Influence of Geometry Relaxation on the Energies of the S_1 and S_2 States of Violaxanthin, Zeaxanthin, and Lutein

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Received: December 19, 2005; In Final Form: February 13, 2006

Precise knowledge of the excitation energies of the lowest excited states S_1 and S_2 of the carotenoids violaxanthin, lutein, and zeaxanthin is a prerequisite for a fundamental understanding of their role in light harvesting and photoprotection during photosynthesis. By means of density functional theory (DFT) and time-dependent DFT (TDDFT), the electronic and structural properties of the ground and first and second excited states are studied in detail. According to our calculations, all-s-*cis*-zeaxanthin and s-*cis*-lutein conformers possess lower total ground-state energies than the corresponding s-trans conformers. Thus, only s-cis isomers are probably physiologically relevant. Furthermore, the influence of geometric relaxation on the energies of the ground state and S_1 and S_2 states has been studied in detail. It is demonstrated that the energies of these states change significantly if the carotenoid adopts the equilibrium geometry of the S_1 state. Considering these energetic effects in the interpretation of S_1 excitation energies obtained from fluorescence and transient absorption spectroscopy shifts the S_1 excitation energies about 0.2 eV to higher energy above the excitation energy of the chlorophyll *a*.

1. Introduction

Carotenoids (Cars) play a crucial role in photosynthesis, since they fulfill several important functions: they serve as additional light harvesting pigments, but, more importantly, they are responsible for photoprotection.[1,2] Carotenoids are well-known to quench dangerous triplet states and to scavenge singlet oxygen, which both always arise as byproducts during normal photosynthesis.[1,2] In addition, it has been shown that the xanthophylls violaxanthin (Vio) and zeaxanthin (Zea) (Figure 1) are key players in a protection mechanism against excess excitation energy that exceeds the capacity of the photosynthetic reaction center.[3] This mechanism is generally called the feedback deexcitation (qE) component of nonphotochemical quenching (NPQ), for which at present no detailed molecular mechanism has been established.[4−7] Recently, it has been suggested that it might be sufficient to replace Vio in its binding pocket of LHC-II by Zea to invoke qE,[8] but it has also been shown that aggregation of LHC-II and possibly formation of chlorophyll (Chl) dimers triggers fluorescence quenching.[9] It is also discussed that other pigment binding proteins such as for example PsbS are the location of qE.[10]

The so-called "molecular gear shift" model[11] for the energies of the involved carotenoids relies on the fact that the conversion of Vio to Zea via the xanthophyll cycle[3] leads to a substantial decrease of the S_1 energy of the Cars. While the S_1 energy of Vio is assumed to lie above the Q_y state of Chl *a* and thus allows only for excitation energy transfer (EET) from Vio to Chl *a*, i.e., light harvesting, the S_1 state of Zea should be below the Q_y state. This switches the direction of the EET process and, thereby, makes quenching of chlorophyll fluorescence by Zea possible. Recent experiments corroborate this simple mechanism.[12] Another discussed scenario is the replacement of lutein (Lut) versus Zea in its binding pocket of LHC-II to make chlorophyll fluorescence quenching possible.[13] Recently, it has also been predicted theoretically and confirmed experimentally that a zeaxanthin radical cation is formed during NPQ, being a hint that electron-transfer (ET) quenching plays a role during NPQ.[14−16] If ET quenching is even the main component of NPQ, the energetic position of the S_1 state is not the key factor for the induction of NPQ. Anyways, it is clear that for a basic understanding of the interplay between qE and light harvesting, knowledge about the precise energetic positions of the excited states of the pigments, and in particular, of the forbidden S_1 states of the involved carotenoids, Zea and Vio as well as Lut (Figure 1), is of prime importance.

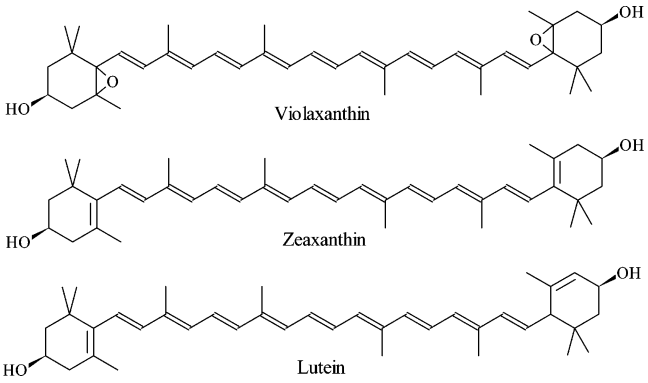

Figure 1. Molecular structure of the xanthophylls violaxanthin, zeaxanthin, and lutein.

Until today, a considerable uncertainty still exists about the actual position of the S_1 energy levels of the Xans.[2] Since the S_1 state exhibits the same spatial symmetry as the ground state (A_g^- in C_{2h} symmetry), it is one-photon forbidden and thus inaccessible for conventional optical spectroscopy. Moreover, this state also poses a considerable challenge to theoreticians due to its highly correlated nature. Recent elaborate experiments

