

Electronic Coherence Provides a Direct Proof for Energy-Level Crossing in Photoexcited Lutein and β -Carotene

Evgeny Ostroumov,¹ Marc G. Müller,¹ Christel M. Marian,² Martin Kleinschmidt,² and Alfred R. Holzwarth¹

¹Max-Planck-Institut für Bioanorganische Chemie, Stiftstrasse 34-36, 45470 Mülheim an der Ruhr, Germany

²Institut für Theoretische Chemie, Heinrich-Heine-Universität, Universitätsstr. 1, 40225 Düsseldorf, Germany

(Received 28 January 2009; published 3 September 2009)

We investigate femtosecond transient absorption dynamics of lutein and β -carotene. Strong oscillations up to about 400 fs are observed, depending on excitation or detection wavelength and solvent. We propose electronic quantum beats as the origin of these oscillations. They provide direct proof for strong coupling of the $1B_u^+$ with another electronic “dark” state predicted by quantum chemical calculations to be the $1B_u^-$ state resulting in a crossing within a dynamic relaxation model. The overall dynamics can be described well by an optical Bloch equation approach.

DOI: 10.1103/PhysRevLett.103.108302

PACS numbers: 82.53.Ps, 87.15.ag, 87.80.Dj

Carotenoids are a large group of molecules with very diverse biological functions. They act as antioxidants, photoreceptors, energy and electron transfer cofactors, and, in particular, as photoprotectors involved in regulatory mechanisms ([1] Chapt. 8). For understanding these diverse functions, a detailed knowledge of the electronic level structure and excited state dynamic properties is essential. However, the excited state properties of carotenoids are very complex and not well understood. Thus, rather controversial interpretations are found in the literature (for a review, see [2]). The “dark” S_1 ($2A_g^-$) and the strongly allowed S_2 ($1B_u^+$) states are generally accepted as the two lowest excited states. The ground state (GS) $\rightarrow S_2(1B_u^+)$ transition gives the strong color to carotenoids while the one-photon transition to the S_1 ($2A_g^-$) state is forbidden. This state plays however an important role in many photoprotection processes [2]. Since the lifetime of the S_2 state is very short—in the range of 100–300 fs—the fluorescence yield of carotenoids is generally very low.

Early theoretical considerations led to the suggestion that there may be additional “dark” electronic states located between the above-mentioned S_1 and S_2 states for longer chain carotenoids [3]. These states might play key roles in the relaxation dynamics of the S_2 and S_1 states, in the electronic interaction of carotenoids with other molecules, and in photoprotective and energy/electron transfer processes. Indeed, a series of steady-state and time-resolved spectroscopic studies by the Koyama group [4,5] provided experimental evidence for the existence of these additional “dark” states ($1B_u^-$, $3A_g^-$), which were proposed to be located below the $1B_u^+$ state for carotenoids of $N \geq 9$ –10 but were ignored or questioned in the interpretation of most spectroscopic and time-resolved data [2,6,7]. Also, some of the experimental observations of Koyama *et al.* have been reinterpreted in terms of vibrationally hot S_1 and S_0 states [6] or as a two-photon absorption to a higher singlet state [8].

For steady-state fluorescence measurements ([1] Chapt. 9, [9]), a mirror-image relationship of absorption

and fluorescence bands was claimed. However, for a number of carotenoids, the fluorescence spectrum deviates quite substantially from a mirror image [10]. From all the available data on carotenoids, it is clear that both temporal and spectral characteristics of fluorescence are much more complex than is generally assumed. Recently, highly damped coherent oscillations in the transient absorption (TA) signals after femtosecond pulse excitation were demonstrated for β -carotene [11]. These oscillations were interpreted as evidence for electronic state coherence and were attributed to the coupling between the S_1 and S_2 states.

