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Excitation energy transfer and carotenoid radical cation formation in light harvesting complexes — A theoretical perspective

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ABSTRACT

Light harvesting complexes have been identified in all chlorophyll-based photosynthetic organisms. Their major function is the absorption of light and its transport to the reaction centers, however, they are also involved in excess energy quenching, the so-called non-photochemical quenching (NPQ). In particular, electron transfer and the resulting formation of carotenoid radical cations have recently been discovered to play an important role during NPQ in green plants. Here, the results of our theoretical investigations of carotenoid radical cation formation in the major light harvesting complex LHC-II of green plants are reported. The carotenoids violaxanthin, zeaxanthin and lutein are considered as potential quenchers. In agreement with experimental results, it is shown that zeaxanthin cannot quench isolated LHC-II complexes. Furthermore, subtle structural differences in the two lutein binding pockets lead to substantial differences in the excited state properties of the two luteins. In addition, the formation mechanism of carotenoid radical cations in light harvesting complexes LH2 and LH1 of purple bacteria is studied. Here, the energetic position of the S_1 state of the involved carotenoids neurosporene, spheroidene, spheroidenone and spirilloxanthin seems to determine the occurrence of radical cations in these LHCs upon photo-excitation. An elaborate pump-deplete-probe experiment is suggested to challenge the proposed mechanism.

1. Introduction

Light-harvesting complexes (LHCs) have been identified in all organisms performing photosynthesis, i.e. plants, purple bacteria and algae [1] The light-harvesting complex LHC-II of green plants, for instance, represents about 30% of the total protein in the chloroplast membranes and is thereby the most abundant membrane protein on Earth [2]. Although LHCs of different organisms vary in structure and pigmentation, they all are responsible for efficient absorption of light and the transport of the excitation energy to the corresponding reaction centers [3,4]. They are also supposed to be involved in excess energy quenching under high-light conditions, a mechanism generally termed non-photochemical quenching (NPQ) [5–11]. Depending on the organism, several other functions are known that are accomplished by the various LHCs [1].

The molecular mechanisms underlying light harvesting and energy quenching are on one hand efficient absorption of light and excitation energy transfer (EET) and on the other hand non-radioactive decay converting excess excitation energy into harmless heat. The efficiencies of these processes are dictated by the interaction of the pigments which are individually tuned via their relative structural arrangement in the light harvesting complexes. Recently determined structures of LHCs reveal indeed complicated arrangements of closely packed pigments hinting at highly optimized molecular mechanisms.

LHC-II of green plants (Fig. 1), for example, is a trimeric protein and each monomer contains fourteen chlorophyll and four carotenoid molecules: two lutein, one violaxanthin and one neoxanthin [12,13]. The light harvesting complex LH2 of purple bacteria (Fig. 1), e.g. from *Rhodopseudomas (Rps.) acidophila*, consists of a circular structure consisting of nine identical subunits each containing one rhodopsin glucoside carotenoid (Car) and three bacteriochlorophyll *a* (BChla) molecules [14,15]. The solution of their molecular structures, the resulting knowledge of the orientation of the pigments and their direct protein environment is a necessary prerequisite for an assignment of experimental spectra and the development of models for EET pathways and possible quenching processes [16–20].

Very often subtle geometric differences can have large influence on the interaction of two pigments and the efficiencies of excitation energy transfer and electron transfer between them, in particular, because the pigments are very tightly packed within light harvesting complexes. Under such circumstances a quantum mechanical treatment of pigment–pigment interactions and of EET and ET processes is strongly required since quantum effects become dominant and "classical" Förster theory is no longer sufficient. However, carotenoids and chlorophylls are from a quantum chemist's perspective already quite large molecules themselves, which limits the available, applicable methods only to a few ones with clear but known limitations in accuracy. Carefully and knowledgably applying these quantum

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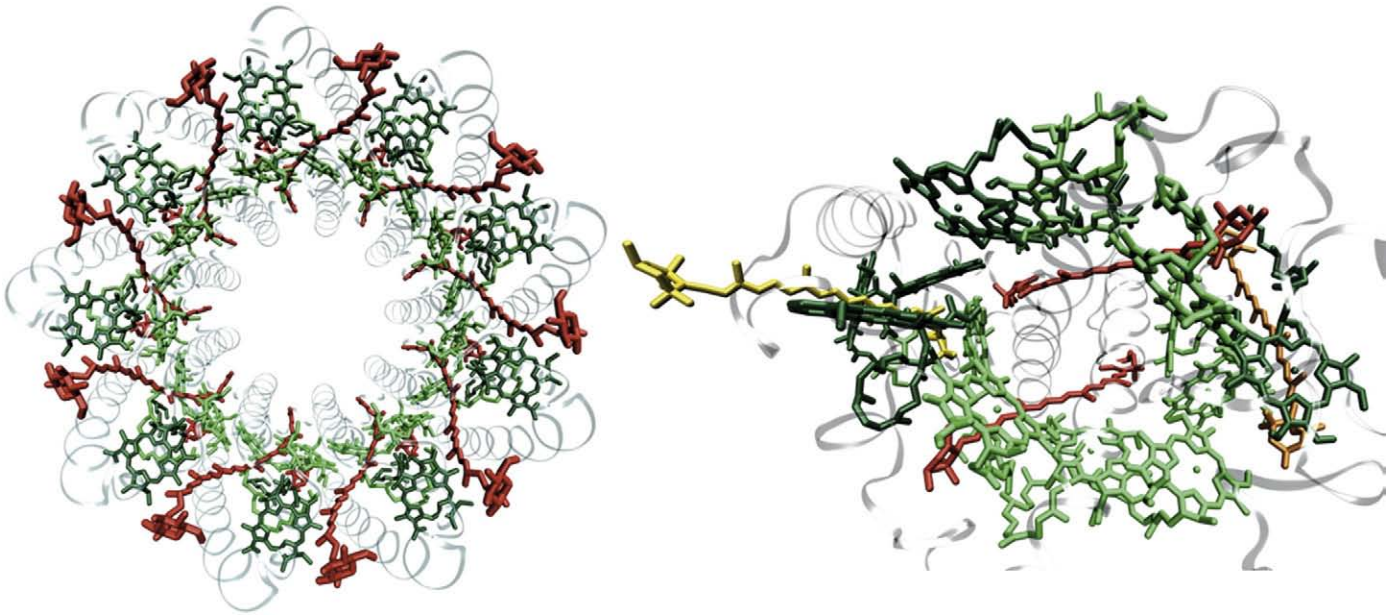

