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ANALYSIS OF FLUORESCENCE DECAYS OF CHLOROPHYLL α
AND β -CAROTENE IN NEMATIC LIQUID CRYSTAL

Key Words: chlorophyll, carotene, fluorescence decay, fractals.

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Abstract

The chlorophyll α (Chl α) and its mixture with β -carotene were embedded in nematic liquid crystal - anisotropic matrix. The decays of fluorescence were

measured at two wavelengths: 680nm and 715nm and analyzed using biexponential and fractal models. Fractal model fits better to decay of fluorescence emitted at 680nm by samples with Chl a alone. For all other cases (decay observed at 680nm and 715nm luminescence of the pigment mixture and 715nm luminescence of Chl a alone) both models describe measured decays with similar adequacy. It seems that β -carotene molecules perturb the regular arrangement of chlorophyll in nematic solvent.

Introduction

Interactions between β -carotene and Chlorophyll a (Chl a) are biologically important because of two processes occurring in photosynthetic organisms : carotenoids are involved in the light harvesting process transferring singlet excitation to chlorophylls and in the protection of chlorophylls against photobleaching¹⁻³. These processes are occurring differently in various organisms and at various stages of thylakoids greening⁴. The interactions between Chl a and carotenoids have been investigated both in living organisms and in simpler model systems⁵. Previously⁵ several spectral properties of Chl a and β -carotene embedded in nematic liquid crystals were reported. The lifetime of

fluorescence(τ) of Chl a in this model system was measured and it was found the Chl a fluorescence decayed slower in the presence of β -carotene than in sample without this addition⁵. This unexpected effect was tentatively explained as disaggregation of Chl a in the carotene presence. Such explanation was very preliminary because, as it follows from absorption spectra, Chl a in LC is weakly aggregated as a result of strong LC-Chl interaction⁵⁻⁸. Further investigations of this effect are reported in the present work. The analysis of the decay of fluorescence, in most cases, is carried under the assumption that decay is the superposition of several exponential components⁹ but for assemblies of molecules of restricted geometry, such as photosynthetic light - harvesting antenna pigment systems or especially oriented in vesicles or in monolayers pigment molecules, rather fractal model should be applied^{10,11}.

Materials and methods

Chl a was purified chromatographically. The β -carotene (Fluka AG, Chem. Fabrik CH-9470 Buchs) was used without further purification. Pigments were dissolved in nematic liquid crystal (LC) mixture of p-methoxybenzylidieno-p-butyylaniline (MBBA) + p-ethoxybenzylidieno-p-butyylaniline (EBBA).

In previous work⁵ β -carotene was partially crystallized. To avoid this crystallization diluted (10^{-5} M) β -carotene solution in chloroform was mixed with LC and chloroform was slowly evaporated. As a result the mono dispersed β -carotene solutions in LC were obtained. The following pigment concentrations were used: Chl a : $1.2 \cdot 10^{-3}$ M ; β -carotene $3.7 \cdot 10^{-4}$ M; concentrations in pigment mixture were : Chl a : $1.4 \cdot 10^{-3}$ M and β -carotene : $9.7 \cdot 10^{-4}$ M. LC cell windows were covered by conducting layer of $In_2O_3:Sn$ and orienting layer of SiO_2 . The LC and pigments were uniaxially oriented around the axis parallel to cell windows.

The details of LC cell construction and samples preparation were given previously⁵.

The fluorescence decays were measured using pico and nanosecond time correlated single photon counting fluorometer built at the Center for Fluorescence Spectroscopy, University of Maryland at Baltimore.

The fluorescence decays of the Chl a and its mixture with monomeric β -carotene embedded in nematic LC cell were analyzed in terms of two models: 1. that fluorescence donor is surrounded by acceptors what is leading to fractal decay and 2. that fluorescence is due to emission of heterogeneous mixture of emitters what leads to biexponential decay.

The instrumental response function was convoluted with the model decay and compare with the experimentally measured decay¹². The nonlinear least squares algorithm based on Marquardt method was used to estimate model parameters. The fit was judged by a value of parameter χ^2 together with visual inspection of residuals¹².

Results and Discussion

If emitting molecules are located in the close proximity of acceptor molecules their fluorescence will decay according to fractal model¹²:

$$F(t) = \alpha \exp(-t/\tau - \beta(t/\tau)^\gamma) + \alpha_{\text{scat}} \quad (1)$$

where τ is the lifetime of donor in the acceptor absence,

γ -stretched exponent value

β -fraction of fractal sites occupied by the acceptor
 α_{scat} -contribution from scattered light.

The second model consideres a heterogeneous mixture of emitting molecules which leads to biexponential decay of fluorescence:

$$F(t) = \alpha_1 \exp(-t/\tau_1) - \alpha_2 \exp(t/\tau_2) + \alpha_{\text{scat}} \quad (2)$$

where t -time, τ_1, τ_2 the lifetimes of two emitting species.

Both models have the same number of adjustable parameters and therefore it is reasonable to compare

the quality of fitting of both models to the same set of experimental data.

