OBSERVATION OF A TRIPLET STATE IN CHLOROPHYLL PROTEIN 668 VIA OPTICALLY DETECTED MAGNETIC RESONANCE

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1. Introduction

We have investigated the photo-excited triplet state properties of a species contained in the water-soluble chlorophyll protein (Cp 668) extracted from the stems of young plants of *Atriplex hortensis* using optically detected magnetic resonance (ODMR). The main absorption band of the material occurs at 668 nm with additional regions of absorption to the blue. Upon illumination in the presence of an electron acceptor such as oxygen, a new absorption band appears at 743 nm, while the A_{668} peak decreases in intensity [1–3]. The fully converted state, Cp 743, is never observed in intact plants, or in plant extracts, so its photoconversion does not seem to be the primary function of this unique water-soluble chlorophyll protein [2–5]. In an effort to understand the photoreactive state of Cp 668 and the involvement of triplet states in chlorophyll proteins the following study was undertaken from which we present the first ODMR results. Some preliminary triplet state data for Cp 668 have been presented [6].

Investigation into the triplet state sublevel characteristics of non-bacterial photosynthetic systems has been limited to chloroplast preparations [7], and various algae [8,9]. In reduced chloroplasts, and in algae, monomeric chlorophyll *a* type transitions were found [7–9]. We shall discuss our findings with reference to the results for non-bacterial systems.

2. Materials and methods

Chlorophyll protein 668 was extracted from freshly collected green stem sections of *Atriplex hortensis* plants by homogenization in a Waring blender in 0.1 M phosphate buffer (pH 7.2). The resulting suspension was filtered through cheesecloth and centrifuged at 10 000 × *g*. Cp 668 protein was precipitated at 0.3–0.7 M ammonium sulfate saturation. The pellet was resuspended in buffer and dialyzed against 100 vol. buffer for 12 h. The protein was additionally purified and concentrated by ultrafiltration using an Amicon Diaflo ultrafiltration cell and a PM-30 filter that retains proteins with mol. wt > 30 000. The resultant sample was centrifuged at 144 000 × *g* for 1 h.

The experimental samples were prepared from the filtrate at A_{668} 0.01. The fluorescence spectrum of Cp 668 exhibits a peak at 676 nm. After irradiation in white light for ~10 min at room temperature, an additional fluorescence peak of comparable intensity appears at 745 nm. Both emission bands have been utilized for the detection of triplet state magnetic resonance. A description of the ODMR technique utilizing fluorescence detection has been published [10].

3. Results and discussion

The observed triplet state ODMR transitions are enumerated in table 1. The D+E microwave transition at 1041 MHz gives rise to the most intense ODMR peak while the D–E resonance at 828 MHz is very weak. No structure is observed on either ODMR peak. The same spectra are observed in both the 668 nm and 743 nm fluorescence peaks. No other ODMR

Table 1
Triplet state ODMR properties of Cp 688

Zero-field transitions (MHz)	Overall triplet decay rate (s^{-1})	Microwave response rates at 1041 MHz (s^{-1})
828 ± 2	295 ± 15	120 ± 10 (μ waves off)
1041 ± 2		250 ± 20 (μ waves on)

transitions were observed over 500–1500 MHz.

Our results seem to indicate that the observed triplet state does not belong to a chlorophyll *a* species, either as the monomer or its dimeric counterpart. Earlier studies of chlorophyll *a* zero-field splitting (ZFS) [11,12] have established chlorophyll *a* transitions to occur at about 720 and 930 MHz (depending on choice of solvents). For chlorophyll *b*, zero-field resonances are found at 837 and 1083 MHz [11], closer to the observed values in the Cp 668 experiments. Triplet exciton theory [13] and experimental verifications [14] have borne out the fact that upon aggregation, the frequency of zero-field resonances should decrease. As can be seen in table 1, our numbers fall well outside the range of chlorophyll *a* transitions, even if the possibility of an aggregated species is considered, leading us to consider the observed triplet state as arising from a chlorophyll *b* moiety.

The overall triplet decay of Cp 668 was measured by the method in [15] and found to be 295 ± 15 s^{-1}. The triplet decay constant for chlorophyll *a* as calculated from its sublevel kinetic data is 719 s^{-1} whereas that of chlorophyll *b* is 291 s^{-1} [10]. Again this points toward the conclusion that the triplet state observed in Cp 668 belongs to a chlorophyll *b* type species.

Transient measurements of the fluorescence intensity (see table 1) with a saturating microwave field of the appropriate frequency alternately turned on and off were also carried out [9]. The low intensity of the D–E transition only enabled us to report reliable kinetic data from one ODMR transition. When coupled with the overall triplet lifetime, a preliminary kinetic scheme is obtained in which τ_z, the lowest lying sublevel, has the largest decay rate, contrary to that observed in all isolated chlorophyll systems [11], and may suggest unusual aggregate properties for the species observed. Obviously, further work is necessary to verify this seemingly anomalous behavior.

It is to be noted that the only optically detected magnetic resonance spectra on a plant preparation reported (chloroplasts from spinach) yielded a triplet state with a ZFS (ODMR frequencies at 723 MHz and 952 MHz) consistent with monomeric chlorophyll *a* [7]. Our chlorophyll protein extract, on the other hand, clearly resembles chlorophyll *b* more closely and is thus presumably due to a species connected with the antenna system of Cp 668. Further, the presence of this triplet species is unaffected by the photoconversion of Cp 668 to Cp 743. In Cp 668 obtained from *Amaranthus* plants, it has been shown that chlorophyll *b* transfers its energy to the 743 pigment [16] but is not itself transformed in the light [17]. The observation of chlorophyll *b* type species, unaffected by the photoconversion of Cp 668 to Cp 743 in our ODMR experiments seems consistent with these energy transfer results. The function of this chlorophyll *b* trap in Cp 668 remains to be resolved, but the present results support an energy transfer role for Cp 668 in vivo.

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