Excited state properties of aryl carotenoids

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Excited-state properties of aryl carotenoids, important components of light harvesting antennae of green sulfur bacteria, have been studied by femtosecond transient absorption spectroscopy. To explore effects of the conjugated aryl group, we have studied a series of aryl carotenoids with conjugated ϕ-ring, chlorobactene, β-isorenieratene and isorenieratene, and compared them with their non-aryl counterparts γ-carotene and β-carotene, which contain β-ring. Changing β-ring to ϕ-ring did not reveal any changes in absorption spectra, indicating negligible effect of the ϕ-ring on the effective conjugation length. This observation is further supported by the carotenoid S_1 lifetimes. In *n*-hexane, the S_1 lifetime of chlorobactene having one ϕ-ring is 6.7 ps, while the S_1 lifetime of the β-ring analog, γ-carotene is 5.4 ps. The same effect is observed for the series β-carotene (two β-rings), β-isorenieratene (one β- and one ϕ-ring) and isorenieratene (two ϕ-rings) whose S_1 lifetimes in *n*-hexane are 8.2, 10.3 and 12.7 ps, respectively. The systematically longer lifetimes of aryl carotenoids show that the additional conjugated C=C bonds at the ϕ-ring do not contribute to the conjugation length. The S_1 lifetimes of aryl carotenoids were slightly shortened in benzene, indicating π–π stacking interaction between the ϕ-ring and benzene.

Introduction

Carotenoids are a family of natural pigments that have attracted a lot of attention due to their well-documented multiple functions in various biological systems. As many of the carotenoid functions, *e.g.* light harvesting and photoprotection, rely on their excited-state properties, a considerable number of experiments carried out in recent years has been devoted to disclose the ultrafast processes following the excitation of a carotenoid molecule (for recent reviews see ref. 1–4). It is now well known that due to symmetry reasons (carotenoids belong to the idealized C_{2h} point group), the transition causing the strong absorption of carotenoids in the blue-green spectral region occurs from the ground state S_0 to a higher excited state, generally denoted S_2. The lower-lying S_1 state, characterized in details during the past decade for some carotenoids,[1] is forbidden for one-photon transitions from the ground state. At the turn of the millennium, it was suggested that other 'dark' states may exist between the S_1 and S_2 states,[3–5] a hypothesis that further complicates the intricate network of relaxation pathways in carotenoids. Although the origin, lifetimes, energies and role in relaxation pathways of these states are not completely clear yet, it seems likely that at least two additional dark states, denoted $1B_u^-$ and S*, may play a role in the excited-state dynamics of carotenoids with the number of conjugated double bonds $N > 9$.[4]

The conjugation length N determines most of the spectroscopic properties of carotenoids, but it was demonstrated that occurrence of specific functional groups, such as conjugated carbonyl group, can significantly affect the excited-state dynamics.[6,7] Therefore, it is of interest to explore potential effect of other functional groups, as for example a conjugated aryl ring (ϕ-ring) that occurs in some naturally-occurring carotenoids. Aryl carotenoids, such as chlorobactene, β-isorenieratene and isorenieratene shown in Fig. 1, are major carotenoids present in chlorosomes of green sulfur bacteria where they act as light-harvesting and photoprotective pigments,[8,9] but also play an important structural role in assembling the chlorosomes.[10–12] It was shown that certain cyanobacteria are also capable of synthesizing aryl carotenoids as a novel aryl carotenoid, synechoxanthin, has been identified in *Synechococcus* sp. PCC 7002.[13] Synthetic aryl carotenoids were employed as energy donors in artificial light-harvesting systems,[14] or as electron donors in artificial reaction centers[15] and dye-sensitized solar cells.[16] Recently, it was also suggested that the ϕ-ring of dihydroxyisorenieratene, an aryl carotenoid from *Brevibacterium linens*,[17] is the structural feature enhancing antioxidative and photoprotective functions of this carotenoid.[18]

Contrary to non-aryl carotenoids, no systematic studies of excited-state properties of aryl carotenoids have been carried out so far. In a study addressing the light-harvesting capability of carotenoids in chlorosomes from *Chlorobium phaeobacteroides* Pšenčík *et al.* showed that the carotenoids (predominantly isorenieratene) transfer energy to bacteriochlorophyll *via* the S_2 pathway with efficiency of 60–70%, while no S_1-mediated channel was detected.[19] The observed S_1 and S_2 lifetimes of ~10 ps and <100 fs would, taking into account the efficiency of

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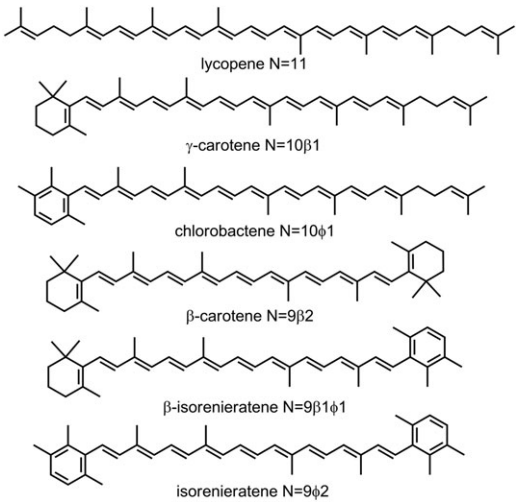

Fig. 1 Molecular structures of lycopene, γ-carotene, chlorobactene, β-carotene, β-isorenieratene and isorenieratene. N indicates conjugation length of the carotenoid, β denotes number of β-ionone rings, ϕ denotes number of the aryl rings.

the S_2 channel, imply that the S_1 and S_2 lifetimes of isorenieratene in solution are comparable to those of β-carotene, suggesting a negligible effect of the two ϕ-rings of isorenieratene.