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10.1021/jp057385y CCC: $33.50
Published on Web 03/14/2006

using fluorescence spectroscopy yielded at ambient temperature S_1 energies for Vio and Zea of 1.84 and 1.80 eV,[17] respectively. Different values were obtained at 77 K in EPA glass with 1.93 and 1.81 eV for Vio and Zea.[18] Transient absorption (TA) spectroscopy gave values of 1.79 and 1.74 eV,[19] respectively. TA measurements on LHC-II complexes reconstituted with only one kind of Xan, revealed that within the experimental error the S_1 energies of Vio, Zea, and Lut have practically the same value of 1.72 eV.[20] Remarkably, almost all reported values are clearly below the Q_y state of Chl *a* (1.84 eV), thus arguing against the proposed molecular "gear-shift" model. However, these results are surprising since excitation energy could in principle always be quenched by Lut and Vio, which are always present in the photosynthetic system, especially in LHC-II, where Lut forms a close-contact pair with Chl2.[8,21] To resolve this issue, it has been argued that changes of the protein environment induced by different carotenoid binding rather than different photochemical properties of the carotenoids are responsible for qE.[9]

In this work, we first investigate different conformers of the carotenoids by means of density functional theory (DFT) and time-dependent DFT (TDDFT) calculations with respect to their relative energies and optical properties, and eventually with respect to their physiological relevance. Furthermore, we demonstrate that geometric relaxation of the carotenoids in the S_1 state leads to a significant energetic contribution that has so far been neglected in the interpretation of the fluorescence and TA measurements. Indeed, considering geometric relaxation the S_1 energies of all carotenoids are shifted above the excitation energy of Chl *a*. The obtained order of S_1 excitation energies Vio > Lut > Zea speaks in favor of the gear-shift model.

2. Theoretical Methods

Our theoretical investigation comprises the optimization of the equilibrium structures of the electronic ground state (S_0) and first and second excited states (S_1 and S_2) of Vio, Zea, and Lut as well as the calculation of the excitation energies at the various optimized geometries. All calculations reported here have been performed with the Q-Chem[22] and TurboMole[23] packages of ab initio programs.

The geometries of the electronic ground states of the Cars have been optimized with standard ground-state DFT[24] using the three-parameter Becke3−Lee−Yang−Parr (B3LYP)[25] and the Becke−Lee−Yang−Parr (BLYP)[26] exchange-correlation (xc) functionals in combination with Dunning's DZP[27] basis set. The equilibrium structures of the excited states have been optimized using the Tamm−Dancoff approximation (TDA)[28] to TDDFT[29,30] with the BLYP functional and DZP basis set. Within the BLYP calculations the resolution-of-the-identity (RI) approximation[31,32] has been employed. We have chosen the BLYP functional as standard in our calculations, since it yields in contrast to B3LYP the correct energetic order of the two lowest excited states, and it has proven previously to reach an accuracy of approximately 0.2 eV for the S_1 excitation energy for linear polyenes[33] and especially carotenoids.[14,15] As will be discussed later, the BLYP functional also gives reasonable Stokes shifts for the S_2 states of the Xans, which are systematically too large when calculated with TDDFT/B3LYP/DZP.

For the calculation of the Stokes and geometric shifts, the geometries of the Cars have been optimized on the potential energy surface of the S_1 and S_2 states, respectively, employing TDA/BLYP/DZP. The Stokes shift ΔS_1 (or ΔS_2) corresponds to the difference between the excitation energy of the excited S_1 (or S_2) state at the equilibrium geometry of the ground state and its excitation energy at the equilibrium geometry of the respective excited state. The geometric relaxation energies Δ_0, Δ_1, and Δ_2 are defined as the difference between the total energies of S_0, S_1, and S_2 states at the equilibrium geometries of the S_1 and S_0 states, respectively. The Δ-values measure the change in energy due to the relaxation of the geometry of the carotenoid in the S_1 state. Obviously, the Stokes shift ΔS_1 is the sum of Δ_0 and Δ_1.

It has been argued previously that the S_1 state exhibits substantial double excitation character and can thus be treated reasonably only with highly correlated theoretical methods explicitly including doubly excited states.[34] It has been shown that in a semiempirical molecular orbital basis higher excited determinants are necessary to correctly describe doubly excited states, since these determinants are in such a treatment required to capture the dynamic electron correlation in doubly excited states.[34] In TDDFT some part of dynamic correlation is already contained by virtue of the xc-functional and is thus unclear whether highly excited determinants are required also in a DFT-based treatment like TDDFT. However, also the electronic ground state of carotenoids contains a large amount of doubly excited states (approximately 40% according to an approximated coupled-cluster (CC2)[35,36] calculation) being a measure for dynamic correlation. If the amount of doubly excited character is approximately the same in the ground state and the S_1 state, these states differ mostly by singly substituted determinants, which are well-contained in TDDFT. This can in the most favorable case lead to a balanced treatment of these states, but more accurate calculations are in principle needed to corroborate this assumption. Since ground-state DFT including dynamic correlation by virtue of the xc-functional describes the geometry of the ground state with reasonable accuracy,[37] and, furthermore, the excitation energy of the forbidden S_1 state of linear polyenes and carotenoids is reasonably reproduced by TDA/BLYP/DZP,[14,15,33] it is our belief that the energy and equilibrium geometry of the S_1 state of carotenoids can sufficiently accurately be described with the chosen TDA/BLYP/DZP approach to make *qualitative* statements. Moreover, errors in the dynamic correlation introduced by the use of TDA can be expected to basically cancel, when differences of total energies at different geometries are calculated. For an accurate *quantitative* prediction of these effects, however, more elaborate quantum chemical calculations would be necessary, but these are at present not feasible due to the large molecular size of the carotenoids.

3. Ground-State Geometries and Energetics

Before we turn to the investigation of the excited-state properties of the xanthophylls, we briefly inspect their ground-state geometries. It is obvious from their molecular structure that Vio, Zea, and Lut can adopt different configurations of the β-ionone orientation with respect to the conjugated polyene chain.

