

Charge-Transfer State as a Possible Signature of a Zeaxanthin–Chlorophyll Dimer in the Non-photochemical Quenching Process in Green Plants

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Quantum chemical calculations of the electronic excited states of a zeaxanthin–chlorophyll dimer show that a zeaxanthin-to-chlorophyll charge-transfer (CT) state lies in the same energy regime as the excited states S_1 of zeaxanthin and Q_y of chlorophyll, which are relevant for the nonphotochemical quenching (NPQ) process in green plants. Comparison with an analogous violaxanthin–chlorophyll dimer is made, and possible quenching mechanisms via excitation energy transfer or electron transfer are discussed. It is pointed out that the strongly allowed absorption of the zeaxanthin radical cation present in the CT state can be experimentally used as signature of the zea–chl dimer during the NPQ process.

Introduction

Under high-light conditions, when the photon flux to the photosynthetic apparatus exceeds the needs of the plant's photosynthetic reactions, the plant protects itself against photooxidative damage through a mechanism generally referred to as nonphotochemical quenching (NPQ). Although NPQ has been phenomenologically documented for years, a fundamental understanding of its physical mechanism remains elusive.^{1–3} Today, it is known that among other factors the conversion of the carotenoid violaxanthin (vio) to zeaxanthin (zea) is a necessary prerequisite for NPQ,⁴ and recent experimental findings suggest that zeaxanthin is directly involved in the process.⁵ One possible molecular mechanism for NPQ involves the formation of a heterodimer between chlorophyll (chl) and zeaxanthin,^{5–7} but no experimental proof has yet been given for its existence.

Recent experimental and theoretical investigations of linked carotenoporphyrins suggest that the quenching of the electronically excited Q_y state of the porphyrin can in principle proceed via two different mechanisms.^{8–10} The first possibility is that the excited Q_y state can decay nonradiatively by excitation energy transfer to the S_1 state of the carotenoid. However, this is only possible if the S_1 state is energetically accessible, i.e., if it is lower in energy than Q_y . Alternatively, the Q_y state can decay via electron transfer quenching, which corresponds to nonradiative decay into an energetically lower-lying charge-transfer (CT) state. Again, this is only possible if the CT state is lower in energy than the originally excited Q_y state of chlorophyll. Fungo et al. pointed out that electron-transfer quenching is possible in linked carotenoporphyrins, when the carotenoid has at least eight conjugated double bonds.⁸

In this report, we show by means of quantum chemical calculations that a charge-transfer (CT) state, in which an electron is transferred from the carotenoid to the chlorophyll, becomes lower in energy than the Q_y and the S_1 states of chlorophyll and zeaxanthin, respectively, when a zea–chl dimer is formed. The presence of the CT state has implications for

possible molecular mechanisms of chlorophyll fluorescence quenching. Moreover, since the CT state is unique to the zea–chl dimer, experimental observation of this state would be a proof for the existence of a zea–chl dimer in NPQ.

Theoretical Methods

Our theoretical investigation comprises the geometry optimization of the individual molecules and the calculation of their electronically excited states. The optimized geometries are used to construct dimers, and the influence of carotenoid–chlorophyll dimer formation on the excited states is studied as a function of intermolecular separation.

Before the composition of a dimer structure, the geometries of vio, zea, and chl were separately optimized at the theoretical level of standard ground-state density functional theory (DFT) employing the Becke3–Lee–Yang–Parr (B3LYP)¹¹ exchange–correlation functional in combination with the 3-21G basis set as employed in the QChem¹² package of programs. The obtained geometrical parameters agree well with known experimental and computed values for zeaxanthin, violaxanthin, and chlorophyll.^{13,14}

The valence-excited states (the states which are localized on one side of the dimer and which are energetically well below the ionization potential) are calculated with the Tamm–Dancoff approximation (TDA)¹⁵ to the time-dependent DFT (TDDFT)¹⁶ approach in combination with the Becke–Lee–Yang–Parr (BLYP)¹⁷ functional, since it has been pointed out previously that TDA/BLYP yields very good results for linear polyenes.¹⁸ In all calculations the 3-21G basis set is used, since the use of larger basis sets is at present prohibitive due to the enormous molecular size of the dimer complex. For calibration purposes, the excited states of zea and chl were initially calculated separately. For example, the calculated values of 2.03 and 2.21 eV for the forbidden S_1 and allowed S_2 states of zeaxanthin, respectively, are in reasonable agreement with the known experimental values of 1.9 and 2.6 eV.¹⁹ Using TDA/BLYP, the excitation energies of the energetically lowest excited states of chl are 2.09, 2.22, and 2.25 eV and correspond to the Q_y and Q_x and a third low-lying state, respectively. They exhibit an

