Fucoxanthin-Pyropheophorbide and Zeaxanthin-Pyropheophorbide Dyads as New Models for Study on Carotenoid-Chlorophyll Excited State Interactions

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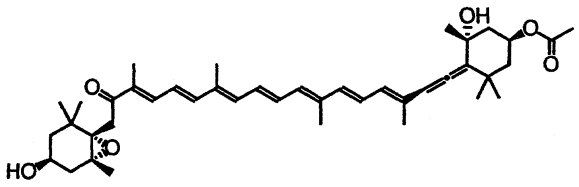
Fucoxanthin-pyropheophorbide dyads, **3c** and **4c**, and zeaxanthin-pyropheophorbide dyads, **3d** and **4d**, were prepared as the first example of natural carotenoid-linked pyropheophorbide. Singlet–singlet energy transfer from carotenoid to pyropheophorbide is more efficient in fucoxanthin-pyropheophorbide dyads than the corresponding zeaxanthin-pyropheophorbide dyads, while the singlet excited state of the pyropheophorbide is quenched more strongly in the zeaxanthin-linked molecules. Marked differences in carotenoid-pyropheophorbide singlet excited-state interactions between fucoxanthin and zeaxanthin strongly suggest their different roles *in vivo*; antenna function for fucoxanthin and energy-dissipation for zeaxanthin. Both carotenoids similarly quench the triplet excited state of the pyropheophorbide through triplet–triplet energy transfer with rates which depend on temperature and the linkage.

In photosynthetic organisms, carotenoids are acting as antenna by transferring light-energy to chlorophyll to promote photosynthesis and as photoprotective pigment by preventing singlet oxygen formation.[1—5] Both these functions involve excitation energy transfer: singlet–singlet from carotenoid to chlorophyll and triplet–triplet from chlorophyll to carotenoid, respectively. To date, more than 600 natural carotenoids have been identified, and their structural and chemical properties have been revealed very diverse. It is conceivable that the manner in which a particular carotenoid function depends on its photochemical properties. Therefore, it is crucial to reveal the excited-state interactions between chlorophylls and respective natural carotenoids for understanding the carotenoid-involved photochemical processes. Fucoxanthin (**1**) that is the major carotenoid in brown algae and probably the most abundant of all natural carotenoids transfers its absorbed energy to chlorophyll with almost 100% efficiency in specific pigment-protein complexes.[6] Recently, its photochemical properties *in vitro* have been unveiled rather favorable for the antenna function: its lowest singlet excited (S_1) state is distinctly long-lived in comparison to normal carotenoids and the $S_1 \rightarrow S_0$ fluorescence emission is observable in a region where chlorophyll has the strong Q_y absorption band.[7,8] However, little is known about the actual mechanism of the efficient energy transfer *in vivo*. Especially the spatial requirements of fucoxanthin relative to chlorophyll still remain unclear. On the other hand, there has been work showing a strong correlation between the content of zeaxanthin and the amount of non-photochemical fluorescence quenching, suggesting another photoprotective role of zeaxanthin via fluorescence quenching of chlorophylls under excess light conditions[3,4] This photoprotection has been postulated in the xanthophyll cycle where light-dependent interconversions of three xanthophyll (zeaxanthin, antheraxanthin, and violaxanthin) via de-epoxidation and epoxidation are thought to adjust the light energies available for photosynthetic reaction center. In this case again, there is no direct evidence that zeaxanthin does quench chlorophyll fluorescence. *In vitro* system either, there is no reliable work concerning the actual excited-state interactions between chlorophylls and natural carotenoids.

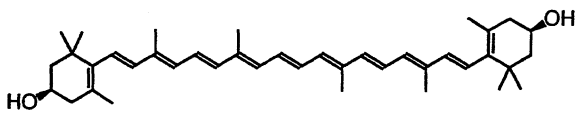
Gust and Moore have demonstrated that covalently

linked carotenoporphyrins and related molecules are very useful to determine the structural requirements for the light-harvesting and photoprotective functions of carotenoids.[9—11] They have explored a range of such molecules where synthetic porphyrins and pyropheophorbides are covalently linked to polyenes that have physical and chemical properties analogous to natural carotenoids. They have revealed that there is strong reactivity-correlation between the singlet–singlet energy transfer from carotenoid to tetrapyrrole and the triplet–triplet energy transfer from tetrapyrrole to carotenoid. Namely, both the antenna function and the photoprotective function have been observed only in the cases where there are very strong electronic couplings between the π-electronic systems of the porphyrin and the carotenoid. However, the carotenoids used in their models have been restricted to a single type of unnatural carotenoids, 7′-apo-7′-aryl-β-carotene,[9—13] thus leaving a direct assessment of the excited-state interactions of chlorophylls and natural carotenoids yet unexplored.

Here fucoxanthin (**1**) and zeaxanthin (**2**) (Fig. 1) were selected as first natural carotenoids to be linked with pyropheophorbide pigments, since they are considered to play very important but possibly different roles in photosynthetic apparatus.[1,7,8] Studies on the electronic interactions of these carotenoids and chlorophyll-type pigments under proximate geometries will be very helpful in understanding the mechanism of the biological functions of natural carotenoids. Fucoxanthin and zeaxanthin are both hydroxylated xanthophylls and their hydroxyl groups are used for making ester-linkage with pyropheophorbide carboxylic acids **3a** and **4a**. Here we report the synthesis of fucoxanthin-pyropheophorbide dyads, **3c** and **4c**, and zeaxanthin-pyropheophorbide dyads, **3d** and **4d**, as the first example of natural carotenoid-linked pyropheophorbide (Fig. 2). Fucoxanthin has a very unique structure bearing sensitive functional groups including secondary and tertiary alcohols, allene, and β,γ-epoxy ketone, while zeaxanthin has essentially the same π-electronic system as that of β-carotene. Dyads **3c,d** and **4c,d** differ in that the carotenoid in the former models is linked to the 17-propionic acid side chain of pyropheophorbide-*a* via an ester bond, whereas in the latter the 3-vinyl group of methyl pyropheophorbide-*a* is replaced by a carboxylic group which in turn is linked to the carotenoid.



fucoxanthin (1)

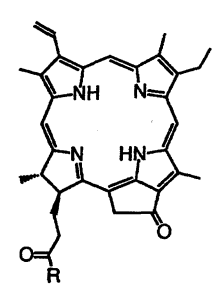


zeaxanthin (2)

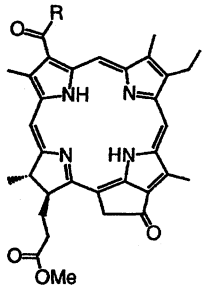
Fig. 1. Structures of fucoxanthin and zeaxanthin.

Results

Synthesis. All the dyads were prepared by esterification of chlorophyll-*a* derivatives with fucoxanthin and zeaxanthin. Fucoxanthin was extracted from brown algae collected at the Sea of Japan. Esterification of the carotenoid was done under mild conditions not to affect its structure. As a condensing reagent we used 2-chloro-1-methyl-pyridinium iodide.[14] When triethylamine was



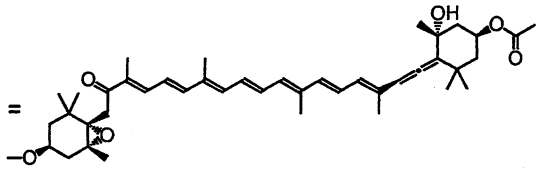
3a-e



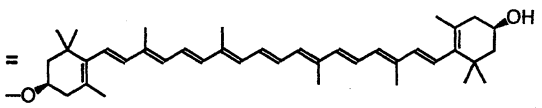
4a-e

a : R = —OH **b : R = —OMe**

c : R =



d : R =



e : R =

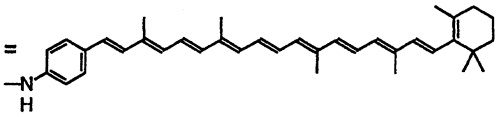


Fig. 2. Structure of model compounds used in this study.

