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Supramolecular aggregates of lycopene

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Abstract

Supramolecular assemblies of lycopene in aqueous tetrahydrofuran and chitosan matrix were studied by using ultraviolet–visible absorption spectroscopy and transmission electron microscopy. It was found that lycopene in aqueous tetrahydrofuran could form H-type molecular aggregates, as manifested by the fact that the main absorption band of lycopene was blue-shifted to 354 nm. Similar results were obtained when lycopene–chitosan complexes were dissolved in water with 1% acetic acid. The main absorption band of the solution, however, was further blue-shifted to 349 nm. The exciton model was used to interpret the spectral shifts of the aggregates. TEM images showed the dentritic, cubic or triangular shapes of the supramolecular assemblies of lycopene in chitosan matrix. A structural model for the supramolecular aggregates was proposed according to the experimental results. It is considered that the supramolecular assemblies form liquid crystals of the nematic type and contain both H-aggregation (more) and J-aggregation (little). The changes in the proportion of H- or J-type aggregation cause the hypsochromic or bathochromic shift of absorption spectrum.

Keywords: Lycopene; Chitosan; Supramolecular assemblies; H-aggregation; J-aggregation

1. Introduction

Carotenoids, a class of natural products, are ubiquitous in plants and animals. They can form supramolecular complexes with some biomacromolecules [1,2]. These supramolecular complexes (also referred to as aggregates) of carotenoids give flowers, fruits and feathers of birds attractive colors which play a key role in reproduction among those plants and animals [3,4]. One of the very important carotenoids is lycopene ($C_{40}H_{56}$) that has a 40-carbon, open hydrocarbon chain including 11 conjugated and two non-conjugated double bonds arranged in a line array. Until recently, lycopene has been increasingly attracting attention due to their protection against oxidation damage and inhibition of cancer development [5–7]. Various investigations have been done to explain the correlation between health-related functions and ingestion of lycopene [8–10], however there are few studies to reveal the supramolecular structure of lycopene aggregates, especially the effects of biomacromolecules (e.g. protein, DNA and polysaccharides, etc.) on the aggregation behavior of lycopene. Chitosan are a family of linear, cationic polymers obtained by deacetylating the chitin, i.e. the most abundant nitrogen-containing organic compound on earth. In this work, the supramolecular aggregates of lycopene and the effect of chitosan on the aggregation behavior were investigated, by which the physical state of lycopene molecules in organisms might be elucidated. Lycopene–chitosan complexes were prepared and different supramolecular assemblies of lycopene in chitosan matrix were analyzed by using UV–Vis

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spectroscopy and transmission electron microscopy (TEM).

Some carotenoids including lycopene, similar to some special dye molecules [11–14], can form supramolecular assemblies under certain conditions [15–18]. These assemblies are often characterized by a significant blue shift in UV–Vis spectrum. The exciton model is widely applied to interpret spectral characteristics of molecular aggregates [19,20] as well as to calculate structural parameters for these aggregates according to the spectral shifts. Therefore, our results were interpreted using molecular exciton model. In addition, a structural model about the supramolecular aggregates was proposed and discussed based on our results and references [17,21,22].

2. Materials and methods

2.1. Preparation of lycopene–chitosan complexes

Lycopene was prepared according to the previous literature [23]. The red crystals were obtained with purity above 90%, which was verified by HPLC and UV–Vis spectroscopy. Chitosan with 90% of *N*-deacetylation was purchased from Yuhuan Ocean Biochemical Co. Ltd (China) and directly used without further purification. The complexes of lycopene and chitosan were prepared as follows: 0.4 g chitosan was dissolved in 5 ml of 1% acetic acid. Then 1.25 ml freshly prepared tetrahydrofuran (THF) solution of lycopene (1.0 mg/ml) was added to the chitosan solution with vigorously stirring. After 30-min stirring, the lycopene–chitosan complexes were precipitated by adding dropwise 0.1 M NaOH until pH of approximately 10 was reached. After removing THF and acid from the complexes by water washing, the complexes of lycopene and chitosan (w/w: 1.25/400) were completely dried under vacuum at 45 °C. Other complexes with different ratios were achieved by changing the volume of lycopene/THF solution. Fresh double distilled water was used throughout the experiment and all the other chemicals were analytical grade unless specified.

