The thermal deactivation of all-*trans* and 15-*cis* beta-carotene-excited states in the ionic liquids without and with methylenoxy group

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Abstract Photoacoustic spectroscopy was used to study thermal (dark) paths of excited state deactivation of β-carotene in ionic liquids. The dark paths of excited state deactivation of all-*trans* and 15-*cis* β-carotene isomers are very important in photosynthesis and medicine. The photoacoustic measurements of β-carotene in traditional solvents are difficult due to fast evaporation of the solvent which leads to concentration fluctuation. Room temperature ionic liquids are good medium for temperature study due to their high thermal stability and low volatility. Dark deactivation of both β-carotene isomers in ionic liquids occurs in a similar way except for an additional path of deactivation of excited state $1^1A_g^+$ ('*cis*' peak). The thermal-excited state deactivation of all-*trans* and 15-*cis* β-carotene isomers is not sensitive to the ionic liquid structure, in contrast to the radiative path.

Keywords All-*trans* β-carotene · 15-*cis* β-carotene · Room temperature ionic liquid · Photoacoustic spectroscopy · Electronic-excited states

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Introduction

Carotenoids are natural pigments which play an important role in multiple vital processes. One of the most studied carotenoids is β-carotene. It can be found in mono-*cis* configuration in photosynthetic reaction centres, where is it responsible for the photo-protection of the photosynthetic apparatus [1–3]. β-carotene is present in all-*trans* configuration in the photosynthetic antenna complexes where it participates in the light harvesting together with chlorophylls [4–6]. The spectroscopic properties of β-carotene, and of carotenoids in general, are defined by the 'core' element of the structure, the symmetrical tetraterpene skeleton consisting of a conjugated system of C=C double bonds. A theoretical description of β-carotene (polyenes)-excited states was proposed by Tavan and Schulten [7] in 1987. The first excited singlet state is labelled $2^1A_g^-$ (S_1) and has the same symmetry as a ground singlet state-$1^1A_g^-$ (S_0). For reasons of symmetry, the transition between these two states is optically forbidden. Strong transition in the blue-green region, from ground state to the second excited state $1^1A_g^-$ (S_0) $\rightarrow$ $1^1B_u^+$ (S_2), is characteristic for carotenoids and the cause of their yellow–red colour. Additionally in case of mono-cis isomers (15-*cis* β-carotene), the transition from $1^1A_g^-$ (S_0) to $1^1A_g^+$ excited state is optically allowed, the so-called '*cis*' peak [8].

Previous studies showed that specially designed ionic liquids (ILs) mimic well the spectroscopic properties of β-carotene in situ [9]. In addition, the high thermal stability of ILs enables temperature-dependent measurements. Room temperature ionic liquids (RTILs) are organic salts with melting point lower than room temperature [10, 11]. They have become very popular in recent years as a 'green' equivalent to standard organic solvents. RTILs are thermally stable, environmentally friendly, recyclable and of

low volatility [12]. The physicochemical properties of RTILs depend on the structure of bulk, organic cations and in most cases inorganic anions, thus some properties like thermal capacities of RTILs one can plan by cations and anions modification [13].

The photoacoustic effect is the production of the pressure modulation around a sample when it absorbs the modulated light [14]. Photoacoustic spectroscopy is a technique providing detailed information, for example, on the photosynthetic system and structure of plants [15]. One can use the photoacoustic spectroscopy to quantify parameters characterizing plants such as: chemical composition, efficiency of photosynthetic oxygen evolution, photochemical energy storage, photosynthetic action spectra, time of photo-thermal signal creation in a photoacoustic cell (heterogeneity of PS II), the coefficient of oxygen diffusion through the cell wall, the sample depth profiling, the light-saturation curve and kinetics of non-photochemical quenching on different plants [15, 16].

The purpose of the present study is (1) determination of the influence of the β-carotene's concentration and (2) the IL's structure on the dark paths of all-*trans* and 15-*cis* β-carotene-excited state deactivations.