used as a base in the esterification of **4a**, acid anhydride **5** was only formed (Chart 1). Small amounts of the dyads were obtained when the reaction temperature was raised higher than 60 °C, but the ^{1}H NMR spectra of the products showed some cis–trans isomerization in the carotenoid moiety. At this temperature the recovered carotenoid showed several separated bands in TLC. An excellent result was achieved when 4-dimethylaminopyridine was used as the base. The reaction proceeded below 40 °C in acceptable yields (15—26%) without formation of the acid anhydride side product. The dyads were identified by their 500 MHz ^{1}H NMR spectra and FAB mass spectra. Assignment of the ^{1}H NMR signals has been done with help of 2D-COSY experiments. A fragile skeleton of fucoxanthin was confirmed intact in both **3c** and **4c** by examining their ^{1}H NMR and high resolution FAB mass spectra (HRMS). The β,γ-epoxy ketone moiety is known to be labile particularly under basic conditions. In these dyads, the C-7 protons between the carbonyl and the epoxy groups appear as a AB-quartet at 2.55 and 3.60 ppm (J=18.3 Hz) in **3c** and 2.75 and 3.81 ppm (J=18.6 Hz) in **4c**, respectively. Changes in the proton chemical shifts ($\Delta\delta$) of the carotenoid upon attachment to the pyropheophorbide are summarized in Tables 1 and 2. Fucoxanthin

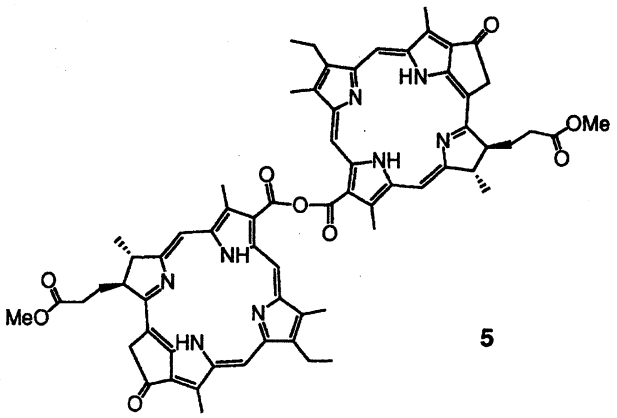
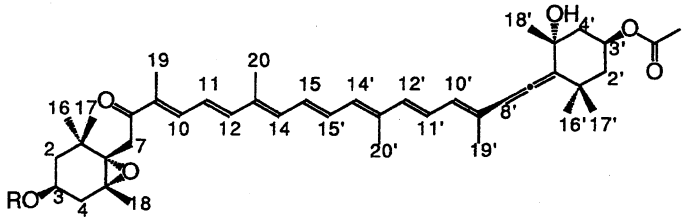


Chart 1.

Table 1. ^{1}H NMR Chemical Shift Changes in Fucoxanthin-Linked Dyads **3c** and **4c**.



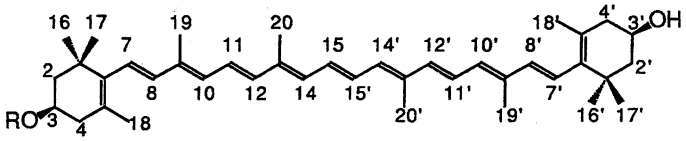
		H_{2ax}	H_{2eq}	H_3	H_{4ax}	H_{4eq}	H_7	H_7	H_{16}	H_{17}	H_{18}
1	(δ)	1.355	1.498	3.818	1.788	2.326	2.600	3.658	1.036	0.964	1.223
3c	(δ)	1.21	1.38	4.81	1.73	2.24	2.55	3.60	0.98	0.83	1.14
	($\Delta\delta$)	−0.15	−0.12	0.99	−0.06	−0.09	−0.05	−0.06	−0.06	−0.13	−0.08
4c	(δ)	1.98	2.14	5.66	2.44	2.96	2.75	3.81	1.33	1.15	1.41
	($\Delta\delta$)	0.62	0.64	1.84	0.65	0.63	0.15	0.15	0.29	0.19	0.19

		H_{10}	H_{11}	H_{12}	H_{14}	H_{15}	H_{19}	H_{20}
1	(δ)	7.150	6.576	6.673	6.414	6.640	1.945	1.992
3c	(δ)	7.09	6.52	6.63	6.39	6.62	1.89	1.98
	($\Delta\delta$)	−0.06	−0.06	−0.04	−0.02	−0.02	−0.06	−0.01
4c	(δ)	7.25	6.62	6.72	6.45	6.65	2.01	2.02
	($\Delta\delta$)	0.10	0.04	0.03	0.04	0.01	0.06	0.03

		$H_{2'ax}$	$H_{2'eq}$	$H_{3'}$	$Ac_{3'}$	$H_{4'ax}$	$H_{4'eq}$	$H_{8'}$	$H_{16'}$	$H_{17'}$	$H_{18'}$
1	(δ)	1.414	1.998	5.384	2.04	1.516	2.288	6.056	1.385	1.072	1.354
3c	(δ)	1.42	2.00	5.38	2.04	1.52	2.28	6.05	1.38	1.07	1.35
	($\Delta\delta$)	0.01	0	0	0	0	−0.01	−0.01	−0.01	0	0
4c	(δ)	1.4	2.0	5.40	2.04	1.5	2.3	6.06	1.39	1.08	1.36
	($\Delta\delta$)	0	0	0.02	0	0	0	0	0	0.01	0.01

		$H_{10'}$	$H_{11'}$	$H_{12'}$	$H_{14'}$	$H_{15'}$	$H_{19'}$	$H_{20'}$
1	(δ)	6.133	6.605	6.351	6.270	6.756	1.815	1.992
3	(δ)	6.12	6.60	6.34	6.26	6.74	1.81	1.97
	($\Delta\delta$)	−0.01	−0.01	−0.01	−0.01	−0.02	−0.01	−0.02
4c	(δ)	6.14	6.62	6.36	6.29	6.77	1.82	2.01
	($\Delta\delta$)	0.01	0.01	0.01	0.02	0.01	0	0.02

Table 2. ^{1}H NMR Chemical Shift Changes in Zeaxanthin-Linked Dyads **3d** and **4d**.



		H_{2ax}	H_{2eq}	H_3	H_{4ax}	H_{4eq}	H_7	H_8	H_{16}	H_{17}	H_{18}
2	(δ)	1.48	1.77	4.01	2.05	2.39	(6.16—6.06)		1.07	1.07	1.74
3d	(δ)	1.40	1.60	4.99	1.95	2.32	(6.1—6.0)		(1.02,0.96)		1.65
	($\Delta\delta$)	−0.08	−0.17	0.98	−0.10	−0.07			(−0.05,−0.11)		−0.09
4d	(δ)	2.16	2.36	5.85	2.71	3.02	(6.27,6.23)		(1.37,1.27)		1.92
	($\Delta\delta$)	0.68	0.59	1.84	0.66	0.63			(0.30,0.20)		0.18

		H_{10}	H_{11}	H_{12}	H_{14}	H_{15}	H_{19}	H_{20}
2	(δ)	6.16	Ca. 6.63	6.36	Ca. 6.25	Ca. 6.65	1.97	1.97
3d	(δ)	6.12	Ca. 6.62	6.34	Ca. 6.24	Ca. 6.62	1.93	1.97
	($\Delta\delta$)	−0.04	Ca. −0.01	−0.02	Ca. −0.01	Ca. −0.03	−0.04	0
4d	(δ)	6.25	Ca. 6.69	6.42	Ca. 6.26	Ca. 6.66	2.05	2.01
	($\Delta\delta$)	0.09	Ca. 0.06	0.06	Ca. 0.01	Ca. 0.01	0.08	0.04

		$H_{2'ax}$	$H_{2'eq}$	$H_{3'}$	$H_{4'ax}$	$H_{4'eq}$	$H_{7'}$	$H_{8'}$	$H_{16'}$	$H_{17'}$	$H_{18'}$
2	(δ)	1.48	1.77	4.01	2.05	2.39	(6.16—6.06)		1.07	1.07	1.74
3d	(δ)	1.46	1.77	3.99	2.02	2.39	(6.2—6.1)		1.07	1.07	1.73
	($\Delta\delta$)	−0.02	0	−0.02	−0.03	0			0	0	−0.01
4d	(δ)	1.48	1.77	4.00	2.05	2.38	(6.14,6.10)		1.08	1.08	1.74
	($\Delta\delta$)	0	0	−0.01	0	−0.01			0.01	0.01	0