2.2. Analytical methods

Lycopene–chitosan complexes were dissolved in 1% acetic acid. Their UV–Vis absorption spectra were recorded on a Hitachi U-2010 PC double-beam spectrophotometer. The size and shape of aggregates of lycopene were observed using a Hitachi JEM-100 transmission electron microscopy. The diluted solutions of complexes (0.01 wt.%) were coated on 200-mesh carbon-coated copper grids and left to dry. Gaussian deconvolution of the absorption spectra was analyzed by using Origin 7.0 software (Originlab Corporation).

2.3. Calculation of spectroscopic properties

M, the electronic transition dipole moment of lycopene, was calculated by Eq. (1) [24]

$$M^2 = 9.184\times10^{-39}\int_{\lambda_1}^{\lambda_2}\varepsilon(\lambda)\,\mathrm{d}\lambda/\lambda \quad (1)$$

where ε is the molar extinction coefficient in $(\mathrm{mol/l})^{-1}$ cm^{-1} and λ is wavelength in nm (λ_1 = 377 nm and λ_2 = 550 nm for lycopene in THF). According to Eq. (1), M was calculated as 1.92 Debye.

3. Results and discussion

3.1. UV–Vis spectra of lycopene in different solvents

Lycopene has 11 conjugated and two non-conjugated double bonds. In such a long conjugated double-bond system, π-electrons are highly delocalized. Therefore, the absorption spectrum of lycopene appears between 360 nm and 550 nm with a characteristic fine structure, as seen in Fig. 1. The second derivative spectrum and gaussian components of the absorption spectrum clearly show the vibrational peak positions. According to the linear combination of atomic orbitals–molecular orbital (LCAO-MO) theory at the Hückel level [25], the generation of the main absorption band is due to the transition of the conjugated π electrons from ground state ($1\ ^1A_g$) to the second excited state ($1\ ^1B_u$).

The solvents affect the spectral properties of lycopene. The main absorption peak (0–1 transition around

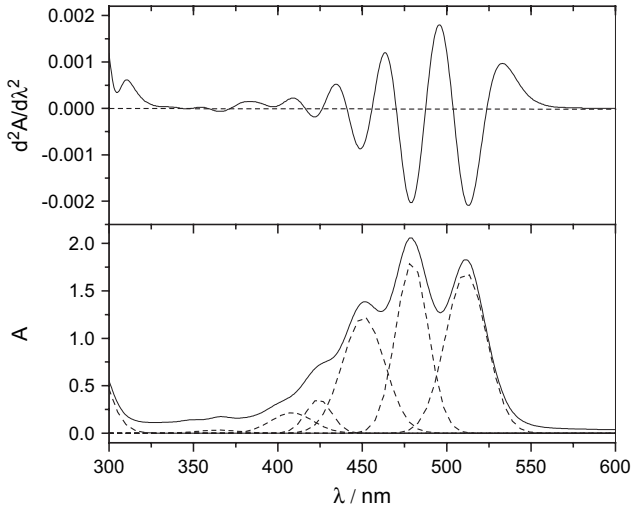

Fig. 1. UV–Vis absorption spectrum of lycopene in tetrahydrofuran. The concentration of lycopene is 1.09×10^{-5} mol/l. The second derivative curve and gaussian deconvolution of the absorption spectrum indicate the vibrational peaks' positions.

479 nm) of lycopene in THF red-shifted by approx. 10 nm compared to it in light petroleum. The possible reason was that THF might be more favorable to the excited sate than the ground state of lycopene [26]. Once it is dissolved in hydrated organic solvent such as THF or acetone, lycopene is susceptible to aggregation due to its highly hydrophobic character. Fig. 2 shows qualitatively the effect of water addition on the UV–Vis spectra of lycopene in THF. The main absorption band blue-shifted to 354 nm when the THF/water mixed solvent was 1/5 (v/v). A large blue shift indicates the formation of H-aggregates (so-called card pack aggregates). This spectral shift is attributed to exciton interactions among closely adjacent lycopene molecules [27].

