

Spectroscopic Characterization of Plant Blue-light Photoreceptors

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Interestingly, in an NMR experiment on a mutant LOV2 domain in which the reactive cysteine has been replaced by alanine, strongly emissive and enhanced absorptive ^{13}C -NMR lines have been observed upon sample illumination [6].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

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