In the case of Vio, two different conformers have been investigated, which are denoted as α-violaxanthin and β-violaxanthin. In both conformers, the β-ionone rings are orientated essentially perpendicular to the conjugated polyene chain (Figure 2). While in α-Vio the epoxy groups point in the opposite direction of the closest methyl group of the polyene chain, in β-Vio they point in the same direction. In other words, the β-ionone rings are rotated by about 180° relative to the polyene chain in the different conformers. Geometry optimization without constraints at the theoretical levels of DFT/BLYP/DZP and DFT/B3LYP/DZP yielded essentially equivalent geometric

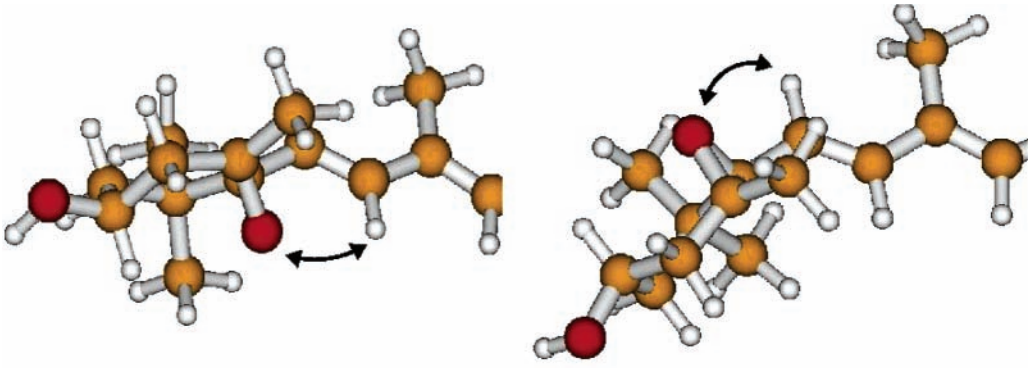

Figure 2. Molecular conformation of the ionone rings of α-Vio (left) and *β*-Vio (right). In the different isomers the *β*-ionone rings are rotated by 180° with respect to the polyene chain, and the interaction of the epoxy rings with the nearest hydrogen atom are indicated by arrows.

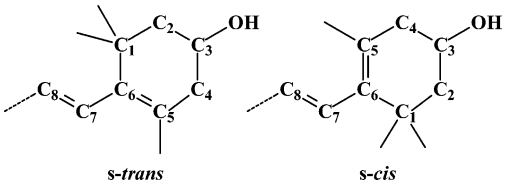

Figure 3. Molecular structure of the s-trans and s-cis configuration of the *β*-ionone ring of zeaxanthin.

parameters for the conformers. The only difference is a slightly less pronounced alternation of the conjugated carbon bonds at the level of DFT/BLYP/DZP, which has been observed previously.[37] At both levels of calculation, α-Vio is more stable than *β*-Vio by 0.27 eV (26 kJ/mol). Having a closer look at the structure of α-Vio compared with *β*-Vio, it is readily apparent that this energy difference is most likely due to an unfavorable interaction of the epoxy oxygens with the α-hydrogen atoms of the polyene chain in *β*-Vio. In α-Vio this interaction does not occur, since the chain is rotated by 180° relative to the *β*-ionone rings (Figure 2). However, although the different orientations of the *β*-ionone rings have a significant influence on the relative energies of the conformers, they affect the energies of the excited states only slightly. Using TDA/BLYP/DZP the S_1 and S_2 states are found at 2.066 and 2.319 eV for α-Vio and at 2.089 and 2.367 eV for *β*-Vio, respectively, at the optimized ground-state S_0 geometry. This only small influence can be attributed to the fact that no functional group of the *β*-ionone ring is directly involved in these $\pi-\pi^*$ excited states, whose corresponding molecular orbitals are strictly located on the conjugated carbon chain.

Turning to zeaxanthin, many different conformers with respect to the relative orientations of the *β*-ionone rings are in principle possible. In general, these conformers can be classified by their double bond configuration as s-trans or s-cis conformers (Figure 3). Calculation of the potential energy surface around the C_6-C_7 single bond reveals that there exist two energetically different s-cis configurations: one with a $C_5-C_6-C_7-C_8$ dihedral angle of 44.3° and one with −47.1° (B and A in Figure 4). The first of these s-cis configurations is 1.2 kJ more stable than the second one and 4.1 kJ more stable than the s-trans configuration (C in Figure 4). Since Zea possesses two *β*-ionone rings, these three different configurations lead to six different conformers with the configurations AA, AB, AC, BB, BC, and CC. Since the *β*-ionone rings are practically noninteracting, the relative energies of the conformers are simply the sum of the relative energies of the *β*-ionone ring configurations. As a consequence, the BB configuration is the most stable one, followed by BA (1.2 kJ/mol), AA (2.4 kJ/mol), BC (4.1 kJ/mol), AC (5.3 kJ/mol), and CC (8.2 kJ/mol).