We report a study of the ultrafast relaxation dynamics and fluorescence of two carotenoids, lutein ($N = 10$) and β -carotene ($N = 11$), under a range of different conditions. A highly oscillatory behavior is observed in the early relaxation dynamics. The relative amplitude and characteristics of the oscillations are strongly dependent on the type of carotenoid, the excitation wavelength, and the solvent. For lutein in the apolar solvents *n*-hexane (HEX) and diethyl ether (DEE), we observe oscillations of extremely large amplitude, about 2–3 times higher than for β -carotene in the time range up to 300–400 fs (Fig. 1). For benzonitrile (BNI), the shape of the oscillations is generally maintained, but the amplitude is reduced by nearly an order of magnitude. The oscillations are only pronounced in the 600–700 nm spectral region—i.e., outside the excitation region and in a range where only excited state absorption (ESA) and stimulated emission (SE) contribute to the signal. They are rather small or absent in the shorter wavelength region where the ESA of the S_1 state is located [Fig. 1(b)]. Another important point is their excitation wavelength dependence [Fig. 1(c)]. After excitation at 495 nm, a well resolved double peak replaces the single oscillation observed for $\lambda_{\text{exc}} = 475$ nm. It is clear that the observed kinetics cannot be described by a simple combination of exponential kinetics. We rather ascribe the oscillations to electronic quantum beats caused by coherent excitation of strongly coupled excited states. For a theo-

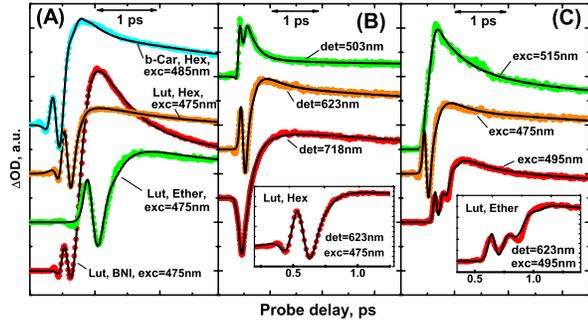


FIG. 1 (color online). Absorption transients of β -carotene in HEX, and lutein in BNI, DEE, and HEX, detection at 623 nm (a); lutein in DEE detected at 503, 623, 718 nm (b); lutein in DEE excited at 475, 495, 515 nm, detection at 623 nm (c). Insets show time-scaled absorption dynamics of lutein excited at 475 nm (b) and 495 nm (c). The thick dots show the measurements and the thin lines the results of global fitting. Femtosecond TA was performed using 2–5 nJ transform-limited pulses of 60 fs width from a tunable optical parametric amplifier at 3 kHz focused to a 120 μ m diameter spot. Absorption changes were detected with a spectral resolution of 0.5 nm in a vertically and horizontally shifted quartz cuvette with a path-length of 1 mm and an OD ca. 0.5–0.8/mm at the excitation wavelength using magic angle polarization between the excitation and probe pulses. The carotenoid concentration was 20 μ M, except for 515 nm excitation where it was 10x higher.

retical description, we thus require a model that includes coherence terms. The simplest possible theory is a solution of the optical Bloch equations [11] in a density matrix approach with elements ρ_{ij} :

$$\begin{aligned}\frac{\partial \rho_{ij}}{\partial t} &= -\frac{i}{\hbar}(H\rho - \rho H)_{ij} - \gamma_{ij}\rho_{ij}; i, j = 1, 2 \\ \frac{\partial \rho_{33}}{\partial t} &= -\gamma_{33}\rho_{33} + \gamma_{11}\rho_{11} + \gamma_{22}\rho_{22} \\ \frac{\partial \rho_{44}}{\partial t} &= -\gamma_{44}\rho_{44} + \gamma_{33}\rho_{33} \\ \frac{\partial \rho_{55}}{\partial t} &= -\gamma_{55}\rho_{55} + \gamma_{44}\rho_{44}.\end{aligned}$$

The Hamiltonian consists of the state energies $H_{11} = H_{22} = E$ and the coupling terms $H_{12} = H_{21} = V$. Relaxation is implemented as rate constants of depopulation γ_{ii} and decoherence $\gamma_{12} = \gamma_{21}$. The equations were solved numerically, and fitting to the experimental data was performed using home-written MATLAB (Mathworks) code. The level scheme and relaxation pathways are shown in the inset of Fig. 2(a).

