

# Spectroscopic Characterization of Plant Blue-light Photoreceptors

Stefan Weber

*Freie Universität Berlin, Fachbereich Physik, Arnimallee 14, 14195 Berlin, Germany*

The phototropic response of higher plants is mediated by the blue-light photoreceptor phototropin. The protein comprises a kinase domain and two topologically similar flavin-mononucleotide (FMN) binding LOV domains, LOV1 and LOV2. Blue-light irradiation of the LOV domains causes a reversible bleaching of the flavin chromophore absorption at 400–500 nm accompanied by a bathochromic shift of a band at 370 nm to 390 nm which has been attributed to the reversible addition of the thiol group of a cysteine in the cofactor-binding pocket to the carbon position C(4a) of FMN. Quite unusually, the flavin triplet state is a reactive intermediate in the photo-induced formation of the FMN C(4a)–cysteine adduct of LOV domains, which undergoes spontaneous fragmentation on a time scale of several minutes at room temperature.

The photo-induced triplet state and the photoreactivity of the FMN cofactor in two LOV domains of plant and algal phototropin have been studied by time-resolved electron paramagnetic resonance (tr-EPR) and UV–vis spectroscopy at low temperatures ( $T \leq 80$  K) [1]. Differences in the electronic structure of the FMN as reflected by altered zero-field splitting parameters of its triplet state [2] could be correlated with changes in the amino-acid composition of the cofactor binding pocket in wild-type LOV1 and LOV2 as well as in mutant LOV domains [1]. Even at cryogenic temperatures, tr-EPR experiments indicate photoreactivity of wild-type LOV, which was further characterized by UV–vis spectroscopy. The absorption maximum of the low-temperature photoproduct of wild-type LOV2 is red-shifted by about 15 nm as compared with the FMN C(4a)–cysteinyl adduct formed at room temperature. In light of these observations we discuss a radical-pair reaction mechanism for the primary photoreaction in LOV domains [1, 3–5].

Interestingly, in an NMR experiment on a mutant LOV2 domain in which the reactive cysteine has been replaced by alanine, strongly emissive and enhanced absorptive  $^{13}\text{C}$ -NMR lines have been observed upon sample illumination [6]. Together with  $^{13}\text{C}$ -ENDOR experiments performed at W-band frequencies, a detailed picture of the electron-spin density distribution on the FMN is obtained.

The work presented here was performed in collaboration with E. Schleicher, R. Kowalczyk, C.W.M. Kay and R. Bittl, in cooperation with the groups of Prof. A. Bacher, Dr. W. Eisenreich, Dr. M. Fischer (TU Munich), Prof. G. Richter (University of Exeter), and Prof. P. Hegemann (HU Berlin). Financial support by the Deutsche Forschungsgemeinschaft (SPP-1051, Sfb-498 (B7)) is gratefully acknowledged.

- [1] E. Schleicher, R.M. Kowalczyk, C.W.M. Kay, P. Hegemann, A. Bacher, M. Fischer, R. Bittl, G. Richter, S. Weber, *J. Am. Chem. Soc.* **126** (2004) 11067–11076
- [2] R.M. Kowalczyk, E. Schleicher, R. Bittl, S. Weber, *J. Am. Chem. Soc.* **126** (2004) 11393–11399
- [3] C.W.M. Kay, A. Kuppig, E. Schleicher, A. Bacher, G. Richter, S. Weber, in “Flavins and Flavoproteins 2002”, S. Chapman, R. Perham, N. Scrutton (Eds.), Rudolf-Weber Agency for Scientific Publications, Berlin (2002), pp. 707–712
- [4] C.W.M. Kay, A. Kuppig, E. Schleicher, H. Hofner, W. Rüdiger, M. Schleicher, M. Fischer, A. Bacher, S. Weber, G. Richter, *J. Biol. Chem.* **278** (2003) 10973–10982
- [5] R. Bittl, C.W.M. Kay, S. Weber, P. Hegemann, *Biochemistry* **42** (2003) 8506–8512
- [6] G. Richter, W. Römisch, E. Schleicher, A. Bacher, W. Eisenreich, M. Salomon, E. Knieb, H. Dürr, W. Rüdiger, V. Massey, F. Müller, S. Weber, in “Flavins and Flavoproteins 2002”, S. Chapman, R. Perham, N. Scrutton (Eds.), Rudolf-Weber Agency for Scientific Publications, Berlin (2002), pp. 719–724