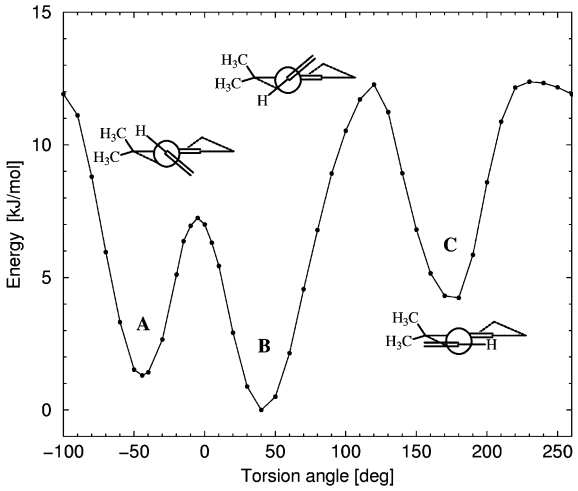

Figure 4. Torsional angle potential for the $C_5-C_6-C_7-C_8$ dihedral angle, i.e., the rotation of the *β*-ionone ring around the C_6-C_7 carbon single bond, and the corresponding Newman projections along this bond are given. Three different *β*-ionone ring conformations correspond to local minima on the potential energy surface leading to six different conformers of zeaxanthin.

This is remarkable since the *π*-system in the s-cis structures is not planar, thus preventing efficient conjugation. On the other hand, in the all-trans conformer the conjugated *π*-system is completely planar allowing for an optimal conjugation. Previous model calculations on *β*-carotene, for which also the all-s-cis conformer is more stable than the all-s-trans conformer by 8.8 kJ/mol, revealed that this can be attributed to reduced ring torsion effects in the s-cis conformer compared to the s-trans form, overcompensating for the more favorable conjugation in the latter one.[38] Due to the structural similarity of *β*-carotene, these findings are also valid for Zea. Nevertheless, the energy difference between the conformers is quite small, and one may ask whether all are present at ambient temperature, i.e., whether all conformers may be relevant for qE. A short glance at the computed minimum energy path for the isomerization from the B (s-cis) to the A (s-cis) and the C (s-trans) configuration reveals that isomerization via torsion of the *β*-ionone ring is unlikely to occur. According to our calculation, there exist rotational barriers of 7.5 kJ/mol from the lowest B configuration to the A configuration and even of 12.5 kJ/mol to the s-trans form. Thus, isomerization via *β*-ionone ring torsion is very slow at ambient conditions, where k*T* is about 2.5 kJ/mol.

In contrast to Vio, the conformation of the *β*-ionone ring has a significant effect on the excitation energies of the S_1 and S_2 states in Zea. Clearly this is due to the fact that one conjugated double bond is located within the ring, which for instance in

TABLE 1: Excitation Energies, Stoke's Shifts, and Geometrical Energetic Shifts of the S_1 and S_2 States at the Optimized Equilibrium Geometries of the Ground State (S_0) and First (S_1) and Second (S_2) States of the Energetically Lowest Conformer of Violaxanthin, All-s-*trans*- and All-s-*cis*-Zeaxanthin (BB Configuration), and s-*trans*- and s-*cis*-Lutein, Respectively[a]

	optimized geometry			Stoke's shift		geometric energy shift at S_1		
	S_0	S_1	S_2	ΔS_1	ΔS_2	Δ_0	Δ_1	Δ_2
				Violaxanthin				
S_1	2.066	1.876	2.018	0.190		0.091	−0.099	0.076
S_2	2.321	2.307	2.260		0.061			
				All-*s*-*trans*-Zeaxanthin				
S_1	1.769	1.598	1.743	0.171		0.080	−0.091	0.092
S_2	2.044	2.056	1.993		0.051			
				All-*s*-*cis*-Zeaxanthin				
S_1	1.885	1.659	1.814	0.226		0.104	−0.122	0.067
S_2	2.086	2.049	2.020		0.066			
				s-*trans*-Lutein				
S_1	1.906	1.739	1.872	0.167		0.081	−0.086	0.081
S_2	2.200	2.200	2.150		0.050			
				s-*cis*-Lutein				
S_1	1.959	1.755	1.898	0.204		0.097	−0.107	0.065
S_2	2.212	2.180	2.151		0.061			

[a] All necessary geometries and corresponding energies have been calculated with TDA/BLYP/DZP and are given in electronvolts (eV).

all-s-*trans*-Zea is in perfect conjugation with the remaining double bonds, while in the all-s-cis conformers the conjugation is diminished due to the out-of-plane torsion described above. Consequently, the S_1 state is found at 1.885 eV for the all-s-cis forms and at 1.769 eV for all-s-*trans*-Zea (Table 1), i.e., 0.116 eV lower, at the theoretical level of TDA/BLYP/DZP. The S_2 state, on the other hand, is only 0.042 eV lower for the all-s-trans conformer. The excitation energies of the S_1 and S_2 states of the mixed cis/trans conformers are found right in the middle between the all-s-cis and all-s-trans conformers. Spectroscopically, no evidence is given for the parallel existence of different conformers in solution,[17−20] and thus one can conclude that probably only the most stable s-cis conformer with both β-ionone rings in the B configuration is present at ambient temperature. However, Zea is produced in the photosynthetic apparatus via enzymatic de-epoxidation of Vio,[3] and the latter is known from the crystal structure of LHC-II to be in the α-configuration (see above).[8,21] The α-configuration does not seem to favor one of the possible conformations of Zea. From that point of view it is in principle possible that also other conformers than the energetically lowest one are produced. Since isomerizations are unlikely due to the involved high-energy barriers, also other isomers might be relevant for the physiological function of Zea in NPQ. Indeed, the so-called orange carotenoid protein (OCP) binds the carotenoid hydroxyechinenone in the s-trans configuration, although the s-cis isomer occurs in solution.[39,40] This however is speculation, and here we will thus focus on the most stable all-s-cis conformer.