		$H_{10'}$	$H_{11'}$	$H_{12'}$	$H_{14'}$	$H_{15'}$	$H_{19'}$	$H_{20'}$
2	(δ)	6.16	Ca. 6.63	6.36	Ca. 6.25	Ca. 6.65	1.97	1.97
3d	(δ)	6.15	Ca. 6.64	6.36	Ca. 6.24	Ca. 6.62	1.97	1.97
	($\Delta\delta$)	−0.01	Ca. 0.01	0	Ca. −0.01	Ca. −0.03	0	0
4d	(δ)	6.17	Ca. 6.65	6.37	Ca. 6.26	Ca. 6.66	1.98	1.99
	($\Delta\delta$)	0.10	Ca. 0.02	0.01	Ca. 0.01	Ca. 0.01	0.01	0.02

has two hydroxyl groups; one is a secondary alcohol at C-3 and the other is a tertiary alcohol at the C-5′. Substantial low-field shifts of the C-3 methine proton in **3c** (0.99 ppm) and **4c** (1.84 ppm) are in accord with the assigned structure of the ester-linkage at the C-3 hydroxyl group. In all the dyads, particular high-field shifts for the protons in the carotenoid moieties were not observed, suggesting extended conformations. The protons of the proximal β-ionone ring in the dyads **4c,d** were considerably shifted to low field due to the ring current effect of the pyropheophorbide, indicating this β-ionone ring being kept in the deshielding region of the macrocycle. The corresponding protons of **3c,d** were slightly shifted to high field.

Absorption and Fluorescence Spectra. Figure 3 shows the absorption spectrum of **3c** in THF solution, together with those of **1** and **3b**. Dyad **3c** features a Soret absorption at 412 nm and Qy absorption at 673 nm for the pyropheophorbide moiety. The strong absorbances in the 450—520 nm region are due mostly to the fucoxanthin. By comparing the absorption spectrum of **3c** with that of **3b**, we have estimated the absorbance due to the fucoxanthin moiety in the dyad

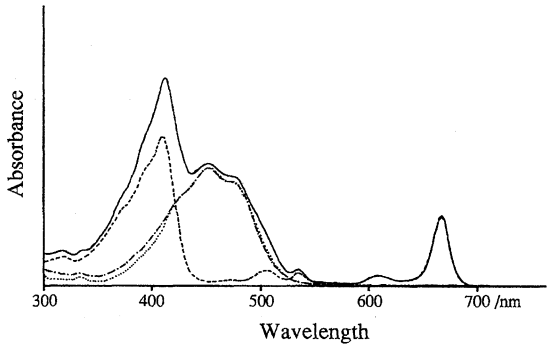


Fig. 3. Absorption spectra of **3c** (—), **3b** (---), and **1** (-·-), and the calculated spectrum of the carotenoid moiety in **3c** (- - -) in THF.

3c that is nearly identical to that of **1**. This analysis indicates that the interchromophore interaction in the ground state is relatively weak. Similarly, the absorption spectra of **3d**, **4c**, and **4d** (Table 3, Fig. 6) were also analyzed as the linear combination of the spectra of the pyropheophorbide and the carotenoid. The fluorescence spectral shapes of **3c,d** and **4c,d** were identical, within experimental error, with those of **3b** and **4b**, re-

Table 3. Absorption and Fluorescence Data in THF

Model	UV-visible λ_{max}/nm[a)]	Fluorescence λ_{max}/nm
3b	412(100), 506(10), 537(9), 609(7), 667(47)	673
3c	412(100), 452(58), 535(8), 608(4), 668(33)	675
3d	413(100), 458(71), 487(62), 537(6), 609(4), 668(32)	675
4b	381(70), 416(100), 514(12), 545(12), 620(6), 682(66)	686
4c	418(100), 544(9), 620(4), 682(47)	686
4d	417(100), 457(85), 485(73), 543(8), 620(3), 681(48)	685

a) Numbers in parentheses indicate the relative extinction coefficients.

spectively (Fig. 4). The fluorescence intensities of the fucoxanthin-linked dyads **3c** and **4c** are virtually the same as those of **3b** and **4b**, while the attached zeaxanthin reduces the fluorescence intensity by 20 and 45% in **3d** and **4d**, respectively, relative to **3b** and **4b**.

Singlet–Singlet Energy Transfer from Carotenoid to Pyropheophorbide. Energy-transfer efficiency from the singlet excited state of carotenoid to pyropheophorbide in THF was estimated by the comparison of the normalized corrected fluorescence excitation spectra with the absorption spectra (Fig. 6 and Table 4).[9,10,15] The A values presented in Table 4 demonstrate that the singlet energy transfer is more efficient in **4c,d** than **3c,d** and that fucoxanthin is more efficient singlet-energy donor to pyropheophorbide than zeaxanthin in both cases. The former result, which is analogous to a trend reported for amide-linked dyads **3e** and **4e**,[12] is likely in accord with expected closer proximity of the chromophores in the **4**-series. Figure 5 shows the transient absorption spectra of fucoxanthin and zeaxanthin, which were assigned due to the $S_n \leftarrow S_1$ absorption on the basis of the previous studies.[8,16,17] By analyzing the time profiles of the transient absorptions of ^{1}(fucoxanthin)* and ^{1}(zeaxanthin)* at 545 and 563 nm, respectively, the S_1-state lifetimes have been determined as 60 ps for fucoxanthin and ca. 8 ps for zeaxanthin.[18]

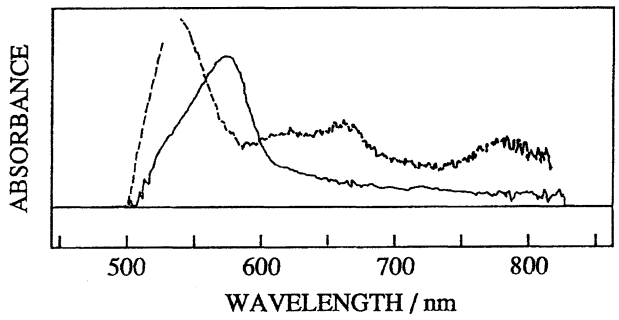


Fig. 5. Transient absorption spectra of fucoxanthin (---) and zeaxanthin (—) in THF for excitation at 532 nm, at 26- and 13-ps delay time, respectively.

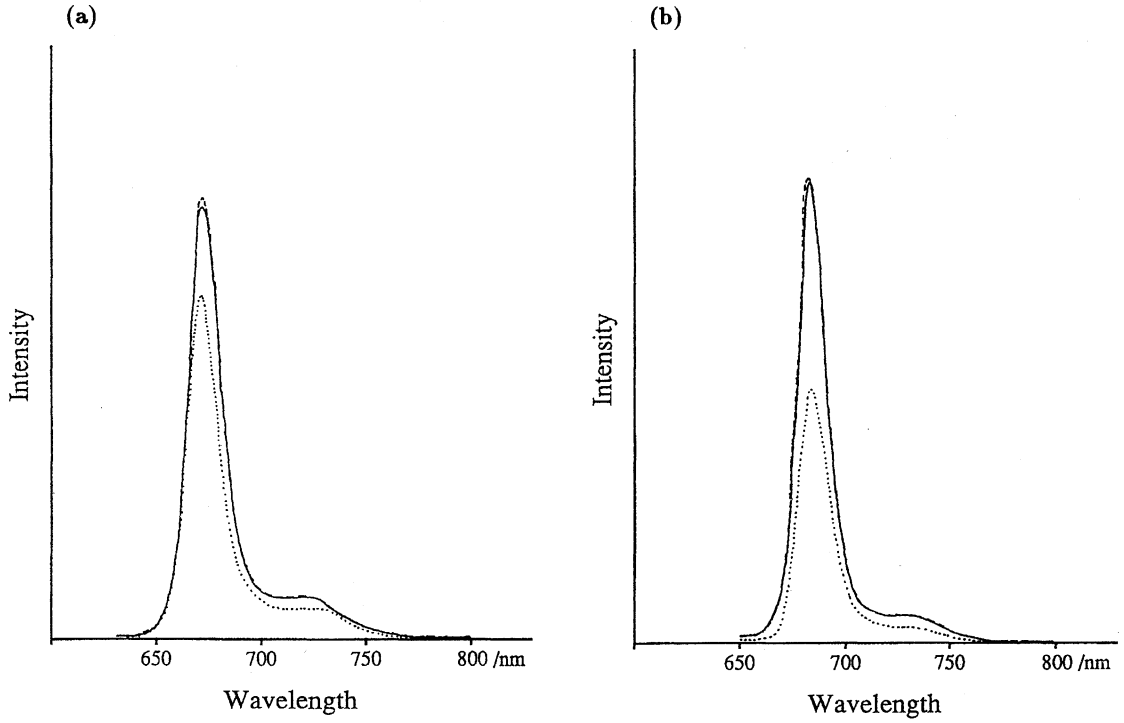


Fig. 4. Steady state fluorescence spectra in THF, (a) **3b** (—), **3c** (---), and **3d** (---); (b) **4b** (—), **4c** (---), and **4d** (---).

