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Spectroscopic properties and temperature induced electronic configuration changes of all-*trans* and 15-*cis* β-carotenes in ionic liquids

G.E. BIALEK-BYLKA[*,**], K. PAWLAK[*], B. JAZUREK[*], A. SKRZYPCZAK[**], and Y. KOYAMA[***]

Faculty of Technical Physics, University of Technology, Nieszawska 13 a, 60-965 Poznan, Poland[*]
Faculty of Chemical Technology, University of Technology, 60-965 Poznan, Maria Sklodowska-Curie Sq. 2, Poland[**]
Faculty of Science and Technology, Kwansei Gakuin University, 669-1337 Sanda, Gakuen 2-1, Japan[***]

Abstract

The clear vibrational structure of fluorescence spectrum of β-carotene in the solvent is reported for the first time at room temperature. This finding is in good agreement with recently discovered covalent 3 $^1A_g^-$ new carotenoid state. The fluorescence yield of β-carotene in ionic liquid (1-methyl-3-octyloxymethylimidazolium tetrafluoroborate) is around hundred times higher than in standard solvent *n*-hexane. The all-*trans* and 15-*cis* β-carotene fluorescence yields in ionic liquid are 1.96±0.03 and 2.53±0.03 %, respectively. The ionic liquid is a very useful tool for modelling photosynthetic system *in situ*. We present the electronic absorption data of β-carotene in ionic liquids (so called neoteric solvents) with special interest in the absorption changes as a function of temperature in the range 0–90 °C (273–363 K). Ionic liquids are also very good medium for temperature study, because they are not changing up to several hundred °C and also not evaporating during heating. The relationship between spectral characteristics of β-carotene in new generation solvents with increasing and decreasing temperature is evaluated. The energy value of the ionic state $1^1B_u^+$ of synthetic β-carotene in ionic liquids exhibits a linear and temperature reversible dependence on temperature up to 30 °C (303 K) and up to 40 °C (313 K) for 15-*cis* and all-*trans* β-carotenes, respectively. This is valid for both 0–0 and 0–1 transitions.

Additional key words: 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate; 15-*cis,* all-*trans* β-carotene; electronic absorption; fluorescence.

Introduction

Hundreds of carotenoid molecules are found in nature (Pfander *et al.* 1987) but crystal structures are reported only for some of them. The β-carotene molecule (both isomers 15-*cis* and all-*trans*) is a non-polar carotenoid with 11 conjugated double bonds (Figs. 1 and 2) and its crystal structure is known (Sterling 1964, Senge *et al.* 1992). The $1^1B_u^+$ (0–0, 0–1, 0–2) transitions represent electronic state S_2 and $1^1A_g^+$, the so called "cis" peak.

The β-carotene molecule is an important compound in photosynthesis, pharmacy, and medicine. In photosynthetic systems β-carotene has two main functions, *i.e.* photoprotection and photon harvesting (Cogdell and Frank 1987, Siefermann-Harms 1987). The 15-*cis* β-carotene is a configuration characteristic and universal in nature for photoprotection and was confirmed for all types of the photosynthetic reaction centres (Koyama 1991, Bialek-Bylka *et al.* 1995, 1996, 1998). The all-*trans* β-carotene molecules present in photosynthetic antennae complexes are responsible for the singlet-singlet energy transfer between β-carotene and chlorophyll *a*. In photosystem 1 complex, efficiency of energy transfer is 30–35 % (Bialek-Bylka *et al.* 1982, Bialek-Bylka and Brown 1986). The β-carotene is an efficient singlet oxygen quencher and free radical trap, being a very important molecule in medicine as an agent against damage of DNA (Krinsky 1968, 1979, Mathews-Roth and Krinsky 1984, Mathews-Roth 1986, Halliwell and Gutteridge 1989, Bendich 1994). The 15-*cis* β-carotene is the best isomer for anti-cancerogenic function and is a more effective trap than planar polyenes such as all-*trans* β-carotene.

