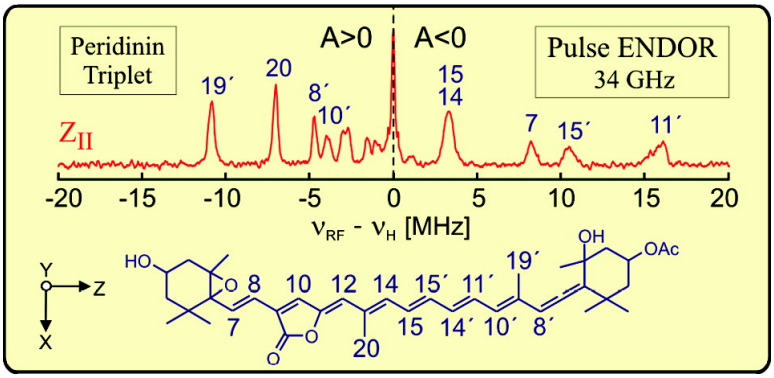
Communication

Spin-Density Distribution of the Carotenoid Triplet State in the Peridinin-Chlorophyll-Protein Antenna. A Q-Band Pulse Electron-Nuclear Double Resonance and Density Functional Theory Study

Jens Niklas, Tim Schulte, Shipra Prakash, Maurice van Gastel, Eckhard Hofmann, and Wolfgang Lubitz

J. Am. Chem. Soc., **2007**, 129 (50), 15442-15443 • DOI: 10.1021/ja077225v



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 11/21/2007

Spin-Density Distribution of the Carotenoid Triplet State in the Peridinin-Chlorophyll-Protein Antenna. A Q-Band Pulse Electron-Nuclear Double Resonance and Density Functional Theory Study

Jens Niklas,[†] Tim Schulte,[‡] Shipra Prakash,[†] Maurice van Gastel,[†] Eckhard Hofmann,[‡] and Wolfgang Lubitz*,[†]

Max-Planck-Institut für Bioanorganische Chemie, Stiftstrasse 34-36, D-45470 Mülheim an der Ruhr, Germany, and Lehrstuhl für Biophysik, Ruhr-Universität Bochum, Universitätsstrasse 150, D-44801 Bochum, Germany

Received September 18, 2007; E-mail: lubitz@mpi-muelheim.mpg.de

Carotenoids fulfill several important functions in nature, such as light harvesting, photoprotection, and dissipation of excess energy, for example, in photosynthetic systems.[1−3] However, little is known about the detailed electronic structure of the short-lived excited states of carotenoids that play a role in these processes. Such information is, in principle, accessible for the carotenoid triplet state from application of time-resolved (pulse) EPR and electron-nuclear double resonance (ENDOR) experiments.[4−7]

In the peridinin-chlorophyll-protein (PCP), an antenna system found in dinoflagellates, the carotenoid peridinin serves as the main light-harvesting pigment.[8] In addition, it protects the protein by quenching chlorophyll (Chl) triplet states, thus preventing the formation of harmful singlet oxygen. The crystal structure of the main form of the PCP trimer (MFPCP) from *Amphidinium carterae* has been determined by X-ray crystallography at a resolution of 2.0 Å (Figure 1).[9] Recently, a heterologous expression system for the N-domain of MFPCP was developed.[10] The refolding of the protein in vitro results in a complex called refolded PCP (RFPCP), with virtually the same structure and spectroscopic properties as MFPCP.[10,11] This offers the possibility of incorporation of modified cofactors into the protein.

Illumination of PCP with red light generates an excited singlet state of Chl *a*, which can undergo intersystem crossing to form the chlorophyll triplet state, ^{3}Chl *a*. The peridinin molecule 614 is in optimal contact with the Chl π-system and is thus able to take over the triplet exciton with high yield within a few ns.[12]

From time-resolved EPR spectroscopy the zero-field-splitting (ZFS) parameters D and E and the *g* values, as well as the kinetics of triplet formation and decay can be obtained.[4,5,7] In Figure 2a the field-swept echo (FSE) Q-band EPR spectrum of triplet peridinin in RFPCP is depicted, which shows a polarization pattern EAEAEA, as a result of intersystem crossing in the Chl *a* molecule and subsequent triplet transfer to the peridinin 614.[12] The ZFS parameters obtained from a simulation are |D| = 48.2 mT and |E| = 4.7 mT.[13] The EPR signal of the peridinin triplet decays at the canonical *X*, *Y*, and *Z* orientations with time constants of 18, 8/44, and 11 μs, respectively.