The fitted theoretical TA signals (thick dots) are overlaid on the experimental TA signals (thin lines) in Fig. 1. The calculated population dynamics of the various states of lutein in DEE ($\lambda_{\text{exc}} = 495$ nm) are shown in Fig. 2(a). All fit parameters are summarized in Table I. The pronounced population oscillations of the two coherently excited states are caused by the large electronic coupling

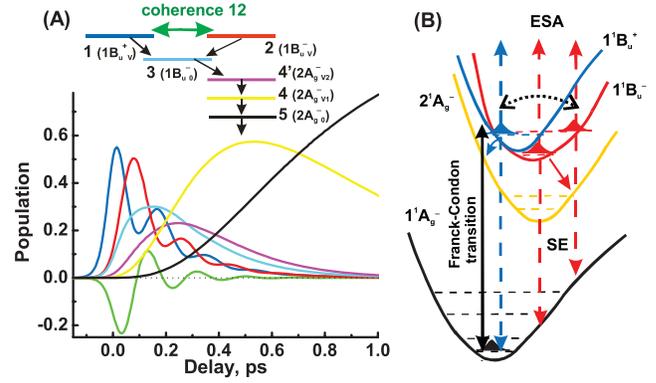


FIG. 2 (color). Population dynamics of lutein in DEE excited at 495 nm (left) calculated using the Bloch equation approach (see text). The kinetic model scheme with assignment to the electronic states is shown (top). Note the oscillatory populations in states 1 and 2. The coherence term is shown in light green.

V_{12} . The relaxation rates of the three highest energy states form the core of the model and describe the evolution in the first 300–400 fs. The model—despite its rather simple form—describes the kinetics remarkably well. This very good agreement (Fig. 1) strongly suggests that the basic features of the excited state level structure and dynamics are described properly. The two highest energy state populations relax with lifetimes in the range of 50–100 fs, in good general agreement with earlier simpler analyses not taking into account coherence terms. The relaxation rates of the two lowest states represent very well the known lifetimes of the vibrationally hot and relaxed S_1 states of lutein and β -carotene [2]. Since coherent coupling was taken into account only for the V_{12} matrix element and the energy gap between the coherently coupled states was assumed to be zero, it is likely that the value for the coupling strength (between 300 and 700 cm^{-1} for lutein in apolar solvents, c.f. Table I) may be somewhat overestimated but is generally considered to be reasonable. Only for relatively strongly coupled excited states we

TABLE I. Values of coupling strength V (cm^{-1}) and relaxation rates (ps^{-1}) (see assignment to the electronic states on the Fig. 2), resulting from the global fitting for the kinetics under different experimental conditions of solvent and excitation wavelength. The rates γ_{44} and γ_{55} agree with literature data for the relaxation on the longer time scale.

sample/solvent	lutein/DEE			lutein/BNI	β -Car/HEX
λ_{exc} (nm)	475	495	515	475	485
V	700	560	330	180	530
γ_{12}	29	11	15	20	12
γ_{11}	6.5	6.8	11	9.0	6.8
γ_{22}	12	4.2	12	7.2	5.9
γ_{33}	13.6	9.4	11.5	...	20
$\gamma_{44}(\gamma_{44}')$	3.1	2 (10.4)	1.7	1.9	6.2
γ_{55}	0.062	0.072	0.071	0.061	0.12

may expect coherent electronic oscillations. The resulting electronic dephasing times of 30–90 fs (Table I) appear to be very reasonable [12] and are fully consistent with our interpretation of the oscillations as manifestations of electronic coherences. Damping is nearly completed already after two oscillation periods [Fig. 2(a)]. From the period of the coherent beating [160 fs, corresponding to 210 cm^{-1} , Fig. 2(a)] for lutein in apolar solvent, it is clear that these signals are not due to vibrational oscillations [13]. The fits indicate stronger interaction energies V_{12} for lutein in apolar solvents, which are however strongly dependent on the exact excitation wavelength, substantially weaker coupling for β -carotene in apolar solvents (HEX, DEE), and further strongly reduced coupling in the highly polarizable BNI [14] for both carotenoids. As compared to a previous study for β -carotene [11] where excitation occurred at the far red edge of the $S_0 \rightarrow S_2$ absorption, the oscillation amplitudes are much higher under our conditions, and the TA signals differ substantially from the present data where excitation has been chosen near the peak of the S_2 absorption band. UV excitation of β -carotene gives no oscillations [15].