According to the point-dipole approximation, the spectral shift from monomer absorption, caused by the neighboring transition dipoles in aggregates, depends on several factors. The following equation gives the relationship between the spectral shift and these factors [20,28]:

$$\nu_A - \nu_M = 2h^{-1}\frac{1}{4\pi\varepsilon_0}\left(\frac{N-1}{N}\right)\left(\frac{M^2}{r^3}\right)\left(\cos\theta - 3\cos^2\alpha\right) \quad (2)$$

where ν_A and ν_M are the main absorption frequencies (s^{-1}) of aggregate and monomer, respectively, N is the degree of polymerization of aggregate, M is the transition dipole moment of monomer (C m), α is the tilt angle between the lines of molecular centers and molecular long axes, θ is the angle between the adjacent molecular planes, r is the separation of molecular centers, h is Planck's constant (6.626×10^{-34} J s), and ε_0 is the permittivity of free space (8.854×10^{-12} C^2 J^{-1} m^{-1}).

To the H-aggregates of lycopene in aqueous THF solvent, the main absorption band undergoes a blue shift from 479 nm to 354 nm. At first approximation [28], for the dimmer we assume that α is 90° and θ is 0°, then we obtained 1.36 Å for the intermolecular distance r. Considering the N-aggregates, if N is large enough (i.e. $N \to \infty$) and r is also equal to 1.36 Å, the tilt angle α is 65.9°. This means that the structure of lycopene aggregates will change with the increase in the degree of polymerization of aggregate (i.e. the size of aggregates).

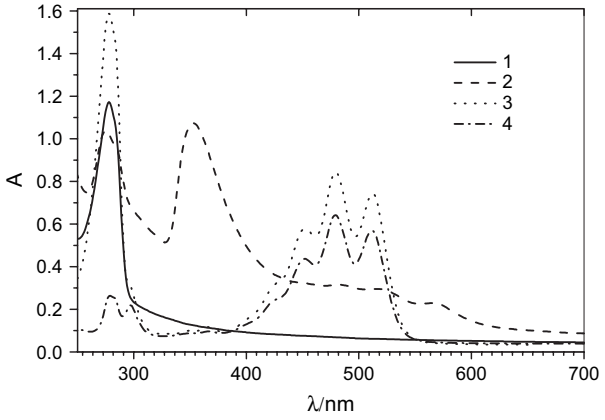

Fig. 2. UV–Vis spectra of pure THF and lycopene in THF and THF–water solvents. 1, Pure THF; 2, THF:water (1:5,v/v); 3, THF:water (3:1,v/v); 4, THF.

3.2. Effect of chitosan on the aggregates of lycopene

During the preparation for the complexes of lycopene and chitosan, the filtrate (THF/water mixed solvent) was colorless after the light red complexes were precipitated. This suggests that there are strong interactions between lycopene and chitosan. Mansoor [29] has reported that chitosan macromolecules could form hydrophobic micro-regions in solution due to the change of the chain configuration. The hydrophobic interaction between lycopene aggregates and the hydrophobic regions might be the driving force for the formation of the complexes.

When the complexes were redissolved in water with 1% acetic acid, the main absorption band further blue-shifted to 349 nm (Fig. 3, curves 2, 3 and 4). According to Eq. (2), the tilt angle α is 66.6° for the aggregates of lycopene in chitosan matrix. This angle is larger than that of the aggregates in aqueous THF solvent. It indicates that the macromolecular chains of chitosan influence the structure of lycopene aggregates. Fig. 3 shows the ratio change of lycopene to chitosan affected the spectral properties of the lycopene–chitosan complexes. When the ratio of lycopene to chitosan was 7.5/400 (w/w), the main absorption band inversely red-shifted to 358 nm. This means the size and structure of lycopene aggregates change.