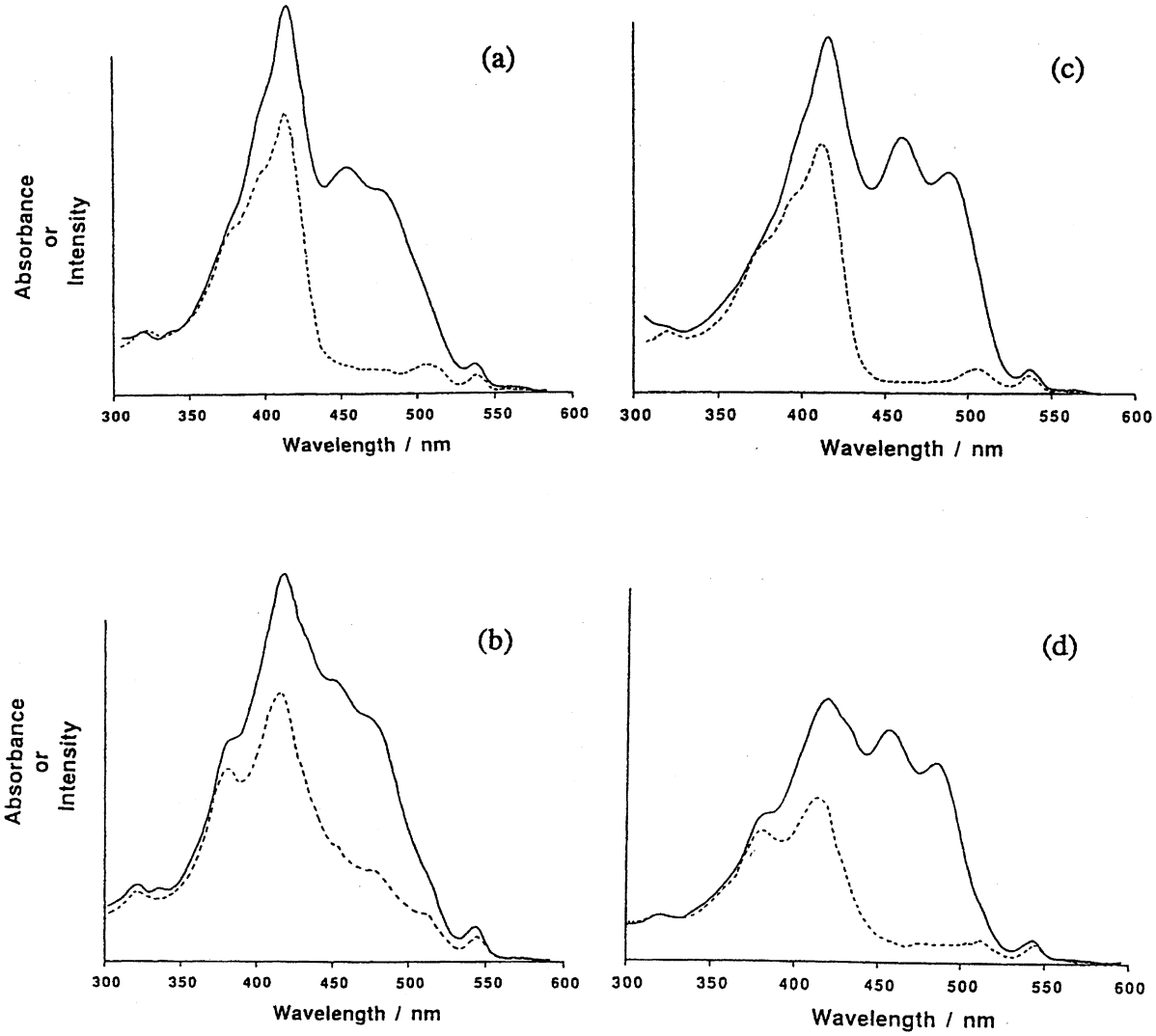


Fig. 6. Absorption spectra (——) and corrected fluorescence excitation spectra (---) of **3c** (a), **4c** (b), **3d** (c), and **4d** (d). The corrected fluorescence excitation spectra were taken by monitoring pyropheophorbide fluorescence at 672 nm for **3c** and **3d** and at 685 nm for **4c** and **4d**.

Table 4. Photophysical Data of Carotenoid-Pyropheophorbide Dyads in THF

Model	A [a]/%	A (77 K)/%[b]	Ω/cm^{-6}mol^{-1} [c]	τ_F/ns [d]	k_{TT}/s^{-1} [d]	τ_C/μs[f]
3c	7	8	6.3×10^{-14}	6.6	7.1×10^{6}	5.6
3d	2	4	4.1×10^{-14}	5.6	6.7×10^{6}	1.9
3e	<5 [g]	—	—	—	ca. 10^{7} [h]	—
4c	38	50	8.0×10^{-14}	6.8	6.0×10^{5}	6.7
4d	8	18	3.4×10^{-14}	3.9	1.1×10^{6}	2.6
4e	53 [g]	—	—	—	7×10^{9} [i]	—

a) Efficiency of the energy transfer from ^{1}(carotenoid)* to pyropheophorbide determined by comparison of the absorption spectra and corrected fluorescence excitation spectra at room temperature. b) Efficiency of the energy transfer at 77 K. c) Spectral overlap between the fluorescence of carotenoid and the absorption of pyropheophorbide, the S_1-fluorescence and the Q_y-absorption for **3c** and **4c**, and the S_2-fluorescence and the Q_x-absorption for **3d** and **4d**. d) Fluorescence lifetime of ^{1}(pyropheophorbide)* at 690 nm for excitation at 660 nm, τ_F of **3b** and **4b** are 6.8 and 7.8 ns, respectively. e) Rate constants of the triplet–triplet energy transfer from ^{3}(pyropheophorbide)* to carotenoid. f) Lifetime of ^{3}(carotenoid)*. g) From Ref. 12, in toluene. h) From Ref. 9, in toluene. i) From Ref. 19.

Therefore, the observed efficient singlet energy transfer in the fucoxanthin-linked dyads may arise mainly from its longer S_1-state lifetime. The singlet–singlet energy transfer process in **4c** has been confirmed in the transient absorption spectroscopy as well as the fluorescence lifetime measurement. Figure 7 shows the transient absorption spectra of **4c** for excitation at 532 nm, where the ratio of the absorbance of carotenoid to that of pyropheophorbide is ca. 0.6. Transient absorption at 557 nm that is due to the ^{1}(fucoxanthin)* decays with a time constant of ca. 30 ps, faster than the decay of the singlet excited state of reference fucoxanthin (60 ps). The fluorescence decay of **4c** at 680 nm has been measured for excitation at 532 or 660 nm. At 660 nm only the pyropheophorbide chromophore is excited, while both the fucoxanthin and pyropheophorbide chromophores are excited at 532 nm. In the case of excitation at 660 nm, the fluorescence exhibits a single-expo-

