Photophysics and photochemistry of phytochrome, a chromoprotein in plants

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Abstract — The photophysical properties of 124-kDa phytochrome from etiolated oat shoots and some aspects of its photochemical reactivity are reviewed in terms of the working scheme depicted in Fig. 3. A review discussing these results in greater detail — with the exception of the interaction of the Pr form of phytochrome with ubiquitin — has been recently published in ref. 1e.

INTRODUCTION
Phytochrome is a light receptor in green plants, which converts absorbed light energy into physiological signals (ref. 1). It thereby exerts photomorphogenic control functions in response to the spectral composition of the light by way of a photochromic Pr \( \Leftrightarrow \) Pfr equilibrium between its physiologically inactive red absorbing (Pr: \( \lambda_{\text{max}} \) 665 nm) and active far-red absorbing (Pfr: \( \lambda_{\text{max}} \) 730 nm) forms.

During the first years of phytochrome research, facile endogenous proteolysis had precluded the isolation of the native-size protein. Rather, partially degraded preparations of ca. 60 kDa and 114/118 kDa (the so-called "small" and "large" phytochromes) were obtained which, however, still exhibited the proper absorption and photochromic ("photoreversible") properties. When isolated from etiolated oat shoots, undegraded native phytochrome has a molecular weight of 124 kDa (the size varies only slightly with the plant source), with a polypeptide chain of 1128 amino acid residues. The protein dissolves as a dimer in aqueous buffers, and electron microscopy has shown the dimer to be Y shaped, with the amino terminal domains occupying one branch each and the carboxyl domains combining to form the third (ref. 4).
Phytochrome possesses a single bilatriene chromophore which is bound covalently to cystein-321 (Fig. 1), and which is responsible for the absorption in the red visible region. The bilatriene chromophores of Pr and Pfr are constitutionally identical, but the C(15) double bond configuration has been established to be different: Pr possesses the 15Z and Pfr the 15E configuration. Configuration and conformation around the other double bonds and around the single bonds of the methine bridges are still unknown.

Our results obtained in studies at physiological temperatures (\( \geq 275 \) K) shall be discussed in terms of the working scheme shown in Fig. 2 for the phototransformation of Pr into Pfr.

PHOTOPHYSICAL PROPERTIES OF Pfr
The stationary fluorescence of the Pr bilatriene chromophore is compatible with the stretched alignment shown in Fig. 1 (ref. 5). Single-photon-timing (SPT) measurements with global data analysis
Fig. 1. Bilatriene chromophore of Pr phytochrome. A "stretched" conformation with \( Z_2 Z Z_2 \) configuration of the double bonds was chosen in analogy to that of the phycocyanobilin chromophores \( \alpha-84 \) and \( \beta-84 \) in C-phycocyanin of cyanobacteria (ref. 2). Furthermore, absorption (ref. 1e) and resonance Raman evidence (ref. 3) is in favour of N-protonation.

Fig. 2. Tentative working hypothesis of the mechanism of the \( P_r \rightarrow P_{fr} \) transformation.

The fluorescence quantum yield of the \( P_{fr}^{1} \) and \( P_{fr}^{2} \) components at 275–293 K is only \( \alpha_f = 3 \times 10^{-3} \) (refs. 6,7), and deactivation proceeds predominantly through nonradiative channels, such as internal conversion back to ground–state \( P_r \) and primary photoreaction(s) such as the \( P_{fr}^{1,2*} \rightarrow P_{fr} \) transformation.

An SPT study of the 10 tryptophan residues, which are situated within the central third of the polypeptide chain of 1128 amino acids (residues 366 – 790), showed that the fluorescence falls into four lifetime classes with distributions ranging from ca. 30 ps to 5 ns at 277 K. The changes in
the decay pattern occurring in the $P_r \rightarrow P_{fr}$ transformation are insignificantly small (Fig. 4). Evidently the central protein domain does not undergo any gross overall conformational change in the reaction (ref. 9).

**THE $P_r \rightarrow P_{fr}$ PHOTOTRANSFORMATION**

Primary photoreactions and the first set of parallel thermal secondary steps

Nanosecond flash photolysis, absorption (refs. 10–12) and time–resolved optoacoustic spectroscopy (refs. 13–15) reveal that in an apparent first step $P_r$ simultaneously affords two products, $I^*$ and
The terminal steps of the Pfr formation

The increase of Pfr 20 ms after the excitation of Pr again proceeds in two phases, which suggests two immediate precursors of Pfr [viz., H2X1,2] with lifetimes of $\tau_1 = 0.03$ s and $\tau_2 = 1$ s at 275 K (ref. 22). Changing from H2O to D2O buffer affects the Pfr formation only slightly, which excludes any kinetic H/D isotope effect.