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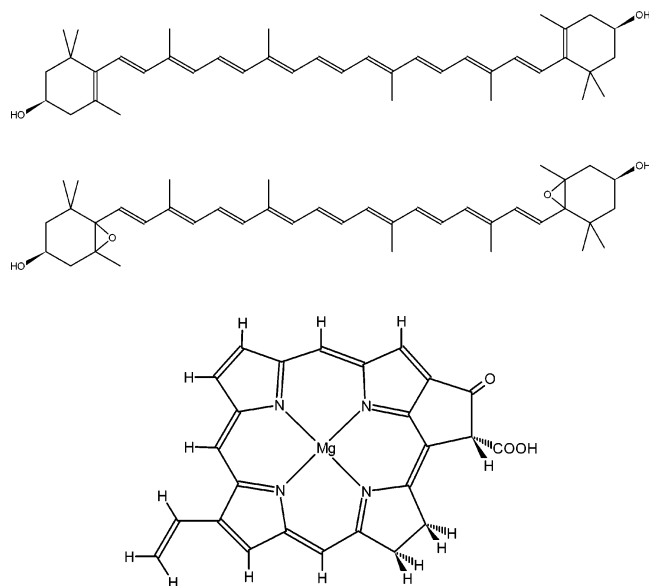


Figure 1. Structure of zeaxanthin (top) violaxanthin (middle) and the chlorophyll model complex used in the calculations (bottom).

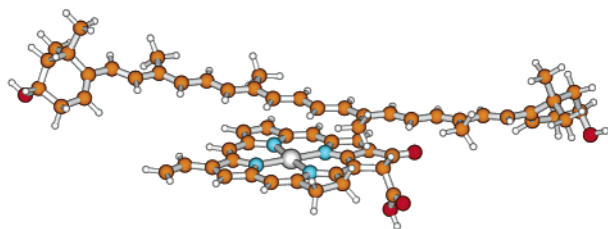


Figure 2. Cofacial-center arrangement of the zeaxanthin-chlorophyll dimer. The intermolecular separation is defined as the distance between the planes of the π systems of zeaxanthin and chlorophyll.

average error of only about 0.3 eV compared to the experimentally determined values of 1.85, 1.91, and 1.95 eV.^{20,21}

Within our calculations we do not use the complete molecular structure of chlorophyll *a*, but a smaller model complex, which is displayed in Figure 2 together with the molecular structure of zeaxanthin. We neglect the side chains of chlorophyll *a*, since they have only minor influence on the excited states of chl which are considered here and which are strictly located at the chlorin ring.

Although TDA and TDDFT yield reliable results for valence excited states, the excitation energies of CT states are underestimated by about 1 eV and CT potential energy curves do not exhibit the correct $1/r$ asymptote.²² The reason for this failure is probably the incorrect long-range behavior of the applied exchange-correlation functionals, which is closely connected to the self-interaction problem.²³ Therefore, a hybrid approach which combines TDA and configuration interaction singles (CIS) is employed for the calculation of the CT states to obtain self-interaction corrected potential energy curves and excitation energies. At first, a ground state DFT/BLYP calculation is performed for the energetically lowest CT state at a zeaxanthin-chlorophyll intermolecular separation of 9 Å by exchanging the HOMO (which is strictly located on zeaxanthin) with the LUMO (which is localized on chl) in the β -spin part of the wave function. At shorter distances the DFT calculation collapses to the electronic ground state. The self-interaction corrected excitation energy is then simply obtained by subtraction of the total energies of the ground and CT state at 9 Å. The total energy of the CT state at 9 Å has the same quality as the total energy of the ground state, since the examined CT state corresponds to a pure

HOMO-LUMO excitation with a transition amplitude of almost exactly 1.0 over the considered zeaxanthin-chlorophyll distance range. The corrected excitation energy at 9 Å is used as an offset for the CT curve calculated with the configuration interaction singles (CIS) method, which is a wave-function-based method and self-interaction-free. Plotting the calibrated CIS curve of the CT state together with the curves of all other excited zeaxanthin and chlorophyll states as obtained with the TDA/BLYP approach yields a self-interaction-free picture of all relevant excited states of the dimer.