Lutein possesses one β-ionone ring that is equivalent to the ones of Zea and one in which the double bond is shifted one bond further not being in conjugation with the remaining 10 double bonds (Figure 1). Therefore, the results for the configurations of the conjugated β-ionone ring can be directly transferred from Zea to Lut. The s-cis conformers in A and B configuration are 2.9 and 4.1 kJ/mol lower in energy than the s-trans isomer, and isomerizations can also be excluded here. Similar to Zea, the energies of the S_1 and S_2 excited states of Lut also depend on the configuration of the conjugated β-ionone ring. In the s-cis conformers, the S_1 and S_2 states are found at 1.96 and 2.21 eV, while in the s-trans form they have excitation energies of 1.91 and 2.20 eV, respectively.

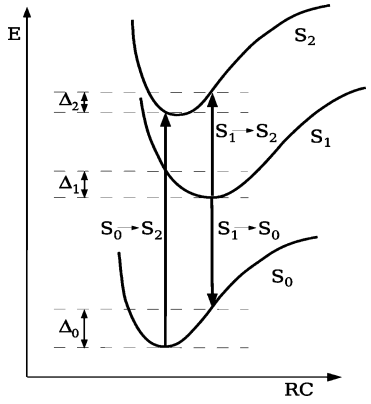

Figure 5. Schematic sketch of the potential energy surfaces of the ground (S_0), first (S_1) and second (S_2) excited states of a carotenoid along an arbitrary geometric relaxation coordinate (RC). Δ_0, Δ_1, and Δ_2 correspond to the energetic changes of the S_0, S_1, and S_2 states, when the geometry of the carotenoid relaxes from the ground-state equilibrium structure into the one of the S_1 state.

4. Analysis of S_1 Energies from Fluorescence and Transient Absorption Spectroscopy

For a basic understanding of the role of carotenoids in photosynthesis comprising light harvesting as well as photoprotection a detailed knowledge of the properties of their energetically low-lying states is of prime importance. The S_2 state of carotenoids is optically allowed (B_{1u} in C_{2h}) and dominates the electronic absorption spectrum. As a consequence the vertical excitation energy of this state is very accurately known. On the contrary, the energetically lowest S_1 state possesses A_g symmetry in C_{2h} and is thus optically forbidden and not directly accessible with conventional one-photon spectroscopy. However, the development of elaborate high-resolution fluorescence spectroscopy as well as transient absorption spectroscopy made the investigation of the S_1 state possible. In these experiments, the optically allowed S_2 state is initially excited, which is displayed as $S_0 \rightarrow S_2$ transition in Figure 5. The S_2 population decays nonradiatively into the S_1 state within a few tens of femtoseconds, whereas the S_1 states of Vio, Lut, and Zea have lifetimes of 24, 14, and 8 ps.[2] During the S_1 lifetime the molecules structurally relax into the equilibrium geometry of the S_1 state, from where they fluoresce very weakly,

since as already mentioned the $S_1 \rightarrow S_0$ transition is optically forbidden in C_{2h}. Nevertheless, there always exist asymmetric vibrational modes that allow for intensity borrowing making very weak fluorescence possible. This weak fluorescence can then be detected in high-resolution fluorescence experiments.[17,18] Another possibility to study the S_1 state is by transient absorption spectroscopy, in which the S_1 population is back-excited into the S_2 state; i.e., the energy of the $S_1 \rightarrow S_2$ transition is measured.[19,20] Most of the current estimates of the S_1 energy of carotenoids rely on these methods, a few on two-photon spectroscopy.[41,42] The energies of the S_1 state are in the case of fluorescence spectroscopy directly given as the energy of the emitted photon, or in the case of TA spectroscopy they have been computed as the difference between the energy of the $S_2 \rightarrow S_0$ transition and the energy of the $S_1 \rightarrow S_2$ transition.

However, if one wants to understand photoprotection by carotenoids and one asks the question whether they can accept singlet excitation energy from chlorophylls, one needs to know the excitation energy of the carotenoids at the equilibrium geometry of their electronic ground state, because all carotenoids are in their electronic ground state before they accept excitation energy. Inspecting Figure 5 carefully, it becomes immediately clear that the S_1 excitation energy obtained from fluorescence experiments does not correspond to this value, because the $S_1 \rightarrow S_0$ transition occurs at the equilibrium geometry of the S_1 states. To obtain the S_1 energy at the ground-state equilibrium geometry one needs to know the Stokes shift of the S_1 state, i.e., how much the ground state and S_1 total energies change upon relaxation into the S_1 equilibrium geometry. Unfortunately, this quantity is experimentally not accessible, and one assumes that it is negligible like it is for the S_2 state,[2] but this shift can in principle be large. The S_1 energy at the ground-state equilibrium geometry is then given as

$$S_1{}^0 = (S_1{}^{eq} \rightarrow S_0) + \Delta_0 + \Delta_1 \qquad (1)$$

where $S_1{}^{eq} \rightarrow S_0$ denotes the energy obtained from the fluorescence experiment, and Δ_0 and Δ_1 correspond to the energy change of S_0 and S_1 upon geometry relaxation being together the Stokes shift of the S_1 state.

