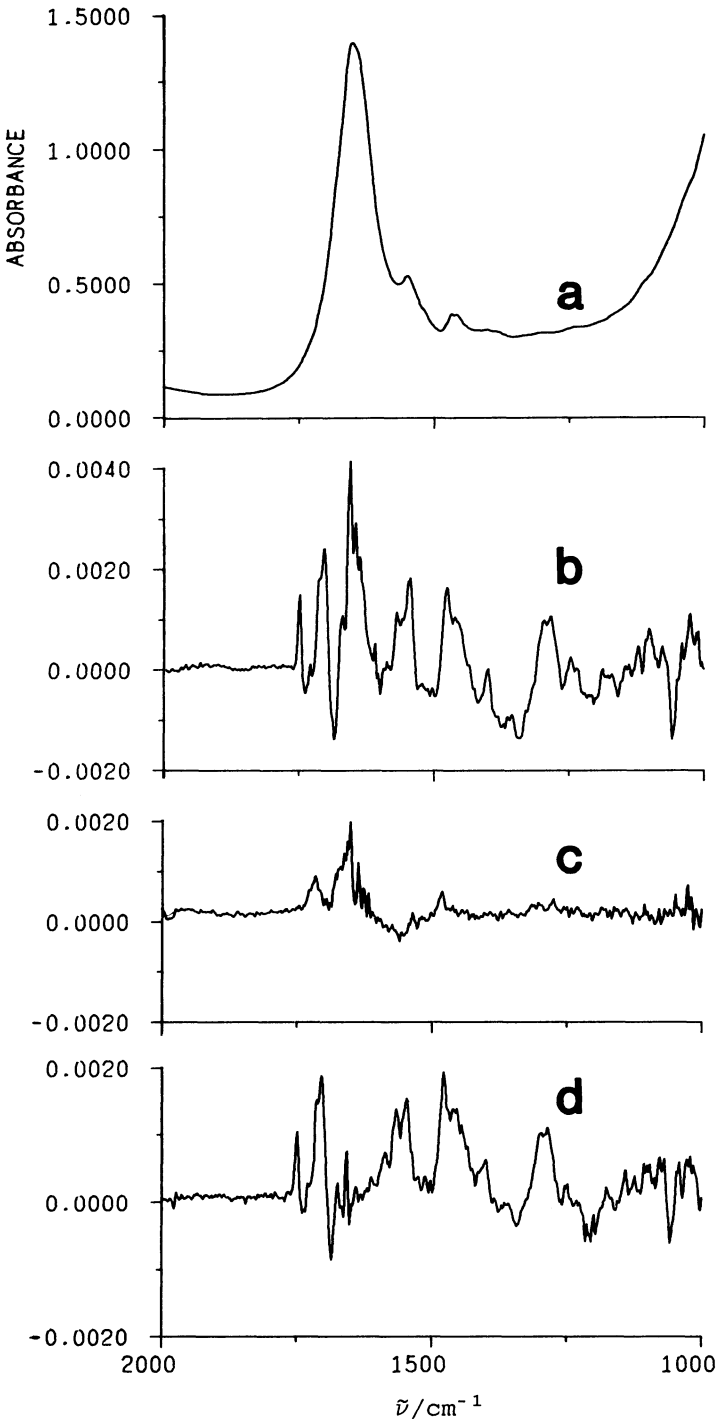

Fig. 1. (a) Infrared spectrum of the reaction centers from a carotenoidless mutant of *Rhodopseudomonas sphaeroides* in H_2O solution; (b) 'Light-minus-dark' difference spectrum in H_2O solution; (c) 'Light-minus-dark' difference spectrum in H_2O solution containing ferricyanide; (d) 'Light-minus-dark' difference spectrum in D_2O solution.

the protein moiety (mostly bands associated with the NH group) but not for bands arising from the pigments which have no exchangeable hydrogens. In fact, the amide II band at 1551 cm^{-1} disappeared and instead the amide II' band appeared at 1458 cm^{-1} in the infrared spectrum observed for the D_2O solution. In spite of such large changes in the original infrared spectrum upon deuteration, the difference spectrum in Fig. 1d retains many features of the difference spectrum in Fig. 1b. However, the following differences are noticeable between Figs. 1b and 1d; (1) the positive peak at 1570 cm^{-1} in Fig. 1b shows a downshift of about 5 cm^{-1} in Fig. 1d and (2) the intensity of the 1547 cm^{-1} band relative to that of the 1477 cm^{-1} band changes. Although precise vibrational assignments of these bands cannot be made at present, it is likely that these bands are associated with the protein moiety. Particularly, the 1570 cm^{-1} band which shifts to 1565 cm^{-1} upon deuteration is considered to have a contribution of the NH group in the protein moiety. Therefore, further studies of such light-induced infrared spectral changes may give a clue to clarify the interactions between the pigment and protein moieties. The bands in the 1680–1620 cm^{-1} region in the difference spectrum of the deuterated RC (Fig. 1d) are also interesting. We note that there are some definite differences in this region between Fig. 1b in the paper by Mäntele et al.[5] and our Fig. 1d. The origin of these differences should be examined further.

It is now imperative to establish reliable assignments for the observed bands in the difference spectra.

References

1) G. Feher and M.Y. Okamura, "The Photosynthetic Bacteria," ed by R.K. Clayton and W.R. Sistrom, Plenum Press, New York (1978), p. 349.
2) J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, J. Mol. Biol., 180, 385 (1984).
3) R.J. Cogdell, Ann. Rev. Plant Physiol., 34, 21 (1983).
4) M. Lutz "Advances in Infrared and Raman Spectroscopy," R.J.H. Clark and E. Hester eds, Wiley-Heyden, London (1984), Vol. 11, p. 211.
5) W. Mäntele, E. Nabedryk, B.A. Tavitian, W. Kreutz, and J. Breton, FEBS Lett., 187, 227 (1985).

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