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Excitation energy transfer from carotenoids probed by femtosecond time-resolved fluorescence spectroscopy

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Fluorescence rise and decay curves of carotenoids were measured in solutions and in pigment protein complexes with a femtosecond time-resolved fluorescence spectroscopy. For linear carotenoids, the S_2 lifetimes showed the maximum value around n = 9-10. The conjugation of a keto-carbonyl group shortened the S_2 lifetime and prolonged the S_1 lifetime. The excitation relaxation dynamics within carotenoids and the excitation energy transfer kinetics from carotenoids to chlorophylls are discussed as a function of molecular structure of carotenoids.

Key words : photosynthesis, carotenoid, excitation energy transfer, femtosecond, dynamics

INTRODUCTION

Carotenoids, in general, show several kinds of biological activities, such as radical scavenging, singlet oxygen trapping, and other protective activities. In photosynthesis, carotenoids have an additional function: they absorb light energy and transfer it to a photochemical reaction center where a light-induced electron transfer reaction takes place. Two kinds of carotenoids are found in photosynthetic organisms; one consists of conjugated polyenes (-(C=C)$_n$-; n is the number of conjugated double bonds) and the other contains a keto-carbonyl group (>C=O) in the conjugated double-bond system. It is well known that the carotenoids in the latter group, such as siphonaxanthin, fucoxanthin and peridinin, work as efficient antenna pigments. We investigate this excitation energy transfer process by the femtosecond time-resolved fluorescence spectroscopy.

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Since carotenoids belong to polyenes with a C_{2h} point group, at least two energetically low-lying singlet states are expected in the visible region. One is closely related to the $2A_g$ (S_1) state which is dipole forbidden from the ground (S_0) state by parity, and the other is related to the $1B_u$ (S_2) state which is allowed for one-photon excitation and contributes significantly to the absorption spectrum of carotenoids. Following excitation of carotenoid to the S_2 state the excitation energy relaxes in two competitive processes; one is the relaxation within the carotenoids and the other, the energy transfer to chlorophyll. Because of presence of the two singlet excited states, two energy transfer pathways are possible in the photosynthetic antenna system: one from the S_2 state just after excitation and the other from the S_1 state following the $S_2 \rightarrow S_1$ internal conversion in carotenoids. The energy transfer pathway is closely related to the molecular structure of carotenoids. Both pathways are operative for carotenoids of conjugated polyenes in bacterial antenna, whereas only the latter is active for carotenoids with a keto carbonyl group.

MATERIAL AND METHOD

Fluorescence rise and decay curves were measured with a fs fluorescence up-conversion system which was performed using a Ti:Sapphire laser pumped with a diode-pumped solid-state laser. The IR pulses were separated into two; one was doubled by a BBO crystal and the second harmonic was used to excite the sample, while the other IR beam served as a gate pulse. The gate pulse traversed a variable delay before being combined with the fluorescence in a 0.5 mm thickness BBO crystal in the type 1 phase matching geometry while the excitation pulse traversed a fixed delay before being focused into a 1 mm sample cell. Each decay curve was individually fit to a single or a double exponential function using an iterative deconvolution method. A Gaussian function fitted to the up-conversion signal from the pure solvent Raman scattering was used as an instrumental response function.

RESULTS AND DISCUSSION

For linear carotenoids, the excited-state lifetimes were strongly dependent on the conjugation length (n) [1]. Figure 1 shows inverses of the S_2 lifetimes as a function of $\Delta E_{21}/h\omega_M$, together with those of β-carotene [2] and spheroidene [3]. The energy gap law of internal conversion is expressed as follow,

$$k_{21} = c \exp(-\gamma \Delta E_{21}/h\omega_M) \quad (1)$$

where k_{21} is the internal conversion rate constant which is given as an inverse of an excited-state lifetime, ΔE_{21} is the S_2 -S_1 energy difference, c is a pre exponential constant related to the electronic coupling matrix element, $h\omega_M$ is the energy of the high frequency acceptor mode, and