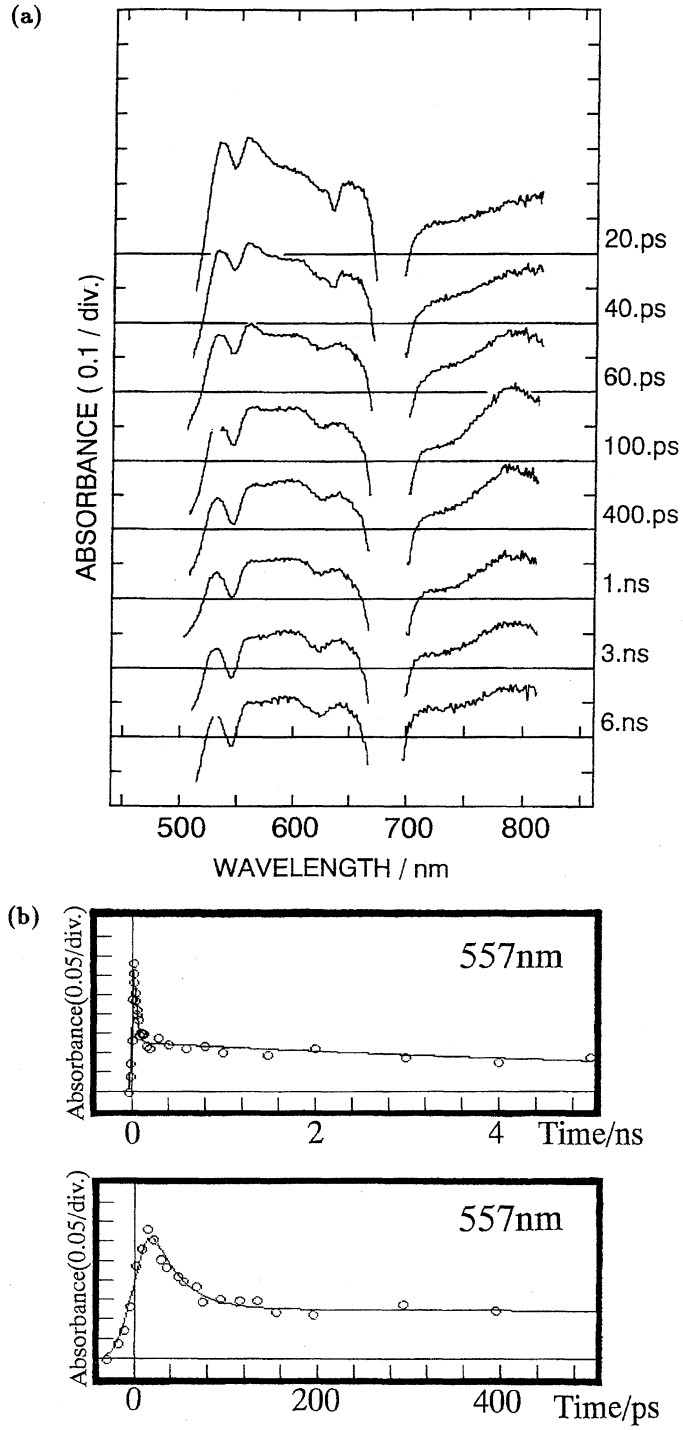


Fig. 7. (a) Picosecond transient absorption spectra of **4c** in THF for excitation at 532 nm. (b) Time profiles at 557 nm.

nential decay with a time constant of 6.8 ns, while in the case of excitation at 532 nm, the fluorescence decay curve contains at increasing component with a time constant of 34 ps and a decaying component with a time constant of 6.8 ns. The observed rise-time constant is in good agreement with the fast decay time constant at 557 nm in the transient absorption spectra. This component has been attributed to the singlet–singlet energy transfer from the fucoxanthin to the pyropheophorbide. However, in the other dyads, we could not detect such a rise-component clearly. In the transient absorption spectra of the other dyads **3c**, **3d** and **4d** for excitation at 532 nm (not shown), the rapid decays of the absorbance at 557 nm for **3c** and 563 nm for **3d** and **4d**, due mostly to the ^{1}(carotenoid)*, were also observed, respectively, with time constants of 60, 10, and 20 ps. In the dyads **3c,d**, the decay time of the ^{1}(carotenoid)* is nearly the same as that of the reference carotenoid, likely in agreement with the observed inefficient singlet–singlet energy transfer from the carotenoid to the pyropheophorbide. It is very difficult to extract the rate of the singlet–singlet energy transfer from the transient absorption spectra of **3c**, **3d**, and **4d** as well as their fluorescence lifetime measurements.

Figure 8 shows the corrected fluorescence excitation spectra of **4b**, **4c** and **4d** taken in rigid MTHF glassy matrix at 77 K. It is evident that the carotenoid to pyropheophorbide singlet–singlet energy transfer still proceeds under these conditions. Similar results were also obtained for the **3**-series. Comparison of these excitation spectra with the corresponding absorption spectra gave the A values at low temperature (Table 4) that indicate the efficiencies of the singlet–singlet energy transfer at 77 K to be similar to or slightly higher than those at room temperature.

Quenching of Pyropheophorbide Fluorescence by Carotenoid. Table 4 also lists the fluorescence lifetimes of the pyropheophorbide measured for excitation at 660 nm. There is a distinct, albeit small, difference in the quenching abilities of the two carotenoids. The attached zeaxanthin shortens the fluorescence lifetime more effectively than the corresponding fucoxanthin; the fluorescence lifetimes of **3d** and **4d** are shorter than those of **3c** and **4c**, respectively. These results are in accord with the steady-state fluorescence studies. Upon lowering temperature, the fluorescence quenching by the zeaxanthin in **4d** become 31% in THF at 173 K and 33% in MTHF at 173 K and 12% in rigid MTHF matrix at 90 K. Thus, the quenching is suppressed at low temperature but its temperature-dependence is weak in the temperature range measured. The fluorescence lifetime of the pyropheophorbide in **4d** was found to be somewhat solvent-polarity dependent and becomes shorter upon increase of the solvent polarity; 4.3 ns in benzene, 3.9 ns in THF, and 2.9 ns in DMF. The shortened fluorescence lifetime of **4d** may indicate the presence of some additional decaying pathway for the singlet excited state of the pyropheophorbide, which seems to be enhanced in polar environments. The transient absorption spectroscopy, however, did not detect any indication for formation of an ion pair (zeaxanthin)$^+$–(pyropheophorbide)$^-$ in solvents of a wide range of polarity (benzene, THF, and DMF) for **3d** and **4d**. Typically, the transient absorption spectra of **4d** in THF (Fig. 9) only show the slightly accelerated intersystem crossing of the singlet excited state of the pyropheophorbide to its triplet.

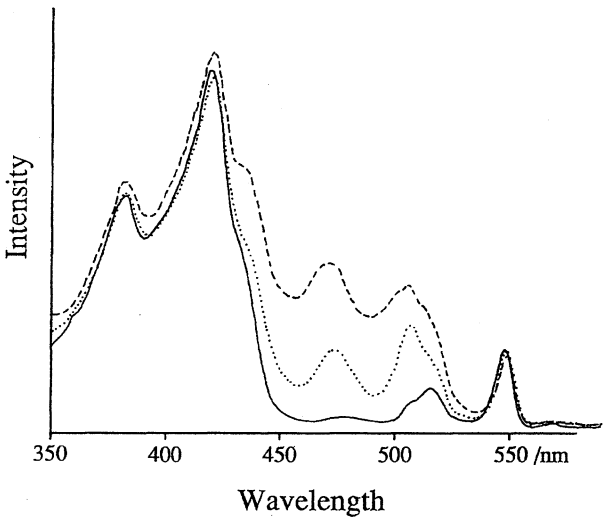


Fig. 8. The corrected fluorescence excitation spectra of **4b** (—), **4c**(---), and **4d** (- - -) in MTHF at 77 K taken by monitoring at 685 nm.

Triplet–triplet Energy Transfer from Pyropheophorbide to Carotenoid. Rates (k_{TT}) of the triplet–triplet energy transfer from pyropheophorbide to carotenoid were determined by monitoring the absorption changes of the triplet states.[19] Transient absorptions were observed at 460 nm for ^{3}(pyropheophorbide)*, at 510 nm for ^{3}(fucoxanthin)*, and at 520 nm for ^{3}(zeaxanthin)*. Figure 10 shows the transient absorption spectra of **3c** in THF for excitation at 532 nm. In the absorption spectrum taken at 20 ns after the photoexcitation, the strong bleachings at 410 and 670 nm are both due to ^{3}(pyropheophorbide)*. These bleachings recover with a common time constant of 0.14 μs and the recovery of these bleachings is accompanied by a rise of a strong absorption of ^{3}(fucoxanthin)* at 510 nm with a time constant of 0.15 μs. On the basis of these observations, we determined the value of k_{TT} in **3c** to be 7.1×10^6 s^{-1}. Triplet–triplet energy transfer rates k_{TT} were determined for other dyads in the same manner (Table 4). Two features are apparent, 1) k_{TT} values in **3c,d** are larger than those in **4c,d** in spite of the expected closer proximity for the latter and 2) the similar k_{TT} values were observed for the two carotenoids. Since the triplet–triplet energy transfer requires electron exchange interactions between donor and acceptor, the former observation implies