The influence of ethylene glycol and ubiquitin

Addition of 20–25% ethylene glycol does not alter the Pr $\Rightarrow$ Pfr photochromicity, and lifetimes and amplitudes of the Pfr1,2 fluorescence components (ref. 9) as well as the absorption decay of 124-kDa Pr1,2 are hardly affected. This is in accord with a confinement of the sequence Pr $\rightarrow$ Ibl to the bilatriene
chromophore–protein domain without any far-reaching assistance by other domains. Interference by ethylene glycol in this domain is reflected by the formation rates of \( I_{700}^1 \) and \( I_{700}^2 \): while the ratio \( I_{700}^1/I_{700}^2 \) is clearly dependent on temperature in the absence of ethylene glycol, it does not vary any more in its presence.

Results like this initiated a search for other agents to modify the dynamics of the complex \( P_r \rightarrow P_f \) transformation, and in particular to affect differentially the various intermediates. The search focused especially on cellular constituents which presumably interact \textit{in vivo} with phytochrome. Ubiquitin, an 8.5–kDa protein claimed to undergo covalent binding \textit{in vivo} to the physiologically active \( P_f \) (ref. 23), has now also been found to interact \textit{in vitro} with the dormant \( P_r \) form in the absence of any other cellular constituent (ref. 24). Association causes dissociation of the protein dimer and formation of ubiquitin–\( P_r \) complexes which contain one \( P_r \) monomer and in which the ubiquitin and \( P_r \) components are not covalently bound. These complexes are still fully photo–reversible. In addition to the monomerization effect, the complexation shifts the 695–nm absorption maximum of \( I_{700}^1 \) differentially to shorter wavelengths by ca. 10 nm, with the shorter–lived component \( I_{700}^1 \) being affected at a lower ubiquitin–\( P_r \) ratio than \( I_{700}^2 \) (Fig. 6). The results altogether point to a reversible interaction of ubiquitin with the protein pocket domain housing the bilatriene chromophore, which thus constitutes — in contrast to previous transformations with non–biological reagents — a tool to differentially monitor the complex reaction dynamics without a permanent chemical change in the domains involved directly.

**The chemical nature of the individual reaction steps**

Photochromicity as well as fluorescence lifetimes, relative amplitudes and quantum yields of \( P_f^1 \) and \( P_f^2 \), which account for \( \geq 99\% \) of the total fluorescence decay amplitude, are the same in \( H_2O \) and \( D_2O \) at 275 and 293 K (ref. 6). The appearance of the \( I_{700}^1 \) absorption does not reveal either a kinetic H/D effect on the efficiency of the primary photoreaction (ref. 12). A proton transfer is therefore unlikely in the photoreaction or any other deactivation of \( P_f^{1,2} \). The most probable process for \( P_r \rightarrow I_{700}^1 \) is therefore a \( Z \rightarrow E \) double bond isomerization.

The subsequent steps leading to \( P_f \) exhibit no more than mere solvent–assisted H/D effects on the reaction rate constants, which excludes rate–determining proton transfer processes.

Step \( I_{700}^{1,2} \rightarrow I_{bl}^1 \) comprizes a conformational relaxation of the chromophore, which leaves room in the still insufficiently explored "grey" zone between \( I_{bl}^1 \) and \( P_f \) for relatively slow reorganizations of the protein structure.

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Fig. 6. Comparison of the difference spectra of \( I_{700}^1 \) at zero time and 275 K for the ubiquitin–free \( P_r \) dimer (□; 124–kDa phytochrome) and for ubiquitin–\( P_r \) 5:1 (■), and component spectra for the ubiquitin–\( P_r \) 5:1 complex (●, \( I_{700}^1 \); ○, \( I_{700}^2 \); △ is a constant function required in the equation

\[
\Delta A(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + \text{constant}
\]

which fits the decay of the \( I_{700}^1 \) absorption. (Taken from ref. 22.)
REFERENCES


7. R.D. Vierstra and P.H. Quail, in ref. 1c, p. 35.