Fig. 1. Structural arrangement of the pigments in the light harvesting complexes LH2 of purple bacteria (left; red: rhodopin glucoside, dark green: B800, light green: B850) and LHCII of green plants (right; yellow: neoxanthin, orange: violaxanthin, red: lutein, dark green: Chl *b*, light green: Chl *a*).

chemical methods to LHCs, we are still in a position to gain valuable insights into possible EET and ET processes between pigment pairs and to identify new aspects in light harvesting and energy quenching. Here we will briefly review and present new theoretical findings on electron transfer quenching or, equivalently, carotenoid radical cation formation in non-photochemical quenching of green plants and in bacterial LHCs.

2. Theoretical methodology

The first step in our quantum chemical investigation of energy and electron transfer processes in light harvesting complexes is the construction of molecular models containing the relevant Car–(B)Chl interactions as in the LHCs of plants and purple bacteria. For this, the known crystal structures of the light harvesting complexes serve as input. The detailed structures of the molecular models are described in later sections. Within our calculations the protein environment is generally neglected unless mentioned otherwise. The relative orientation of the pigments is constrained to the one as in the pigment protein while all other geometrical parameters of the individual pigments are optimized using Kohn–Sham density functional theory with the well-known B3LYP [21] exchange-correlation functional and 6–31G* basis set as implemented in the Q-Chem 3.0 package of ab initio programs [22].

Since the interaction of the pigments and its influence on their excited states is of primary interest, it is useful to compare the excited states of the pigments at large intermolecular separation with those obtained at small intermolecular distance. Therefore, the derived Car-(B)Chl model complexes have been used to calculate the potential energy curves of the lowest relevant electronic excited states along an intermolecular distance coordinate using the Tamm–Dancoff approximation (TDA) [23] of time-dependent density functional theory (TDDFT) [24,25] in combination with the BLYP [26] xc-functional and the small 3–21G basis set. This methodology has proven previously to yield reasonable results for the excitation energies for the S_1 state of carotenoids as well as for the Q_y state of Chls [27–29] owing to fortuitous cancellation of errors [30]. For calibration purposes, the excited states of the individual pigments were calculated and compared with literature data. For example, the calculated values of 2.03 eV 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 [31–34]. Using TDA/BLYP, the excitation energies of the energetically lowest excited states of Chl *a* are 2.09, 2.22, 2.25 eV exhibiting an average error of only about 0.2 eV compared to the experimentally determined values of 1.85, 1.91 and 1.95 eV [35].

However, excited charge transfer (CT) states suffer from electron transfer self-interaction in TDDFT and are given at much too low excitation energies and with a wrong asymptotic behavior with respect to the chosen distance coordinate [24,36]. A more reliable estimate of the potential energy curve of the energetically lowest ET state has thus been applied utilizing the hybrid approach devised in Ref. [37]. In this approach the potential energy curves of CT excited states are computed with the configuration interaction singles (CIS) method and a long-range off-set for these curves is calculated with conventional ΔDFT. These curves can then be plotted together with the curves of the local excited states obtained with TDDFT.

3. Results and discussion

3.1. The role of carotenoid radical cations in non-photochemical quenching of green plants

Non-photochemical quenching (NPQ) is a fundamental photosynthetic process by which plants protect themselves against over-excitation of the photosynthetic apparatus under excess light conditions when the absorbed sun light exceeds the turn-over capacity of the reaction center [5–11]. Generally, NPQ is a complicated multi-step process occurring on different time-scales [38], however, here we are only concerned with the fastest response of the photosynthetic apparatus, the so-called high-energy state quenching usually referred to as qE component of NPQ. Although qE is well documented and ample empirical data is available, the detailed molecular mechanism as well as its location in the plants photosynthetic system is still matter of ongoing debate.

At present, four major mechanisms of qE are discussed: (1) zeaxanthin (Zea) replaces violaxanthin (Vio) in the LHC-II binding pocket and induces quenching without conformational change; (2) Zea quenches by formation of some quenching complex [39,40],

possibly directly from the S_1 state [41–43] of via electron transfer from Zea to chlorophyll, i.e. via carotenoid radical cation formation [44–48]; (3) a subtle conformational change in the lutein (Lut) binding pocket occurs and switches LHC-II into a quenched state where Lut acts as terminal quencher [37]; (4) a subtle change in the chlorophyll packing induces formation of a chlorophyll pair and quenching via a Chl–Chl CT state [35,49]. The conformational changes required for the latter two mechanisms can be induced by aggregation of LHC-II [50–52].

In earlier quantum chemical investigations of chlorophyll fluorescence quenching by the xanthophyll cycle carotenoids violaxanthin (Vio), antheraxanthin (anthera) and zeaxanthin (Zea) we could show that Zea can in principle act as quencher by energy as well as by electron transfer. In the latter an electron is transferred from the carotenoid to the chlorophyll, however, this is only possible when a Zea–Chl *a* complex is formed during qE. Vio, the carotenoid present under normal light conditions was shown not to be able to quench excess excitation energy [44–46]. A subsequent experiment has corroborated our findings identifying the signature of a carotenoid radical cation during qE [47]. Although the carotenoid radical cation was tentatively assigned to Zea, it may well be that is stems from some other carotenoid species present in the photosynthetic apparatus, for example lutein (Lut).