Materials and methods

The β-carotene was solubilized in ionic liquids after *n*-hexane (Merck) evaporation under nitrogen gas. In one particular experiment where the concentration of all-*trans* β-carotene (Fig. 1a) (Fluka-Chemica) was 80 μM after *n*-hexane evaporation, β-carotene was solubilized in *N,N*—dimethylformamide (DMF, Chempur) followed by the

incorporation into ILs, DMF: IL ratio was 1:10. The 15-*cis* β-carotene (Fig. 1b) was purified and analysed using HPLC method [1, 17] prior to the measurements.

The room temperature ionic liquids were synthesised as previously described [18, 19]. The chemicals 1-methylimidazole, 1-bromooctane, chloromethyloctyl ether, sodium tetrafluoroborate, acetonitrile, dichloromethane and heptane were obtained from Sigma-Aldrich. 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate (IL 1) and 1-methyl-3-octylimidazolium tetrafluoroborate (IL 2) were prepared in two reactions. In the first one, 1-methyl-3-octyloxymethylimidazolium chloride was obtained in the Menschutkin reaction of 1-methylimidazole and chloromethyloctyl ether. The reaction was carried out for 1 h in acetonitrile in 293 K, and the product was purified by extraction with heptane in 343 K. Chloromethyloctyl ether readily hydrolysed in a present of small amount of water to form HCl, which in turn gives the 1-methylimidazolium hydrochloride. The separation of the quaternization product and hydrochloride is practically impossible. For this reason, quaternization reaction was conducted under strictly anhydrous conditions. The product of 1-methyl-3-octyloxymethylimidazolium chloride was hygroscopic compound with the yield 98.9 %. The first reaction of 1-methyl-3-octylimidazolium tetrafluoroborate synthesis was the Menschutkin reaction of 1-methylimidazole and 1-bromooctane. The reaction was carried out for 24 h in acetonitrile at 354 K. The product of 1-methyl-3-octylimidazolium bromide was purified by extraction with heptane at 343 K with 94.7 % yield.

The second reaction was metathesis reaction between our products and NaBF$_4$. 1-methyl-3-octyloxymethylimidazolium chloride or 1-methyl-3-octylimidazolium bromide was dissolved in water, and sodium tetrafluoroborate was added

Fig. 1 The structure of **a** all-*trans* β -carotene, **b** 15-*cis* β-carotene, **c** 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate (IL 1), **d** 1-methyl-3-octylimidazolium tetrafluoroborate (IL 2)
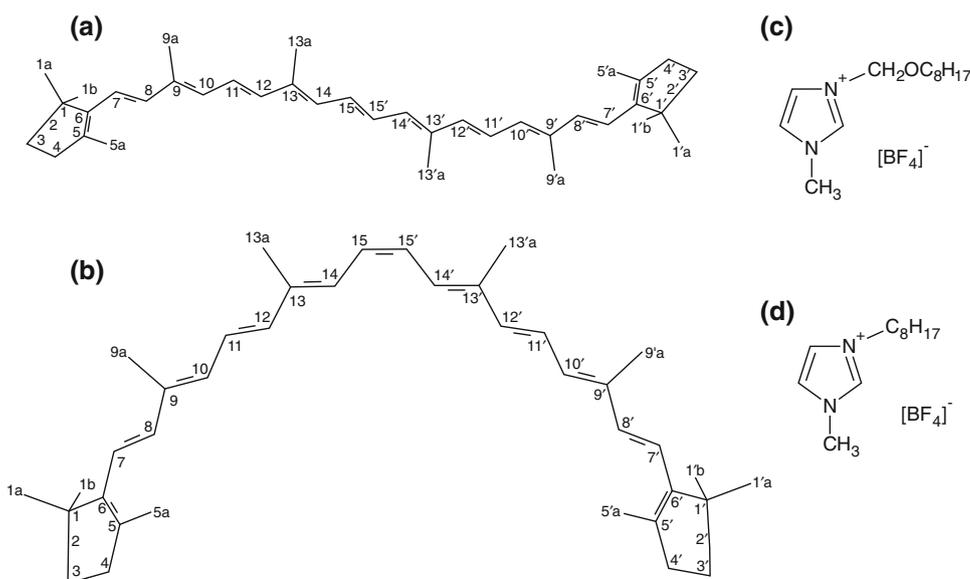
with 20 % molar excess. The reaction was stirred for 1 h, and the upper aqueous phase was carefully decanted. After solvent removing in vacuum, product was once again dissolved in anhydrous dichloromethane in order to filter educed NaCl or NaBr and excess of used NaBF$_4$.