The ionic liquids are organic salts with melting points under 100 ºC and often also even lower than room temperature (Welton 1999, Sheldon 2001, Dupont *et al.* 2002). The ionic liquids are thermally stable in the temperature range from –96 to +200 °C (183–473 K) and they are non-flammable. The other properties are very good dissolution in most organic and inorganic compounds and no measurable vapour pressure. The

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Fax: (+48) 61 6653178, e-mail: bialek-bylka@wp.pl
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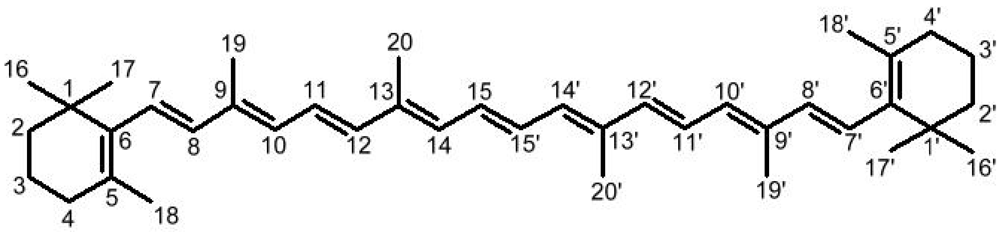

Fig. 1. The structure of all-*trans* β-carotene.

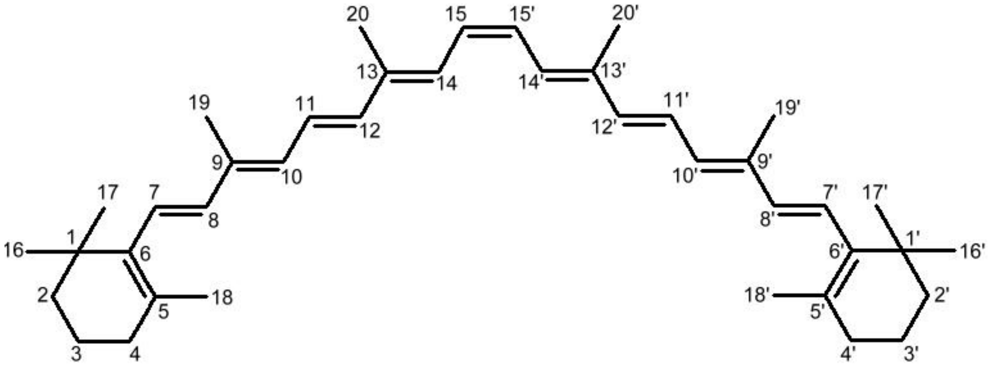

Fig. 2. The structure of 15-*cis* β-carotene.

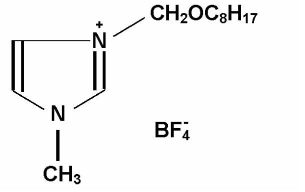

Fig. 3. The structure of 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate.

ionic liquids are safe for environment and are called green solvents. They are also good catalysts for many chemical reactions. The ionic liquids also do not evaporate during heating which allows for easy separation of many organic molecules by distillation or crystallization without loss of solvent. The physical and chemical properties of ionic liquids can be optimized in order to obtain specific application. Thanks these properties ionic liquids are a very good medium for temperature studies. One can change the chemical properties of ionic solvents by introducing in the ionic liquid structure different ions like for example imidazolium cation as was done in our work. The ionic liquid we used is 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate ($MOMI^+BF_4^-$) and its molecular structure is shown in Fig. 3.

Our study should increase understanding of various aspects of photosystem structure and function *in vivo*. The specific interaction of β-carotene isomers with ionic liquid ($MOMI^+BF_4^-$) differs in the ground and excited states. The absorption and fluorescence spectroscopic properties of β-carotene isomers in ionic liquids are important in photosynthetic model system studies. Our fluorescence data of β-carotene in $MOMI^+BF_4^-$ confirmed the recently discovered new carotenoid state $3\ ^1A_g^-$ (Onaka *et al.* 1999, Koyama *et al.* 2004).