Fig 1 shows the comparison of decay curves measured for Chl a with and without carotene addition. The fluorescence measured at 680nm for the sample with admixture of β -carotene decays much slower than for Chl a alone (Fig.1a). The difference in the decay of fluorescence measured at 715nm (Fig.1b) is much smaller than that for 680nm emission. With carotene the decays at both wavelengths of observation are very similar but without carotene F680 decays faster than that at 715nm. These results are in qualitative agreement with previously reported for similar samples but with partially crystallized carotene⁵. The recovered parameters obtained from the fitting of both models to the experimental data are gathered in Table 1. In a case of fluorescence collected at 680nm the fractal model better describes decay than biexponential model, but for a sample with carotene the improvement of fit by introducing fractal model is much lower. For fluorescence observed at 715nm both models give similar fit, but χ^2 is much higher for the sample with carotene than for Chl alone. It seems that the introducing more exponential components for this case can improve fitting because in such sample to

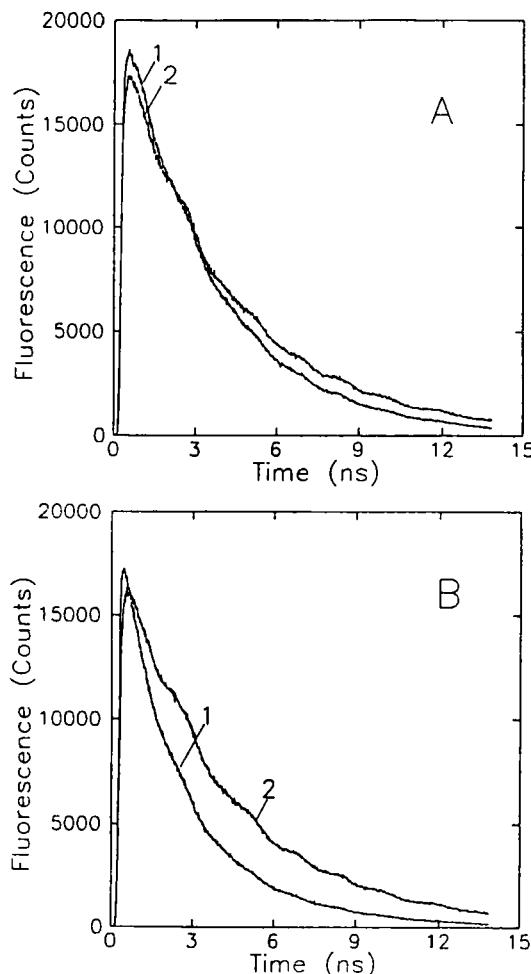


Fig. 1 Decays of fluorescence of Chl a (curves 1) and Chl a with β -carotene (curves 2) $\lambda_{\text{exc}} = 380\text{nm}$. A- $\lambda_{\text{flu}} = 680\text{nm}$; B- $\lambda_{\text{flu}} = 715\text{nm}$

TABLE 1

Analysis of fluorescence decay. $\lambda_{exc} = 380$ nm, car - β -carotene.

Sample	λ_f nm	Biexponential model						Fractal model					
		α_1	τ_1 ns	α_2	τ_2 ns	α_{scc}	χ^2	α	τ ns	β	γ	α_{scc}	χ^2
Chl a	680	3.8	2.94	2.80	0.79	0.20	3.7	8.8	3.75	1.08	0.37	0.15	2.4
Chl a	715	5.8	3.30	1.18	0.57	0.01	2.7	12.1	3.46	0.70	0.09	-0.01	2.6
Chl a + car	680	4.6	4.13	0.95	1.10	-0.02	4.7	6.7	4.40	0.43	0.25	-0.08	4.2
Chl a + car	715	4.8	4.18	1.10	1.10	0.03	4.1	6.2	4.95	0.43	0.50	0.02	4.1

715nm emission can contribute more than two types of emitters. Emission at 680nm is characteristic for monomeric Chl a in LC, whereas to the fluorescence at 715nm besides the contributions from vibronic shoulder of monomeric form contributes also emission of aggregated or solvated forms of pigment¹³.

The value of γ when Chl a fluorescence decay was analysed in terms of formula (1) is about 0.37 very close to 1/3 value theoretically predicted for two dimensional system with acceptor molecules randomly distributed around the donor. Examples of such two dimensional systems are molecular crystals, monolayers adsorbed on solid substrate etc.¹⁰. In our LC cell nematic and pigments are uniaxially oriented around

the axis parallel to the cell window⁵. Practically all positions of Chl a ring surfaces around the axis are represented¹⁴.

The Q_y transition moment of Chl a is directed at about 32° degree with respect to LC axis¹⁵. Because of this anisotropic arrangement of pigment molecules our sample can not be treated as random two-dimensional system. In our model not all Chl a molecules are in the same situation. In a case when two adjacent molecules have parallel orientation of their rings very strong interaction between them can occur, and can be formed statistical "special pair" observed in other model systems^{16,17}. In such a case energy trap, having different level the of the excited singlet state could be formed¹⁰. The delayed emission can be observed in such systems and the rather high thermal deactivation of excitation could be expected. The β -carotene addition preserves the traps formation because the β -carotene molecules can be located in between Chl a molecules perturbing their regular arrangement. It was shown that the degree of Chl a orientation in a sample with β -carotene is lower than that in LC cells with Chl a alone⁵. The quenching of delayed luminescence of Chl a in LC by β -carotene was

also previously observed⁵. When number of traps decreases the singlet excitation is not quenched by traps and as a result the fluorescence lifetime increases. Data concerning thermal deactivation yields⁵ are difficult to interpret because of high yields of β -carotene thermal deactivation. But in a region of Chl a absorption addition of β -carotene causes the decrease in the yield of thermal deactivation.

The proposed explanation is in agreement with the lack of changes in the Chl a absorption due to β -carotene addition, with the changes in thermal deactivation yield with the quenching of delayed luminescence of Chl a by carotene and with the analysis of decay of fluorescence presented in this work.

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