The hypothesis of minor effect of the ϕ-ring was recently supported by transient absorption measurements on dihydroxy-isorenieratene in ethanol, yielding S_1 lifetime of 12 ps.[20] This aryl carotenoid has structure of the conjugated chain identical to that of isorenieratene (Fig. 1), thus has more conjugated C=C bonds than β-carotene. Yet, its S_1 lifetime is longer than that of β-carotene. This seemingly contradictory observation was explained by calculations showing that twisting of the ϕ-ring out of the main conjugation plane isolates the ring from the rest of the conjugation, making the effective conjugation even shorter than for β-carotene.[20] However, a shorter S_1 lifetime of 7.8 ps was found for a synthetic aryl carotenoid derived from β-isorenieratene, having one ϕ-ring and one β-ring.[14] Since this study was carried in toluene, the shorter S_1 lifetime may indicate that the ϕ-ring may be involved in π–π stacking interaction that stabilizes the ring and makes the effective conjugation longer.

It is thus obvious that excited-state properties of the aryl carotenoids cannot be simply derived from the number of conjugated double bonds. Instead, the structure of the particular aryl carotenoid and likely also the solvent properties may alter the effective conjugation significantly. Here we present a systematic transient absorption study of three aryl carotenoids, chlorobactene, β-isorenieratene and isorenieratene (Fig. 1) that occur in chlorosomes of green sulfur bacteria. By comparing their excited-state properties with their non-aryl counterparts in a few solvents, we determine the effective conjugation lengths of these carotenoids and identify the effect of the aryl ring on the observed excited-state dynamics.

Materials and methods

Lycopene and β-carotene were purchased from Sigma-Aldrich, and γ-carotene from Carotenature. These carotenoids were used without further purification.

Chlorobactene was isolated according to Klinger *et al.*[11] Pigments were extracted with a mixture of acetone and methanol, 7 : 2, from a green sulfur bacterium *Chlorobaculum tepidum* (formerly known as *Chlorobium tepidum*) and further purified by high performance liquid chromatography (HPLC). HPLC was performed at room temperature on a semi-preparative SGX C_{18} reverse-phase column (7 μm particle size, 8 × 250 mm, Tessek, Czech Republic) using a Waters HPLC system consisting of Pump Controller 600, Delta 600 injection system and a PDA 996 detector (Waters, USA). Pigments were eluted with 100% solvent A (methanol) for the first 10 min followed by a 3 min linear gradient to 100% solvent B (methanol : ethyl acetate, 68 : 32). The solvent B was then used for an isocratic elution of remaining nonpolar pigments. After the last pigment, column was allowed to re-equilibrate in solvent A for 10 min prior to the next injection. The flow rate was 2 ml/min.

Isorenieratene and β-isorenieratene were isolated from *Chlorobium phaeobacteroides* according to the methods described previously,[21] by HPLC on a C_{18} reverse-phase column (4.6 × 250 mm, 5 μm particle size, non-Endcapped Zorbax ODS, Agilent, USA) at a flow rate of 1 ml/min. Pigments were eluted by a linear gradient from 100% solvent A (methanol) to 100% solvent C (methanol : hexane, 4 : 1) for the first 20 min and then isocratically by the solvent C until all pigments were eluted. Dried carotenoids were stored in the dark at −76 °C, and prior to experiments dissolved in *n*-hexane, cyclohexane and benzene (Sigma Aldrich, spectroscopic grade) to yield optical density ~0.5 at absorption maximum in a 1-mm cuvette.

Femtosecond transient absorption experiments were carried out using pulses provided by amplified femtosecond laser system Integra-i (Quantronix). The output beam (2.1 mJ, 130 fs, 795 nm, 1 kHz repetition rate) was split into two beams used as excitation and probe. A white light generated in a 2-mm sapphire plate was used as a probe beam. Prior to the sample, the visible broadband white light was collimated and divided into two identical beams, one of which overlaps at the sample with the excitation beam, while the other serves as a reference. After the sample, both reference and probe beams were sent to a spectrograph, and detected by a pair of 1024 diode-arrays. Excitation pulses were generated in an optical parametrical amplifier (TOPAS, Light Conversion). In all measurements, the mutual polarization of the pump and probe beams was set to the magic angle (54.7°) using a Berek compensator placed in the pump beam path. The sample was placed in a rotating quartz cuvette to prevent thermal and photochemical degradation.

All kinetic traces collected by the diode-array detection system were fitted globally by DAFit software (Pascher Instruments). This approach allows for better determination of the time constants of the excited state processes and for assignment of spectral profiles of the intermediate excited-state species.[22] The data were fitted to a sum of exponentials, including numerical deconvolution of the FWHM of the response function, and a fourth degree polynomial describing the chirp. To visualize the excited state dynamics, we assume that the excited system evolves according to a sequential, irreversible scheme A → B, B → C, C → D,...