In the TA measurements, the initial excitation $S_0 \rightarrow S_2$ is vertically out of the equilibrium geometry of the ground state and thus corresponds to the vertical excitation energy of the S_2 state. Then the S_1 state is probed at its equilibrium geometry and the $S_1 \rightarrow S_2$ transition energy is measured. To obtain information about the S_1 state energy at the S_0 equilibrium geometry, one now needs to know how the S_1 and S_2 states change upon relaxation into the S_1 equilibrium geometry. Indeed, according to Figure 5, the S_1 energy at the ground-state equilibrium geometry is in the case of a TA measurement given as

$$S_1{}^0 = (S_0 \rightarrow S_2) - (S_1{}^{eq} \rightarrow S_2) + \Delta_2 + \Delta_1 \qquad (2)$$

Here, $S_0 \rightarrow S_2$ is the experimentally well-known vertical excitation energy of the S_2 state at the equilibrium geometry of S_0, while $S_1{}^{eq} \rightarrow S_2$ corresponds to the energy determined by TA spectroscopy at the S_1 equilibrium structure. Δ_2 and Δ_1 describe how the energy of S_2 and S_1 change upon geometry relaxation of the S_1 state. Until today, only S_1 energies are given that neglect the geometry relaxation effects of the S_0, S_1, and S_2 states.

From eqs 1 and 2 it becomes also clear that fluorescence and TA measurement should not find equivalent values for the S_1 energy if the geometric relaxation effects are neglected.

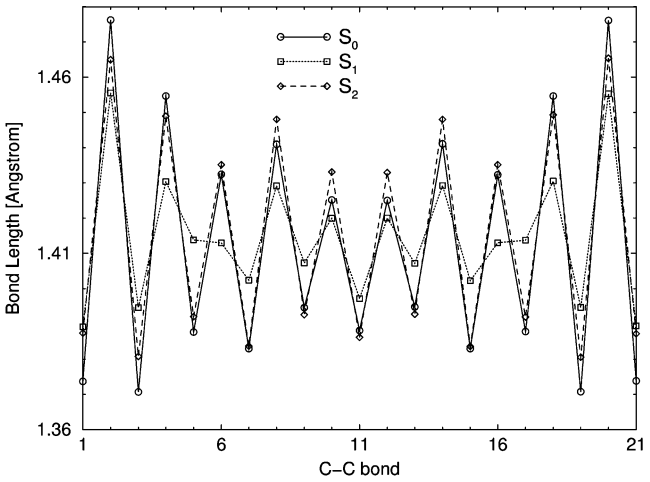

Figure 6. Bond length alternation in the conjugated carbon chain of all-s-*cis*-zeaxanthin in the ground-state S_0 and the first S_1 and second S_2 excited states. The conjugated carbon bonds are numbered successively starting with the double bond in one β-ionone ring.

Equating (1) and (2), setting $S_1{}^{TA} = (S_0 \rightarrow S_2) - (S_1{}^{eq} \rightarrow S_2)$ and $S^F{}_1 = (S_1{}^{eq} \rightarrow S_0)$ one obtains

$$S_1{}^{TA} - S_1{}^{F} = \Delta_0 - \Delta_2 \qquad (3)$$

i.e., the difference in the TA and fluorescence measurements is at least as large as the difference of the geometric relaxation effects of the S_2 and S_0 states upon geometry relaxation into the S_1 equilibrium geometry. However, the experimental discrepancies can be expected to be much larger due to the intrinsic experimental errors.

In the following section we will use TDDFT to investigate the geometric relaxation effects for the three states of interest S_0, S_1, and S_2 and thereby obtain estimates for Δ_0, Δ_1, and Δ_2. These values will finally be used to extrapolate from the measured $S_1{}^{TA}$ and $S_1{}^{F}$ values to the S_1 excitation energy $S^0{}_1$ at the equilibrium geometry of the electronic ground state, which are also compared with calculated values for the $S_1{}^0$ excitation energy.

5. Excited-State Properties

For the calculation of the geometric shifts, the geometries of α-Vio, s-*cis*- and s-*trans*-Lut, and all-s-*cis*- (BB configuration) and all-s-*trans*-Zea have been optimized on the ground-state potential energy surface as well as for the S_1 and S_2 excited states. The only differences between the optimized geometries of the different electronic states of each carotenoid occur along the conjugated polyene chain, and all other geometrical parameters change practically not. As example, the optimized bond lengths of the conjugated carbon chain of all-s-*cis*-Zea are displayed in Figure 6. It is apparent that the equilibrium geometries of the electronic ground state and of the S_2 state differ only slightly. In the terminal region the alternation pattern is slightly weakened, while it is slightly enhanced in the middle region of the polyene chain upon transition from S_0 to S_2. This is in agreement with the well-known Franck−Condon active symmetric stretch vibration of carotenoids, which is responsible for the characteristic shape of the corresponding $S_0 \rightarrow S_2$ absorption peak. It is furthermore in agreement with the small experimental Stokes shift observed for the S_2 state,[2] which implies only small geometric changes. The optimization of the S_1 equilibrium geometry reveals a pronounced bond length equilibration of the conjugated chain especially in the middle

TABLE 2: Excitation Energies of the S_1 and S_2 States of the Energetically Lowest Conformers of Vio, Zea, and Lut (S_1^{eq}, S_1 Excitation Energy at Its Equilibrium Geometry; S_1^0 and S_2^0, Excitation Energies of S_1 and S_2 at the Ground-State Equilibrium Geometry of the Ground-State S_0

	S_1^{eq}			S_2^0		S_1^0		
carotenoid	TA[a]	F[b]	calc[c]	abs[d]	calc[e]	calc[e]	TA + shifts[f]	F + shifts[g]
α-violaxanthin	1.79[h]	1.93[j]	1.87	2.63[j]	2.32	2.07	1.97	2.12
	1.70[i]	1.84[k]					1.88	2.03
all-*s*-*cis*-zeaxanthin	1.74[h]	1.81[j]	1.66	2.60[j]	2.09	1.89	1.93	2.03
	1.71[i]	1.80[k]					1.90	2.04
s-*cis*-lutein	1.74[i]		1.76	2.62[l]	2.21	1.96	1.94	

