

Real-time tracking of phytochrome's ring D orientational changes during Pr photoisomerization: Two Pr isoforms with different photoisomerization yields

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Abstract. Phytochromes' ring D orientational changes are tracked during Z-to-E photoisomerization by polarization resolved femtosecond visible pump-infrared probe spectroscopy. Two distinct Pr isoforms Pr-I and Pr-II exhibit photoisomerization yields of 3% and 29%, respectively.

1 Introduction

Photoisomerization of the phycocyanobilin (PCB) chromophore is the basis of the light sensing and signaling response of Cph1 phytochrome. Phytochromes are a family of dimeric chromoproteins that absorb light and regulate numerous photoresponses in plants, bacteria, and fungi.[1]

Two thermally stable ground states conformers sensing red (Pr form) and far-red light (Pfr form) can be interconverted by photoexcitation. Excitation of the Pr form with ZZZssa (C₅-Z, C₁₀-Z, C₁₅-Z, C₅-syn, C₁₀-syn, C₁₅-anti)[2] PCB chromophore geometry initiates an ultrafast Z-to-E photoisomerization of phytochromes' ring D around C₁₅=C₁₆ methine bridge resulting in the Lumi-R photoproduct.

Two time constants of about 3 ps to 16 ps and 25 ps to 40 ps are reported for the initial processes.[3] The low photoproduct quantum yield of 7% to 16% in phytochromes was proposed to result from photoreceptor heterogeneities.[4] Recently, two Pr isoforms were identified by NMR spectroscopy.[5]

2 Materials and Methods

We used polarization resolved femtosecond visible pump-infrared probe spectroscopy to determine the orientation of the $\nu(\text{C}=\text{O})$ vibrational modes of the PCB chromophore on a femtosecond time scale in the Pr* and Pr state.[6]

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We investigated $^{13}\text{C}/^{15}\text{N}$ labelled Cph1 Δ 2 protein with non-labeled PCB chromophore, shifting all $\nu(\text{C}=\text{O})$, $\nu(\text{C}=\text{C})$, and $\nu(\text{C}=\text{N})$ stretching vibrations of the protein out of our spectral window of 1670 – 1745 cm^{-1} .

The data were acquired by excitation of less than 3% of the Cph1 Δ 2 sample at 660 nm ($S_0 \rightarrow S_1$ transition). The signals were recorded with a 2x32 element MCT array detector at a spectral resolution of 1.5 cm^{-1} . Parallel and perpendicular polarization signals with respect to pump pulse polarization were recorded at the same time.

3 Results

Isotropic transients are presented in Figure 1. Transients are fitted with two time constants of 30 ps and 4.7 ps. Two distinct bleaching signals at 1701 cm^{-1} and 1708 cm^{-1} display two Pr isoforms with different photoreaction quantum yields (Figure 1B).

By analysis of the relative angles between electronic transition dipole moment (tdm) and vibrational tdm's measured at 1701 cm^{-1} and 1708 cm^{-1} , we were able to assign the two orientations to the Pr-II and Pr-I isoform, respectively. The isoforms exhibit different ring D orientations presented in Figure 2 allowing for assignment of the bleaching signals.

In addition, rotation of ring D was observed in the electronic excited state of Pr (Pr*). The measured polarization resolved signals indicate 16% of the excited chromophores reach a 90° twisted transition state after 21 ps.

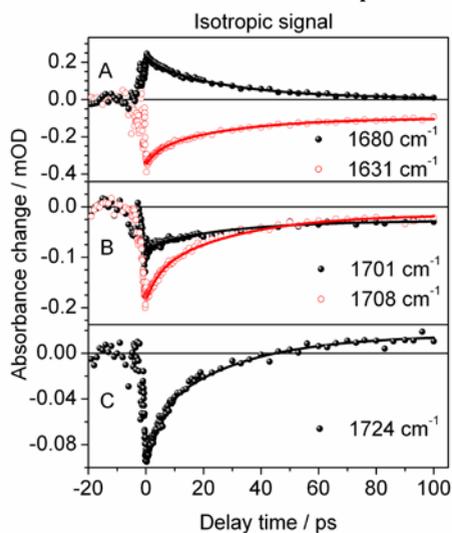


Fig. 1. Transient dynamics at frequency positions of marker bands calculated for isotropic conditions. A) Bleaching signal of $\nu(\text{C}_{15}=\text{C}_{16})$ and $\nu(\text{C}_{17}=\text{C}_{18})$ stretching vibrations (open red circles), absorption of $\nu(\text{C}_{19}=\text{O})^*$ stretching vibration in the electronically excited state. B) Bleaching signals of $\nu(\text{C}_{19}=\text{O}^a)$ and $\nu(\text{C}_{19}=\text{O}^b)$ stretching vibrations in the Pr state (solid and open red circles, respectively). C) Bleaching signal of $\nu(\text{C}_1=\text{O})$ stretching vibration and $\nu(\text{C}_{19}=\text{O})$ absorption of lumi-R.

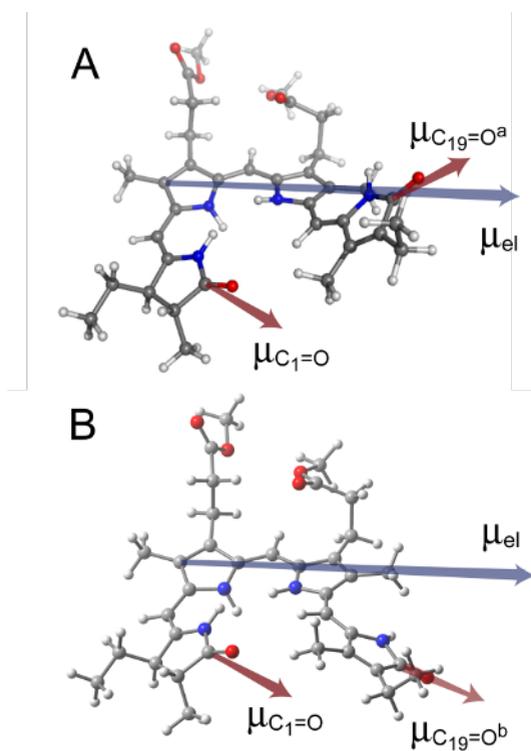


Fig. 2 . PCB chromophore geometries with Pr $S_0 \rightarrow S_1$ transition dipole moment μ_{el} (black arrow) and vibrational tdms of $\nu(C_{19}=O)^a$ of Pr-II and $\nu(C_1=O)$ (red arrows). A: PCB^a geometry of Pr-II, B: PCB^b geometry of Pr-I, Note, tdm arrows can point in either direction.

The overall photoisomerization is best explained by a single rotation around $C_{15}=C_{16}$ methine bridge in the Pr*. The Pr-I isoform shows a quantum yield of 3%, while the Pr-II isoform exhibits a much higher quantum yield of 29%. The Pr-II isoform has a hydrogen bond between His290 and ring D and is more twisted than the Pr-I isoform. These data suggest an efficacy increase of the photoreaction by side chain assisted pre-twisting.

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