Recently, the crystal structures of LHC-II from spinach and from pea leaves were published both being practically identical revealing the detailed arrangement of the pigments (Fig. 1) [12,13]. In particular, the binding site of Vio was identified to lie close to the surface of LHC-II thus being easily accessible. Since the conversion of Vio to Zea via the so-called xanthophyll cycle is known to be a prerequisite for efficient qE [53], it is reasonable to assume that Vio may be replaced by Zea in LHC-II during the induction time of NPQ being sufficient to induce quenching [13]. Moreover, Vio in the LHC-II binding pocket interacts strongly with the spatially close Chl *b* (Chl 609 according to the nomenclature of Ref. [13]) of the neighboring LHC-II monomer allowing for potentially fast energy transfer. This mechanism is appealingly simple: Zea once replacing Vio in its binding pocket quenches chlorophyll excitation energy from Chl 609. The natural question to ask now is whether Zea can actually quench chlorophyll fluorescence when it is bound to the binding pocket of LHC-II.

As first step of our theoretical investigation [54], molecular model complexes for the quenching site have been constructed by cutting violaxanthin, the spatially closest Chl 609 molecule as well as the amino acid residue Tyr24 out of the crystal structure of LHC-II (Fig. 2) [12,13]. Tyr24 is coordinated to the magnesium atom of Chl 609 by the main chain carbonyl oxygen and influences the energetic position of its excited states. In the computations the phenol ring of Tyr24 and the phythyl chain of Chl 609 are neglected, since their influence on the excited electronic states is negligible. Based on the Vio–Chl 609–Tyr24 model complex, also Zea–Chl 609–Tyr24 model complexes have been constructed to mimic the situation when Vio is replaced by Zea in its binding pocket. For this objective, we simply deleted the epoxy oxygens in the β-ionone rings of Vio, which implies that the LHC-II binding pocket imposes the same geometrical constraints on Zea as on Vio. Unconstrained geometry optimizations of all model complexes have been performed. Although the intramolecular geometrical parameters, e.g. bond lengths and angles, adjusted slightly, the relative orientation of the pigments remained essentially unchanged compared to the one in the crystal structure. All model complexes exhibit the general structure like the Vio–Chl 609–Tyr24 complex shown in Fig. 2. The optimized model complexes serve as input for the theoretical investigation of possible excitation energy quenching pathways. The obtained potential energy curves are displayed in Figure for the Vio–Chl 609–Tyr24 and Zea–Chl 609–Tyr24 complexes. The curves have been calculated using the methodology described in Section 2 along an intramolecular distance coordinate *R* defined in Fig. 2.

Analysis of the obtained potential energy curves reveals that the Q_y state of Chl 609 is lower in energy than the S_1 state of Zea and lower than the S_1 of Vio (Fig. 3). As a consequence excited Chl 609 molecules cannot transfer excitation energy neither to Vio nor to Zea, hence, excitation energy transfer quenching cannot occur according

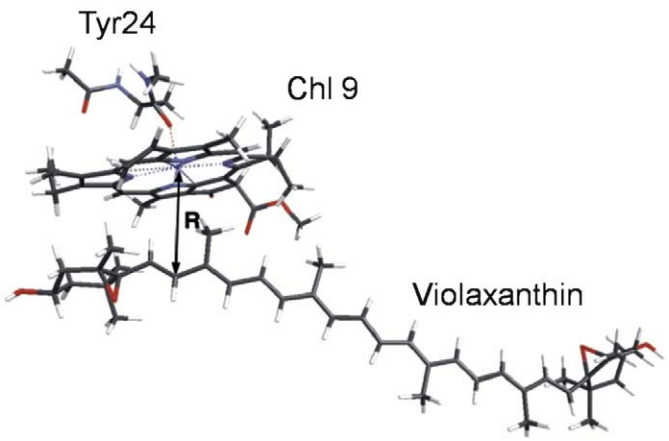

Fig. 2. Structure of the Vio–Chl 609–Tyr24 complex reflecting the relative orientation of the pigments in the Vio binding pocket of LHC-II. *R* corresponds to the intermolecular distance coordinate along which the potential energy curves have been calculated.

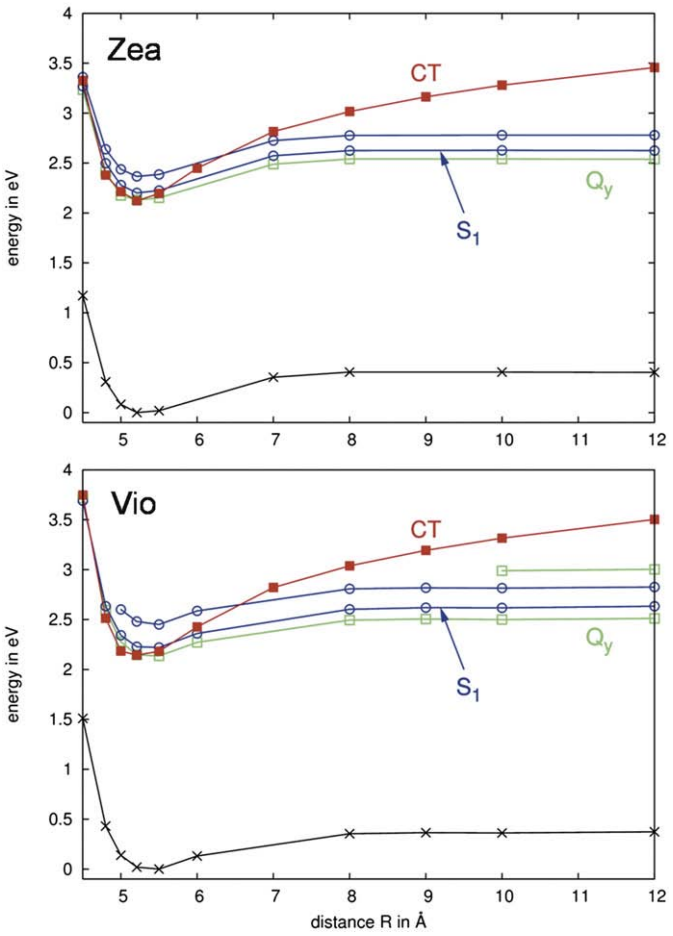

Fig. 3. Calculated potential energy curves of the Zea–Chl 609–Tyr24 (top) and Vio–Chl 609–Tyr24 (bottom) model complexes in the orientation of the crystal structure of LHC-II along the intermolecular separation coordinate *R* (see Fig. 2). Green lines (open squares) correspond to Chl a states, blue ones (open circles) to carotenoid states, while red lines (full squares) refer to excited CT states.