γ can be related to the displacement of the potential surfaces in the S_2 and S_1 states, respectively. For

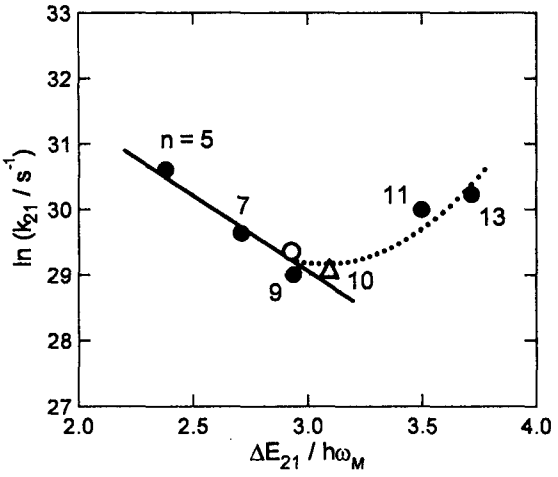

Figure 1 Internal conversion rate constants in a natural logarithmic scale against $\Delta E_{21}/h\omega_M$. Respective points correspond to linear carotenoids (closed circle), β-carotene (open circle, Ref. [2]), and spheroidene (triangle, Ref. [3]).

polyenes, the C=C stretching mode has been assigned to be the dominant acceptor mode for the internal conversion. Contrary to what the energy gap law (Eq. 1) predicts, the logarithm of k_{21} does not linearly depend on ($\Delta E_{21}/h\omega_M$) (Fig. 1); as n was increased, the $S_2 \rightarrow S_1$ internal conversion rate, the inverse of lifetime, decreased and then increased again with a longer conjugation length, showing the minimum value around n = 9-10. This trend was in striking contrast to that of the $S_1 \rightarrow S_0$ internal conversion which was simply accelerated with the increase of n except for n = 3 reported elsewhere [4]. For the $S_2 \rightarrow S_1$ internal conversion, the other factors, such as the displacement of the potential surfaces (γ), the density of states, or both should be taken into account.

Since the energy transfer from carotenoid to chlorophyll competes with the intramolecular relaxation process within carotenoid, the carotenoids that have longer lifetimes in their excited states are more suitable for the energy donor. In this sense, the

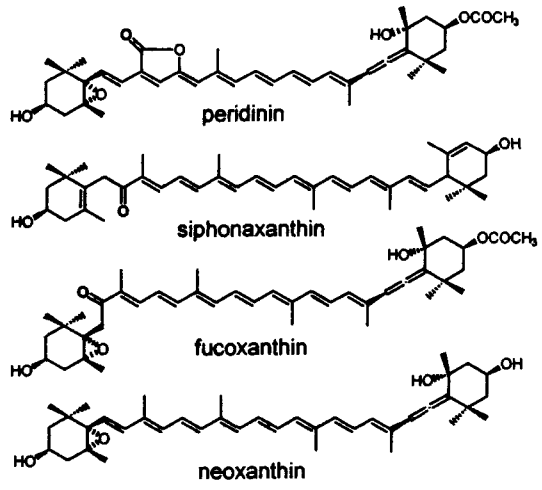

Figure 2 Molecular structures of carotenoids.

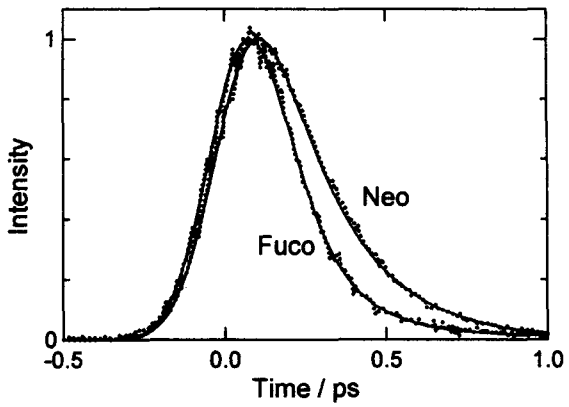

Figure 3 S_2 fluorescence decay curves of neoxanthin (Neo) and fucoxanthin (Fuco) in *n*-hexane.

carotenoids with n = 9-10 are expected to be most probable to work as antenna pigments, and the energy transfer via the S_2 state is observed for spheroidene (n = 10) [3] and lutein (n = 9+1 (one C=C bond is located in a β-end group)) [5] and predicted for neurosporene (n = 9) [6].

