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Spectral and nonlinear optical properties of chlorophyll b depends on distortion of two-dimensional electron configuration along one axis

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Abstract

This paper reports investigation on spectra and optical nonlinearity of chlorophyll b (Chl b) and Cu/Zn-substituted chlorophyll b (Cu/Zn-Chl b). Compared with Chl b, absorption spectra of Cu-Chl b in acetone showed that its B-band (Soret band) and Q-band were blue-shifted 43 nm and 6 nm, respectively; B-band of Zn-Chl b was blue-shifted 25 nm and its Q-band red-shifted 16 nm, respectively. Optical nonlinearities of the Cu/Zn-Chl b and Chl a/b with same absorptive coefficient were examined with 532 nm picosecond pulses. The results showed that optical nonlinear refractive (n_2) of Chl b was larger than that of Chl a and the Cu/Zn-Chl b. The influence of Cu/Zn on π-electron distribution of the porphyrin cycle along X and Y axes was analyzed according to the results of the spectra and the optical nonlinearity. Contribution of the π-electron distribution variation on the axes to the optical nonlinear refractive was discussed.

Keywords: Chlorophyll a; Chlorophyll b; Cu/Zn-substituted chlorophyll; Optical nonlinearity; Optical nonlinear refractive index

1. Introduction

Chlorphylls (Chls) are the most important light-harvesting pigments for photosynthetic organisms including oxygen-evolving organisms, such as cyanobacteria, red algae and green plants, and photosynthetic bacteria that contain bacteriochlorophylls (BChls). Chls/BChls not only function as antenna pigments, but also directly take part in converting sun light into chemical energy, the key reaction to sun-light fixation in all of the world from ancient to present [1–4]. In the oxygen-evolving photosynthetic cells, Chls situate in thylakoid membranes in the form of pigment-protein complexes. In the complexes, Chls distribute in clusters; they, therefore, are close enough to have intermolecular dipole–dipole interaction or exciting-energy coupling, known as the general means of energy transfer in the primary process of photosynthesis, between one another [4–9]. Unfortunately, Chls are not stable in vitro at room temperature, and especially exposed under light. This weak point makes them not convenient

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in practical use. But when the central metal Mg cation of Chls is substituted with Zn/Cu cations, the weak of their unstable characteristics is eliminated. Just because of this, Zn-chlorophyll a and b (Zn-Chl a and Zn-Chl b) have been used to substitute Chlorophyll a and b in reconstruction of Chlorophyll a/b light-harvesting pigment-protein complexes in vitro. Furthermore, results from the investigation of the interaction between the apoproteins and Chls provide valuable data for the field of the molecular recognition and the assembly of synthetic receptors [10].

Chlorophyll a (Chl a) and b (Chl b) are a kind of natural porphyrin derivatives with central metal magnesium (Mg) cation in organisms [11]. They have macrocyclic π conjugated-electron systems. Chls, like common porphyrin and its derivatives, have two absorption regions that locate respectively in 380–480 nm, known as B band, and 580–680 nm, named Q band. The coordinated Mg cation in center, the unsymmetrical distribution of the π conjugated-electron delocalization along X and Y axes and the fifth ring make Chl a and b have two pairs of splitting electronic states, B_x, B_y, Q_x and Q_y, and specific strong absorption in Q band [11,12]. Obviously, the substitution of the central Mg with Cu/Zn may inevitably bring about the spectral variation of chlorophyll a and b, leading to some variations of corresponding photophysical characteristics of the Chls. In addition, Chl a in the concentration of 10^{-5} M, like the artificial porphyrin derivatives which are took as a model of two dimension molecules in the optical nonlinearity field, exhibited the large magnitude of third-order optical nonlinearity, and the modulation of the transmitting pulse light beam [13]. The organic materials with π-conjugated electron configurations in one or two dimensions have been considered as promising candidates in practical uses for photo- and electro-illumination device owing to their favorable photophysical characteristics [14–24].

In this work, the influence of central metal substituted by Cu/Zn on π-electron distribution of the porphyrin cycle along its X and Y axes was analyzed compared with Chl b and pheophytin b (Pheo b). Contribution of the π-electron distribution variation to the optical nonlinear refractive indexes of the Chls was discussed according to the results of their spectra and optical nonlinearity. It is concluded that for the Chls, magnitude of their third-order optical nonlinearity is dependent mainly on the net distortional extent of the Chl π-electron configurations on one of the two axes.