Materials and methods

Ionic solvent (1-methyl-3-octyloxymethylimidazolium tetrafluoroborate) syntheses were done according to Pernak *et al.* (2001).

Preparation of 1-methyl-3-octyloxymethylimidazolium chloride: An anhydrous solution of 0.1 mol 1-methylimidazole in acetonitrile was added to 0.1 mol of chloromethyloctyl ether. The reaction was carried out for 1 h and then the solvent was evaporated in vacuum. Product was purified by extraction with heptane at 70 °C (343 K). The final product was a hygroscopic compound with the yield of 92.5 %.

Preparation of 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate: To 0.09 mol 1-methyl-3-octyloxymethylimidazolium chloride dissolved in 30 cm^3 of methanol, 0.095 mol of sodium tetrafluoroborate was added. The reaction was completed by heating at 55 °C and stirring for 24 h. After removing the solvent in vacuum, the product was once again dissolved in anhydrous acetone in order to filter small amounts of NaCl and excess of $NaBF_4$. The product was a colourless liquid with the yield of 98 %.

Sample of β-carotene in ionic solvent was prepared after *n*-hexane evaporation under nitrogen gas. Synthetic 15-*cis* β-carotene was purified and analyzed in complete darkness by HPLC method (Tsukida *et al.* 1982, Bialek-Bylka *et al.* 1995). All-*trans* β-carotene, a very stable isomer, was used for study after spectroscopic analysis without further purification.

Absorption spectra were measured with a UV/VIS *Lambda 20 Perkin Elmer* spectrophotometer with temperature device. Fluorescence spectra were measured with an *F-4500 Hitachi* fluorometer.

Results and discussion

The experimental system with high fluorescence quantum yield at room temperature of β-carotene in ionic liquid ($MOMI^+BF_4^-$) was established. The fluorescence quantum yield of all-*trans* β-carotene in this ionic liquid was around 100 times higher than in *n*-hexane but for 15-*cis* β-carotene it was even higher than 100. A possible interpretation of the drastic enhancement of fluorescence is a complete overlap of the $1\,^1B_u^+$ and $3\,^1A_g^-$ states that block the $1\,^1B_u^+ \rightarrow 3\,^1A_g^-$ internal conversion. Then, the mixed $1\,^1B_u^+/3\,^1A_g^-$ state becomes long-lived, and enhances fluorescence substantially. In β-carotene (effective $n = 10.5$), the ionic $1\,^1B_u^+$ and covalent $3\,^1A_g^-$ states are located close-by and mixed with each other (Koyama *et al.* 2004). According to the selection rules the transition from $1\,^1B_u^+$ to the ground $1\,^1A_g^-$ state is active but from the $3\,^1A_g^-$ state is forbidden, however, due to the electronic mixing it can become partially allowed.

The fluorescence quantum yield of all-*trans* β-carotene S_2 state in *n*-hexane is 2×10^{-4} (Shreve *et al.* 1991, Andersson *et al.* 1992) and of S_1 state is 4×10^{-6} (Wasielewski and Kispert 1986, Wasielewski *et al.* 1989). From our measurements and fluorescence quantum yield (Φ) calculations (Parker 1968, Lakowicz 1983) based on cumarin (Φ = 98 %) as a standard the all-*trans* β-carotene fluorescence quantum yield in ionic liquid is $\Phi\pm\Delta\Phi = 1.961\pm0.025$ %. But in *n*-hexane it is $\Phi\pm\Delta\Phi = 0.017\pm0.001$ %, what is in agreement with literature data (Shreve *et al.* 1991, Andersson *et al.* 1992). For the 15-*cis* β-carotene in ionic liquid the fluorescence quantum yield is $\Phi\pm\Delta\Phi = 2.526\pm0.032$ % but in *n*-hexane it is 0.015 ± 0.002 %. In ionic liquids the fluorescence quantum yield of 15-*cis* β-carotene is higher than for all-*trans* β-carotene in spite of almost the same for both isomers fluorescence quantum yield in *n*-hexane.