The arrows represent increasingly slower monoexponential processes and the time constants of these processes correspond to lifetimes of the species A, B, C, D,... The spectral profiles of the species are called evolution-associated difference spectra (EADS), and although they do not correspond to the pure spectra of the excited state species, they provide valuable information about the time evolution of the whole system.[22]

Results

Absorption spectra of all studied carotenoids in *n*-hexane are shown in Fig. 2. In the top panel, the linear carotenoid lycopene is compared with γ-carotene and chlorobactene, both having the conjugation extended to terminal rings. This extension is accompanied by characteristic changes in absorption spectrum. The 0–0 band of the S_0–S_2 transition of lycopene peaking at 502 nm is shifted to 492 nm for γ-carotene, which has the same number of conjugated C=C bonds, but one bond is in the *s-cis* orientation at the β-ring (Fig. 1). The resolution of vibrational bands is diminished for γ-carotene due to increase of conformational disorder induced by the conjugation extended to the β-ring.[23] Absorption spectrum of the aryl carotenoid chlorobactene is nearly identical to that of γ-carotene, suggesting that two additional conjugated C=C bonds at the ϕ-ring have negligible effect on the S_0–S_2 transition. This is further underlined in Fig. 2 (bottom) that compares absorption spectra of β-carotene, β-isorenieratene and isorenieratene. These carotenoids have the same linear conjugated backbone consisting of 9 C=C bonds that is extended to two β-rings (β-carotene), one β- and one ϕ-ring (β-isorenieratene), and two ϕ-rings (isorenieratene). Thus, although these carotenoids have formally 11, 13 and 15 conjugated C=C bonds, respectively, their absorption spectra are identical. The resolution of vibrational bands is the same and the 0–0 band of the S_0–S_2 transition peaks at 480 nm for all three carotenoids. To test if any solvent-induced effects may occur in aryl carotenoids, we have measured absorption spectra of all carotenoids in *n*-hexane, cyclohexane, and benzene. Whereas these solvents have comparable polarity, they differ significantly in polarizability, and the aryl ring of benzene may be involved in a π–π stacking interaction with the terminal rings of carotenoids. The solvent dependence of absorption spectra of β-carotene and isorenieratene is shown in Fig. 3. The red shift of absorption spectra results from dispersive interactions in solvents with larger polarizability. The positions of the 0–0 bands of the S_0–S_2 transition for all carotenoids are summarized in Table 1.

Although the effect of the ϕ-ring on the S_0–S_2 transition is negligible, differences induced by the ϕ-ring appear in transient absorption spectra. Fig. 4 shows transient absorption spectra taken at 2 ps after excitation into the 0–0 band of the S_0–S_2 transition (Table 1). At this time delay, the relaxation processes associated with the S_2–S_1 internal conversion[24] and vibrational cooling in the S_1 state are mostly finished,[25–27] and the transient spectra are dominated by the S_1–S_n transition from the relaxed S_1 state. The main feature observable in the transient absorption spectra is the change

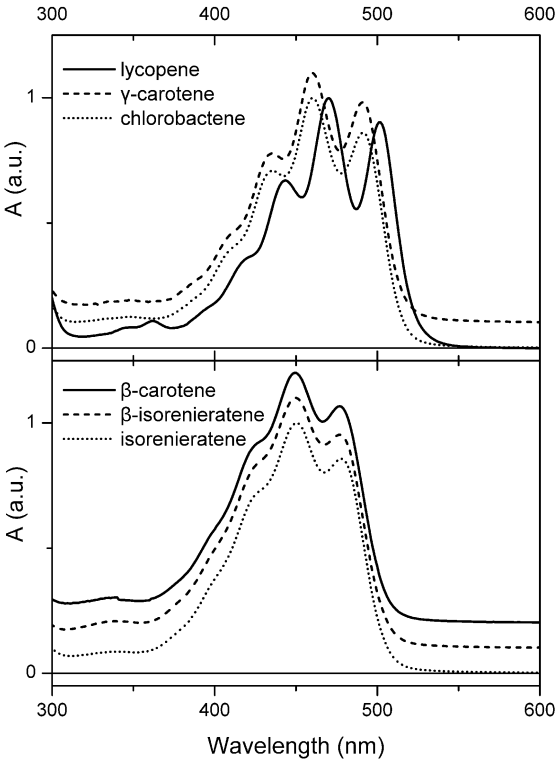

Fig. 2 Steady-state absorption spectra of lycopene, γ-carotene, chlorobactene (top), β-carotene, β-isorenieratene and isorenieratene (bottom) in *n*-hexane at room temperature. All the spectra were normalized to the absorption maximum. Data in the graph were moved vertically by 0.1 to distinguish the spectra of the carotenoids.

of width of the S_1–S_n band. In the lycopene, γ-carotene, chlorobactene series (Fig. 4a), the transient absorption spectra peak at the same wavelength, 556 ± 1 nm, but addition of a terminal ring significantly broadens the S_1–S_n band. Although this is a well-known phenomenon related to the larger conformational disorder induced by the terminal rings, it must be noted that there is a mild difference depending on whether β-type or ϕ-type ring is attached to the conjugated system. While in *n*-hexane the S_1–S_n bands of γ-carotene and chlorobactene have about the same width, the S_1–S_n band of the aryl carotenoid chlorobactene is further broadened in benzene (Table 1). This effect is further enhanced in the series of carotenoids with two terminal rings, β-carotene, β-isorenieratene and isorenieratene (Fig. 4b). Both β-isorenieratene and isorenieratene have their S_1–S_n bands significantly broader than for β-carotene even in *n*-hexane, indicating that the ϕ-ring may influence conformational disorder in the excited state, although the ground state disorder is not affected (Fig. 2). Similar changes are found in benzene, although the effect is weaker than in *n*-hexane, most likely due to significant broadening of the β-carotene S_1–S_n band in benzene (Table 1, Fig. 5).