2. Materials and methods

2.1. Preparation of chlorophyll a and chlorophyll b

Chlorophyll a and b were prepared by silica column chromatography from green leaves [13,25]. Chl a/b were extracted in mixture of acetone and ethanol (1:1 v/v), and then transferred into petroleum ether by extraction. The silica column, onto which the properly concentrated Chl a/b extractive in petroleum ether was loaded, was eluted in steps by mixture of petroleum ether and ethyl acetate with the eluent polarity increasing. In order to ensure purity of the prepared Chls, the chromatographic Chls passed through the silica column once more, and the purity was examined by thin-layer silica chromatography. In the meantime, the samples were also examined by photospectroscopy in acetone solution.

2.2. Preparation of Zn- and Cu-chlorophylls

When the purified Chl b solution in ethonal was being stirred, drop 36% HCl (w/w) into it until the green solution changed into brown. Add zinc/copper acetate crystal into the brown solution until it was saturated; then the solution was incubated at 60 for about 2 h. After the brown solution changed its color into green again, Zn/Cu cations were coordinated in the center of the pheophytin b (Pheo b). The Zn- and Cu-chlorophylls transferred in petroleum ether were purified by the silica column chromatography in the same way as the Chl b.

2.3. Measurements

The Z-scan [13,26,27] was used to determine the magnitude and sign of third-order nonlinearities in our experiment. It was performed with TEM_{00}

mode and Q-switched mode-locked Nd:YAG laser which is 532 nm 200 ps (FWHM) pulse train with seven pico-pulses whose intervals are 7.5 ns. In the present experiment, optical intensity $I_0 = 26.4$ MW/cm^2 per pulse. For elimination lenssing effects, CS_2 was employed to calibrate the experimental system by its circular pattern of focusing and defocusing spots.

Absorption spectra of the Chls in acetone at room temperature were measured at room temperature using the UV-3400 Spectrophotometer (HITACHI).

3. Results

Absorption spectra of chlorophyll a/b in acetone were presented Fig. 1. Compared with the Q_y and B_x bands present in the absorption spectrum of Chl b, the Q_y bands of Chl a shifted to red 24 nm, and the B_x bands of it shifted to blue 16 nm. These data show that for chlorophyll a, the energy of the B_x band rose and that of the Q_y bands decreased. It implied that there are reduction in the extent of the-conjugated π-electron delocalization along X axis and increase in that along Y axis. Absorption spectra of the Cu/Zn-chlorophyll b in acetone were shown in Fig. 2. Compared with the Q_y and B_x bands present in the absorption spectrum of Chl b, similarly the Q_y bands of Cu- and Zn-chlorophyll b shifted to red 6 nm and 16 nm, respectively, and the B_x bands of them shifted to blue 43 nm and 25 nm. The Q_y/B_x bands of pheophytin b were also red/blue-shifted 18 nm/46 nm. Moreover, the peak of $Q_x(0,0)$ band of pheophytin b was distinguished at about 532 nm. These data revealed a fact that for Chl b, whenever its central Mg was lost or substituted by Cu/Zn, it was commonly caused that the energy of the B_x band rose, implying a decrease in the extent of the conjugated π-electron delocalization along X axis and that the energy of the Q_y band fell, indicating an increase in the degree of the conjugated π-electron delocalization along Y axis.

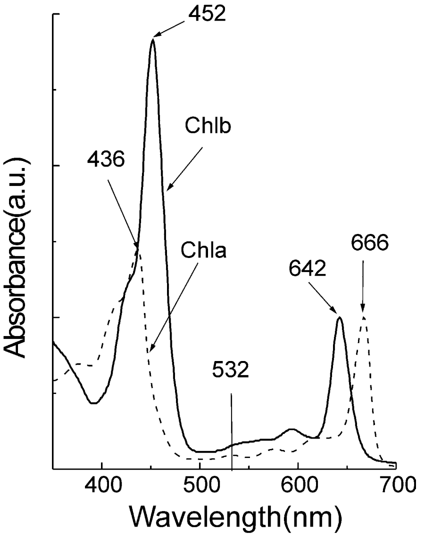

Fig. 1. Absorption spectra of Chl a and Chl b in acetone.