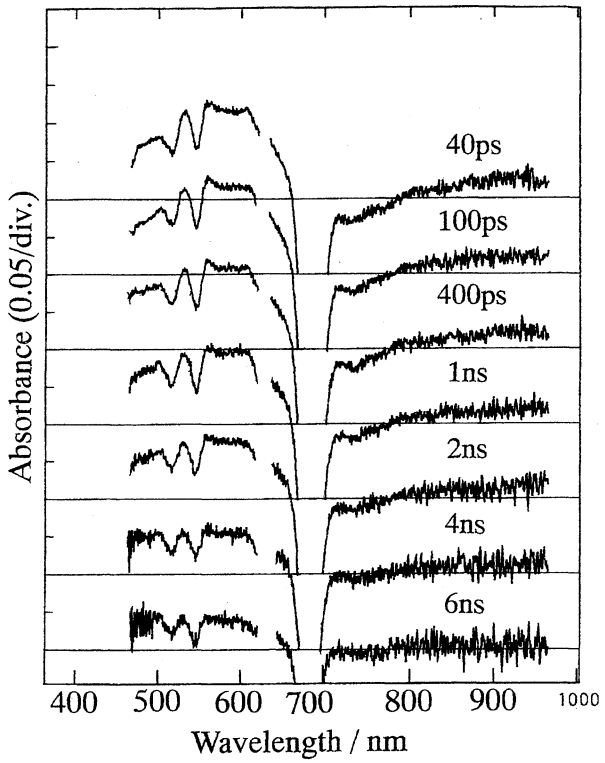


Fig. 9. Picosecond transient absorption spectra of **4d** in THF for excitation at 625 nm.

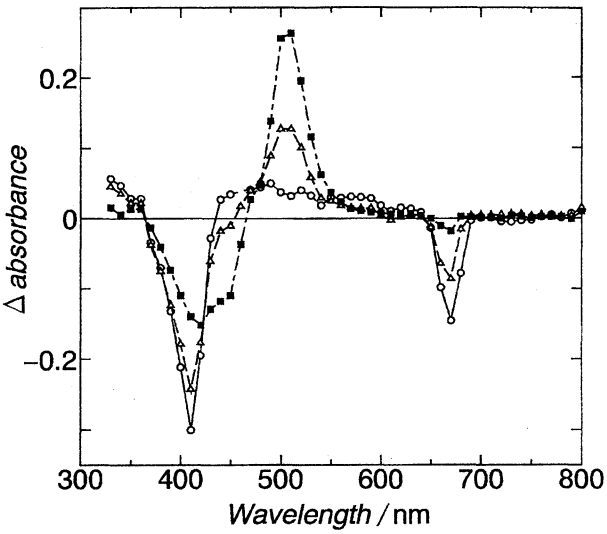


Fig. 10. Nanosecond transient absorption spectra of **3c** in THF for excitation at 532 nm at delay times of 20 ns (○), 120 ns (△), and 800 ns (■).

larger electronic interaction between the pyropheophorbide and the carotenoid in **3c,d**. But the through-bond electronic exchange interactions in **3c,d** are expected to be small and must be smaller than those in **4c,d**. Accordingly, a large portion of electron exchange interaction in **3c,d** should be supplied probably via through-space interaction in spatially folded conformation where the π-electronic systems of the pyropheophorbide and the carotenoid are kept in a close proximity. Such folded conformation is acquired by intramolecular motion of the large π-systems, as suggested in **3e**.[19] In **4c,d**, the molecular structures are less flexible and may prevent to some extent intramolecular motion from bringing the π-systems of the two chromophores into close contact. Plot of $\ln k_{TT}$ vs. $1/T$ from 172 to 295 K was found to be linear for both **3c** and **4c** and the activation energies determined from the slopes were 175 and 100 meV for **3c** and **4c**, respectively (Fig. 11). In rigid MTHF glassy matrix at 90 K, no effective triplet energy transfer was observed in either case. Since the pyropheophorbide to the carotenoid triplet–triplet energy transfer itself is exoenergetic, the observed activation energies seems to stem from the required intramolecular motion of the large π-systems.

The lifetime of the triplet excited state of the carotenoid has been also determined (Table 4). The triplet excited state of the fucoxanthin is slightly longer-lived in comparison to that of the zeaxanthin in both cases. The triplet decay rates of the carotenoid are not much influenced upon the attachment to the pyropheophorbide chromophore.

Discussion

The antenna functions of carotenoids have been examined in a variety of carotenoporphyrins and carotenopyropheophorbides.[9—13] Since the lifetime of the singlet excited state of carotenoid is usually very short, <10 ps, the singlet–singlet energy transfer from carotenoid to chlorophyll or porphyrin needs a very large rate constant to be competitive with the intrinsic very fast decay of the singlet excited state of carotenoid. Therefore, it is quite reasonable that the energy transfer from carotenoid to tetrapyrrole has been only effective in models where the electronic exchange interaction between carotenoid and acceptor is very large.

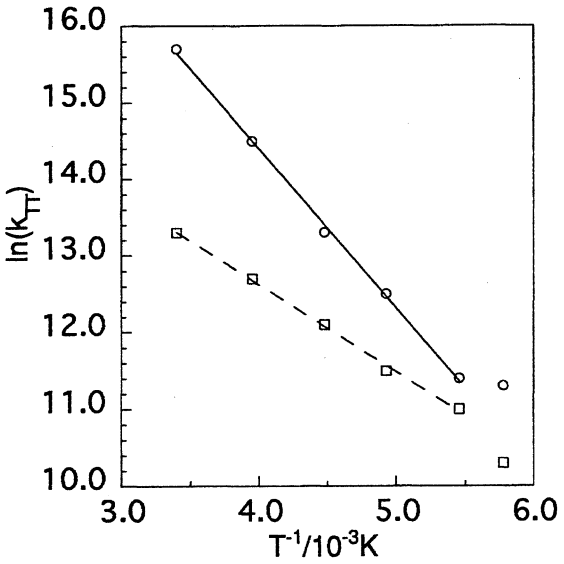


Fig. 11. Temperature dependence of k_{TT} of **3c** (○) and **4c** (□) in THF.

The triplet–triplet energy-transfer reactions are very useful for evaluation of such electron exchange interactions, since the triplet–triplet energy transfer usually proceeds via electron exchange (Dexter) mechanism.[20] The observed k_{TT} rates indicate that electron exchange interaction in **3c**,**d** and **4c**,**d** is both much smaller in comparison to that in **4e**. If we consider that the observed decrease in k_{TT} upon lowering temperature is due mainly to suppression of thermal motion of the large π-electron system, net through-bond electronic interaction should be much smaller in **3c**,**d** and **4c**,**d**. By contrast, the observed large k_{TT} value in **4e** indicates much larger electronic interaction between the two π-systems of the carotenoid and the pyropheophorbide.[10] This is consistent with the molecular structure of **4e**, in which the π-electronic system of the carotenoid is extended to the 7′-phenyl moiety and is connected with the π-electronic system of the pyropheophorbide via an amide linkage, thereby contributing to the large through-bond electron exchange interaction. Therefore, it seems very plausible that the efficient singlet–singlet as well as triplet–triplet energy transfer reported for **4e**[10] stem from this large through-bond electron exchange interaction. In this respect the relatively efficient singlet–singlet energy transfer observed for **4c** with efficiency comparable to that in **4e** is remarkable, since the triplet–triplet energy transfer indicates a only small electron exchange interaction in the former model. Intramolecular molecular motion may have a chance to increase the electron exchange interaction between the two chromophores, but the short lifetime of the singlet-excited state of carotenoid precludes contribution of such molecular motions in the carotenoid to pyropheophorbide singlet energy transfer. In **4c**, there are as many as 5 saturated carbon atoms between the two π-systems and no appreciable stacked conformation. Under these conditions, through-bond and through-space electronic exchange interactions are both normally too small to allow the observed singlet–singlet energy transfer via electron exchange mechanism. In addition, the efficiency of the singlet–singlet energy transfer is still high in rigid MTHF glassy matrix at 77 K, suggesting the static nature of the energy transfer, since large molecular motion should be frozen under these conditions as revealed by the observed no triplet–triplet pyropheophorbide to carotenoid energy transfer.