The observed large oscillations in lutein cannot occur primarily between the traditionally assumed S_2 and S_1 excited states for several reasons. First, the high frequency oscillation requires strong electronic coupling in the order of several hundred cm^{-1} which would not be possible for the large $S_2 - S_1$ electronic energy gap of $\sim 7000\text{ cm}^{-1}$. Second, the quantum beats are damped with decoherence times well below 100 fs while the strong ESA of the S_1 state only rises with lifetimes of ca. 600 fs in the $\sim 530\text{--}560\text{ nm}$ region. Thus another electronic state must be located very close to the S_2 state with a crossing near the Frank-Condon (FC) region. If this is the case, the observed oscillations provide the most direct evidence for the existence of the earlier proposed lower-lying $1B_u^-$ state [3,4]. However, since the observed kinetics is rather unusual, we sought for an independent confirmation of the existence of the $1B_u^-$ state nearby the S_2 ($1B_u^+$) state. Thus, experimental fluorescence spectra were studied in detail and high level quantum mechanical calculations were performed using a method suitable to tackle the complex excited state structure of carotenoids [16–18]. Technical parameters of the calculations were the same as in recent work on carotenoids with phenolic end groups [18].

In Fig. 3, absorption and fluorescence steady-state spectra of β -carotene and lutein are shown together with the calculated theoretical fluorescence spectra, utilizing the Stepanov relationship [19]. In the absence of other electronic states close to the S_2 ($1B_u^+$) state, the fluorescence spectra are expected to show good mirror image relationship with the absorption. However, Fig. 3 reveals that the fluorescence in its largest part does not arise from the strongly absorbing S_2 state, but must arise from a lower-lying state, which at the same time can not be the tradi-

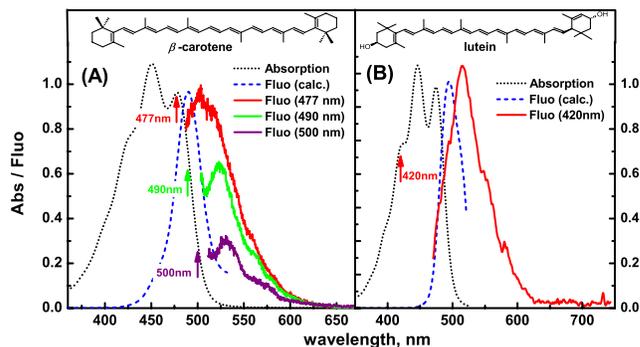


FIG. 3 (color online). Absorption (dotted line) and fluorescence spectra of β -carotene in HEX (a) and lutein in DEE (b) together with the theoretical fluorescence spectra (dashed line) calculated from the absorption using the Stepanov relationship [19]. Note the large deviations of experimental and calculated spectra. The chemical structures of β -carotene and lutein are shown at the top. The carotenoid concentration for fluorescence was 1–5 μM .

tional “dark” S_1 state. This new state which is responsible for most of the fluorescence apparently does not have a strong absorption, but has a broad strongly red-shifted fluorescence that extends, in particular, for lutein, up to 700 nm. For β -carotene, a strong excitation wavelength dependence of the fluorescence maximum is demonstrated [Fig. 3(a)]. We consider this fluorescence behavior as clear evidence for the existence of an additional state slightly below the S_2 state. The long tail of the fluorescence band of this state can be explained by a pronounced shift of the excited state potential surface(s) vs the nuclear coordinates of the electronic GS.