The natural carotenoids which contain a keto-carbonyl group, peridinin, siphonaxanthin, and fucoxanthin, have 8 C=C bonds and 1 C=O bond in their conjugation systems. Carotenoids show dual emissive characteristics, depending on their structural parameters, such as the conjugation length of C=C bonds and a conjugation of a C=O bond. The conjugation of a keto-carbonyl group to the C=C conjugated double bonds dramatically changes the spectral properties. When one C=C bond located at an end of conjugated double bonds in all-*trans* neoxanthin is replaced by one C=O bond (fucoxanthin), the origin of fluorescence changes; S_2 emission is predominant for neoxanthin, while S_1 emission for fucoxanthin. This fluorescence behavior is closely related to the excitation relaxation dynamics of carotenoids. Figure 2 shows S_2 fluorescence decay curves of neoxanthin and fucoxanthin in *n*-hexane. Fucoxanthin has a shorter S_2 lifetime (120 fs) and a longer S_1 lifetime (40 ps, not shown) than neoxanthin does (210 fs and 20 ps, respectively), showing that the conjugation of a keto-carbonyl group shortens the S_2 lifetime and prolongs the S_1 lifetime. These results clearly indicate that the energy transfer via S_1 state is profitable for the keto-carbonyl carotenoids.

Recently, it has become possible to examine the excitation relaxation dynamics of carotenoids and the excitation energy transfer from carotenoids in the pigment protein complexes whose three-dimensional structures were resolved: spheroidene in the LH-1 and LH-2 light-harvesting complexes [3], lutein and neoxanthin in the LHC II of higher plants [5], peridinin in peridinin-chlorophyll *a*-protein (PCP) [7], and so on. In the former two cases, the S_2 lifetime of carotenoids were extensively reduced to less than 100 fs in pigment protein complex, indicating an additional excitation relaxation process, i.e. the energy transfer via the S_2 state. On the other hand, peridinin exhibited almost the same fluorescence decays in PCP as in solution. Therefore, it was concluded that the efficient energy transfer occurs only from the forbidden S_1 state of peridinin to the S_1 state of chlorophyll [7]. Our most recent study revealed that the S_2 fluorescence decay lifetime of siphonaxanthin is 125 fs in the LHC II of a

green alga [unpublished data]. This lifetime value is in good agreement with that in solution (Fig. 2), indicating that the S_2 energy transfer is inefficient for siphonaxanthin in LHC II. Although it is known that both lutein and siphonaxanthin work as efficient antenna pigments in LHC II, the energy transfer pathways are different in these two systems, suggesting that the energy transfer routes from carotenoid to chlorophyll do not depend on structure of the pigment protein complexes but on the structure of carotenoids. As well as the prolonged S_1 lifetime, the keto-carbonyl group brings about a partially allowed property to the S_1-S_0 transition through a perturbation to the symmetry of conjugated π-electron system [6,7]. These two effects, the prolonged lifetime and the partially allowed transition, may cause an effective energy transfer via the S_1 state for the keto-carbonyl carotenoids.

In solution, the excited state dynamics within the S_2 state strongly depend on the molecular structure of carotenoids [1,2,8,9]. In the case of linear carotenoids, intramolecular vibrational redistribution (IVR), vibrational relaxation (VR), and internal conversion were clearly discriminated [8,9]. On the other hand, the IVR process was not resolved in carotenoids containing a keto-carbonyl group due to a time constant shorter than the time-resolution of apparatus (~30 fs) [9]. For further studies on the energy transfer from carotenoid to chlorophyll, relationship between the energy transfer efficiency and the relaxation dynamics within the excited states of carotenoids should be discussed.

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