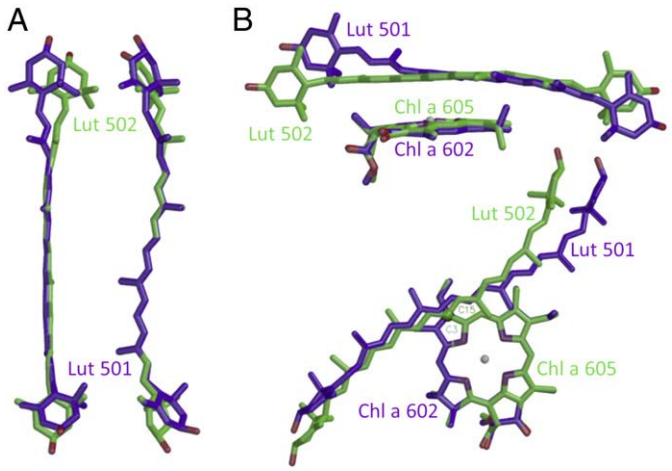

Fig. 4. (A) Overlay of the structures of Lut 501 and Lut 502 in side and top view. (B) Overlay of the Lut 501–Chl 602 and Lut 502–Chl 605 model complexes in side and top view.

to our calculations. However, for both complexes Vio–Chl 609–Tyr24 and Zea–Chl 609–Tyr24, a CT excited state, in which an electron is transferred from the carotenoid to Chl 609 becomes very low in energy around the equilibrium distance of 5–5.5 Å in the protein, and is practically degenerate with the Q_y state of Chl 609 at the applied level of calculation. The accuracy of approximately 0.1–0.3 eV of the applied theory is certainly not sufficient to exclude the possibility of electron transfer quenching. Nevertheless, one can definitely conclude that both carotenoids have identical properties within the binding pocket of LHC-II under the assumption that the geometry of the pocket does not largely change upon replacement, since the calculated curves of both complexes are essentially equivalent. Consequently, Zea cannot quench Chl 609 of LHC-II, since Vio does not quench it.

One may want to speculate that during the induction time of NPQ, Chl 609 may also be replaced by a Chl *a* molecule and this might change the energetic situation in the Vio binding pocket. In fact, it appears unlikely that Chl *b* plays a dominant role in the quenching since they are known to transfer their excitation energy very rapidly to Chl *a* [18,20]. Calculation of the potential energy curves of the corresponding Vio–Chl *a*-Tyr24 and Zea–Chl *a*-Tyr24 complexes (not shown), however, corroborate the above finding that both Zea and Vio possess equivalent properties in the Vio binding pocket of LHC-II independent of the nature of Chl 609. The similarity of the excited state energies of Vio and Zea in the geometry of the LHC-II binding pocket is due to restricted torsion of the β-ionone ring of the carotenoids which is located directly above Chl 609. This restriction forces the particular torsion angle of Zea to be practically locked at 90°, thus diminishing the overlap between the β-ionone double bond of Zea with the conjugated double bond backbone. Hence, the effective conjugation length of Zea in this constrained geometry is only about 9.5 compared to 9 of Vio explaining the negligible changes upon conversion.

Recent experimental investigations of the excited state dynamics of isolated trimeric LHC-II complexes containing either Vio or Zea in the binding pocket of LHC-II are in agreement with our theoretical findings [55]. Femtosecond Pump-probe experiments on these complexes revealed neither spectral differences nor changes in lifetime of the excited state absorption signal of the initially excited Q_y states of the present chlorophylls [51]. Both the Vio and Zea containing LHC-II complexes exhibit the same excited state dynamics and their chlorophyll fluorescence is thus unquenched. In conclusion, Zea is not capable of quenching isolated LHC-II complexes if it replaces Vio in its binding pocket. As a consequence, this originally proposed simple mechanism for qE of NPQ is not sufficient to invoke efficient quenching and additional factors must be included, for example additional interaction with other pigments or pigment proteins as outlined in Ref. [51].

Recently, another mechanism of qE has been suggested based on elaborate resonance raman and transient absorption spectroscopy, in which one of the central lutein (Lut) molecules is supposed to be the terminal quencher [37], i.e. the excess energy is transferred from a nearby Chl molecule to Lut which eventually dissipates it as heat. It is assumed that LHC-II complexes can switch from an unquenched state under normal light conditions into a quenched state triggered by intense light and the built up of the trans-membrane pH gradient. It is argued further that both states differ by subtle conformational changes only, and furthermore, that the known crystal structures actually display the latter quenched state of LHC-II. The molecular structure of LHC-II monomers exhibits pseudo-C_2 symmetry (Fig. 1), and two central lutein molecules Lut 501 and Lut 502 which are in close contact with Chl 602 and Chl 605, respectively. Chl 602 and Chl 605 form strongly coupled Chl dimers with the spatially very close lying Chl 607 and Chl 612, respectively, of which the first is a Chl *a* and the latter a Chl *b*. Due to the different coupling partners and the different protein environment, the structures of Lut 501 and Lut 502 deviate slightly as well as the relative spatial arrangement between Lut 501 and Chl 602 differs slightly from the one between Lut 502 and Chl 605 (Fig. 4).