The products 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate (IL 1) and 1-methyl-3-octylimidazolium tetrafluoroborate (IL 2) were purified by extraction with two times distilled water. The yield of (IL 1) and (IL 2) was 84.6 and 84.2 %, respectively.

All obtained ILs are air-stable under ambient conditions and may be handled under normal laboratory conditions. Their purities were confirmed by thin-layer chromatography (TLC Merck silica gel 60 F254 plates) and elemental analysis (Elementer Analzensystem Vario EL III). Water content after drying in vacuum was determined by Karl-Fischer coulometric titration method (C20 Coulormetric KF Titrator, Mettler Toledo). The water content was 156 and 132 ppm for (IL 1) and (IL 2), respectively.

In this study two RTILs were used: 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate (IL 1) (Fig. 1c) and 1-methyl-3-octylimidazolium tetrafluoroborate (IL 2) (Fig. 1d).

The photoacoustic spectra were recorded with a home-made spectrometer [14, 20] at the University of Gdansk.

Light from xenon arc lamp (OSRAM, 450 W) was modulated with the MS9 modulator (made in Poland). For the wavelength selection in the UV and visible range the manual and automatic, respectively, a monochromator (Carl Zeiss, SPM-2) controller was used. The monochromatic and modulated light beam was directed to a sample located in a closed-type photoacoustic cell. The molecules exposed to the modulated light can deactivate a part of the absorbed energy in the form of the modulated heat (thermal deexcitation). Modulated temperature changes in the sample/gas border induced the pressure oscillation in the gas. The fluctuations were converted to an electrical signal by a microphone (Bruel and Kjaer, type 4146). Further, the signal was selected and amplified by a lock-in amplifier (SRS, type SR850) and subjected to the computer for analysis.

A spectrophotometer Lambda 20 Perkin-Elmer with Peltier temperature control system PTP-1 was used to record UV/VIS absorption spectra.

Results and discussion

The absorption and photoacoustic spectra of all-*trans* (Fig. 2) and 15-*cis* β-carotene (Fig. 3) in *n*-hexane and ionic liquids, with and without methylenoxy group, were