The size and shape of aggregates of lycopene in chitosan matrix are shown in Fig. 4. The nanocrystals of

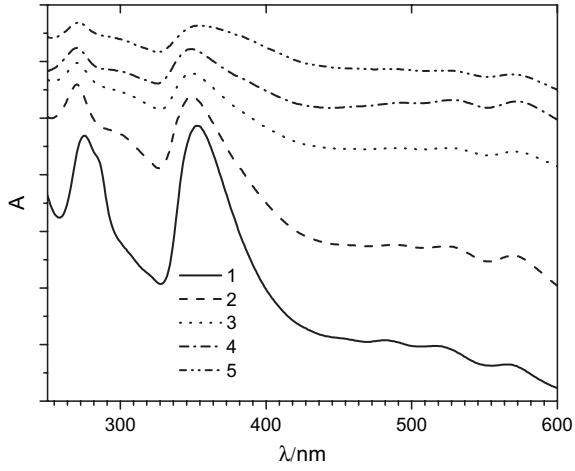

Fig. 3. UV–Vis spectra of lycopene in THF–water (1:5, v/v) solvents (1) and lycopene–chitosan complexes in 1% acetic acid in water with the ratio of lycopene and chitosan (w/w): (2) 1.25/400, (3) 2.5/400, (4) 5/400, (5) 7.5/400.

lycopene with size ranging from 20 nm to 40 nm arrange in a dendritic structure by self-assembly. With the increase in the ratio of lycopene to chitosan up to 7.5/400, however, the shape of the supramolecular aggregates changed into cubic or triangular shape and their average size increased to 100 nm. The unusual dendritic structures of aggregates mean that the macromolecular chains of chitosan influence the crystallization of lycopene and induced the formation of supramolecular assemblies. The TEM images about the changes of aggregates in shape and size due to chitosan are consistent with the results obtained using spectral method.

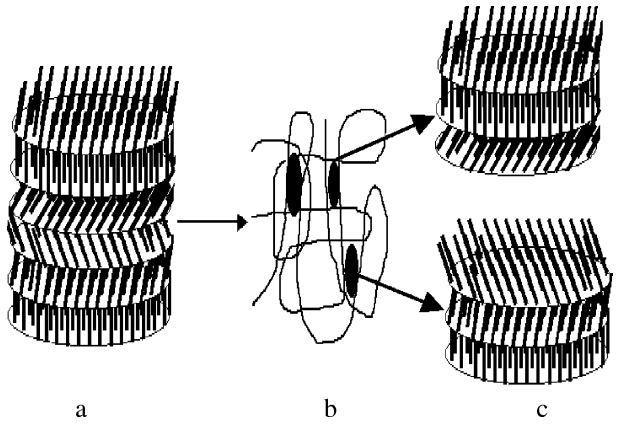

Fig. 5. A proposed structural model of liquid crystals of nematic type for the supramolecular aggregates of lycopene in water-miscible solvents (a) and the structural transformation of aggregates in lycopene–chitosan complexes (b, c).