Similarly to the steady-state absorption spectra, the transient absorption spectra of the carotenoids are red-shifted in solvents with larger polarizability (Fig. 5). The red shift is caused by stabilization of the S_n state, because the S_1 state has negligible dipole moment and its energy remains essentially

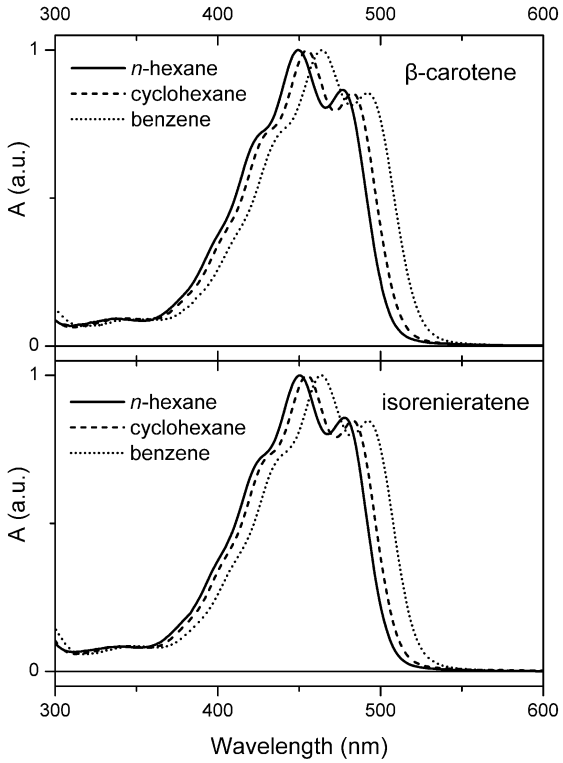

Fig. 3 Steady-state absorption spectra of β-carotene (top) and isorenieratene (bottom) in *n*-hexane (solid line), cyclohexane (dashed line) and benzene (dotted line) at room temperature. All the spectra were normalized to the absorption maximum.

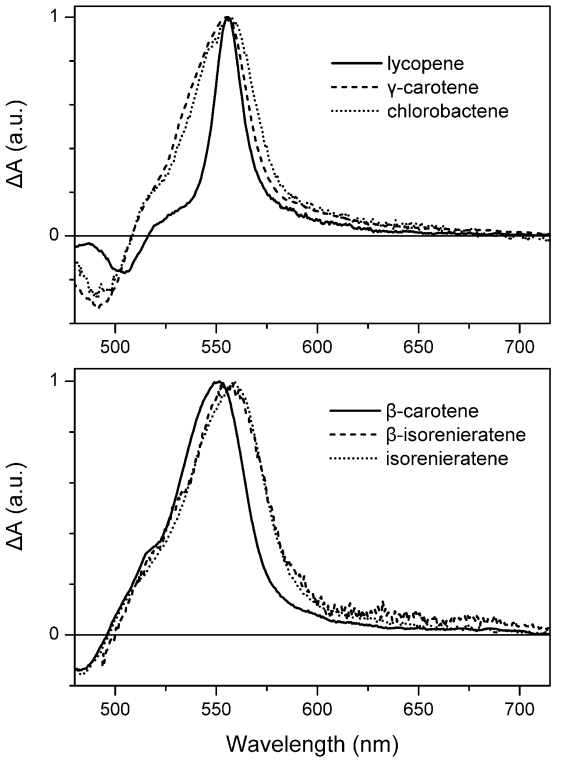

Fig. 4 Transient absorption spectra of lycopene, γ-carotene, chlorobactene (top), β-carotene, β-isorenieratene and isorenieratene (bottom) in *n*-hexane taken 2 ps after excitation. All the spectra were normalized to the maximum band intensity.

unaffected by the solvent polarizability.[28] However, besides the polarizability-induced shift, transient absorption spectra of the carotenoids with two terminal rings exhibit in *n*-hexane a slight red shift of the S_1–S_n band maximum with an increasing number of ϕ-rings (Fig. 4b, Table 1). Although it is tempting to interpret this red shift as due to prolongation of the effective conjugation length due to the ϕ-rings, absence of the red shift in absorption spectra prevents such interpretation. This is further supported by kinetics recorded at the maxima of the S_1–S_n transition that monitors the S_1 lifetime (Fig. 6). The S_1 lifetime becomes progressively longer as the number of the ϕ-rings in the conjugated system increases. Thus, the ϕ-ring makes the S_1 decay in *n*-hexane longer, implying that, despite addition of the conjugated C=C bonds at the ϕ-ring, its presence shortens the effective conjugation length. This trend is especially obvious for the series β-carotene, β-isorenieratene, isorenieratene, that have the S_1 lifetimes in *n*-hexane of 8.2, 10.3 and 12.7 ps, respectively. In carotenoids with a single terminal ring, γ-carotene and chlorobactene, the prolongation of the S_1 lifetime is less pronounced; chlorobactene has

Table 1 Summary of S_1 state lifetimes (τ_{S1}), excitation wavelengths (λ_{exc}), energies of 0–0 band of S_2 state, energies of S_1–S_n band maxima and widths of S_1–S_n bands (measured as a full width at half maximum of the S_1–S_n band) of all studied carotenoids.

Carotenoid	Solvent	τ_{S1} ps	λ_{exc} nm	S_1–S_n max		S_1–S_n FWHM	S_0–S_2 (0–0)	
				nm	cm^{-1}	cm^{-1}	nm	cm^{-1}
Lycopene	*n*-hexane	3.9	502	556	17 990	566	502	19 930
	benzene	4.5	520	576	17 360	611	519	19 250
γ-Carotene	*n*-hexane	5.4	492	555	18 020	1206	492	20 340
	benzene	6.1	513	579	17 270	1210	509	19 650
Chlorobactene	*n*-hexane	6.7	491	557	17 960	1205	492	20 320
	benzene	5.8	512	579	17 270	1359	509	19 640
β-Carotene	*n*-hexane	8.2	478	551	18 150	1279	479	20 870
	benzene	9.4	492	564	17 740	1529	495	20 210
β-Isorenieratene	*n*-hexane	10.3	479	556	18 000	1544	480	20 850
	benzene	10.1	491	570	17 550	1642	496	20 170
Isorenieratene	*n*-hexane	12.7	479	559	17 900	1433	480	20 850
	benzene	11.0	492	568	17 590	1923	494	20 220
	cyclohexane	12.7	481	559	17 900	1697	485	20 640