Fig. 2 The absorption (*solid line*) and photoacoustic (*open circles*) spectra of all-*trans* β-carotene ∼30 μM in **a** IL 1, **b** IL 2 and **c** *n*-hexane, normalized at maximum ∼460 nm
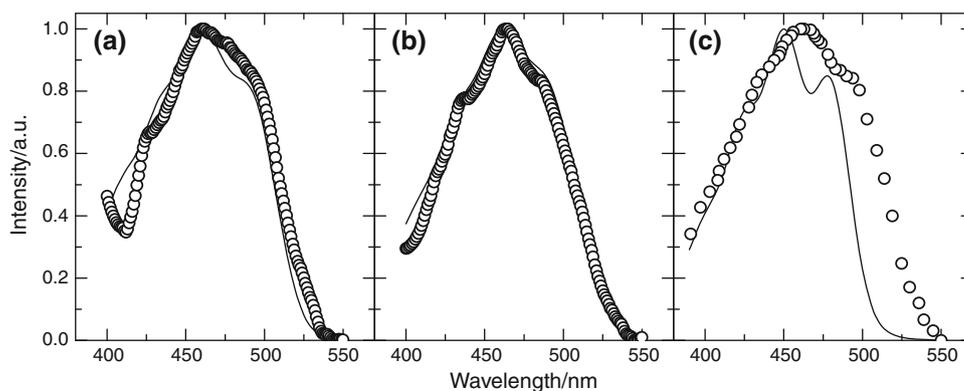
Fig. 3 The absorption (*solid line*) and photoacoustic (*open circles*) spectra, normalized at maximum ∼455 nm, of 15-*cis* β-carotene ∼30 μM in **a** IL 1, **b** IL 2 and **c** *n*-hexane
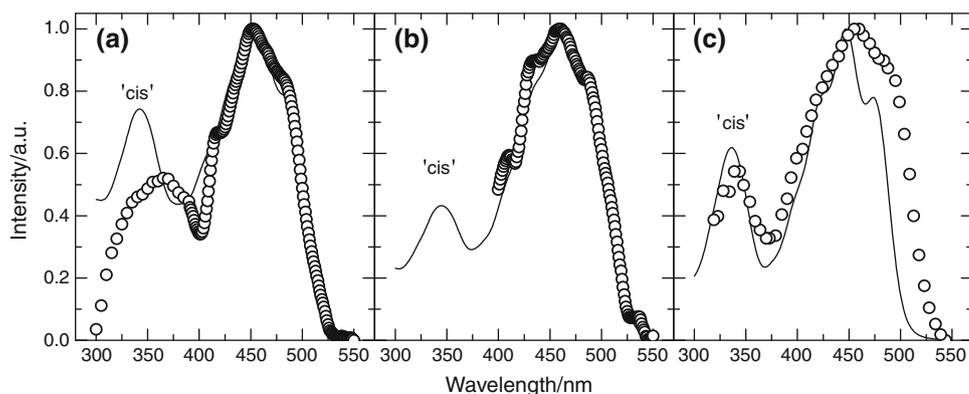
Fig. 4 The difference spectra of crystal photoacoustic signal (*open circles*) and: the monomeric (**a**) or the aggregated (**b**) forms of all-*trans* β-carotene in IL 1. The solid and dashed lines indicate the calculated spectra and Gaussian components: I—525 nm, II—560 nm, III—616 nm, respectively
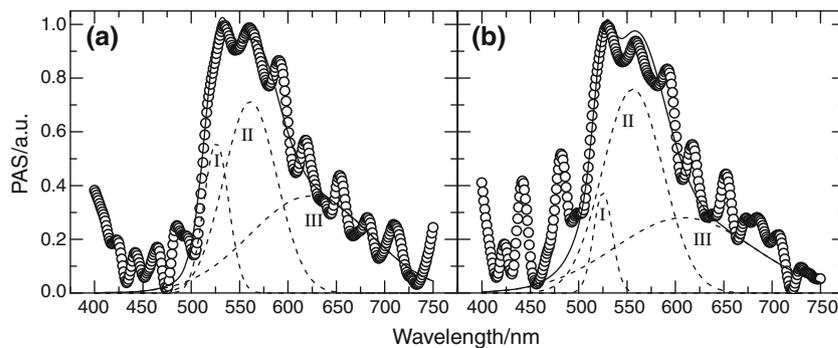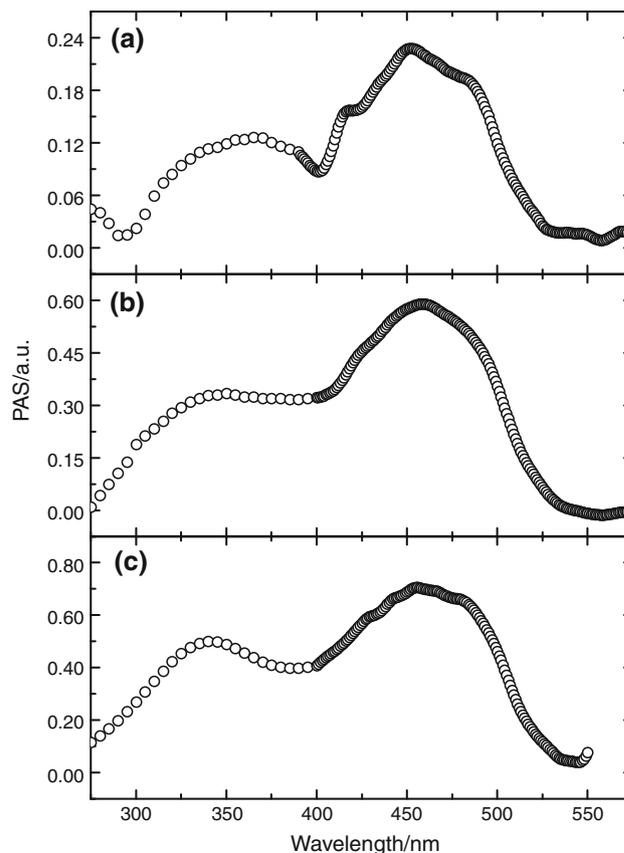

