Archives of Biochemistry and Biophysics 650 (2018) 49–58

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Unified analysis of optical absorption spectra of carotenoids based on a stochastic model

Chiasa Uragami[a], Keisuke Saito[b], Masayuki Yoshizawa[c], Péter Molnár[d], Hideki Hashimoto[a,*]

[a] School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda, Hyogo 669-1337, Japan
[b] JST-PRESTO and School of Engineering, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan
[c] Graduate School of Science, Tohoku University, 6-3 Aramaki-aza-aoba, Aoba-ku, Sendai, Miyagi 980-8578, Japan
[d] Department of Pharmacognosy, University of Pécs, Faculty of Pharmacy, H-7624 Pécs, Rókus u. 2, Hungary

ARTICLE INFO

Keywords:
Carotenoid
Optical absorption spectra
Stochastic model
Raman spectra
Huang-Rhys factor

ABSTRACT

The chemical structures of the carotenoid molecules are very simple and one might think that their electronic features are easily predicted. However, there is still has so much unknown information excepting the correlation between the electronic energy state and the length of effective conjugation chain of carotenoids. To investigate the electronic feature of the carotenoids, the most essential method is measuring the optical absorption spectra, but simulations based on the resonance Raman spectra are also an effective approach. For this reason, we studied the optical absorption spectra as well as resonance Raman spectra of 15 different carotenoid molecules each possessing a cyclic end-group, recorded in tetrahydrofuran (THF) solutions at room temperature. The whole band shapes of the absorption spectra of all these carotenoid molecules were successfully simulated using a stochastic model and Brownian oscillators. The parameters obtained from the simulation make it possible to discuss the intermolecular interaction between carotenoids and solvent THF molecules quantitatively.

1. Introduction

Carotenoids are ubiquitous pigments in photosynthesis [1,2]. They absorb the blue-green spectral region of sunlight and transfer the captured energy to chlorophylls that have scant absorption in this spectral range. This singlet-singlet type excitation energy transfer enhances the overall efficiency of photosynthetic light reaction. Carotenoids also serve as the scavenger of the excess amounts of light exposure. In this case, triplet-triplet type energy transfer from chlorophylls to carotenoids is the important role. In the light-harvesting pigment-protein complexes from purple photosynthetic bacteria, carotenoids show the additional role of structural stabilization of the pigment-protein complexes [3]. All of these roles are essential for photosynthetic organisms to survive on the earth.

Carotenoids are able to accomplish the above roles of energy transfer because their electronic features are defined by their linear conjugated polyene structure, or more precisely by their electronic structures in low-lying singlet excited states [4–7]. Predicting the electronic structure of the carotenoids would appear to be easily done by modern molecular physics, because of their simplicity of chemical structure. However, this is not always true because of the involvement of optically forbidden singlet excited states [8]. Nevertheless, it is relatively well known that how the energy of transition from the S_0 ground state to the optically allowed S_2 second singlet excited-state tightly depends on the length of the conjugation chain as well as on the polarity of the solvent [9]. The spectral and functional variety of the natural carotenoids is expected to arise from the presence of cyclic end-groups and the several functional groups, but to understand how these features modulate carotenoid electronic energy states requires plenty of additional information. To figure out how the carotenoids perform their biological function *in vivo*, it is necessary to consider the interaction between the carotenoid molecule and the surrounding molecules. In biological systems the situation is even more complicated because of the presence of the pigment-protein interaction. Carotenoids in natural systems exist in the very anisotropic environments, specially binding to the protein makes this problem more difficult [10].