[a] Determined from experimental. $S_1 \rightarrow S_2$ transient−absorption. [b] Determined from experimental. $S_1 \rightarrow S_0$ fluorescence. [c] Calculated with TDA/BLYP/DZP at equilibrium geometry of the S_1 state. [d] Experimental. UV/vis $S_0 \rightarrow S_2$ absorption data. [e] Calculated with TDA/BLYP/DZP at equilibrium geometry of S_0. [f] Calculated with eq 2 using the experimental $S_1 \rightarrow S_2$ and $S_0 \rightarrow S_2$ data and the calculated geometric shifts from Table 1. [g] Calculated with eq 1 using the experimental $S_1 \rightarrow S_0$ data and the calculated Stoke's shift from Table 1. [h] Taken from ref. 19. [i] Taken from ref. 20. [j] Taken from ref. 18. [k] Taken from ref 17. [l] Taken from ref 43.

region of the polyene. This is in agreement with expectation, but in this case one has to be careful with quantitative statements due to the possibly doubly excited character of the S_1 state and the lack of those in the applied TDDFT method. However, as discussed in section 2, it is unclear what quality the geometry would have, but as we will see later, one obtains a reasonable and consistent picture of the S_1 and S_2 excited states using the optimized geometries for the calculation of the geometric shifts.

Turning first to Vio, TDA/BLYP/DZP yields values for the S_1 and S_2 excitation energies of 2.07 and 2.32 eV at the ground-state equilibrium geometry, respectively. At the S_2 equilibrium geometry, the S_2 excitation energy changes to 2.26 eV revealing a small Stoke's shift (ΔS_2) of only 0.06 eV (Table 2). Experimentally the S_2 state of Vio shows a Stokes shift of approximately 0.03 eV.

The S_1 state on the contrary exhibits a large Stokes shift (ΔS_1) of 0.19 eV, since its excitation energy is only 1.88 eV at its equilibrium geometry, which agrees reasonably with the experimental values obtained from fluorescence and TA measurements of 1.70−1.93 eV (Table 2). The geometric shifts of the individual states, which correspond to the change of the total energies of the individual states when the geometry is relaxed from the S_0 to the S_1 equilibrium geometry, are calculated to be 0.091, −0.099, and 0.076 eV for S_0, S_1, and S_2 (Table 2). If one now uses the experimentally determined S_1^{eq} values from fluorescence and TA experiments, S_1^F and S_1^{TA}, respectively (Table 2), and the calculated geometric shifts according to eqs 1 and 2 (Table 1), one can calculate a "semiexperimental" value for the S_1 excitation energy at the ground-state equilibrium geometry. One obtains values of 1.88−2.12 eV, which compare well with the calculated value S_1^0 of 2.07 eV (Table 2).

For all-s-*cis*-Zea, the S_1 and S_2 excitation energies have been calculated to be 1.89 and 2.09 eV at the ground-state equilibrium structure, 1.66 and 2.05 eV at the S_1 optimized structure, and 1.81 and 2.02 eV at the S_2 optimized structure (Table 1). As a consequence, the S_2 state exhibits a small calculated Stokes shift ΔS_2 of 0.07 eV, which compares well with the experimentally observed one of 0.03, while the Stokes shift of the S_1 state is as large as 0.23 eV. Again, the calculated S_1^{eq} value of 1.66 eV is in reasonable agreement with the measured values of 1.71−1.81 eV. The geometric shifts for Zea are 0.104, −0.122, and 0.067 eV for Δ_0, Δ_1, and Δ_2 (Table 1), respectively. Employing these shifts in eqs 1 and 2 together with the experimentally determined S_1^{TA} and S_1^F values one obtains a semiexperimental value for the S_1 excitation energy of 1.90−2.04 eV at the ground-state equilibrium geometry (Table 2). The corresponding value computed with TDA/BLYP/DZP is 1.89 eV. For completeness, the computed values for all-s-*trans*-Zea are also given in Table 1, but are not discussed in detail, since this conformer is supposed to be physiologically not relevant. If it does possess physiological relevance, its S_1 state can be expected to be about 0.1 eV lower than the one of the all-s-cis conformers.

A similar picture is obtained for lutein. At the equilibrium geometry of the ground state, TDDFT/BLYP/DZP yields excitation energies of 1.96 and 2.21 eV for the S_1 and S_2 states, respectively. Relaxing the structure to the S_1 equilibrium geometry, the S_1 and S_2 excitation energies are 1.76 and 2.18 eV, while at the S_2 optimized geometry they are 1.90 and 2.15 eV, respectively (Table 1). From these values the Stokes shift is derived to be 0.06 eV for the S_2 state and 0.20 eV for the S_1 state. The corresponding geometric shifts are 0.097, −0.107, and 0.065 eV for S_0, S_1, and S_2 (Table 1), respectively. As already found for Zea and Vio, the calculated Stokes shift of the S_2 state agrees with the experimentally observed one. Again, using the geometric shifts together with the experimentally known S_1^{TA} value in eqs 1 and 2, one obtains the S_1 excitation energy at the ground-state equilibrium geometry being approximately 1.94 eV. TDA/BLYP/DZP yields 1.96 eV for this energy. In Table 1, the corresponding values for s-*trans*-Lut are also listed.