Results and Discussion

NPQ is a dynamic process, which occurs in photosystem II (PS II) and protects green plants from photodamage. It is invoked after a few minutes of high light illumination, and it is known that zeaxanthin is essential for NPQ.²⁴ During this initiation time, a heterodimer between the carotenoid zeaxanthin (zea) and chlorophyll (chl) might be formed.⁵⁻⁷ So far, no experimental proof for the existence of a zeaxanthin-chlorophyll dimer during NPQ has been given, and consequently, no data are available for the molecular structure of PS II in the so-called “quenched” state when the NPQ process is active. Thus, the first step of a theoretical study of the zeaxanthin-chlorophyll dimer is the assumption of a reasonable geometrical arrangement of zeaxanthin and chl. We investigated several vertical and cofacial arrangements, of which the cofacial ones turned out to be energetically more favorable. For all cofacial arrangements, in which the π -systems slightly overlap, the obtained results were essentially independent of the specific geometrical arrangement, and thus we focus here on a cofacial arrangement in which the chl is right above the center of mass of zeaxanthin (Figure 1) or, analogously, violaxanthin.

For dimers in which the π -systems do not overlap, electron-transfer quenching is generally not accessible. Since the cofacial-center structure is found to be the energetically most stable one in the gas phase, from this perspective, it is a good candidate to be formed in the “quenched” state. Of course, within the protein environment of PSII, the possible geometrical arrangements are strongly sterically restricted.

Under normal light conditions, i.e., when NPQ is not active, violaxanthin is present in PSII and the excitation energy of antenna chlorophylls in the Q_y state is funneled to the reaction center. In the “quenched” state of PS II, i.e., when NPQ is active, violaxanthin is converted to zeaxanthin, and the electronically excited Q_y states of chlorophylls have a new decay channel which allows for rapid thermal dissipation of excess excitation energy. The fact that PS II can switch on NPQ under high-light conditions implies that a new faster nonradiative decay channel becomes available under high-light conditions. One possible mechanism follows from the conversion of violaxanthin to zeaxanthin, which coincides with an increase of the number of conjugated double bonds in the carotenoid from nine double bonds in violaxanthin to 11 double bonds in zeaxanthin. As a consequence the energy of the S_1 state is expected to drop, but at present the experimental evidence on the relative S_1 energies of zeaxanthin and violaxanthin is equivocal,^{19,25} and current experimental results suggest that both molecules have S_1 states at higher energy than the chl Q_y .²⁵ A second possibility is to make a new nonradiative decay channel accessible via structural changes in PSII during the induction time of NPQ, which, in turn lead to a lowering of the S_1 state of zeaxanthin or other electronic states below the Q_y state of chl. An obvious possibility to bias the excited states of single molecules is to form aggregates, or, in the simplest case, dimers, which is assumed to be the case for zeaxanthin and chl. To investigate the influence of the formation of a dimer complex on the excited states of chl and zeaxanthin, we calculated the excited states of zeaxanthin