In order to investigate the interaction between pigment and surrounding molecules, measurement of the optical absorption spectra is of essential importance. Since the optical absorption occurs when the irradiated light energy is in resonance with the transition energy of the pigment molecules, the observed absorption spectra sensitively reflects a perturbation of the electronic properties of the pigment molecule

Abbreviations: MeOH, methanol; Et_2O, diethyl ether; THF, tetrahydrofuran; HR, Huang-Rhys; FWHM, Full-width at half maximum

* Corresponding author.
E-mail addresses: chiasa.uragami@kwansei.ac.jp (C. Uragami), ksaito@appchem.t.u-tokyo.ac.jp (K. Saito), m-yoshizawa@m.tohoku.ac.jp (M. Yoshizawa), peter.molnar@aok.pte.hu (P. Molnár), hideki-hassy@kwansei.ac.jp (H. Hashimoto).

https://doi.org/10.1016/j.abb.2018.04.021
Received 28 February 2018; Received in revised form 6 April 2018; Accepted 30 April 2018
Available online 04 May 2018
0003-9861/

perturbed by the surroundings. There have been many previous studies on optical absorption spectra of carotenoids [9–21]. Combining the measurement of resonance Raman excitation profiles and the analysis in terms of the Albrecht theory the absorption spectra of β-carotene were simulated [11–13]. The absorption spectra of carotenoids were sometimes correlated with the polarity or polarizability of solvents [8,14–16]. The correlation between the frequency of the C=C stretching vibration of carotenoids and the energy of absorption transition has been known for decades, starting just several years after the first laser Raman spectroscopy on carotenoids by Rimai et al. [17]. Similarly, the correlation between the C=C stretching and the conjugation length has been highlighted for decades in numerous reviews [18–20]. Recently, this interesting issue was re-visited by the group of Robert [21]. A good linear relationship between the frequency of C=C stretching mode and the energy of absorption transition of carotenoids with or without end cyclic structures has been interpreted by introducing the idea of effective conjugation length of carotenoids [21,22]. There is a report on even another theoretical approach that correlates solvent polarizabilty and the transition energies of a carotenoid peridinin in different solvents [23]. In this report, a similar theoretical approach to this presented paper (the cumulant expansion technique) has been employed and the solvent polarizability has been discussed in terms of the Lippert-Mataga equation.

In this study, we have applied the generally accepted approach to interpret the shape of absorption spectra based on the stochastic theory using the Brownian oscillator model. Sue and Mukamel originally proposed this idea, and used stochastic Gaussian model to interpret solvation dynamics in coherent and spontaneous Raman spectroscopy of β-carotene [24]. More recently, the group of McHale has explored this approach to β-carotene in non-polar solvent [25]. They used the Brownian oscillator model, and examined the effects of temperature on the absorption line-shape. The work of Kelley emphasized the importance of Raman excitation profiles in tandem with absorption spectra to quantify displacements, and to distinguish broadening mechanisms [26]. This is actually true, but laborious work is required to apply this approach to a number of carotenoids. Therefore, we have adopted a more simple approach, and examined 15 different carotenoids. These carotenoids are classified into the following 4 structural categories in their structural point of view. **Group1:** Carotenoids with epoxide groups in their terminal end-rings where the π electron conjugation is restricted in polyene chain (**1**, **2**, **4**, **5**, **8** in Fig. 1). **Group 2:** Carotenoids whose π electron conjugation is extended into terminal end-rings (**3**, **6**, **7**, **9**, **10** in Fig. 1). **Group 3:** Carotenoids whose π electron conjugation is coupled to keto-carbonyl groups (**11**, **12**, **13**, **14** in Fig. 1). **Group 4:** A carotenoid that has both carbonyl groups and diacetate structure (**15** in Fig. 1). We found a new systematic relationship between the carotenoid-solvent interaction by simulating the band-shape of absorption spectra using a stochastic model.

2. Materials and methods

2.1. Isolation and preparation of the studied carotenoids

Fig. 1 shows the chemical structure of carotenoids used in this study. Except for β-carotene (**9**), canthaxanthin (**11**) and astaxanthin (**13**), all other carotenoids were isolated and purified from the corresponding plant materials. All-*trans*-β-carotene was purchased from Wako Pure Chemicals Japan and purified by twice recrystallization from benzene solution. All-*trans*-canthaxanthin and all-*trans*-astaxanthin were gift from Kuraray Chemical Company Japan were used as received.