Therefore, these results tend to suggest that some contribution of the Förster mechanism[21] in the intramolecular singlet–singlet energy transfer from the fucoxanthin to the pyropheophorbide in **3c** and **4c**. We thus attempted to estimate the rate (k_F) of the energy transfer via the Förster mechanism by Eq. 1,

$$k_F = \frac{9000\ (\ln 10)\kappa^2}{128\pi^5 n^4 N \tau_D^0 R^6} \int f_D(\nu)\varepsilon_A(\nu)\nu^{-4}d\nu, \qquad (1)$$

where n is the refractive index of the solvent (1.41 for THF), κ^2 is the orientation factor which is assumed to be 2/3, N is Avogadro's number (mol^{-1}), R is the center-to-center distance (cm),[22] $\varepsilon_A(\nu)$ is the molar extinction coefficient of the pyropheophorbide acceptor ($cm^{-1}M^{-1}$), $f_D(\nu)$ is the spectral distribution of the fluorescence of carotenoid donor normalized such that $\int f_D(\nu)d\nu=1$, and τ_D^0 is the natural fluorescence lifetime of the donor state (s). We calculated the overlap integrals between the S_1-fluorescence of fucoxanthin and the Q_y-absorption bands of pyropheophorbide in **3c** and **4c** to be 6.3 and 8.0×10^{-4} $cm^{-6}\,mol^{-1}$, respectively. Since the corrected fluorescence spectrum of fucoxanthin is available only up to 800 nm and the fluorescence spectrum has a tailing beyond this wavelength, the estimated values of the spectral overlap integral contain some ambiguity. Using these parameters, the k_F values and the efficiency of the singlet–singlet energy transfer from fucoxanthin to pyropheophorbide have been estimated, 8×10^8 s^{-1} and 4×10^{-2} for **3c** and 5×10^9 s^{-1} and 0.2 for **4c**. The estimated energy-transfer efficiencies seems to be in good agreement with the observed trend.

It should be noted here that even in the zeaxanthin-linked dyads **3d** and **4d** some portion of singlet excitation energy of the polyene is actually transferred to the pyropheophorbide. Strictly dipole forbidden nature of the zeaxanthin $S_1\rightarrow S_0$ transition requires short-range electron exchange (Dexter) mechanism, while the long-range dipole–dipole (Förster) mechanism may be possible for the energy transfer from the zeaxanthin S_2 state. In considering the Dexter mechanism, it may be important to note that the carotenoid and the pyropheophorbide in the zeaxanthin-linked dyads **3d** and **4d** are connected by smaller number of saturated carbons compared with the corresponding fucoxanthin-linked dyads **3c** and **4c**. This structural difference may bring about somewhat larger electronic coupling in **3d** and **4d** than **3c** and **4c** via through-bond interaction. This electronic coupling may facilitate the singlet–singlet energy transfer from the dipole forbidden zeaxanthin S_1-state via Dexter mechanism. As the other and additional pathway, the energy transfer from the dipole-allowed zeaxanthin S_2-state may be possible. For β-carotene or zeaxanthin, the fluorescence from the S_2 state has been recently reported.[5,8,17,23—26] In fact, the S_2 fluorescence of zeaxanthin is indeed observed in THF, which has the maximum at 535 nm and has spectral overlap with the high energy Q_x transition of the pyropheophorbide. We thus calculated the spectral overlap in the equation (1); 3.8 and $3.1\times10^{-14}cm^{-6}\,mol^{-1}$ for **3d** and **4d**, respectively. These overlap integrals in turn lead to an estimation of k_F values of $1—2\times10^{10}$ s^{-1} and $4—7\times10^{10}$ s^{-1} for **3d** and **4d**, respectively. These rates, however, predict nearly 0 and only 0.5—1% efficiencies for the singlet energy transfer from the zeaxanthin S_2 excited state since the lifetime of the S_2 state is very short (ca. 200 fs).[8,27,28] This analysis indicates that the energy

transfer from the S_2 state by the Förster mechanism has only small contribution to the observed efficiency of the zeaxanthin to pyropheophorbide singlet energy transfer in **3d** and **4d** and the S_1 state of the zeaxanthin is probably a state responsible for the energy transfer.

The mechanism of the quenching of the singlet excited state of the pyropheophorbide by the zeaxanthin is somewhat unclear at the present stage. The energy levels of the lowest singlet excited states are estimated on the basis of the absorption and fluorescence spectroscopic data to be 1.85 and 1.81 eV for ^{1}(**3b**)* and ^{1}(**4b**)*, respectively. The one-electron oxidation potentials of zeaxanthin and fucoxanthin are 0.16 and 0.35 V vs. E^0 (ferrocene/ferrocenium ion) in CH_3CN, and the one-electron reduction potentials of **3b** and **4b** are −1.46 and −1.32 V, respectively. Thus the energy levels of hypothetical ion pair (carotenoid)$^+$–(pyropheophorbide)$^-$ in THF are estimated to be 1.82, 1.63, 1.97, and 1.78 eV for **3c**, **3d**, **4c**, and **4d**, respectively, by the Born equation.[27] The hypothetical ion pair state thus seems to be lying slightly lower than that of the singlet excited state of the pyropheophorbide in the zeaxanthin-linked dyads **3d** and **4d**. One might envision the fluorescence quenching by intramolecular electron transfer from the zeaxanthin to the singlet excited state of the pyropheophorbide.[28] But we could not detect the relevant ion pair state in the transient absorption spectroscopy. It may be possible that a subsequent charge recombination in (carotenoid)$^+$–(pyropheophorbide)$^-$ is too fast to allow the detection of such an ion-pair state. Otherwise, lower lying ionic states may be mixed with the singlet excited state of the pyropheophorbide, thereby enhancing a nonradiative decay to the ground state. Such a nonradiative decay enhancement has been recently reported for other covalently linked dyads.[29] In either polar quenching mechanism (electron transfer or mixing with lower lying ionic states), the attached fucoxanthin will be a less efficient quencher, since its oxidation potential is more positive in comparison to that of zeaxanthin.

Alternatively, the fluorescence quenching by zeaxanthin may be accounted for by considering the reverse singlet–singlet energy transfer from the ^{1}(pyropheophorbide)* to the zeaxanthin. On the basis of the absorption and emission spectral data, the energy level of ^{1}(fucoxanthin)* is estimated to be 2.00 eV,[7,8,30] being much higher than those of ^{1}(**3b**)* and ^{1}(**4b**)*, while the location of the $S_1(2^1Ag)$ state of zeaxanthin has been still unsettled.[5] Recently ca. 1.80 eV or a lower value has been proposed for β-carotene on the basis of extrapolation of the energy levels of shorter polyenes.[31,32] Given the excitation energy of the S_1-state of zeaxanthin lower than that of ^{1}(pyropheophorbide)*, the fluorescence quenching by zeaxanthin involves the singlet–singlet energy transfer from pyropheophorbide to zeaxanthin. Such fluorescence quenching is not available for fucoxanthin. The observed small temperature dependence in the fluorescence quenching seems to be in line with the energy transfer mechanism rather than the electron transfer mechanism. The decrease in the quenching efficiency upon lowering temperature suggests the possibility of a slightly higher location of the S_1-rate of zeaxanthin relative to that of ^{1}(pyropheophorbide)*. Even under these situations, the decay of the ^{1}(pyropheophorbide)* in **3d** and **4d** can be enhanced by uphill intramolecular energy transfer to the attached zeaxanthin followed by the very rapid internal conversion of ^{1}(zeaxanthin)* to the ground state. Similar fluorescence quenching of porphyrin by carotenoid has been observed in related synthetic carotenoporphyrins.[13] It may be appropriate to note here that we can change the S_1 energy level of pyropheophorbide or chlorophyll pigments by chemical modifications such as introduction of suitable substituents and alteration of π-electronic system. Therefore we will be able to estimate the real location of the elusive, nonemissive 2^1 Ag state of carotenoids by the model approach described here, provided that only intramolecular energy transfer between carotenoid and tetrapyrrole affects the lifetime of the singlet excited state of tetrapyrrole. Such possibilities are currently being vigorously explored in our laboratories.