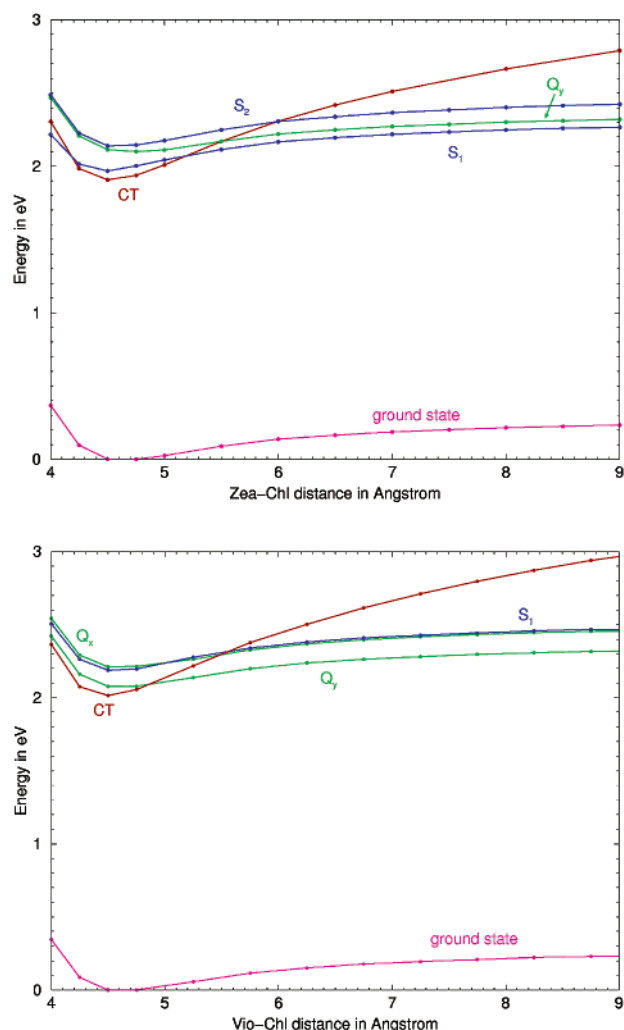


Figure 3. Potential energy surfaces of the ground (magenta) and excited states (red, zeaxanthin-to-chl CT; green, chl states; blue, zeaxanthin states) of the zeaxanthin–chlorophyll dimer (upper part) and the violaxanthin–chlorophyll dimer (lower part) along the distance coordinate between the carotenoid and chlorophyll.

and chl along the distance coordinate between the isolated systems. For comparison, we performed the same calculation also for the violaxanthin–chlorophyll dimer.

We successively decreased the distance between the carotenoid and chl in the cofacial center arrangement starting at a distance of 9 Å until 4 Å is reached. For each point, the excited states were calculated, and the resulting potential energy curves are displayed in Figure 3. In the case of the zeaxanthin–chl dimer (upper part of Figure 3), the excitation energies of the S_1 and S_2 states of zeaxanthin (blue curves) as well as the Q_y state of chlorophyll (green curve) have values of 2.00, 2.14, and 2.07 eV at 4.75 Å, respectively, which is in reasonable agreement with the values for the individual parts (see Theoretical Methods). At the equilibrium distance, however, the energetically lowest excited state is a CT state with an excitation energy of about 1.88 eV. According to our calculations, the S_2 , Q_y , and S_1 states are lower in energy than the CT state until a zeaxanthin–chl distance of 6 Å is reached. Then the CT state crosses the S_2 , Q_y , and S_1 states to become the energetically lowest state of the dimer for an intermolecular distance smaller than 5.2 Å. Analysis of the CT state shows that it corresponds to an excitation of an electron from the highest occupied molecular orbital (HOMO), which is strictly located at zeaxanthin, to the lowest unoccupied orbital (LUMO), which has only contributions at chl (Figure 4).

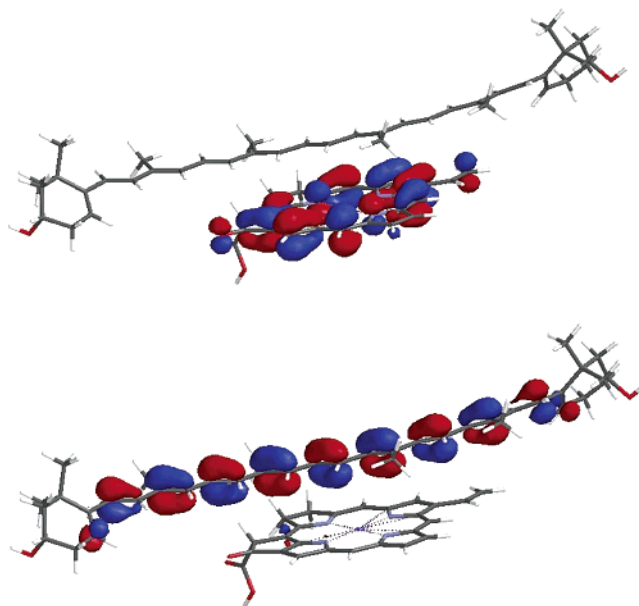


Figure 4. HOMO (lower part) and LUMO (upper part) of the zeaxanthin–chlorophyll dimer. The energetically lowest CT state corresponds to a pure HOMO–LUMO transition. Blue and red correspond to the different phases of the molecular wave functions.