recorded. The photoacoustic spectra of β-carotene in *n*-hexane are broader and shifted towards longer wavelength in comparison to the absorption spectrum. This effect is due to the crystallization of the samples measured in n-hexane and is not observed in the samples solubilized in ionic liquids. Ionic liquids are thermally stable within a wide range of temperature that allows to perform the photoacoustic measurements in a liquid phase. In the photoacoustic spectrum, the '*cis*' peak of 15-*cis* β-carotene in IL 1 is shifted towards lower energies in comparison to absorption.

The PAS signal intensity of the all-*trans* β-carotene $1^1B_u^+$ energy state in IL 1 has been changed from 0.11 to 0.21 with the pigment concentration increased from 25 to 35 µM. As expected, the thermal excited state deactivation of all-*trans* β-carotene increased with the increase of the sample concentration. Also with the increase of the β-carotene concentration, the effect of aggregation occurs followed by crystallization. Recently [21] we presented the fluorescence emission data showing that the β-carotene aggregates/crystals are characterized by a lower fluorescence emission in comparison to monomers, thus more energy is deactivated by thermal pathways than by the fluorescence. The PAS signal of the $1^1B_u^+$ energy state decreased (0.11) with the crystallization of the all-*trans* β-carotene (80 µM). At the same time in the red region part of the spectrum, a relative higher non-radiative deactivation occurs in comparison to the monomeric and aggregated forms. The difference spectra of the photoacoustic signal of the crystalized and the monomeric (Fig. 4a) or the aggregated (Fig. 4b) forms of all-*trans* β-carotene decomposed into the Gaussian components indicate three maxima at I—525 ± 1 nm, II—560 ± 3 nm and III—616 ± 5 nm.

The photoacoustic spectra of the 15-*cis* β-carotene in the IL 1 at the different concentrations (Fig. 5).

The photoacoustic spectra (Fig. 5) of the 15-*cis* β-carotene isomer in IL 1 indicate the increase of the photoacoustic signal (0.23 → 0.71) proportionally to the sample concentration increased 30–60 µM, similarly as it is for the all-*trans* β-carotene isomer. Additionally, the increase of the 15-*cis* β-carotene concentration induced the blue shift 365–340 nm of the '*cis*' peak.