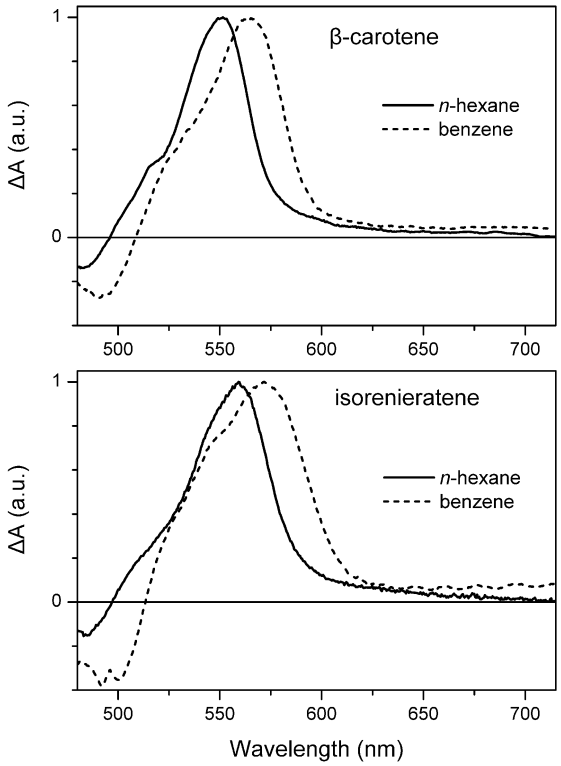

Fig. 5 Transient absorption spectra of β-carotene and isorenieratene obtained 2 ps after excitation in two different solvents (*n*-hexane and benzene). All the spectra were normalized to the maximum band intensity.

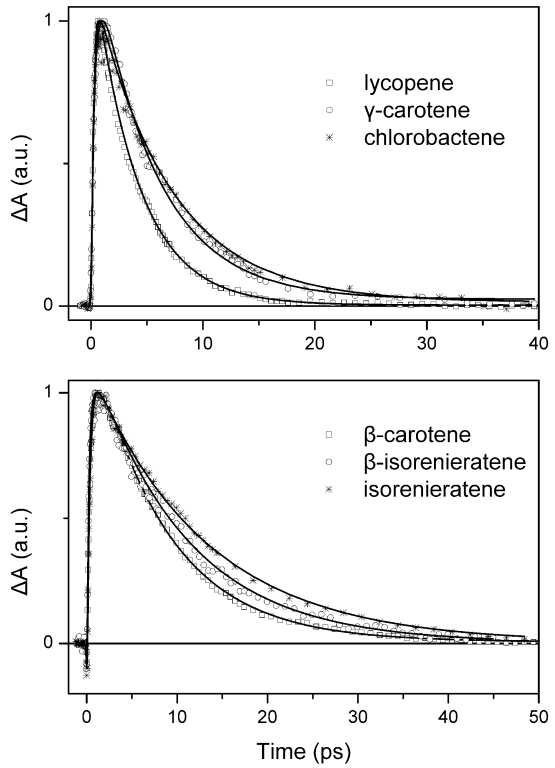

Fig. 6 Kinetics recorded at the maximum of the S_1–S_n band of lycopene, γ-carotene, chlorobactene (top), β-carotene, β-isorenieratene and isorenieratene (bottom) in *n*-hexane. All the kinetics are normalized to the maximum. Solid lines represent fits from global analysis.

a lifetime of 6.7 ps while its counterpart lacking the ϕ-ring, γ-carotene, has a shorter lifetime of 5.4 ps.

Thus, the presence of the conjugated ϕ-ring prolongs the S_1 lifetime in *n*-hexane. For the carotenoids with two terminal rings, the same trend is reproduced also in benzene. Yet, the effect of the ϕ-ring is less pronounced as the S_1 lifetimes in benzene yields 9.4, 10.1 and 11.1 ps for β-carotene, β-isorenieratene, and isorenieratene, respectively. From the S_1 lifetimes summarized in Table 1 it is obvious that while for carotenoids without the ϕ-ring the S_1 lifetime in benzene is always longer than that in *n*-hexane, opposite situation occurs for aryl carotenoids. For isorenieratene having two ϕ-rings the S_1 lifetimes in *n*-hexane and benzene are 12.7 and 11.1 ps (Fig. 7), while for β-carotene with two β-rings the corresponding S_1 lifetimes are 8.2 and 9.5 ps. The same trend, though with smaller magnitude, is observed for chlorobactene and γ-carotene (Table 1). The S_1 lifetime of chlorobactene in benzene (5.8 ps) is shorter than in *n*-hexane (6.7 ps). Reverse situation occurs for γ-carotene whose S_1 lifetimes in *n*-hexane and benzene are 5.4 and 6.1 ps. This clearly shows that benzene interacts with the terminal rings of aryl carotenoids, making their S_1 lifetimes shorter than in *n*-hexane.