Quantum chemical calculations using a parallelized version of the combined DFT/MRCI method [17] performed for lutein and β -carotene show that upon geometry relaxation in the $1B_u^+$ potential well, the multi-configurational state of $1B_u^-$ symmetry drops below the S_2 state and gains a substantial transition dipole moment as compared to the transition dipole for the GS geometry (see Table II). For lutein in apolar solvent, which comes very close to the *in vacuo* situation assumed for the theoretical calculations, the crossing of the $1B_u^+$ and $1B_u^-$ states is predicted to be close to the potential minimum of the $1B_u^+$ state. For β -carotene, the crossing is predicted to occur a bit further away from the potential energy minimum. At the intersection of these potential energy surfaces, strong nonadiabatic coupling between the $1B_u^-$ and $1B_u^+$ states is expected. This situation allows coherent excitation and the resulting strong oscillations. Thus, all our experimental observations as well as the results of the theoretical calculations support the notion that the relaxed $1B_u^-$ state in lutein is located just below the $1B_u^+$ state, in agreement with earlier suggestions [20]. A conical intersection and dynamical level crossing between these states is predicted (Fig. 2). The exact energy differences of these two states will be deter-

TABLE II. Calculated energetic positions (cm^{-1}) and oscillator strengths $f(r)$ of the $1B_u^+$ (S_2), $1B_u^-$, $2A_g^-$ (S_1), and $3A_g^-$ states at the S_0 (FC transition) and the $1B_u^+$ state minimum geometries (m.g.).

Compound	$1B_u^+/f(r)$	$1B_u^-/f(r)$	$2A_g^-$	$3A_g^-$
Lutein/ S_0 m.g.	19 875/3.70	22 238/0.17	16 651	26 987
Lutein/ $1B_u^+$ m.g.	18 863/2.19	17 857/1.64	12 554	23 224
β -carotene/ S_0 m.g.	19 510/3.66	21 371/0.31	16 040	25 795
β -carotene/ $1B_u^+$ m.g.	18 324/3.15	17 096/0.83	11 922	22 019

mined by N and by the solvent since the location of the $1B_u^+$ state strongly depends on solvent polarizability [14]. This leads us to the electronic level structure and dynamic relaxation model shown in Fig. 2. Excitation pulses with energies corresponding to the FC transition of the $1B_u^+$ state do not excite substantially the $1B_u^-$ state since it is located above the $1B_u^+$ state and has a small transition dipole. For lutein in apolar solvent, the most favorable situation is realized for the observation of coherent oscillations since the FC region of $1B_u^+$ and the crossing of the $1B_u^+$ and $1B_u^-$ states are energetically very close. These features explain the pronounced dependence of the oscillations on the excitation wavelength and the solvent. Note that quantum chemical calculations for these carotenoids place the $3A_g^-$ strictly above the $1B_u^+$ state at all nuclear geometries studied in contrast to previous suggestions [4].

We can exclude coherent artifacts or other causes as the origin of the oscillations. The pure solvents did not give such signals under identical conditions, and such oscillations were not observed on the same apparatus for a very wide range of other compounds and conditions. Also, the oscillations are only observed strongly if the carotenoids are excited close to the maximum of the first strong absorption band, which corresponds to the crossing region of the two states. Vibrational coherences in the GS are excluded since they have much higher frequencies (above 1000 cm^{-1}) which could not be excited by our pulses, they would not depend critically on the solvent, their dephasing times are more than an order of magnitude larger than those observed here, and they occur in different wavelength regions [13].

The complex development of the TA and steady-state fluorescence features as well as the calculations strongly suggest the existence of the $1B_u^-$ state that has been mostly ignored in the interpretation of ultrafast dynamics so far. The predicted large increase of the transition dipole moment of the $1B_u^-$ state in the relaxed conformational state is consistent with the experimental observation that the main part of the fluorescence of lutein and β -carotene do not derive from the $1B_u^+$ state. The model explains the broad red-shifted fluorescence spectrum of these carotenoids as a consequence of the strong excited state potential shifts of the excited states vs the GS. Thus, our results cannot be explained in terms of the often invoked tradi-

tional S_2 - S_1 - S_0 model, and a much more sophisticated dynamic relaxation model is necessary (Fig. 2). Our conclusions should apply generally for longer chain carotenoids and are highly relevant for the understanding of the photochemical and photoprotection functions of carotenoids.

The present work has been performed as a project of the SFB 663 (B2, C1) and is printed at its instigation with financial support provided by the Deutsche Forschungsgemeinschaft.

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