Third-order nonlinearity of the Chls was measured by Z-scan [13]. The nonlinear refractive index of the chlorophyll b/a and the Cu/Zn-chlorophyll b, including the pheophytin b, was shown in Table 1. Considering the effect of linear absorption coefficient α, the nonlinear refractive index of them was divided by the linear absorption coefficient α at 532 nm. Comparing the magnitude of $N_2 = n_2/\alpha$, we concluded that: (1) N_2(Pheo b) > N_2(Chl b) > N_2(Cu-Chl b) and N_2(Zn-Chl b); (2) N_2 (Chl b) > N_2(Chl a). Chlorophylls are two-dimensional compounds in structure. In addition, the self-defocusing and self-focusing patterns of laser beam for the Chls were different from that of one-dimensional compounds with conjugated π-electron systems and small molecules with no conjugated π-electron system. As shown in Fig. 3,

Table 1
The third-order nonlinear refractive indexes of Chl a, Chl b, Phe b, Cu-Chl b and Zn-Chl b in acetone

Samples	n_2	α at 532 nm	$N_2 = n_2/\alpha$
Cu-Chl b	1.33	0.28	4.75
Chl b1	2.47	0.28	8.82
Zn-Chl b	1.40	0.322	4.35
Chl b2	2.60	0.321	8.1
Phe b	3.12	1.105	2.82
Chl b3	2.87	1.103	2.6
Chl b4	2.70	0.46	5.88
Chl a1	1.31	0.46	2.84
Chl b5	2.97	0.92	3.23
Chl a2	2.42	0.92	2.63

the pattern of laser beam with TEM_{00} mode was changed to be ellipse or quasi-cross pattern. But for the one-dimensional compounds and the small molecules, such as polyethylene derivatives and CS_2, the self-defocusing and self-focusing patterns of the laser beam transmitting them are usually circular (the results not shown). These phenomena reveal that in the path of the high-field laser beam, the nonlinear refractive index distribution of the polarized Chls created a quasi-cylinder, but not a thin lens as those of the one-dimensional compounds and the small molecules.

4. Discussion

Among the Chls included in this work, Chl b has the highest Q_y-band energy and the lowest B_x-band energy (Fig. 2); therefore, its conjugated π-electron delocalization along X axis is the largest in degree, but the smallest along Y axis. This feature determines that Chl b may relatively hold a conjugated-π-electron configuration with the least asymmetry with respect to the axes X and Y on its two-dimensional molecular plane. On analogy of the same way, Pheo b has the largest asymmetrical conjugated-π-electron configuration; therefore, its polarization is the easiest on the axis Y. Compared with Cu-Chl b, Zn-Chl b is more asymmetrical in its conjugated-π-electron distribution. The 532 nm laser employed in the experiment is not within the two strong resonant bands B_x and Q_y, while it may fall in the region of some weak Q_x vibration bands. In this case, the third-order optical nonlinearity created by the 532 nm laser beam in the Chl solutions includes two contributions from off-resonance and the weak resonance of Q_x bands [18,20,21]. However, redistribution of the electron densities is always related with the polarized direction of laser beam. Fig. 3 demonstrated that the nonlinear polarization induced by the high intensity occurred preferable on one of the two axes of the two-dimensional Chl molecules. For the Chls, the most possibly polarized direction is on the Y axis, parallel to the π-electron delocalization of the lower-energy Q_y bands. The magnitude of the nonlinear refractive index induced in Chls should be determined by distortion degree of their electronic structures. Compared with Chl b, Cu-Chl b and Zn-Chl b have the configurational distortion smaller than Chl b based on their nonlinear refractive indexes. On the contrary, the

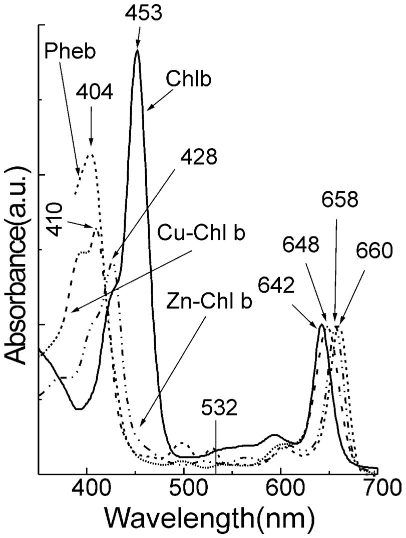

Fig. 2. Absorption spectra of Chl b, Cu-Chl b, Zn-Chl b and Pheo b in acetone.