Further details about excited state dynamics were obtained from global fitting analysis. The EADS of chlorobactene and isorenieratene in *n*-hexane obtained from global analysis are shown in Fig. 8. Minimum of three time components is needed to fit the data. The first EADS is generated by the excitation pulse and represents a spectrum of the initially excited S_2 state, which consists of the ground state bleaching and stimulated emission (SE) from the S_2 state. This first EADS decays in all carotenoids in less than 130 fs to form the second EADS (dashed line in Fig. 8) that is reminiscent of the S_1–S_n band, but it is significantly red-shifted. Because of these features we assign the second EADS to the spectrum of a hot S_1 state that further relaxes on a time scale 200 fs (chlorobactene) and 400 fs (isorenieratene). This time scale is typical for the vibrational relaxation in the S_1 state of carotenoids,[25–27] further supporting our assignment. The reason for differences in the S_1 vibrational relaxation time constant, which for the carotenoids studied here varies from 200 to 600 fs (data not shown, the longest time constant was found in β-isorenieratene in benzene), is unclear. Yet, previous studies showed that the decay of the hot S_1 state is sensitive to both carotenoid structure and solvent.[26,27] The hot S_1 state decays to produce EADS that is dominated by the S_1–S_n band and is thus assigned to the EADS of the relaxed S_1 state. This EADS decays to zero with a time constant corresponding to the S_1 lifetimes discussed above. It should be noted that although the transient absorption spectra of some carotenoids in our series exhibit a hint of shoulder in the spectral region where signal of the S* state may be expected (the higher-energy part of the S_1–S_n band), none of the carotenoids studied here required the fourth decay component to account for distinct dynamics in this spectral region.

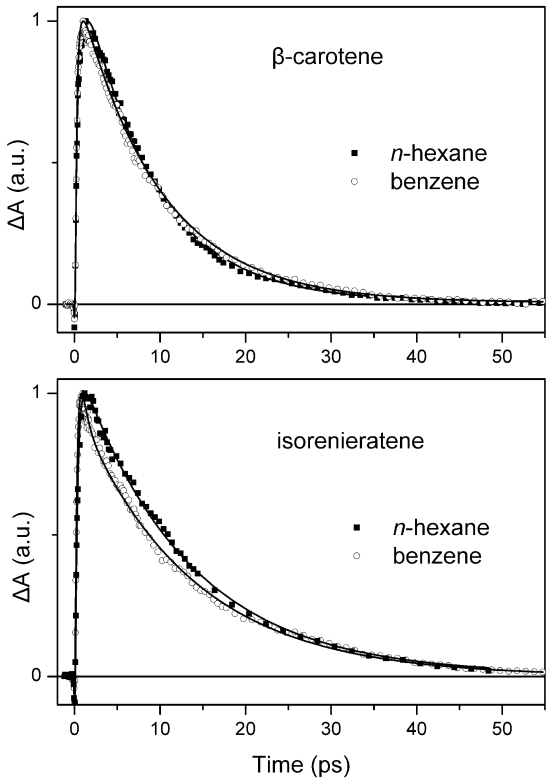

Fig. 7 Kinetics recorded at the maximum of the S_1–S_n band of β-carotene (top) and isorenieratene (bottom) in *n*-hexane (full squares) and benzene (open circles). All the kinetics are normalized to the maximum. Solid lines represent fits.

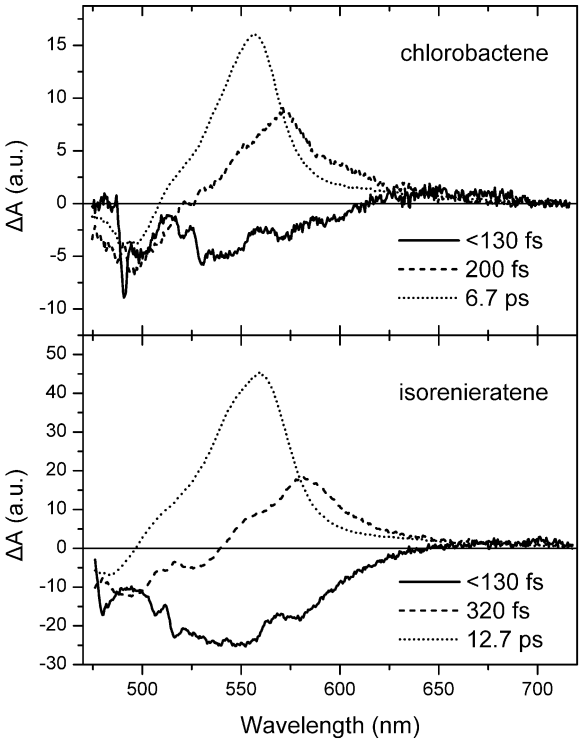

Fig. 8 Evolution associated difference spectra of chlorobactene and isorenieratene in *n*-hexane.

Discussion

Carotenoids used in this study represent an optimal set for exploring influence of the ϕ-ring on excited-state dynamics. Comparing spectroscopic properties of three aryl carotenoids having either one (chlorobactene and β-isorenieratene) or two (isorenieratene) ϕ-rings attached to the main conjugated chain with carotenoids having the same structure except the ϕ-ring is replaced by the β-ring (γ-carotene and β-carotene) allows for identification of effects caused by the presence of the conjugated ϕ-ring. In addition to the reference carotenoids with β-rings, linear carotenoid lycopene lacking any terminal rings is also used as an additional reference.

The most important observation is that the effective conjugation length, N_{eff}, of aryl carotenoids cannot be deduced from the formal number of conjugated C=C bonds in molecular structure of the particular aryl carotenoid. For carotenoids without the ϕ-ring, it is a well-established fact that addition of a conjugated C=C bond always increases N_{eff}. Although specific orientation of the C=C bond is important as *s-cis* orientation increases N_{eff} by only 0.5,[29] a general rule is that $N_{\mathrm{eff}}(\mathrm{C{=}C})_n \leq N_{\mathrm{eff}}(\mathrm{C{=}C})_{(n+1)}$ for two non-aryl carotenoids with n and $n + 1$ C=C bonds in their conjugated system. Our results show that this rule is violated in aryl carotenoids.

