Much work has been done on synthetic catalysts with transacylase activity,[7] but significant success has been achieved more in the acylation than in the deacylation step. It appears therefore that the present investigation constitutes a decisive step toward a hydrolytic synzyme.[8] In future studies, structural modifications will be performed, in the hope of making the catalyst capable of substrate binding and recognition.

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Primary Process of Phytochrome: Initial Step of Photomorphogenesis in Green Plants

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The primary reaction rate of phytochrome, the chromoprotein which triggers photomorphogenetic processes in plants, is measured by picosecond transient absorption spectroscopy. Excitation of the red-absorbing form (Pr) of pea large phytochrome induces rapid depletion of the original absorption followed by recovery of the absorption and appearance of the primary photoproduct at about 695 nm. Both time constants are 24 ps, indicating that the product is formed directly from the excited state of the Pr form. The most likely primary reaction of phytochrome to express its function has been considered to be *Z-E* photoisomerization of the tetrapyrrole chromophore, the rate of which is 2 orders of magnitude slower than photoisomerization of rhodopsins.

Phytochrome[1] is a chromoprotein in green plants, which acts as a photoreceptor for a variety of morphogenetic and developmental responses including the regulation of the expression of light-sensitive genes. Modulation of these photoresponses is achieved by the photo-transformation of the phytochrome molecule from the red-absorbing form (Pr) to the far-red-absorbing form (Pfr).[2] Although the primary events following photon absorption and subsequent conformational changes of protein for expression of function have been attracting much interest, they are still obscure. In fact, the conversion from its excited state to the primary intermediate is not clearly understood,[3] although a number of time-resolved fluorescence measurements have been applied to study the excited-state relaxation processes.[4] This is in remarkable contrast to the other two photoreceptive pigments, rhodopsins and photosynthetic reaction centers, whose mechanisms have been better characterized.

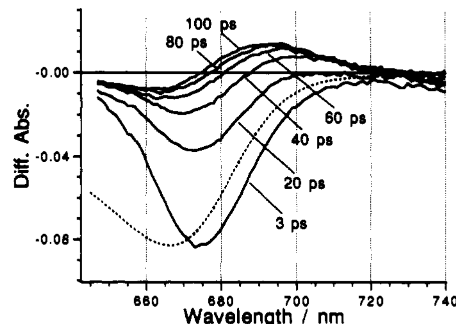

Figure 1. Transient absorption spectra of the Pr form of phytochrome following excitation with a sub-picosecond pulse (wavelength: 600 nm; pulse width: 0.8 ps; energy: 16 μJ; diameter of laser beam: 1.6 mm). The static absorption spectrum is also shown (---) after being multiplied by −0.12 (percentage of excitation). The spectrum at 100 ps is ascribed to the difference spectrum between the primary product and the original phytochrome.

The recent development of generation of ultrashort optical pulses has enabled us to directly observe the primary events of the photoreceptive pigments of the biological systems. In fact, a number of time-resolved spectroscopies using optical pulses shorter than 1 ps have been applied to rhodopsins[5] and photosynthetic reaction centers[6] to investigate their primary cis-trans isomerization or electron-transfer processes, respectively. From the results, we now know that rapid reactions take place (~0.2 ps for the former or 2-3 ps for the latter) from the initially excited states, so that the primary reaction overcomes the other relaxation processes back to the original state, and the highly efficient biological systems are realized.

In this communication, we present an approach to determine the primary reaction pathways of phytochrome in the picosecond regime by applying transient absorption spectroscopy. We use pea large phytochrome type A as the sample, which shows essentially the same photoreaction pathways as that of intact phytochrome type A despite its lack in the 6-kDa N-terminal fragment.[7] The apparatus for obtaining transient absorption spectra is a double-beam spectrometer linked with a sub-picosecond laser.[8] The large pea phytochrome in HEPES buffer is flowed

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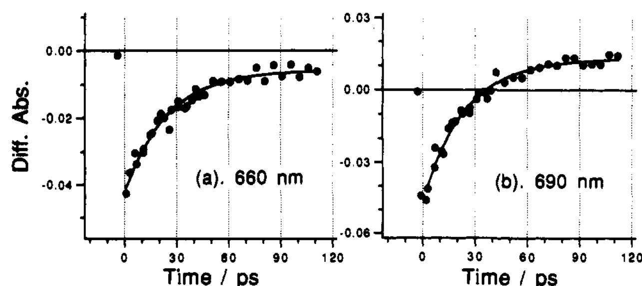