To address the questions whether the luteins can act as quencher in LHC-II and whether the different structures and relative orientations of the luteins have a significant influence on the excited states of the pigments and thus on the electron and excitation energy transfer capabilities, we performed quantum chemical calculations of the excited states of molecular model complexes in strict analogy to above. For this objective, we constructed suitable model complexes by cutting the Lut molecules as well as the corresponding neighboring Chl molecules together with the amino acid to which the Mg atom is coordinated out of the LHC-II crystal structure. Unfortunately, it is not possible to also include Chls 607 and 612 into the calculations due to the enormous size of those complexes. This gives rise to the two model complexes shown in (Fig. 5): Lut 501–Chl 602–Asn 183 and Lut 502–Chl 605–His 68. After constrained geometry optimizations in which the relative orientations of the pigments are conserved, these complexes are subjected to excited state calculations as described in the previous section.

The calculation of the excited states of the Lut 501–Chl 602 complex along the distance coordinate defined in Fig. 5 revealed that in the geometrical arrangement of the Lut1 binding site, Lut 501 has a slightly lower S_1 excitation energy than the Q_y excitation energy of Chl 602, i.e. excitation energy transfer from Chl 602 to Lut 501 can thus be in principle possible. However, these states are practically degenerate and a definite conclusion about the relative energetic position of these states and a clear-cut statement about EET processes are certainly beyond the accuracy of our theoretical methodology. In particular, when the effect of the missing, strongly coupled Chl 607 is taken into account, one can expect the excitation energy of Chl 602 to further decrease due to excitonic coupling. Thus, it is very likely that the Q_y

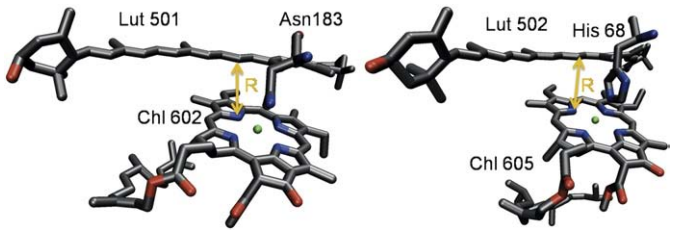

Fig. 5. Structures of the Lut 501–Chl 602–Asn 183 and Lut 502–Chl 605–His 68 model clusters resembling the relative orientations of the pigments in the Lut1 and Lut2 binding sites of LHC-II. *R* defines the distance coordinate for our computations.

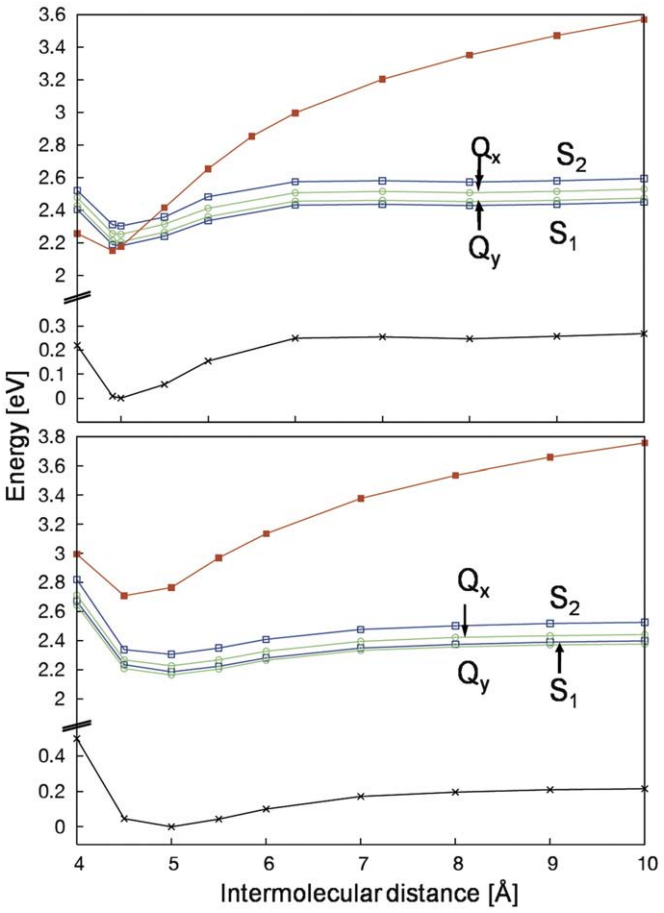

Fig. 6. Calculated potential energy curves of the Lut 501–Chl 602–Asn183 (top) and Lut 502–Chl 605–His68 (bottom) model complexes in the orientation of the lutein binding sites of LHC-II along the intermolecular separation coordinate R. Green lines (open circles) correspond to Chl *a* states, blue ones (open squares) to carotenoid states, while red lines (full squares) refer to excited CT states.

state actually lies below the S_1 state of Lut 501. The same holds for the charge-transfer excited state, which is seen in Fig. 6 to drop slightly below the S_1 and Q_y state at an intermolecular separation of 4 Å, the distance found in the protein. This suggests that electron transfer quenching may be possible for Lut 501 by transferring an electron to Chl 602. However, for the reasons above, a final definite conclusion is not possible based on our results alone.

Turning to Lut 502, it is readily apparent from Fig. 6 that electron transfer quenching is not possible in the Lut 502–Chl 605 complex, since the charge-transfer excited state stays clearly above all valence excited states, which is certainly due to slightly worse overlap of the π-systems of Lut 502 and Chl 605 than between Lut 501 and Chl 602. Also, the S_1 state of Lut 502 is energetically slightly above the Q_y state of Chl 605 suggesting that excitation energy transfer quenching cannot occur within this pigment pair. However, the energetic difference between these states is tiny at the theoretical level employed, and thus a conclusive statement is just impossible. In addition, also here one may speculate that the inclusion of Chl 612 in the calculation would result in a decrease of the Q_y excitation energy making EET quenching even more unlikely.

It is certainly not surprising that the subtle structural differences between the Lut 501–Chl 602 and Lut 502–Chl 605 leave the vertical excited valence states of the Luts and Chls practically unchanged, however, it is striking that the charge-transfer excited state responsible for ET quenching is strongly affected, and that only Lut 501 can probably quench via ET quenching. Although the accuracy of the theoretical methodology is not high enough to draw definite conclusions whether the lutein molecules in LHC-II can quench excess excitation energy or not, or in other words, whether the lutein molecules can be the terminal quencher during qE, we can nevertheless conclude that if at all then Lut 501 is the quencher.