The violation of this rule is best demonstrated when comparing the S_1 lifetimes of β-carotene (two β-rings) and isorenieratene (two ϕ-rings) in *n*-hexane. Although isorenieratene has four additional conjugated C=C bonds in its conjugated chain, the S_1 lifetime of 12.7 ps is significantly longer than 8.2 ps measured for β-carotene. Since the longer S_1 lifetime suggests shorter effective conjugation,[1] it means that adding the four conjugated C=C bonds in the two ϕ-rings actually decreases the effective conjugation. Translating this observation into numbers, nine linear C=C bonds and two C=C bonds in *s-cis* orientation of β-carotene gives $N_{\mathrm{eff}} \sim 10$,[29] but nine linear C=C bonds and six C=C bonds located at the two ϕ-rings of isorenieratene suggests $N_{\mathrm{eff}} \sim 9.5$, as indicated by the 12.7 ps lifetime that is not far from 15 ps measured for lutein.[30,31] A very similar lifetime of 12 ps was found in dihydroxyisorenieratene in ethanol.[20] This carotenoid has the same structure as isorenieratene except two hydroxyl groups at the ϕ-rings.

β-Isorenieratene, possessing one β- and one ϕ-ring underlines the role of the ϕ-ring; its S_1 lifetime of 10.3 ps falls between the two extremes, demonstrating that magnitude of the shortening of the effective conjugation is proportional to the number of ϕ-rings in the conjugated system. This hypothesis is further strengthened by comparison of γ-carotene (β-ring) and chlorobactene (ϕ-ring). Conjugated system of γ-carotene has 10 linear C=C bonds and one C=C bond in *s-cis* configuration at the β-ring. Such a system suggests $N_{\mathrm{eff}} \sim 10.5$, which is also supported by its S_1 lifetime of *n*-hexane (5.4 ps). Replacing the β-ring by the ϕ-ring in chlorobactene, the S_1 lifetime becomes longer, yielding 6.7 ps, again demonstrating the unusual effect of the ϕ-ring.

Having established that adding the C=C bonds located at the ϕ-ring to the conjugated system actually decreases the effective conjugation length, let us now turn to explanation of

this phenomenon. It is known that β-ring of β-carotene is twisted by 42–47° in respect to the main conjugated backbone, because of repulsion between the methyl group at the β-ring and hydrogen atoms in the backbone.[32,33] Since this twist partially isolates the C=C bond in the *s-cis* configuration at the β-ring (this C=C bond adds only 0.5 to the effective conjugation length), similar mechanism could explain the results observed here. For example, a large torsional angle of 83°, which is induced by a specific binding site in protein xanthorhodopsin, leads for the carotenoid salinixanthin to a total isolation of the C=C bond at the terminal ring from the rest of the conjugated system.[34] However, recent quantum chemical calculations on dihydroxyisorenieratene showed that the torsional angle of the ϕ-ring is 37°,[20] thus solely the magnitude of the twist cannot explain the effective isolation of the C=C bonds located at the ϕ-ring. Although dihydroxyisorenieratene has additional hydroxyl groups at the ϕ-ring in contrast to isorenieratene measured here, this difference should not affect the value of the torsional angle, because in zeaxanthin, which has the hydroxyl groups at the β-ring, the torsional angle is essentially identical to that of β-carotene.[35]

Thus, although the twist of the terminal ring in isorenieratene is comparable to that in β-carotene, the specific structure of the ϕ-ring isolates the C=C bonds at the ϕ-ring from the rest of the conjugated chain, making the effective conjugation of isorenieratene shorter than that of β-carotene. The most likely explanation is that the conjugation within the ϕ-ring promotes the isolation of the ring from the rest of the conjugated system even if the twist of the ring is relatively small. Such effect can be understood if we assume that delocalization of π-electrons within the ϕ-ring is nearly perfect, and the twist of the ring prevents significant perturbation of this 'local' conjugated system by the main linear conjugation, resulting in the ϕ-ring having the same effect as the β-ring. This hypothesis is supported by comparison with a synthetic analog of dihydroxyisorenieratene whose ϕ-ring lacks methyl groups. Quantum chemical calculations showed that absence of the methyl groups leads to a significant planarization of the whole conjugated system as the torsional angle is only 8°.[20] Consequently, the local conjugated system of the aryl ring is connected with the rest of the conjugation, which is manifested by $\sim 1000\ cm^{-1}$ red shift of the absorption spectrum.

It is therefore the combination of the torsional angle and effective local conjugation within the ϕ-ring that enhances the isolation of the ϕ-rings, resulting in the seemingly contradictive conclusion that carotenoids with ϕ-rings have shorter effective conjugation than those with β-rings. Thus the ϕ-rings of aryl carotenoids contribute only marginally to the effective conjugation length. This is in agreement with fluorescence detected magnetic resonance data,[36] which revealed that the conjugated system in chlorobactene is longer than in isorenieratene, and this can be accomplished only by ignoring the conjugation within the rings.

Yet, presence of the ϕ-rings affects some excited-state properties of aryl carotenoids. Absorption spectra of the carotenoids shown in Fig. 2 exhibit systematic decrease of resolution of vibrational bands when going from linear lycopene to one-ring γ-carotene and chlorobactene, and the least resolved vibrational bands are observed in two-ring β-carotene, β-isorenieratene and isorenieratene. This effect is due to increase of conformational disorder in the ground state induced by the presence of terminal rings.[23] While absorption spectra of carotenoids with β- and ϕ-rings are identical, the S_1–S_n bands in transient absorption spectra shown in Fig. 4 are different (see also Table 1); the S_1–S_n band of isorenieratene is broader than that of β-carotene, indicating that although conformational disorder in the ground state remains unaffected by the ϕ-ring, situation changes in the excited state.