2.2. A general isolation procedure of carotenoids from the corresponding plant materials (fruits, flowers, leaves)

The plant materials were extracted three times with MeOH and once with Et_2O. The methanolic solutions were combined and were transferred into toluene/hexane (~1:1) mixture. This solution was evaporated and the residue was dissolved in Et_2O. The ethereal solutions were combined and this "total extract" was saponified by 30% KOH-MeOH in heterogeneous phase overnight. After washing alkali-free the ethereal solution was evaporated, dried by anhydrous Na_2SO_4 and the residue was partitioned between MeOH/H_2O (9:1) mixture and hexane in a separatory funnel. This procedure resulted in two fractions: fraction of hypophasic carotenoids [(containing more than one OH group and epoxy group(s)] and epiphasic carotenoids (carotenoid hydrocarbons and carotenoids containing only one OH group). After the usual work-up [27] the fraction containing the hypophasic carotenoids was evaporated and crystallized from toluene/hexane (~1:5) mixture. The fraction of epiphasic carotenoids was evaporated and crystallized from toluene/MeOH (~1:5) mixture.

The separation of hypophasic (polar) carotenoids was carried out by column liquid chromatography (CLC) using $CaCO_3$ (Ph. Hg. VI.; Biogal, Hungary; precipitated pharmaceutical) as adsorbent and toluene/hexane mixtures as eluent. After the identification (UV/VIS spectroscopy) and the usual work-up the separated carotenoids were crystallized from a toluene/hexane (~1:5) mixture. The purity control of the crystalline samples was determined by HPLC. The identification of pure crystalline samples was completed by NMR, IR, CD and MS methods.

2.3. Preparation of the acetylated carotenoids

Capsorubin diacetate (**15**), violaxanthin diacetate (**5**) and *syn-syn* violaxanthin diacetate (**4**) were prepared from the pure crystalline capsorubin [(**14**, isolated from red paprika, *Capsicum annuum*)], violaxanthin [(**8**, isolated from the blossoms of yellow pansy, *Viola tricolor*)] and *syn-syn* violaxanthin [(**2**, prepared from zeaxanthin diacetate; zeaxanthin (**10**) was isolated from the berries of *Lycium halimifolium* or *Lycium barbarum*)] using the well-known acetylation procedure [27].

2.4. Isolation of the other carotenoids from natural sources

Capsorubin (**14**) and capsanthin (**12**) were isolated from red paprika (*Capsicum annuum*) using the general isolation procedure in our laboratory [28–31]. Zeaxanthin (**10**) was isolated from the ripe red berries of *Lycium halimifolium* or *Lycium barbarum* [32]. Violaxanthin (**8**) was isolated from the blossoms of yellow pansy [27,33]. Lutein (**7**) was isolated from the flowers of *Helianthus annuus*, *Taraxacum officinale* and from the leaves of *Acer campestre* [27,34]. Antheraxanthin (**6**) was isolated from the pollen sacs (anthers) of *Lilium candidum*, from the pollen sacs and petals of *Lilium tigrinum* and from the leaves of *Acer campestre* [27,35–37]. *Syn*-antheraxanthin (**3**) and *anti*-antheraxanthin (**6**) as well as the diastereoisomers of violaxanthin [(*syn-syn*- (**2**) and *syn-anti*-violaxanthin)] were prepared from zeaxanthin-diacetate [(prepared from zeaxanthin (**10**) using the well-known acetylation procedure)] by the well-known method of epoxidation, using monoperphthalic acid in absolute ether solution [38,39]. Lutein-5,6-epoxide (**1**) was isolated from the flowers of *Helianthus annuus* and from the flowers of *Chelidonium majus* [34,40].

2.5. Spectroscopic measurements

Optical absorption spectra of carotenoids in tetrahydrofuran (THF) solutions were recorded on JASCO V-670 spectrophotometer at room temperature. These solutions were placed in a quartz cuvette to record the Raman spectra. Resonance Raman spectra were recorded using 514.5 nm excitation light from an Ar^+ laser (ILT Model 5500A, Japan Laser Inc.,) at room temperature. The 90° scattering from each sample was focused onto a Raman spectrometer (SpectraPro 2300i, Acton Research Corporation, USA) equipped with a liquid nitrogen cooled CCD camera (LN/CCD-576-E/1, Roper Scientific, USA). The spectral