The vibronic structured fluorescence spectra for both β-carotene isomers in ionic liquid (in comparison to in *n*-hexane) are blue-shifted (Fig. 4). Maximum of the fluorescence spectrum of all-*trans* β-carotene in *n*-hexane is at 522 nm which is in agreement with literature values (Mimuro *et al.* 1992). The absorbance of β-carotene (of comparable concentration) in *n*-hexane is around 3 times higher than in ionic liquid for both isomers. The experimental data of the molar extinction coefficients of all-*trans* and 15-*cis* β-carotenes in ionic liquid and *n*-hexane were calculated and listed in Table 1.

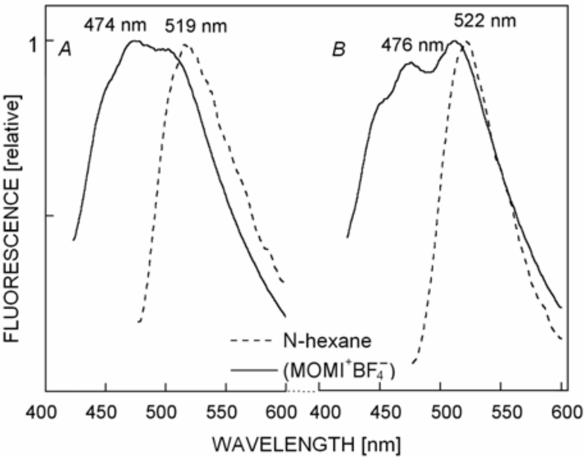

Fig. 4. Fluorescence spectra (normalized at fluorescence maxima) of 15-*cis* (*A*) and all-*trans* (*B*) β-carotene in ionic liquid ($MOMI^+BF_4^-$) and in *n*-hexane. $\lambda_{exc} = 413$ nm.

Table 1. The molar extinction coefficients of all-*trans* and 15-*cis* β-carotenes in ionic liquid ($MOMI^+BF_4^-$) and *n*-hexane at 20 °C (293 K).

β-carotene isomers	λ_{max} [nm] *n*-hexane	 $MOMI^+BF_4^-$	molar extinction coefficient [mol^{-1} cm^{-1}] *n*-hexane	 $MOMI^+BF_4^-$
all-*trans*	450	461	139 000	46 153
15-*cis*	448	456	103 200	28 037

The differences Δλ nm at 20 °C (293 K) between values of λ_{max} of absorption spectra of all-*trans* and 15-*cis* β-carotenes in ionic liquid was 5 nm, bigger than that in *n*-hexane (2 nm). Molar extinction coefficient at 450 nm of all-*trans* β-carotene in *n*-hexane was 139 000 mol^{-1} cm^{-1} and that of 15-*cis* isomer was 103 200 mol^{-1} cm^{-1} (Weedon 1965, Tsukida *et al.* 1982, Britton and Young 1993).

At room temperature in concentration range of 10^{-5}–10^{-6} mol, the absorbance of β-carotene (both isomers) was a linear function of concentration in both the ionic liquid and *n*-hexane. The solubility of β-carotene molecules was limited in ionic liquid especially in the case of 15-*cis* β-carotene. The 15-*cis* β-carotene in the experiment with the same ionic liquid but with a different substituent, $-CH_2OC_2H_5$ (position 3 of imidazolium ring) instead of $-CH_2OC_8H_{17}$, was completely un-dissolved. Samples were first prepared in *n*-hexane and after evaporation of *n*-hexane they were transferred to a very small (mm^3) amount of dimethylformamide, and finally to ionic liquid. Absorbance and fluorescence intensities of 15-*cis* and all-*trans* β-carotenes in ionic liquid and *n*-hexane are presented in Table 2.

