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Infrared Studies of the Phototransformation of Phytochrome

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Infrared spectral changes caused by red light irradiation have been observed for pea phytochrome. The results obtained seem to contain useful information for elucidating structural changes of the chromophore and its environment occurring in the phototransformation of phytochrome.

Phytochrome is a chromoprotein consisting of two equivalent subunits, which controls red and far-red photoreversible reaction in green plants.[1] Each subunit has a molecular weight of about 124000, and contains a tetrapyrrole chromophore similar to biliverdin. The most prominent feature of phytochrome is its photoreversible transformation between the red light absorbing form (Pr) and the far-red absorbing form (Pfr).

Studies on the phototransformation of phytochrome have been made by using spectroscopic methods such as ultraviolet-visible absorption, fluorescence, and circular dichroism measurements.[2] Recently, resonance Raman studies have also been reported.[3,4] In this letter, we wish to report the red-light-induced infrared spectral changes of the so-called 'large' phytochrome[5] which lacks 51 amino acid residues from the N-terminus of each subunit. ('Large' phytochrome shows essentialy the same photoreversible transformation as the native one, and it is more soluble than the latter.) An advantage of using infrared spectroscopy is that infrared light in the region below 2000 cm^{-1} induces neither phototransformation nor any other photoreactions, and therefore can be used safely for spectral measurements.

'Large' phytochrome was extracted from 7-day-old etiolated pea seedlings (*Pisum sativum* cv. Alaska), purified by a method similar to that of Tokutomi et al.[6] using mAP-4 monoclonal anti-pea-phytochrome antibody,[7] treated with ammonium sulfate, and resuspended in phosphate buffer. This solution was freeze-dried, and H_2O or D_2O was added to the freeze-dried sample at least 1 hour before infrared measurment.

Infrared measurements were made on an FT-IR spectrophotometer (JEOL JIR-5500) equipped with an MCT detector (JUDSON). The sample solution was placed between two CaF_2 plates with a spacer (6 μm and 13 μm for H_2O and D_2O solutions, respectively). The temperature of this cell was maintained at 4 °C. Actinic light from a tungsten-halogen lamp was used for inducing the phototransformation after passing through a water layer of 3-cm thickness and a bandpass filter with transmittance peak at 660 nm and a bandwidth of 17 nm. A Ge filter was put in front of the MCT detector.

Under continuous red-light irradiation, a photostationary state is reached, where Ibl (bleached intermediate) as well as Pfr exists.[6] The infrared difference spectra (1800-1500 cm^{-1}) in H_2O and D_2O solutions between the photostationary state and Pr are shown in the Figure, where the positive and negative bands correspond, respectively, to the photostationary state and Pr. The bands whose wavenumbers are indicated could be observed reproducibly, but bands around 1650 cm^{-1} in H_2O solution were less reliable.

Although definite vibrational assignments of the observed bands are not possible at present, most of them are probably due to the chromophore. (The structure of the chromophore in Ibl is thought to be identical to that in Pfr.[2]) If this view is correct, it is likely that (1) the bands near 1700 cm^{-1} arise from the lactam CO stretch, (2) the bands near 1600 cm^{-1} from the antisymmetric stretch of the CO_2^- group, and (3) the 1733 cm^{-1} band from the CO stretch of the COOH group. The pyrrole ring modes may also be associated with the bands in the 1640-1500 cm^{-1} region. Another possibility for the origin of the 1733 cm^{-1} band is that it is due to the COOH group in the amino acid side chain of the protein.

Studies on model compounds of the chromophore are now in progress to obtain a basis for definite band assignments.

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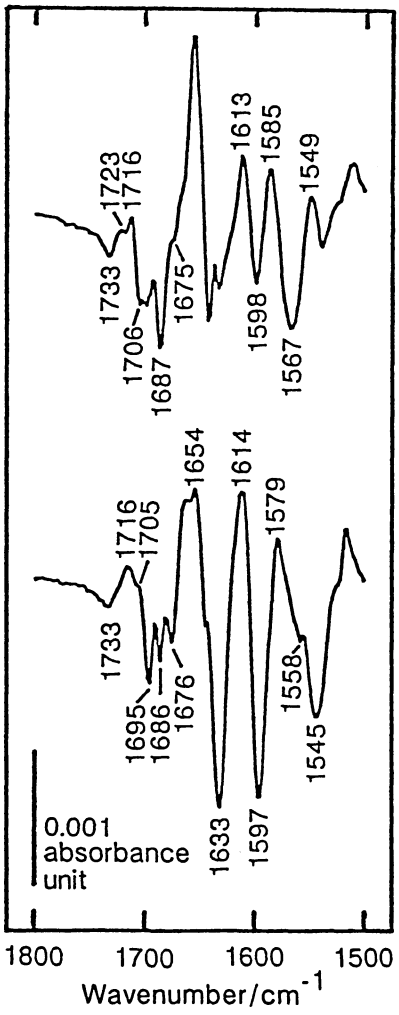

Fig. 1. Infrared difference spectra of 'large' phytochrome between photostationary state and Pr in H_2O (top) and D_2O (bottom).

References

1) "Phytochrome and Photoregulation in Plants," ed by M. Furuya, Academic Press, Tokyo (1987).

2) K. Schaffner, S. E. Braslavsky, and A. R. Holzwarth, "Photophysics and Photochemistry of Phytochrome," in "Advances in Photochemistry," ed by D. H. Volman, G. S. Hammond, and K. Gollnick, John Wiley & Sons, Inc., New York (1990), Vol. 15, pp. 229-277.

3) S. P. A. Fodor, J. C. Lagarias, and R. A. Mathies, *Photochem. Photobiol.*, **48**, 129 (1988).

4) D. L. Farrens, R. E. Holt, B. N. Rospendowski, P.-S. Song, and T. M. Cotton, *J. Am. Chem. Soc.*, **111**, 9162 (1989).

5) K. T. Yamamoto, "Phytochrome and Photoregulation in Plants," ed by M. Furuya, Academic Press, Tokyo (1987), Chap. 2.5.

6) S. Tokutomi, Y. Inoue, N. Sato, K. T. Yamamoto, and M. Furuya, *Plant Cell Physiol.*, **27**, 765 (1986).

7) A. Nagatani, K. T. Yamamoto, M. Furuya, T. Fukumoto, and A. Yamashita, *Plant Cell Physiol.*, **25**, 1059 (1984).

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