Application of ENDOR spectroscopy[6,16] allows determination of the hyperfine coupling constants (hfcs) of the magnetic nuclei ($I > 0$) interacting with the strongly coupled triplet electron spins ($S = 1$). From the assigned hfcs, for example of the protons ($I = {}^1/_2$) of peridinin, a map of the spin-density distribution of the triplet electrons over the molecule can be obtained. However, the short lifetime and low triplet yield often prevents the application of ENDOR spectroscopy to triplet carotenoids.[17] We have therefore used pulse Q-band (34 GHz) ENDOR spectroscopy, a specially

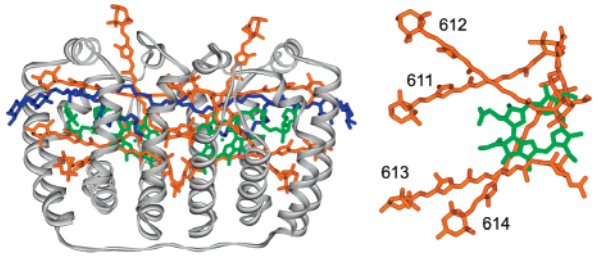


Figure 1. Left: Crystal structure of a MFPCP monomer (PDB entry 1PPR).[9] Each PCP monomer consists of two pseudosymmetric domains (N- and C-domain), each of which contains one lipid (blue), one Chl *a* (green), and four peridinin molecules (orange) that are in van der Waals contact with the Chl to promote efficient energy transfer. Right: Chl *a* (truncated phytyl chain) and peridinin cofactors in the N-domain; for numbering see ref 9.

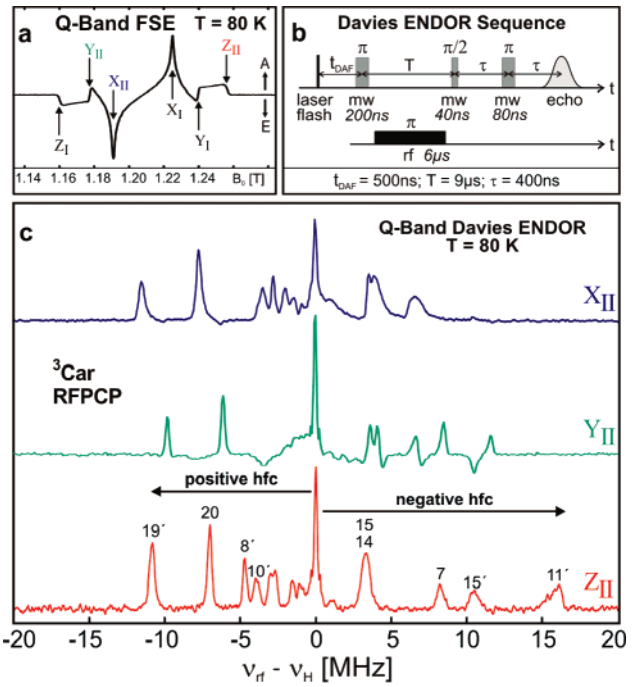


Figure 2. (a) Q-band FSE EPR spectrum of peridinin triplet in RFPCP (A = absorption, E = emission); (b) Davies ENDOR[14] pulse sequence; (c) Q-band ^{1}H ENDOR spectra recorded at the three canonical orientations X_{II}, Y_{II}, Z_{II}, which are marked with arrows in the EPR spectrum of panel a, using the conditions in panel b. At the proton Larmor frequency ν_H a narrow and intense line is visible resulting from nuclear transitions in the $M_S = 0$ manifold. The frequency axis gives the deviation from ν_H in the respective spectra. The concentration of RFPCP was about 200 μM, and the excitation wavelength was 630 nm.[15]

[†] Max-Planck-Institut für Bioanorganische Chemie.
[‡] Ruhr-Universität Bochum.