Table 2. Fluorescence intensity and absorption of 15-*cis* and all-*trans* β-carotenes in ionic liquid ($MOMI^+BF_4^-$) and *n*-hexane at 20 °C (293 K).

Solvent	15-*cis* β-carotene		all-*trans* β-carotene	
	absorbance	fluorescence	absorbance	fluorescence
$MOMI^+BF_4^-$	0.3 *456 nm*	257.9 *474 nm*	0.6 *461 nm*	179.2 *476 nm*
n-hexane	1.1 *448 nm*	2.8 *519 nm*	1.8 *450 nm*	3.6 *522 nm*

The absorption maxima λ_{max} ($1\ ^1B_u^+$, transition 0–1) of both isomers all-*trans* and 15-*cis* of β-carotenes in the ionic liquid represent bathochromic shifts of 11 and 8 nm in comparison with data in *n*-hexane. In the ground state there is not a strong interaction (dispersive and ionic) of β-carotene with ionic solvent.

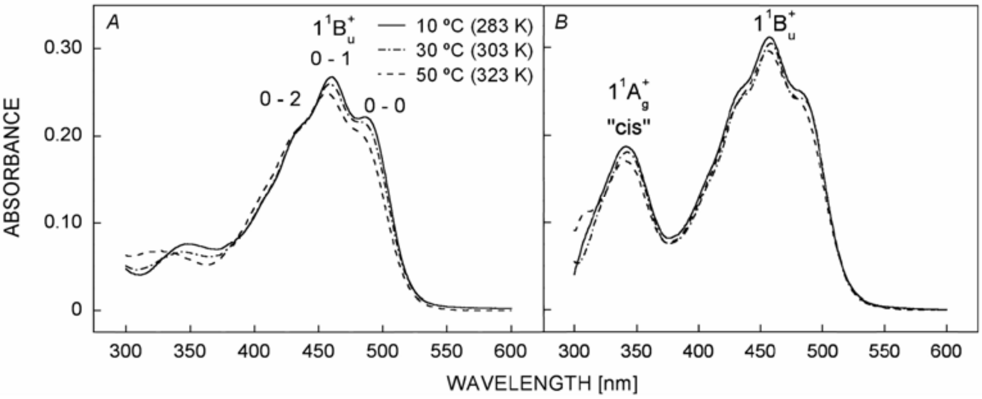

Fig. 5. Absorption spectra of all-*trans* (*A*) and 15-*cis* (*B*) β-carotenes in ionic liquid ($MOMI^+BF_4^-$) (*A*: 3.2×10^{-6} mol, *B*: 4.3×10^{-6} mol) as a function of temperature.

The ionic $1\ ^1B_u^+$ state is much more sensitive to the polarity of the solvent than the covalent $3\ ^1A_g^-$ one. Therefore, the $1\ ^1B_u^+$ state should be substantially shifted to the lower energy; but the $3\ ^1A_g^-$ state might rather remain unaffected (or shifted much less, depending on the electronic mixing). As a result, a large reorganization of the state ordering and state mixing is expected to take place upon the substantial change in the solvent polarity from *n*-hexane to $MOMI^+BF_4^-$.

The Stokes' shifts (Δλ) for both 15-*cis* and all-*trans* β-carotenes in ionic liquid are around 4 times smaller than in *n*-hexane. In ionic liquid Δλ for 15-*cis* and all-*trans* β-carotenes are 18 and 15 nm, respectively, but in *n*-hexane 71 and 72 nm (±1 nm), respectively. Data for all-*trans* β-carotene in *n*-hexane are in agreement with literature (Van Riel *et al.* 1983) and with data for other organic solvents such as ethanol (Δλ = 76±2 nm) and chloroform (Δλ = 77±2 nm).