Future theoretical efforts will have to address the limitations of our current methodology. It is planned to also include the second strongly coupled chlorophyll molecules, which certainly do have an influence on the excited states of Chls 602 and 605. Also the complete neglect of the protein environment needs to be addressed and we are currently working on including the electric field of the protein in the excited state calculations. Further quantum chemical calculations are under way too further investigate the possibilities of excitation energy quenching in LHC-II. We are studying the possibility of excitation energy quenching through strongly coupled chlorophyll pairs as has been experimentally suggested [35,38].

3.2. Carotenoid radical cation formation in light harvesting complexes LH2 and LH1 of purple bacteria

The architecture of the light harvesting complex LH2 of purple bacteria is different from the structure of LHC-II. It exhibits a circular structure consisting of similar subunits each possessing one carotenoid (Car) and three bacteriochlorophyll *a* (BChl) molecules embedded into two polypeptide chains (Fig. 1) [14,15]. Two of the BChl molecules (B850) are oriented perpendicular to the ring plane forming a strongly coupled ring while the third BChl molecule (B800)

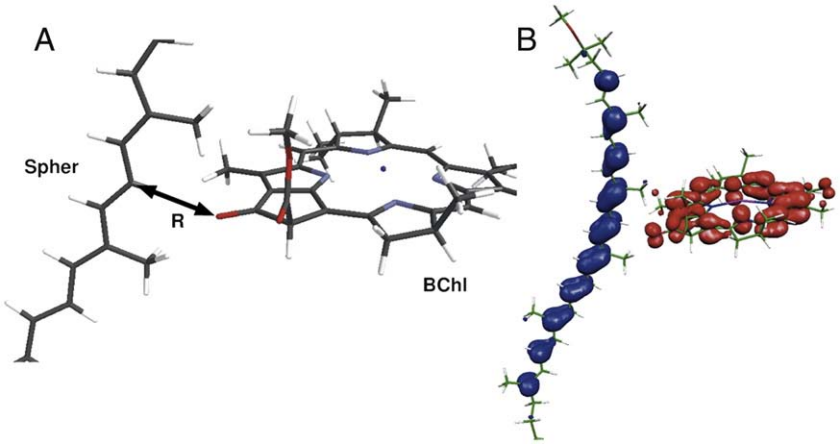

Fig. 7. (A) Definition of the distance coordinate in the investigated Car–B800 complexes resembling the relative orientation in LH2 complexes. (B) Relative orientation of the Cars and B800 in the model complexes as well as detachment density (blue) and attachment density (red) of the excited charge transfer state.

is oriented parallel and only weakly coupled to the neighboring B800s. The Car molecules span the height of the LH2 complex (one in each subunit) and are coupled to both BChl rings. The knowledge of the molecular structure of LH2 triggered a number of theoretical and experimental investigations of its light harvesting function [56–62]. In contrast to LHC-II of green plants, the carotenoids in the bacterial LH2 are largely involved in light-harvesting. In this context, experiments on reconstituted LH2 complexes with different carotenoids have shown that the efficiency of Car to BChl excitation energy transfer (EET) via S_2 does not significantly change with conjugation length of the carotenoid [62–64]. On the other hand, the transfer efficiency via the S1 state changes largely [53,55].

Recently, photo-initiated electron transfer (ET) from Cars to B800 molecules, i.e. formation of carotenoid radical cations has been discovered in LH2 complexes of *Rhodobacter (Rb.) sphaeroides* after excitation of the S_2 state of spheroidene [65]. Furthermore, it has been discovered that the efficiency of carotenoid radical cation formation depends on the conjugation length of the carotenoid present in reconstituted LH2 complexes from *Rb. Sphaeroides* [66]. In reconstituted LH2 complexes containing neurosporene (Neuro, nine double bonds), the formation efficiency is about 10%–15%, for spheroidene (Spher, ten double bonds) a value of 5%–8% was found while for spheroidenone (Spherone) no cation radical formation was observed. This behavior is the opposite of what one would expect, since electron transfer should be facilitated with increasing chain length due to the decreasing ionization potential of the Car. From the experiments, two possible precursor states were suggested, a vibrationally excited S_1 state and an energetically higher ET state, were proposed. However, the detailed molecular mechanism of the ET processes in LH2 complexes remain to be established.

To shed some more light onto the mechanism of radical cation formation in LH2, we investigated the excited state properties of various Car–BChl complexes in analogy to the LHC-II investigations outlined above [67]. For this objective, we first constructed molecular models for the reconstituted LH2 complexes that capture the interaction between the B800 and carotenoid molecules based on the crystal structure of the LH2 complex of *Rps. acidophila* [14,15], since the LH2 complex of *Rb. sphaeroides* is expected to have a very similar molecular structure. Cutting out the Car and the spatially closest B800 molecule from the crystal structure, one arrives at computationally feasible molecular model complexes with a general structure displayed in Fig. 7. The B850 BChls can be neglected since the electron acceptor in the Car radical cation formation is undoubtedly B800 [61,62]. The end groups of rhodopin glucoside, the carotenoid present in *Rps. acidophila*, have been replaced by those of Neuro, Spher, and Spherone, respectively, and constrained geometry optimizations have been performed to incorporate spatial restrictions which the neglected protein backbone imposes on the Car and BChl molecules [63]. The optimization procedure results in three model complexes: Neuro–B800, Spher–B800 and Spherone–B800, which were used to compute potential energy curves of the relevant excited states along an intermolecular distance coordinate *R* (Fig. 7), which reveals the influence of the interaction of the pigments onto their excited states. The excited states were calculated following the procedure outlined above and for more details see Ref. [63].