10.1021/ja077225v CCC: $37.00

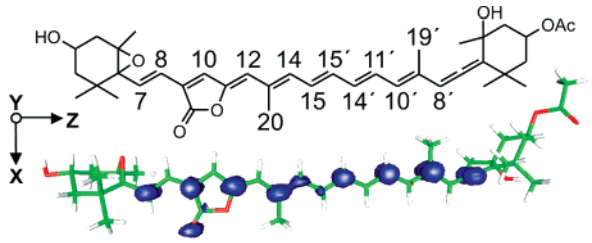


Figure 3. Molecular structure of the carotenoid peridinin (IUPAC numbering) and spin density plot of peridinin 614 in its excited triplet state. The orientation of the ZFS tensor axes *X*, *Y*, and *Z* according to the DFT calculations is also given.

Table 1. ^{1}H Hyperfine Coupling Constants (MHz) of Peridinin Triplet in RFPCP Obtained from ENDOR Spectroscopy and DFT Calculations. A Tentative Assignment to Molecular Positions (Figure 3) Is Provided.

	experimental				DFT calculation			
position[a]	A_x	A_y	A_z	a_{iso}	A_x	A_y	A_z	a_{iso}
8′	3.5	1.8	4.7	3.3	2.9	1.4	4.1	2.8
19′*	11.5	9.8	10.8	10.7	10.0	8.0	8.8	8.9
10′	2.8	1.2	3.9	2.6	1.9	0.2	3.1	1.7
11′	−6.6	−11.7	−16.0	−11.4	−4.1	−9.8	−12.7	−8.9
15′	−3.9	−8.5	−10.5	−7.6	−2.6	−7.7	−9.2	−6.5
15	−1.0	−4.1	−3.3	−2.8	−0.5	−3.8	−3.3	−2.5
14	−1.0	−3.6	−3.3	−2.6	−1.1	−5.1	−5.0	−3.7
20*	7.7	6.1	7.0	6.9	6.7	5.0	5.9	5.8
7	−3.6	−6.7	−8.2	−6.2	−3.0	−7.4	−9.1	−6.5

[a] The asterisks mark the magnetically equivalent protons of the rotating methyl groups. All hfc tensor axes are collinear to the ZFS tensor axes within 20°.

designed resonator with large sample access and illumination slits, and a sequence with optimized microwave (mw) and radiofrequency (rf) pulses for our experiments.[15] The increased sensitivity and spectral resolution of this setup enabled the study of carotenoid triplet states with good signal-to-noise ratio.

^{1}H ENDOR spectra of triplet peridinin in RFPCP are shown in Figure 2c.[18] According to the ENDOR resonance condition $\nu_{ENDOR} = |\nu_H - M_S A|$ the hfcs including their signs, relative to that of D, can directly be obtained from the spectra.[6,16] The hfc tensors are deduced from orientation selected spectra recorded at different magnetic fields, for example, corresponding to the canonical orientations of the **D** tensor (X_{II}, Y_{II}, and Z_{II} in Figure 2c; see also Figure S1 in Supporting Information).

At least 13 proton hyperfine couplings have been resolved, comparable to the number of protons in the conjugated part of the peridinin (see Figure 2c and Figure 3). This confirms that the triplet is localized on one specific peridinin molecule at low temperature (80 K).[12]

The two large *positive* hfcs with small anisotropy can be safely assigned to the methyl group protons (positions 19′, 20); the five largest *negative* hfcs show a large anisotropy (cf. Figure 2c), they are assigned to α-protons directly attached to the peridinin π-system. A tentative assignment of the measured larger couplings to molecular positions in peridinin is achieved by a comparison with DFT calculations[19] of the hfc tensors (see Table 1). The calculated spin-density plot is depicted in Figure 3.

This study has provided detailed information about the ^{1}H hfc tensors and the spin density distribution of the excited triplet state of the carotenoid peridinin in the RFPCP antenna. The combined EPR/ENDOR experiments described here can be extended to elucidate the electronic structure of other short-lived carotenoid triplet states, opening new perspectives in the investigation of the functional details of these important molecules on a molecular level with atomic resolution.