In order to elucidate the temperature induced β-carotene electronic configuration change effect, experiments with both isomers of β-carotene were performed in ionic liquids in the region of temperature of 10–90 ºC (283–363 K). The absorption spectra of all-*trans* and 15-*cis* β-carotenes in ionic liquid ($MOMI^+BF_4^-$) as a function of temperature, 10 ºC (283 K), 30 ºC (303 K), and 50 ºC (323 K), are presented in Fig. 5. All measurements started at 10 ºC (283 K) because at 0 ºC (273 K) the ionic liquid was solidified and strong light-scattering effect was present during spectroscopic measurements.

In the physiological temperature range of 10–40 ºC (283–313 K), β-carotene absorbance reversibly changes up to 30 ºC (303 K) and 40 ºC (313 K) in 15-*cis* and all-*trans* isomers, respectively. In *n*-hexane, the temperature induced electronic configuration changes of β-carotene isomers are not so much sensitive as in ionic liquids.

Table 3 shows the refractive indexes of 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate as a function of temperature (during cooling ionic liquid) in the

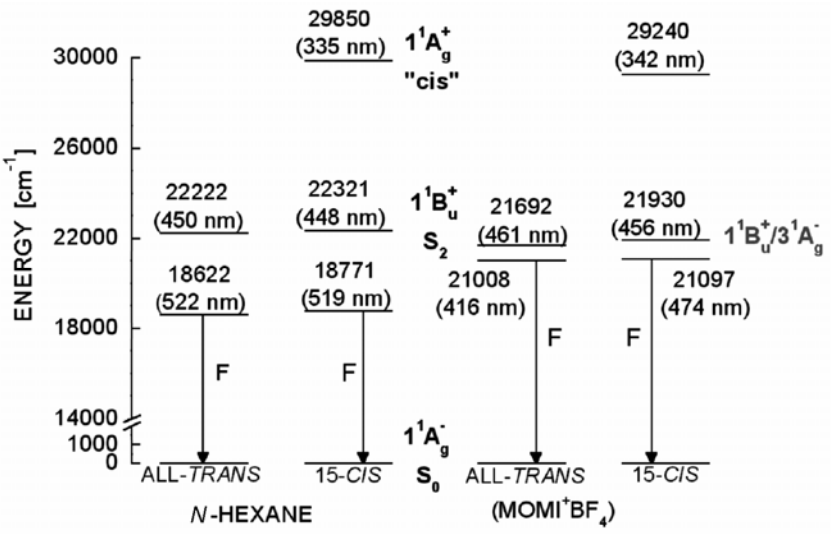

Fig. 6. Energy diagram for the absorption and emission (F – fluorescence) of all-*trans* and 15-*cis* β-carotenes in ionic liquid ($\mathrm{MOMI^+BF_4^-}$) and in *n*-hexane; $1B_u^+/3A_g^-$ mixed state.

Table 3. The value of refractive indexes n (Δn = ±0.001) of ionic liquid ($\mathrm{MOMI^+BF_4^-}$) as a function of temperature °C (K).

Temperature °C (K)		n±0.001
40.0	(313 K)	1.430
34.0	(307 K)	1.432
30.0	(303 K)	1.433
25.0	(298 K)	1.438
20.0	(293 K)	1.440

temperature range of 40–20 °C (313–293 K) measured with the Abbe refractometer. The fluorescence and absorption data of all-*trans* and 15-*cis* β-carotenes in ionic liquid and *n*-hexane are summarized in Fig. 6.

There exists a specific interaction of β-carotene and ionic liquid ($\mathrm{MOMI^+BF_4^-}$) different in the ground and excited states. In the ground state there was no strong interaction (dispersive and ionic) of β-carotene with ionic solvent but in the excited state of β-carotene in ionic liquid a strong electrostatic interaction is suggested.

The 15-*cis* and all-*trans* β-carotenes in ionic liquid seem to be interesting models of photosynthetic system considering mechanisms of the excited state deactivation such as fluorescence and energy transfer.

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