Analysis of the computed potential energy surfaces for the Neuro–B800, Spher–B800, and Spherone–B800 complexes (Fig. 8) reveals the experimentally observed decrease in excitation energy of the carotenoid states with increasing conjugation length, which are in acceptable agreement with the best known experimental values with an error of 0.1–0.2 eV. The Q_y state of B800, however, deviates by as much as 0.6 eV from its experimental value of 1.55 eV. Most notably, two charge-transfer excited states are identified which at large distances *R* are both higher in energy than any calculated valence excited state in any studied Car–B800 model complex. At shorter intermolecular distances only the energetically lower excited CT state

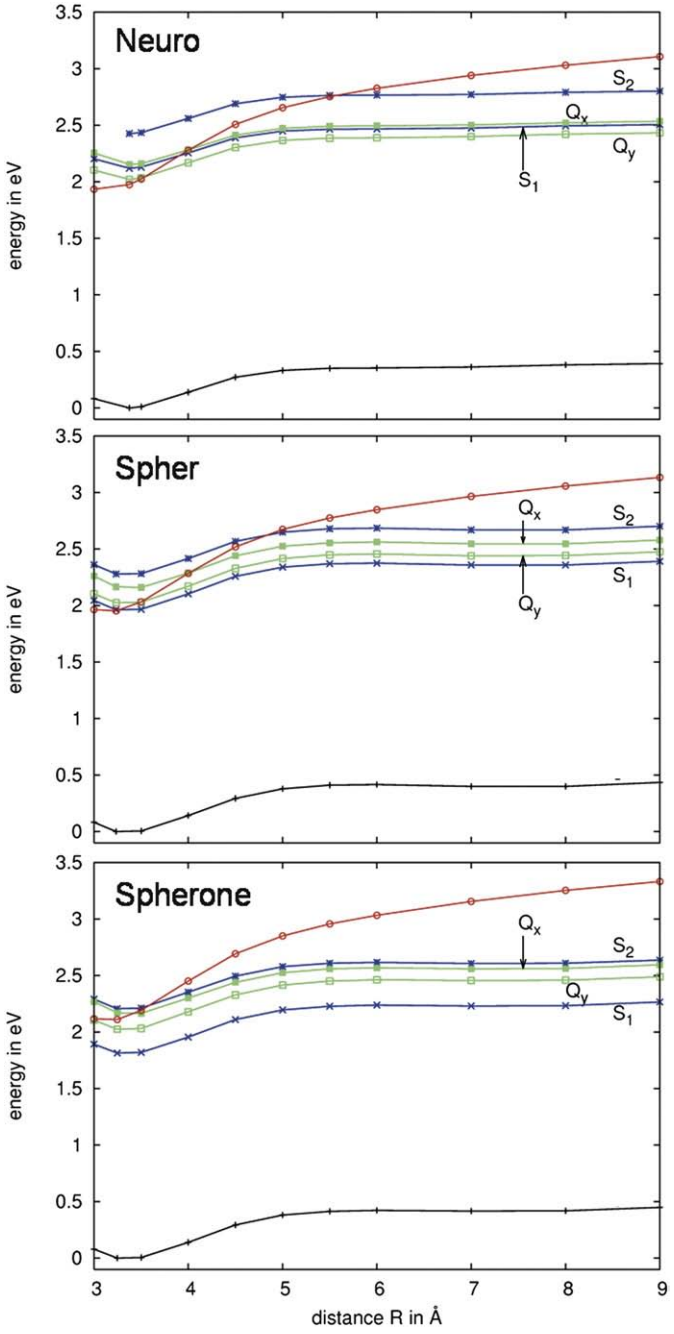

Fig. 8. Potential energy curves of the investigated Car–B800 complexes along the intermolecular distance coordinate: Neuro–B800 (top), Spher–B800 (middle), Spherone–B800 (bottom) at the theoretical level of TDA/BLYP/3–21G. Green lines correspond to B800 states, blue lines to Car states, red lines to charge-transfer excited states, black lines to electronic ground states.

exhibits several crossings with the valence excited states for each Car, which are necessary prerequisites to make efficient CT possible. Therefore the higher-lying CT state can be excluded as precursor for the carotenoid radical cation. Since the calculated excitation energy of Q_y of B800 are significantly too high compared to the experimental values, one can safely assume that in reality no crossing of Q_y and the lowest CT state occurs in any of the three complexes. The corresponding detachment and attachment densities of that CT state (Fig. 7) reveals that the detachment density is located entirely on the Car, while the attachment density has contributions only on the B800. Consequently, this state corresponds to an excited state in which an electron is transferred from the Car to the B800, thus being

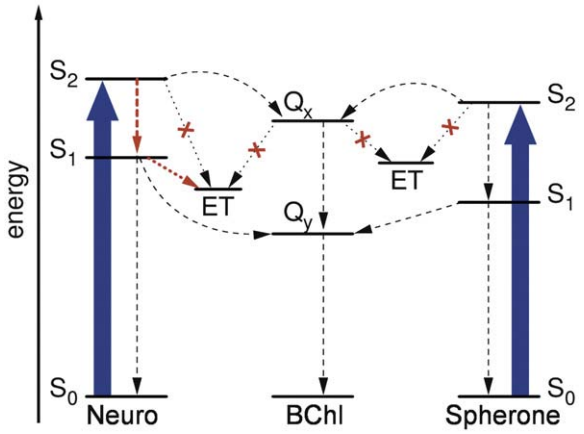

Fig. 9. Schematic energy level diagram for the EET and ET processes in the Car–BChl complexes.

responsible for the experimentally observed Car radical cation and a B800 radical anion.