Figure 2. Kinetic absorption changes of phytochrome at 660 (a) and 690 nm (b). ● and — indicate experimental results and fitting curves, respectively. The kinetics is mainly probing the recovery of the ground-state depletion (660 nm) and the appearance of the primary product (690 nm), both of which are well fit by a single exponential with the same time constant of 24 ps. It is noted that this time constant (24 ps) is obtained throughout the entire 650–720 nm wavelength region, indicating that all primary processes including decay of Pr*, recovery to original Pr, and appearance of the primary product can be described by a single kinetic component.

through a microcell at 2 °C (2-mm light path) and irradiated with far-red light during experiments to convert the Pfr produced back to the Pr form. The λ_{max} of the sample is located at 666 nm, and the absorbance at λ_{max} is 0.59 in a 2-mm path length. The specific absorption ratio (A_{666nm}/A_{280nm}) is 0.98. The present experiments are carried out under the low excitation conditions (12% of molecules are excited), in which the absorption decrease is proportional to the excitation energy.

Upon excitation of the Pr form of the phytochrome sample with a sub-picosecond pulse (600 nm, 0.8 ps), we observed rapid depletion of the original absorption in the 650–740 nm wavelength region (Figure 1, at 3 ps). The spectral differences between the transient absorption at 3 ps and the original absorption (broken line) imply that the transient spectrum contains an excited-state absorption (mainly <670 nm) and a stimulated emission (mainly >670 nm). In other words, the excited phytochrome molecule is still in its excited state (Pr*) at 3 ps. The depletion signal recovers, accompanied by the appearance of the product absorption of positive signal at 675–730 nm. Kinetic changes at 660 (mainly probing ground-state recovery) and 690 nm (probing product rise as well as ground-state recovery) yield the same time constant of 24 ± 2 ps (see Figure 2), indicating that the rise time of the product is 24 ps as are the lifetime of Pr* and ground-state recovery time. Thus, the primary reaction rate of the present phytochrome sample is determined to be $(24 \text{ ps})^{-1}$.

Time-resolved fluorescence measurements on a sample with same preparation (114-kDa pea phytochrome in buffer) previously determined the lifetime of Pr* to be 34 ps, which was the predominant component (97%) among three.[4c] The difference of the lifetimes may be due to time resolution of the apparatuses (0.8 ps for the current study vs 40 ps for the previous study). In the fluorescence studies, intact phytochrome displayed a slightly larger lifetime of Pr* (39 ps for 121-kDa pea phytochrome),[4c] suggesting that the native chromophore may possess a longer lifetime. Our present observation is considerably different from the previous picosecond absorption study, in which the negative transient absorption at 665 nm increases in the first 25 ps and decays to zero by 50 ps (ref 3). The difference is unclear, as the present results clearly show instantaneous bleaching and recovery in 24 ps at 660 nm that coincide with the product formation time (Figure 2).

The spectral change is complete in approximately 100 ps (Figure 1). The difference spectrum is similar to that between Pr and lumi-R, which has been reported to be the primary intermediate.[9] The detailed spectral analysis will be given elsewhere.[10] Although there is no direct proof of the structures of the intermediates, generation of the primary product should be due to the conformational change of the chromophore, probably photoisomerization from the 15-*Z* to 15-*E* form.[11] It should be noted that isomerization of rhodopsins, such as rhodopsin,[5d] bacteriorhodopsin,[5a–c] and halorhodopsin,[5e] takes place in the sub-picosecond regime. In these molecules, the reaction time (200 fs for rhodopsin[5d]) indicates that an essentially barrierless transition is realized in formation of the product and effective competition with fast intramolecular relaxation. It is generally accepted that fast reaction results in highly efficient photoisomerization ($\phi = 0.67$ for rhodopsin). In contrast, our results suggest that the photoisomerization of phytochrome is 2 orders of magnitude slower than that of rhodopsins. It is interesting to compare the rate with that of rhodopsins. The rate presently obtained is "slow", whereas the efficiency of the functional photoreaction (Pr → Pfr) is never low. The quantum yield has been reported to be 0.5 or higher.[12] Thus the present work indicates a greater variety of mechanisms of photoreceptive pigments in nature.

Finally, the present transient absorption study has provided the primary reaction rate for phytochrome, which will help lead to an understanding of its reaction mechanism. It has also opened the question of how the system can control its efficient primary reaction even with a slow rate constant.

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Synthesis and X-ray Crystal Structure of $[(i\text{-}Pr_2N)_2P(H)CP(N\text{-}i\text{-}Pr_2)_2]^+CF_3SO_3^-$: A Carbene, a Cumulene, or a Phosphaacetylene?

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The recent isolation of stable (phosphanyl)(silyl)carbenes ($R_2PCSiMe_3$)[1] has prompted considerable discussion of the best description of their ground states[2] (Scheme I). Calculations[3] led to the conclusion that they were best formulated as multiply bonded λ^5-phosphaacetylenes B or phosphorus vinyl ylides C,

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