Briefly summarizing our findings, semiexperimental values have been deduced for the excitation energies of the S_1 states of Vio, Zea, and Lut at the S_0 equilibrium geometry. Depending on the accuracy of the experimentally determined S_1^{eq} values from transient absorption and fluorescence spectroscopy, we yielded values of 1.88−2.12 eV for Vio, where the lowest value is based on an experimental value determined by transient absorption spectroscopy on reconstituted LHC-II complexes with only one carotenoid present. Since this value is significantly lower than the others, and it is difficult to assess this discrepancy here, we exclude this value from further discussion. Most likely the S_1 excitation energy is slightly above 2 eV, the average of the three largest values is 2.04 eV. For Zea values for the S_1 excitation energies of 1.90−2.04 were obtained at the ground-state equilibrium geometry; the average is 1.97 eV. For lutein only one experimental value was available, resulting in a S_1^0 excitation energy for Lut of 1.94 eV. This value, however, stems from the same experiment on reconstituted LHC-II complexes[20] as the lowest experimental values of Vio and Zea, and the value for Vio is clearly the lowest reported experimental value. Comparing the Zea and Lut values from only this experiment (1.90 eV for Zea and 1.94 eV for Lut), one can assume that the S_1^0 value of Lut is also about 0.04 eV higher than the one for Zea, placing it at 2.01 eV when geometric relaxation effects are included.

6. Summary and Conclusions

Our calculations on the ground-state structures of various conformers of Vio, Zea, and Lut have shown that many different β-ionone ring configurations are in principle possible. The high energy barriers involved in β-ionone ring rotation in Zea and Lut prevent isomerization of the conformers at ambient temperature. In the crystal structure of LHC-II, Vio is in the energetically lowest configuration found, which is not related to a particular zeaxanthin conformer. Thus, one cannot conclude whether deepoxidation of Vio will lead to all-s-*cis*-Zea or all-s-*trans*-Zea. However based on the lower ground-state energy of the all-s-cis conformer, the all-s-trans conformer seems unlikely to be physiologically relevant for light harvesting and photoprotection. The excitation energies of the S_1 and S_2 states of the all-s-trans conformer are significantly lower than the ones of the all-s-cis conformer. Still, an experimental investigation of the conformation of Zea naturally occurring in the photosynthetic apparatus would be of great interest, since if the deepoxidation of Vio does for some reason form the s-trans isomer of Zea, which is then appropriately locked in some binding pocket, such an arrangement could invoke quenching of chlorophyll fluorescence. This could be another mechanism of NPQ. And indeed, the so-called orange carotenoid protein (OCP) binds the carotenoid hydroxyechinenone in the s-trans configuration, although the s-cis isomer occurs in solution.[39,40]

Furthermore, the detailed investigation of the excited-state properties of Vio, Lut, and Zea clearly demonstrates that inclusion of geometric relaxation in the determination of S_1 energies from fluorescence and transient absorption measurements shifts the excitation energies approximately 0.2 eV to higher energies at the equilibrium geometry of the electronic ground states. This has consequences for the role of the carotenoids in light harvesting and photoprotection. In the photosynthetic apparatus all pigments are in their ground state before they are either directly photoexcited or before they receive excitation energy from a neighboring pigment via some energy-transfer process. Thus, the $S_1{}^0$ energy at the equilibrium geometry of the electronic ground state is the decisive quantity that determines whether excitation energy transfer from chlorophyll to carotenoids is possible or not. Experimentally, these values are not accessible due to the very low oscillator strength of the $S_0 \rightarrow S_1$ transition. Therefore, experimental accessible $S_1{}^{eq}$ values have been combined with calculated geometric shifts to obtain semiexperimental $S_1{}^0$ values for all investigated carotenoids. The obtained values for Vio (2.04 eV), Lut (2.01 eV), and Zea (1.97 eV) are all higher than the Q_y state of Chl *a* (1.84 eV). Our calculations also place the excitation energy of the S_1 state of Zea above the Chl *a* Q_y state, making energy transfer from Chl *a* to Zea at first glance impossible and thus arguing against the gear-shift model for NPQ. However, our approximate calculations do not allow for a quantitative interpretation that far, since the error in the calculated excitation energies is on the order of 0.2 eV. The error in the calculated Stokes shifts, which are used to calibrate the experimental excitation energies, might be smaller, but then for the calculation of the semiexperimental $S_1{}^0$ excitation energies, the error in the experimental values for the excitation energies comes into play, which is about 0.05 eV. Earlier experiments on polyenes have shown that in these systems the S_1 state exhibits Stokes shifts in the order of 300 cm^{-1},[44−46] which is much smaller than the ones obtained in the reported calculations. It is however difficult to assess where the origin of this discrepancy lies. A discussion about the Stokes shift of the S_1 state of spheroidene can be found in ref 47. Concluding, it cannot unambiguously be determined whether the S_1 states of the investigated Xans lie above or below the Chl Q_y state, but it is clear that they are higher than the previously given purely experimental values. Thus, it may still be possible that the gear-shift model as outlined in the Introduction is relevant for the high energy state quenching component qE of NPQ, since favorable electrostatic interactions or geometric distortions of Zea in the binding pocket can significantly change the S_1 excitation energy. But this is at this state mere speculation, and such effects shall be the topic of future research.

Acknowledgment. A.D. gratefully acknowledges scientific discussions with Prof. Josef Wachtveitl and continuing support by Dr. Christine Hettmann-Dreuw. A.D. is financially supported by the Deutsche Forschungsgemeinschaft as an "Emmy-Noether" fellow. Computer time has been generously provided by the Center of Scientific Computing (CSC) of the University of Frankfurt.

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