Comparison of the curves for the different complexes shows as the most significant difference that the lowest CT state of the Neuro–B800 complex is clearly lower in energy than the S_1 state of neurosporene at the equilibrium intermolecular distance in LH2 of 3.2 Å, while the CT state in the Spher–B800 complex is essentially degenerate with the S_1 state at the applied level of theory. In the Spherone–B800 complex, however, the CT state is clearly above the S_1 state. Remembering the experimental results that carotenoid radical cation formation has been observed for Neuro, less for Spher and no formation at all for Spherone containing LH2 complexes, and in addition knowing that the radiationless transition from the initially excited S_2 state to the S_1 state is very fast, one may speculate that the energetic position of the S_1 state is the relevant quantity for the occurrence of the carotenoid radical cations. Based on our findings, we thus suggest that the formation of the carotenoid radical cations in LH2 proceed via the population of the S_1 state according to the general kinetic scheme depicted in Fig. 9. In other words, it seems that carotenoid radical cations are formed only when the S_1 of the carotenoid state is populated and when the corresponding CT excited state is lower in energy that S_1.

The proposed mechanism explains the dependence of radical cation formation on the conjugated chain length of the Cars in LH2 and suggests the proposed vibrationally hot S_1 to be the precursor of the carotenoid radical cations. It is important to note that this mechanism is also in agreement with experimental findings for NPQ in plants [47,48]. There, carotenoid radical cation formation has only been observed when Zea was present possessing an energetically lower excited S_1 state than Q_y of Chl *a* according to our calculations above, and Zea exhibits the energetically lowest S_1 state among the carotenoids present in the photosynthetic apparatus. Nevertheless, our theoretical considerations have been done on the basis of simplified molecular models of LH2 complexes and comparison with experimental findings [61,62]. To challenge our proposed mechanism, we suggest a pump-deplete-probe experiment, in which the Car S_2 state should be excited and after a certain delay time, the S_1 state should be depleted by a second laser pulse at the S_1 excited state absorption of the Car. Then the radical cation signature should be probed. If our mechanism is correct, the radical cation signal will be diminished when the S_1 state is depleted.

The light harvesting complex LH1 is structurally closely related to LH2 [68,69]. It is a larger circular arrangement of sixteen identical subunits and is missing the weakly coupled ring of B800 BChl *a* molecules. The LH1 complex of the bacterium *Rhodospirillum rubrum* contains the carotenoid spirilloxanthin (Spirillo) with thirteen conjugated double bonds exhibiting an S_1 state with very low excitation energy and with a low ionization potential as the only carotenoid species. According to the trends observed for the LH2 complexes and the carotenoid radical cation formation mechanism outlined above, no radical cation should be formed in the LH1 complex of *R. rubrum* upon excitation of the S_2 state of Spirillo.

To further investigate this possibility, we have constructed model complexes reflecting the relative arrangement of Spirillo and the coupled BChl *a* pair within LH1. However, it is again possible to include only one of the BChl *a* molecules in our calculations, and since electron transfer, i.e. carotenoid radical cation formation, is strongly distance dependent, we have chosen to include the spatially closest BChl *a* in the model (Fig. 10). Following the same theoretical methodology as outlined above, we have computed the vertical excited states of this model complex along the intermolecular distance coordinate *R* (Fig. 10). Not surprisingly, the obtained potential energy curves are very similar to the ones for Spherone (Fig. 8). Most notably, the gap between the S_1 state of Spirillo and the CT state leading to carotenoid radical cation formation is even larger, since the S_1 excitation energy has a value of only 1.42 eV at the employed level of theory and the BChl has a larger distance from Spirillo in LH1 than B800 has from Spherone in LH2. Hence one can exclude the formation of spirilloxanthin radical cations in LH1 upon excitation of the S_2 state.

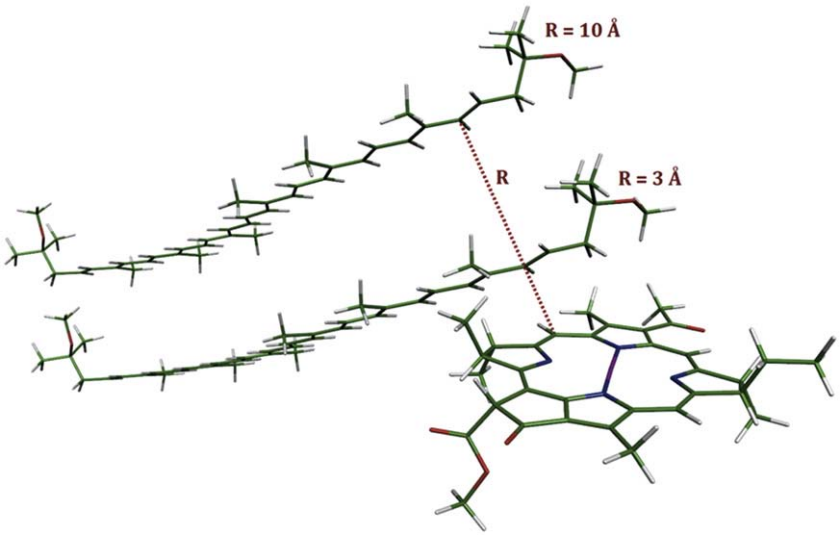

Fig. 10. Structure of the Spirillo–BChl a model complex shown at intermolecular separation of 3 and 10 Å.

4. Concluding remarks

Our theoretical investigations employing quantum chemical methodology allow for a qualitative understanding of the interaction of pigments in light harvesting complexes and for an investigation of excitation energy and electron transfer between them. Nevertheless, although the accuracy of the applicable methods is enough to draw qualitative conclusion, experimental data is required for quantitative analysis. At the same time, experimental spectra of LHCs are complicated and congested and theoretical support is needed for a meaningful interpretation.

Our computations support the following hypotheses with respect to qE of NPQ: (1) Zeaxanthin can act as a quencher in qE by excitation energy transfer and electron transfer, if it is strongly coupled to a chlorophyll molecule. (2) Simple replacement of Vio in the binding pocket of LHC-II by Zea is not sufficient to trigger quenching. (3) If one of the two central luteins of LHC-II acts as terminal quencher during qE, then it is Lut 501 due to structural differences in their binding pockets.

Furthermore, our study of carotenoid radical cation formation in reconstituted LH2 and LH1 of purple bacteria suggests that the excitation energy of the S_1 state of the involved carotenoids determines the occurrence of the radical cation. The latter, however, requires further experimental verification.

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