Consequently, the CT state corresponds to an excited state, in which an electron is transferred from zeaxanthin to chl resulting in a zeaxanthin radical cation and a chlorophyll radical anion. The additional electrostatic attraction between the cation and the anion in the CT state is the reason that this state drops in energy more rapidly than the S_2 , Q_y , and S_1 states (Figure 3) and that the CT state finally becomes the energetically lowest state. This suggests that the Q_y excitation energy is transferred from the Q_y state of the chlorophyll to the CT state by electron transfer from zeaxanthin to chl. The CT state finally decays nonradiatively into the ground state by thermal dissipation and back transfer of an electron. The role of zeaxanthin in this scenario is on one hand structurally to form the dimer and on the other hand electronically to provide the electron, which is transferred to the chlorophyll. It is worth noting that the S_1 state of zeaxanthin is lower than the Q_y state of chl at infinite distance; i.e., chlorophyll fluorescence quenching via excitation energy transfer from Q_y to S_1 is in principle possible over the complete distance range. In summary, two competing chlorophyll fluorescence quenching paths are in principle accessible, if a zeaxanthin–chl dimer with an intermolecular distance of less than 5.5 Å is formed during NPQ. The Q_y state can decay via excitation energy transfer into the S_1 state of zeaxanthin and via electron transfer from zeaxanthin to chl into the CT state. This is in agreement with experimental observations of chlorophyll fluorescence quenching in linked carotenoporphyrins via electron transfer from the carotenoid to the porphyrin.^{8–10} Indeed, test calculation of the excited states of one of the investigated carotenoporphyrin molecule¹⁰ with our hybrid approach clearly shows the existence of a CT state energetically lower than the Q_y state of the porphyrin and the S_1 state of the carotenoid. This substantiates the existence of the additional chlorophyll fluorescence quenching path by electron transfer and at the same time corroborates the validity of our theoretical approach.

Comparing to a cofacial vio–chl dimer (lower part of Figure 3), the S_1 energy of vio is clearly above the Q_y state of the chlorophyll over the complete distance range; i.e., the quenching channel via excitation energy transfer is not accessible. However, at short distances, smaller than about 4.8 Å, a CT state analogous

to the one of the zeaxanthin-chl dimer becomes lower in energy than the Q_y state. As a result, a quenching channel for the violaxanthin-chlorophyll dimer via electron transfer quenching is in principle accessible at these short distances. In view of the fact that NPQ does not occur, when only violaxanthin and no zeaxanthin is present in PS II, it seems unlikely that electron transfer quenching is an efficient quenching pathway in the investigated systems. This, of course, is not a final conclusion, since NPQ is a very complex and complicated process. It is, for example, just as possible that violaxanthin does not form a violaxanthin-chl dimer in PS II but zeaxanthin does. Or, equally well, a violaxanthin-chl dimer simply does not reach a short enough intermolecular distance to invoke electron transfer quenching due to sterical hindrance in the protein. In addition, one can expect that the energies of the CT states drop relative to the "neutral" states in the polar protein environment with a given dielectric constant, since the CT states possess a very large static dipole moment. Consequently, the quenching mechanism via electron transfer will be energetically more favorable in the protein than our gas-phase calculations suggest. However, a remaining theoretical task is the calculation of rates for electron transfer and excitation energy transfer. The latter can be further subdivided into a long-range regime and a coupling regime (excitonic coupling),⁷ which both are intrinsically accounted for within our chosen theoretical approach.

The proposed molecular mechanism for chlorophyll fluorescence quenching by zeaxanthin has been found under the assumption that a cofacial zeaxanthin-chlorophyll dimer is formed during the induction time of the NPQ process (see above). Currently, no experimental confirmation of the formation of the dimer has been given. However, the CT state identified above can be a key for a conclusive answer to this question, since the CT state consisting of a zeaxanthin radical cation and a chlorophyll radical anion is only energetically accessible when a cofacial dimer is formed. It can thus serve as a spectroscopic signature of the zeaxanthin-chl dimer in the quenched state of PS II. Explicitly, we suggest to experimentally search for typical electronic absorptions of the radical cation of zeaxanthin, which according to a calculation at the theoretical level of TDA should be located around 750 and 1250 nm when PS II is in the quenched state. A similar experiment was recently performed by Polívka et al., who identified a spheroidene radical cation in the light-harvesting complex of purple bacteria by transient absorption spectroscopy after excitation of spheroidene.²⁶ We suggest to excite the Q_y state of the chlorophylls of PS II while NPQ is active and to probe the electronic absorption spectrum in the region of the carotenoid radical cation absorption.

In general, we expect that charge-transfer states of the type characterized here are relevant in many biological processes where dimers or, in general, oligomers are present, e.g., photoinitiated electron transfer^{27,28} or excitation energy transfer,⁸⁻¹⁰ since the energy of the CT state is very sensitive to the distance between the acceptor and donor sites and drops rapidly when they approach each other due to the additional electrostatic

attraction. Consequently, CT states naturally cross the "other" excited states at certain distances and, thus, may provide a natural mechanism for efficient energy or electron transfer.