3.3. Structural model of the supramolecular aggregates of lycopene

Some carotenoids like β-carotene, lutein and luteindiester containing long conjugated double bonds are considered to be rod-like and rigid. They are able to self-assemble nematic liquid crystal phase in vivo or in solution [17,28], which causes the aggregates to have strong optical anisotropy. If carotenoid molecules have chiral centers, they will be aggregated into a chiral nematic liquid crystal phase. The carotenoid molecules located in the adjacent thin two-dimensional layers will align at a slight angle to one another. In this structure, the two-dimensional layers (like ideal H-aggregation) twist and form a continuous helical configuration. The strong circular dichromism (CD) effect can be observed [30]. Because lycopene also is a rod-like and rigid molecule, it is reasonable to infer that lycopene can form a nematic liquid crystal in aqueous organic solvent (Fig. 5a). Due to the lack of chiral centers, however, the aggregates of lycopene could not form helical pattern. Each two-dimensional layer (ideal H-aggregate) probably arranges at a random tilt angle. So the tilt angle α of a whole aggregate follows statistics rule and depends on the number of two-dimensional layers. Considering the aggregates from another point of view, lycopene molecules in neighboring two layers can be thought to form head to tail aggregation (i.e. J-aggregation). We think that there actually exist two different types of aggregation in the supramolecular aggregates of lycopene. The changes of the proportion of H-type or J-type aggregation caused the hypsochromic or bathochromic shift of absorption spectrum. When a layer-packed structure is destroyed (e.g. by the size decrease of aggregates), the proportion of J-aggregation will be decreased and the main absorption band will have a blue shift. Otherwise, it will cause a red shift. Ray and Misra [21] reported that the UV–Vis absorption spectra of the multilayer lycopene–stearic acid LB film have a fairly sharp peak at 348 nm, which indicates the formation of aggregates of lycopene. In view of the structure of the LB film, it is obvious that the H-aggregates assembling in the two-dimensional layers stack above and below. A whole aggregate of lycopene should contain both H-type and J-type considering the interaction of lycopene molecules located in the adjacent layers. Therefore, the aggregation model in LB film supports the model we proposed in this paper. In addition, Wegmann et al. [31] also found that the type of aggregates transformed from

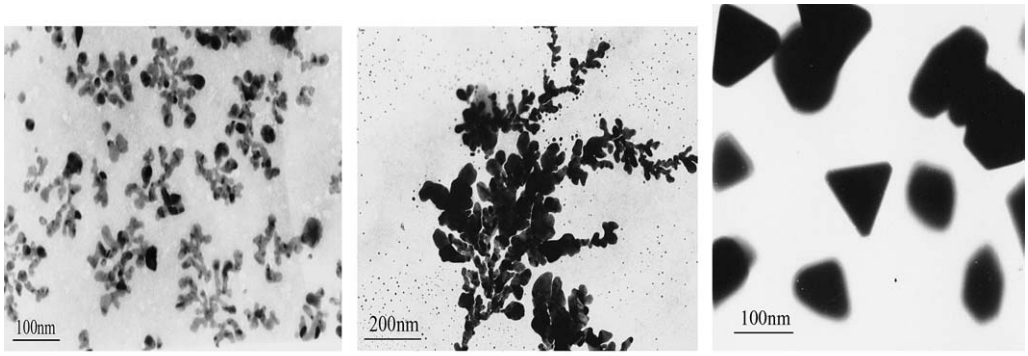

Fig. 4. TEM images of supramolecular assemblies of lycopene. The ratios of lycopene to chitosan (w/w) are (a) 1.25/400, (b) 5/400 and (c) 7.5/400.

J-type to H-type due to the decrease in the size of nanoparticles of lycopene. In other words, the model we proposed gave another evidence that the type of aggregation varies with the size of aggregates.

Lycopene molecules could form smaller size aggregates in the chitosan matrix due to the effect of the macromolecular chains of chitosan, thus causing the proportion of J-aggregation to decrease and making the tilt angle change from 65.9° to 66.6° compared with the aggregates in aqueous organic solvent (Fig. 5b, c). Therefore, the characteristic main band in UV–Vis absorption spectrum further blue-shifted. In addition, the main absorption band inversely red-shifted to 358 nm when the ratio of lycopene and chitosan is 7.5/400 (w/w). This phenomenon is caused by the size-effect of aggregates of lycopene and the increase in the proportion of J-aggregation.

4. Conclusions

In our current work, we found the supramolecular aggregates of lycopene in aqueous solvent and chitosan matrix are different according to their UV–Vis absorption spectra and TEM images. This finding will help us deeply understand the related physical chemistry and photochemistry about lycopene, even its biological functions. We proposed a model to explain the difference of the supramolecular aggregates. Using this model, we think that the spectral shifts of aggregates depend on the proportion of H-type or J-type aggregation. The changes in the structure of aggregates cause the hypsochromic or bathochromic shift.

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