Acknowledgment. We thank D. Carbonera, M. Di Valentin, S. Ceola (University Padova), and F. Neese (University Bonn) for helpful discussions, G. Klihm (MPI Mülheim) for technical assistance, and Silke Johanning (University Bochum) for providing MFPCP. This work has been supported by the DFG (SFB 663, TP A7 and SFB 480, TP C6) and the Max Planck Society.

Supporting Information Available: Sample preparation, experimental details, details of the DFT calculations, and additional ENDOR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Polivka, T.; Sundström, V. *Chem. Rev.* **2004**, *104*, 2021.
(2) *The Photochemistry of Carotenoids*; Frank, H. A., Young, A. J., Britton, G., and Cogdell, R. J., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1999.
(3) Frank, H. A.; Cogdell, R. J. In *Carotenoids in Photosynthesis*; Young, A. J., Britton, G., Eds.; Chapman & Hall: London, 1993; pp 252−326.
(4) Stehlik, D.; Möbius, K. *Annu. Rev. Phys. Chem.* **1997**, *48*, 745.
(5) Angerhofer, A. In *The Photochemistry of Carotenoids*; Frank, H. A., Young, A. J., Britton, G., Cogdell, R. J., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1999; pp 203−222.
(6) Kemple, M. D. In *Multiple Electron Resonance Spectroscopy*; Dorio, M. M., Freed, J. H., Eds.; Plenum Press: New York, 1979; pp 409−436.
(7) Levanon, H.; Norris, J. R. *Chem. Rev.* **1978**, *78*, 185.
(8) Polivka, T.; Hiller, R. G.; Frank, H. A. *Arch. Biochem. Biophys.* **2007**, *458*, 111 and references therein.
(9) Hofmann, E.; Wrench, P. M.; Sharples, F. P.; Hiller, R. G.; Welte, W.; Diederichs, K. *Science* **1996**, *272*, 1788.
(10) Miller, D. J.; Catmull, J.; Puskeiler, R.; Tweedale, H.; Sharples, F. P.; Hiller, R. G. *Photosynth. Res.* **2005**, *86*, 229.
(11) Schulte, T.; Hofmann, E. Personal communication 2007.
(12) Di Valentin, M.; Ceola, S.; Salvadori, E.; Agostini, G.; Carbonera, D. *Biochim. Biophys. Acta*, published online **2007**, http://dx.doi.org/10.1016/j.bbabio.2007.09.002.
(13) The sign of D was determined to be negative. This was achieved by analysis of the ENDOR spectra. For further details see Supporting Information.
(14) Davies, E. R. *Phys. Lett.* **1974**, *47A*, 1.
(15) For further experimental details see Supporting Information.
(16) (a) Lubitz, W.; Lendzian, F.; Bittl, R. *Acc. Chem. Res.* **2002**, *35*, 313. (b) Di Valentin, M.; Kay, C. W. M.; Giacometti, G.; Möbius, K. *Chem. Phys. Lett.* **1996**, *248*, 434. (c) Lendzian, F.; Bittl, R.; Telfer, A.; Lubitz, W. *Biochim. Biophys. Acta* **2003**, *1605*, 35. (d) Kay, C. W. M. *J. Am. Chem. Soc.* **2003**, *125*, 13861. (e) Lendzian, F.; Bittl, R.; Lubitz, W. *Photosynth. Res.* **1998**, *55*, 189.
(17) A preliminary internal report on Mims ENDOR at X-band of triplet peridinin in MFPCP has been given by S. Parrish and A. Angerhofer (NHMFL Annual Research Review 1999, pp. 22−23).
(18) The ^{1}H ENDOR spectra of MFPCP are very similar (data not shown).
(19) The DFT calculations were performed for the peridinin molecule 614 (without taking into account the other cofactors or the protein environment). All calculations were spin-unrestricted calculations using the BP functional. Peridinin was geometry optimized in the triplet state. ZFS tensor and ^{1}H hyperfine tensors were calculated with the ORCA program package. Further details are provided in the Supporting Information.

JA077225V