This observation is in accordance with the notion that certain conformational changes occur during the relaxation processes in carotenoids.[25,31,37] Calculations of equilibrium geometries of dihydroxyisorenieratene in the S_0 and S_2 states indeed showed that while the twist of the ϕ-ring in the ground state is 37°, the equilibrated structure in the S_2 state has a smaller torsion angle of 28°.[20] Since the relaxation dynamics in the S_2 state remains unclear and experiments exploring excitation wavelength dependence suggested a possibility of S_2–S_1 relaxation occurring from the vicinity of the Franck–Condon region prior to geometry relaxation in the S_2 state,[37] a possible scenario is that the molecules with different degree of the S_2 geometry relaxation decay to the S_1 state. The S_1 state has, based on the broad S_1 emission, rather flat potential surface, thus distribution of torsional angles generated in the S_2 state is preserved at room temperature even in the relaxed S_1 state. Although there is no information about change of torsional angle upon excitation of carotenoids with β-rings, the results presented here suggest that the ϕ-ring of aryl carotenoids generates broader distribution of conformers in the S_1 state.

Specific properties of the ϕ-rings in generating conformers in the S_1 state may also explain the absence of the S* state in these carotenoids. Although the origin of the S* state is still an unsettled issue,[4] there is accumulating evidence pointing to the S* being associated with an excited state of a specific carotenoid conformation.[27,31] The fact that the ϕ-ring causes larger conformation disorder in the S_1 state, but prevents population of the S* state points to importance of terminal rings and their specific structure in facilitating the small structural changes that occur during the S_2–S_1 relaxation. While the β-ring favors the S* state population presumably associated with a stable minimum at the S_1 potential surface,[27] the ϕ-ring rather generates a broad distribution of conformers. This would imply that energy barrier separating various conformations is smaller when the ϕ-ring is present. Although this hypothesis needs to be verified by calculations, it is worth noting that significant difference between the barrier height separating the *s-cis* and *s-trans* conformations has been found in the ground state of two carotenoids with different terminal rings.[38]

The presence of the ϕ-ring also causes specific solvent effect. The S_1 lifetimes summarized in Table 1 show that for carotenoids without the ϕ-ring, the S_1 lifetime in benzene is systematically longer than in *n*-hexane. Although origin of this phenomenon is unknown, the same effect of benzene was observed in β-carotene analogs.[28] On the other hand, opposite change of the S_1 lifetimes occurs for aryl carotenoids; the S_1 lifetimes are always shorter in benzene, suggesting an interaction between benzene and ϕ-ring. Since the aryl carotenoids

exhibit specific behavior in benzene, we may suspect that the π–π stacking interaction indeed plays a role in photophysics of aryl carotenoids. The identical S_1 lifetimes of isorenieratene in *n*-hexane and cyclohexane (Table 1) strengthen this hypothesis. The ϕ-ring allows for the π–π stacking because the methyl groups of the ϕ-ring are in plane of the ring, in contrast to the out-of-plane methyls of β-ring, which prevents close contact between the β-ring and benzene. Thus, we propose that the π–π stacking interaction between benzene and the ϕ-ring likely stabilizes the position of the ring in respect to the main conjugation, making the effective conjugation slightly longer in benzene. Yet, it must be noted that even in benzene the effective conjugation of isorenieratene remains shorter than that of β-carotene.

Since aryl carotenoids occur mainly in chlorosomes of green sulfur bacteria, it is interesting to ask whether their specific properties are important for proper functioning of chlorosomes as light-harvesting antenna. It was demonstrated that *Chlorobaculum tepidum* mutants lacking the enzyme converting γ-carotene to chlorobactene form chlorosomes very similar to those of the wild type,[12] suggesting that the ϕ-ring is not crucial for assembling the chlorosomes. Nevertheless, recent experiments on self-assembled aggregates mimicking chlorosomes showed that the aryl carotenoid chlorobactene markedly promotes aggregate formation as compared with β-carotene.[11,39] This indicates that although the aryl ring is not likely to be important for large-scale organization of chlorosomes, local interactions between carotenoids and bacteriochlorophylls are affected by the presence of ϕ-ring. Thus, the ϕ-ring might be important to rationalize the efficient energy transfer from the short lived S_2 state of carotenoids to bacteriochlorophylls in chlorosomes,[19] as well as for photoprotective quenching of BChl triplet states by triplet–triplet energy transfer to carotenoids.[36,40–42] Both processes critically depend on distance and mutual orientation of the involved pigments. As there are no proteins in the chlorosome interior, π–π stacking between the aryl rings of carotenoids and conjugated system of bacteriochlorophylls might provide the means ensuring the required distance and orientation between bacteriochlorophylls and carotenoids. For this purpose the aryl carotenoids are the best choice, as the ϕ-ring alone represents a conjugated system whose planarity facilitates interaction with π-system of a molecule nearby. It is worth noting that the S_1 lifetimes of isorenieratene and β-isorenieratene in benzene measured here are close to the S_1 lifetime of $\sim$10 ps measured in chlorosomes of *Chlorobium phaeobacteroides*,[19] which contain a mixture of these two carotenoids. This observation further strengthens the proposed hypothesis that it is the π–π interaction, which shortens the S_1 state lifetime, both in benzene and chlorosomes, and provides an evidence for importance of π–π stacking between carotenoids and bacteriochlorophylls in chlorosomes.

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