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References and Notes

- (1) Demmig, B.; Winter, K.; Krüger, A.; Czygan, F.-C. *Plant Physiol.* **1987**, *84*, 218.
- (2) Horton, P.; Ruban, A. V.; Walters, R. G. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1996**, *47*, 665.
- (3) Müller, P.; Li, X.-P.; Niyogi, K. K. *Plant Physiol.* **2001**, *125*, 1558.
- (4) Gilmore, A. M. *Physiol. Plant.* **1997**, *99*, 197.
- (5) Ma, Y.-Z.; Holt, N. E.; Li, X.-P.; Niyogi, K. K.; Fleming, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4377.
- (6) Gilmore, A. M.; Hazlett, T. L.; Govindjee. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2273.
- (7) van Amerongen, H.; van Grondelle, R. *J. Phys. Chem. B* **2001**, *105*, 604.
- (8) Fungo, F.; Otero, L.; Durantini, E.; Thompson, W. J.; Silber, J. J.; Moore, T. A.; Moore, A. L.; Gust, D.; Sereno, L. *Phys. Chem. Chem. Phys.* **2003**, *5*, 469.
- (9) Cardoso, S. L.; Nicodem, D. E.; Moore, T. A.; Moore, A. L.; Gust, D. *J. Braz. Chem. Soc.* **1996**, *7*, 19.
- (10) Hermant, R. M.; Lidell, P. A.; Lin, S.; Alden, R. G.; Kang, H. K.; Moore, A. L.; Moore, T. A.; Gust, D. *J. Am. Chem. Soc.* **1993**, *115*, 2080.
- (11) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
- (12) Kong, J. et al. Q-Chem 2.0: a high-performance *ab initio* electronic structure program package. *J. Comput. Chem.* **2000**, *21*, 1532-1548.
- (13) Vasilev, S.; Orth, P.; Zouni, A.; Owens, T. G.; Bruce, D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8602.
- (14) Sundholm, D. *Chem. Phys. Lett.* **1999**, *302*, 481.
- (15) Hirata, S.; Head-Gordon, M. *Chem. Phys. Lett.* **1999**, *314*, 291.
- (16) Casida, M. E. In *Recent Advances in Density Functional Methods*; Chong, D. P., Ed.; World Scientific: Singapore, 1995; Part I, pp 155-192.
- (17) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098.
- (18) Hsu, C.-P.; Hirata, S.; Head-Gordon, M. *J. Phys. Chem. A* **2001**, *105*, 451.
- (19) Josue, J. S.; Frank, H. A. *J. Phys. Chem. A* **2002**, *106*, 4815.
- (20) Fragata, M.; Norden, T.; Kurusev, T. *Photochem. Photobiol.* **1988**, *47*, 133.
- (21) Thomas, L. L.; Kim, J.-H.; Cotton, T. M. *J. Am. Chem. Soc.* **1990**, *112*, 9378.
- (22) Dreuw, A.; Weisman, J. L.; Head-Gordon, M. *J. Chem. Phys.* **2003**, submitted.
- (23) Polo, V.; Kraka, E.; Cremer, D. *Mol. Phys.* **2002**, *100*, 1771.
- (24) Demmig-Adams, B.; Adams, W. W.; Heber, U.; Neimanis, S.; Winter, K.; Krüger, A.; Czygan, F.-C.; Bilder, W.; Björkman, O. *Plant Physiol.* **1990**, *92*, 293.
- (25) Polívka, T.; Herek, J. L.; Zigmantas, D.; Akerlund, H.-E.; Sundström, V. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4914.
- (26) Polívka, T.; Zigmantas, D.; Herek, J. L.; He, Z.; Pascher, T.; Pullerits, T.; Cogdell, R. J.; Frank, H. A.; Sundström, V. *J. Phys. Chem. B* **2002**, *106*, 11016.
- (27) Häberle, T.; Hirsch, J.; Pöllinger, F.; Heitele, H.; Michel-Beyerle, M. E.; Anders, C.; Döhling, A.; Krieger, C.; Rückemann, A.; Staab, H. A. *J. Phys. Chem.* **1996**, *100*, 18269.
- (28) Worth, G. A.; Cederbaum, L. S. *Chem. Phys. Lett.* **2001**, *338*, 219.