Fig. 5 The photoacoustic spectra of 15-*cis* β-carotene at different concentrations: **a** 30 µM, **b** 50 µM **c** 60 µM in IL 1

Based on the absorption and the photoacoustic results, the data of the thermal deactivation of the excited states (TD) were calculated. The procedure of the –CH₂O– group introducing into the cation structure of the ionic liquid caused the increase of around 30 % (state$1^1B_u^+$) of the TD of the all-*trans* β-carotene (30 µM), whereas the TD of the 15-*cis* β-carotene (30 µM) showed no significant change. The crystalized form of the all-*trans* β-carotene deactivates around 30 % of the absorbed energy by the thermal pathways, whereas in the aggregate and the monomer forms: 15 and 10 %, respectively. The TD for $1^1B_u^+$ and $1^1A_g^+$ energy
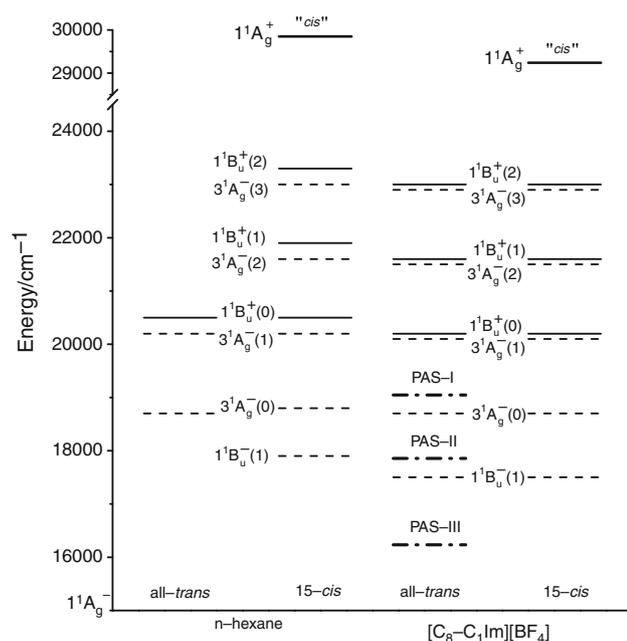
Fig. 6 The energy levels of β-carotene isomers in *n*-hexane and RTILs (*solid* and *dashed lines*, respectively) obtained from our previous data [23] and PAS measurements (*dashed dotted lines*)

states of 15-*cis* β-carotene depend on the sample concentration linearly.

The non-radiated (dark) and radiated excited state deactivation signal can be measured by the photoacoustic and fluorescence spectroscopy, respectively. Figure 6 presents the energy levels of all-*trans* β-carotene in RTILs and in the standard solvent (*n*-hexane) for comparison. Three energy levels PAS-I (525 nm), PAS-II (560 nm) and PAS-III (616 nm) were calculated from the photoacoustic measurements (Fig. 4) of all-*trans* β-carotene in the ionic liquids. The energy levels named PAS-I and PAS-II on the energetic scheme (Fig. 6) are the energy states $3^1A_g^-(0)$ and $1^1B_u^-(1)$, respectively. The small shift of energy (PAS measurements) in comparison to the data (fluorescence technique) obtained in our previous papers [9, 23] is due to the higher concentration of β-carotene (necessary for PAS measurements) and the lower signal/noise ratio. The energy level PAS-III is close to the value of energy state $1^1B_u^-(0)$ reported by Sashima et al. [22].

Conclusions

The thermal deactivation of the excited states of all-*trans* β-carotene depends on the presence of the methylenoxy group in the ionic liquid structure. The amount of absorption energy converted into heat depends on the β-carotene concentration. The non-radiative deactivations of both β-carotene isomers in the ionic liquids occur via

$1^1B_u^+$ state but in the case of 15-*cis* β-carotene by an additional path $1^1A_g^+$ ('*cis*' peak) transition.

In the case of the 15-*cis* isomer, the thermal deactivation of $1^1B_u^+$ and $1^1A_g^+$ energy states linearly depends on the sample concentration in the investigated concentration range. Additionally, the increase of the 15-*cis* β-carotene concentration caused the blue shift of the '*cis*' peak.

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