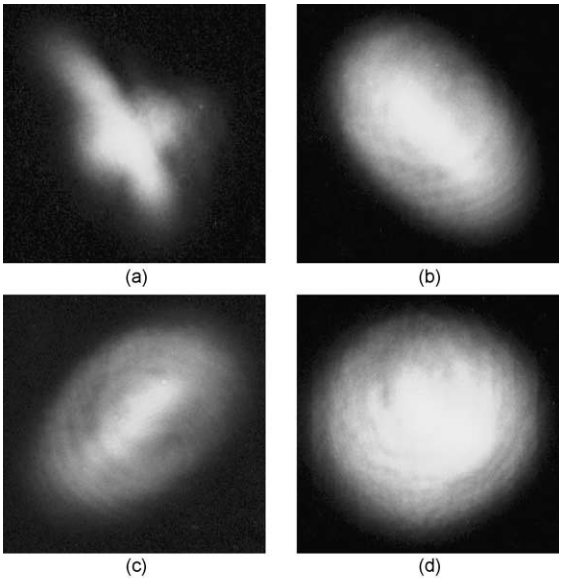

Fig. 3. The defocusing and self-focusing patterns of the laser beam at different Z positions; a and c: negative Z positions near the peak; b: a positive Z position near the valley; d: far field positions.

highest N_2 of Phe b evidences that its molecular distortion is the largest. It can be concluded, accordingly, that the central metal, its existence or not and its size, may obviously affect on the magnitude of the molecular distortion of the Chls. Between Chl a and Chl b, both of which coordinate Mg in their centers, the molecular configuration distortion of the Chl b is larger than that of Chl a (see the data in Table 1) although Chl a has the more easily polarized Q band than Chl b. Here, the flexibility of Chl b electron configuration, which originates from the less asymmetry of its electron distribution [18,20,21] on axes *X* and *Y*, makes it represent a larger integral variation of the electron configuration distortion on the Y axis. But for Chl a, the relatively larger asymmetry of its electron distribution on axes *X* and *Y*, which is created by its conjugated π-electron delocalization of the lower energy Q_y band in a higher degree on the Y axis and that of the higher energy B_x band in an lower degree, leads to its giving a smaller integral variation of the electron configuration distortion on the Y axis although it has the initial electron configuration of easier polarization.

The Chls in concentration of 10^{-5}–10^{-6} M exhibited pretty symmetry *Z*-scan curve in shape at 532 nm, demonstrating that the imaginary one from the nonlinear absorption nearly gave contribution to the Chl optical nonlinearity in the present work by contrast to the real part. Moreover, only the real part, not the imaginary one, of the nonlinearity, N_2, was considered here. In addition, 532 nm light leis within the Q_x band, a weak band, for the $Q_x(0-1)$ of Chla and Chlb situates at 533 nm and 549 nm, respectively (chlorophylls 1991). Therefore, excited states of Q_x band have their contribution to the optical nonlinearity of Chla/b. Considering that the excited states of Q_x band will relax to Q_y band in very short time on fs scale, the excited states in Q_y band make the real contribution of the excited state to the optical nonlinearity of the Chls. Ratio of $\epsilon_{\max}$ (643nm) to $\epsilon_{\max}$ (532nm), however, was about 6.2 for the Chl b with concentration about 10^{-5} M in acetone. When ratio of the 532nm oscillator strength (*f*), which estimated by the formula $f \approx \epsilon_{\max} \Delta\nu / 2.5 \times 10^8$[28], of the Chl b to the 643nm one was less than 1/6, $f\,(532\text{ nm})/f\,(643\text{ nm}) < 1/6$. In this case, the limited population of the exited molecules that the 532 nm laser beam created generated only the partial optical nonlinearity from the resonance band. The other part of the optical nonlinearity doubtless originated mainly from off-resonance bands.

In conclusion, for molecules with two-dimensional conjugated-π-electron configurations like the Chls, magnitude of their third-order optical nonlinearity is dependent not only on their original electron structures which determine how easy they may polarized or not under high intensive light fields, but also mainly on the net distortional extent of their π-electron configurations under high intensive light fields. In addition, because the two-dimensional molecules with macrocyclic π-conjugated electron systems, similar to chlorophylls, is induced to create a quasi-cylinder but not thin lens under intensive light fields, *Z*-scan technology is not very suitable to investigate third-order optical nonlinearity of such kind molecules.

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