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MAS NMR study of the photoreceptor phytochrome

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Summary

The protein phytochrome is responsible for light-induced physiological changes (photomorphogenesis) in plants. This chromoprotein also occurs in bacteria, cyanobacteria and fungi. The activity of phytochromes relies on an open-chain tetrapyrrole chromophore which is covalently bound to a cysteine residue *via* a thioether linkage at the C3¹ or C3² position of the side chain of ring **A**. In the case of Cph1Δ2, the three methine bridges of the PCB chromophore adopt a *ZZZssa* geometry in the biologically inactive Pr state. Upon absorption of red light, the C15=C16 double bond undergoes a *Z*-to-*E* photoisomerization leading to the conversion into the active Pfr state *via* three intermediates. The thermally stable Pfr state is converted back to Pr upon absorption of far-red light. The back-reaction presents two intermediates, called Lumi-F and Meta-F. The first step of the back-reaction is the *E*-to-*Z* isomerization of the C15=C16 double bond. The aim of this thesis is to provide an understanding of the light-induced chromophore mechanics and its coupling to the intramolecular signal transduction pathway. To this end, MAS NMR spectroscopy is applied to Cph and plant Phy proteins.

The study of the free PCB may be useful for understanding its complex behavior inside the protein matrix. **Chapter 2** deals with the investigation of the free PCB in the microcrystalline state. ¹H, ¹³C and ¹⁵N MAS NMR spectroscopy reveals that two forms of PCB moieties, called A and B, are present in equal proportions in the microcrystal. In addition ¹⁵N CP/MAS data show that solely the nitrogen atom of ring **B** is unprotonated. The structure of PCB in the microcrystalline sample is determined by a combination of MAS NMR and quantum mechanical calculations. It is shown that the PCB moieties form **D**–**D'** dimers linked by hydrogen-bonding interactions between the nitrogen of ring **D** and the carbonyl group of ring **D'** and *vice versa*.

In **Chapter 3**, both parent states, Pr and Pfr, have been studied by ^1H , ^{13}C and ^{15}N CP/MAS NMR in the Cph1 Δ 2 and *phyA*65 phytochrome sensory modules containing an u- ^{13}C , ^{15}N -, ^{13}C 5- or ^{15}N 21-PCB labeled bilin chromophore. 2D homo- and heteronuclear experiments allowed for the assignment of the ^{13}C and ^{15}N chemical shifts in both states. The chromophore in Cph and plant Phy shows similar features in their respective Pr and Pfr states. The ^{15}N chemical shift analysis reveals that all four nitrogen atoms are protonated in both states. Differences in ^{13}C chemical shift reflect the changes of the electronic structure of the cofactor at the atomic level as well as its interactions with the chromophore binding pocket. The ^{13}C data are interpreted in terms of a strengthened hydrogen-bond at the ring **D** carbonyl in the Pfr state. The red shift of the maximum of absorption in the Pfr state is explained by the increasing length of the conjugation network beyond ring **C** including the entire ring **D**. The enhanced conjugation within the π -system stabilizes the strained chromophore in the Pfr state. Concomitant changes at the ring **C** propionic side chain and the ring **D** carbonyl are explained by a modification of their hydrogen-bonding to His-290. These and other conformational changes may lead to modified surface interactions.

Chapter 4 describes the photochemically induced Pfr $\rightarrow$ Pr back-reaction. The light-triggered Pfr $\rightarrow$ Pr conversion is followed at low temperature by MAS NMR spectroscopy. Both Lumi-F and Meta-F intermediates have been thermally trapped in the magnet and all four states, Pfr, Lumi-F, Meta-F and Pr have been characterized. The mechanical process of the back-reaction involves the bond rotation around the C15 methine bridge, which occurs in two distinguishable steps: (i) In the Lumi-F state, the C15=C16 double bond is photoisomerized, (ii) the rotation along the C14-C15 single bond occurs during the transformation to Meta-F. The structural analysis reveals that the chromophore adopts a distorted conformation in Lumi-F. The Meta-F intermediate shows features similar to Pr, however, the final hydrogen-bonding interactions of the ring **D** nitrogen are still to be formed and a protein re-arrangement around ring **A** still has to take place. A model for the back-reaction is presented.

Tetrapyrrole moieties have been extensively studied by liquid-state NMR spectroscopy. In particular, the members of the 2,3-dihydrobilindione family are good model compounds for the chromophore in phytochrome. In **Chapter 5**, the ^{13}C chemical shifts of such compounds are compared to those of PCB

in its free state and as chromophore in phytochrome. In this way, information about the chromophore/protein interactions resulting from PCB assembly and upon the $\text{Pr} \rightarrow \text{Pfr}$ conversion is obtained. First, the change in ^{13}C chemical shifts in PCB caused by its assembly in the protein shows that rings **B** and **C** are symmetrical in the protein matrix, suggesting that the positive charge is delocalized over the two inner rings in the Pr state. Second, the chromophore assembly into the protein imposes new constraints to the chromophore. The strongest effect is located around the C15 methine bridge, which adopts a (*Z*, *anti*) geometry in the protein. The pattern of change in ^{13}C chemical shift caused by the *Z*-to-*E* isomerization in protonated model compound in solution is entirely different from that one observed upon the $\text{Pr} \rightarrow \text{Pfr}$ conversion. This demonstrates that the chromophore/protein interactions drastically influence the electronic structure. Finally, future perspectives for MAS NMR on phytochrome are discussed. For instance, distance and angle measurements by MAS NMR can provide information about the change in geometry of the chromophore throughout the photocycle. In addition, MeLoDi-type of NMR experiments can be used to investigate the chromophore/protein interaction at various stage of the photocycle. In this way, the photo-triggered activity of the chromophore can be related to the signal transduction in phytochrome.