In summary, fucoxanthin-pyropheophorbide dyads nicely reproduce the antenna function through efficient carotenoid to pyropheophorbide singlet energy transfer as well as inefficient quenching of the ^{1}(pyropheophorbide)*, while the observed poor singlet–singlet energy transfer and substantial quenching of the ^{1}(pyropheophorbide)* in the zeaxanthin-pyropheophorbide seems to be unfavorable for the antenna function but might be suitable for the excitation-energy dissipation. These results reveal a marked difference in the carotenoid-pyropheophorbide singlet excited-state interactions, suggesting,their different biological roles *in vivo*.[25] The rates of the triplet–triplet energy transfer are virtually the same in fucoxanthin- and zeaxanthin-linked pyropheophorbide but depends on the conformational flexibility. Covalently linked natural carotenoid-chlorophyll molecules will offer a new opportunity for detailed studies on the geometric, energetic, and in particular structural requirements of carotenoids for the biological functions of carotenoids in photosynthesis.

Experimental

UV-visible spectra were recorded with a Shimadzu UV-3000 spectrometer and steady-state fluorescence and fluorescence excitation spectra were taken on a Shimadzu RF-502A spectrofluorimeter both at room temperature. Efficiencies of the singlet–singlet energy transfer from the ^{1}(carotenoid)* to the pyropheophorbide were determined as follows; (1) the ratios of the normalized corrected excitation spectrum to the absorption spectrum were determined at an interval

of 10 nm in a range of 440—540 nm and (2) the obtained values were averaged to get the energy transfer efficiency (A).[9,10,15] ^{1}H NMR spectra were recorded on a JEOL GX-400 spectrometer (operating at 400 MHz) or on a JEOL α-500 spectrometer (operating at 500 MHz), chemical shifts being reported in the δ scale in ppm relative to Me_4Si. Mass spectra were recorded on a JEOL HX-110 spectrometer, and the positive-FAB (fast atom bombardment) ionization method was used, accelerating voltage 1.5 kV, Xe atom as the primary ion source. The FAB matrix was 3-nitrobenzyl alcohol/chloroform. Cyclic voltammetry was performed at Pt electrode on Huso Electrochemical System HECS 312B DC-pulse polarogragh and Huso Electrochemical System HECS 321B Potential sweep unit.

Fluorescence lifetimes were measured on 10^{-4} M air-saturated solutions (1 M=1 mol dm^{-3}) by using a picosecond pulsed laser and a picosecond time-correlated single-photon counting system.[33] The laser system was a combination of a mode-locked Nd^{3+} : YAG laser (Spectra Physics 3800), a pulse compressor (Spectra Physics 3690), and a cavity dumped DCM laser (Spectra Physics 3500). The pulse widths of the dye laser was 2 ps (fwhm). The instrumental response function was measured with a pulse width of 30 ps from the scattering light of an aqueous vesicle solution. The fluorescence decay profiles were analyzed by a nonlinear least-squares iterative deconvolution method.

Picosecond transient absorption spectra were measured on 10^{-4} M nitrogen-bubbled solutions by means of a microcomputer-controlled double-beam picosecond spectrometer with a repetitive, mode-locked Nd^{3+} : YAG laser[34] or with a picosecond dye laser (Quantel PTL 10) pumped by the second harmonics (532 nm) of a mode-locked Nd^{3+} : YAG laser (Quantel YG501C).[35] The second harmonic of the Nd^{3+} : YAG laser pulse (24 ps pulse duration) or the 620 nm output of the dye laser (8 ps pulse duration) was used for excitation.

A Q-switched Nd^{3+} : YAG laser (Quantel YG 580) with second harmonic output (532 nm, 15 nm pulse duration) was used for measurements of nano-second transient absorption spectra. The absorption changes of the excited solution were measured using a spectrograph (CT-25C, Japan Spectroscopic Co., Ltd.) equipped with a grating blazed at 1000 nm and with a photomultiplier R3896 (Hamamatsu Photonics). The photocurrent was amplified with a wide-band width amplifier, CLC140 (Comlinear, DC-600 MHz). Solutions of the dyad compounds (ca. 10^{-4} M) were deaerated by bubbling with argon. The details of data-acquitition and temperature control have been described elsewhere.[36]

Synthesis of Dyads 3c,d and 4c,d. Preparative separations were performed by flash column chromatography on silica gel (Merck, Kieselgel 60 H, Art 7736). For synthetic use, dichloromethane was refluxed with and distilled from CaH_2 under nitrogen and stored with molecular sieves 3A. Fucoxanthin was extracted from thali of a brown alga, Dilophus Okamurai with methanol and purified as described.[7] Zeaxanthin in a pure state was kindly gifted from Roche Japan Co. (Tokyo) and was used without any purification. The dry cells of *spirulina maxima* were purchased from Nippon-Ink Co., Ltd. Methyl pyropheophorbide-*a* was isolated from *spilulina* and purified as described[37] and was oxidized to methyl pyropheophrobide-*d* by the literature method.[29] We first attempted the oxidation of methyl pyorpheophorbide-*d* to 3-devinyl-3-carboxy-pyropheophorbide-*a* methyl ester **4a** by the reported method ($KMnO_4$),[38] but we could not get **4a** in a pure state. We thus employed a combination of sulfamic acid and $NaClO_2$[39] for the oxidative transformation with some modification. In order to suppress the chlorination at the δ-position, 1,3-dimethoxybenzene was added as a scavenger.

3-Devinyl-3-carboxy-pyropheophorbide-*a* Methyl Ester 4a. To a solution of pyropheophorbide-*d* methyl ester in THF (60 ml) were added sulfamic acid (3.6 mmol) in water (5 ml) and resorsinol dimethyl ether (76 mmol). The resulting solution was stirred for 10 min. Then $NaClO_2$ (0.46 mmol) in water (5 ml) was added to this mixture. After 1 h, the solution was poured into water and extracted with AcOEt. The organic layer was washed with water, then saturated NaCl solution, dried over Na_2SO_4 and evaporated. It was purified by chromatography with $CHCl_3$-1% MeOH as eluent and crystalized to give 144 mg of **4a** (67%). ^{1}H NMR ($CDCl_3$) δ=10.52 (s, 1H, *meso*-H), 9.64 (s, 1H, *meso*-H), 8.87 (s, 1H, *meso*-H), 5.36 (d, J=20.0 Hz, 1H, 13^2-H), 5.20 (d, J=19.5 Hz, 1H, 13^2-H), 4.59 (m, 1H, 18-H), 4.39 (m, 1H, 17-H), 3.80 (s, 3H, methyl), 3.74 (s, 3H, methyl), 3.74 (q, 2H, 8-ethyl), 3.62 (s, 3H, 17-Me ester), 3.33 (s, 3H, methyl), 2.8, 2.6, 2.3 (m, 1H, 1H, 2H, 17-CH_2CH_2), 1.85 (d, J=7.3 Hz, 3H, 18-Me), 1.73 (t, J=7.8 Hz, 3H, 8-ethyl), −2.1 (br s, NH).

Dyad 3c. To a 20 mL flask were added 11.7 mg (22 μmol) of pyropheophorbide-*a* (**3a**), 14.0 mg (21 μmol) of fucoxanthin, 10.2 mg (40 μmol) of 2-chloro-1-methylpyridinium iodide, 10.6 mg (87 μmol) of dimethylaminopyridine and 10 mL of dichloromethane. After being refluxed for 3 h under argon shaded from light, the solution was chromatographed on silica gel with chloroform. Recrystallization from dichloromethane and hexane gave 5.6 mg (23%) of dyad **3c**. The ^{1}H NMR data (Tables 1 and 2) of **3c,d** and **4c,d** were based on the reported chemical shifts for fucoxanthin.[40] Mp 136—139 °C. ^{1}H NMR ($CDCl_3$) δ=9.52 (s, 1H, 10-H), 9.41 (s, 1H, 5-H), 8.56 (s, 1H, 20-H), 8.02 (dd, J=11.6 Hz, J=17.7 Hz, 1H, 3-vinyl), 7.09 (d, J=10.9 Hz, 1H, 10C–H), 6.74 (dd, J=13.1 Hz, J=13.1 Hz, 1H, 15′C–H), 6.63 (d, J=14.7 Hz, 1H, 12C–H), 6.62 (m, 1H, 15C–H), 6.60 (m, 1H, 11′C–H), 6.52 (m, 1H, 11C–H), 6.39 (d, J=12.2 Hz, 1H, 14C–H), 6.34 (d, J=14.7 Hz, 1H, 12′C–H), 6.30 (d, J=18.9 Hz, 1H, 3-vinyl), 6.26 (d, J=12.2 Hz, 1H, 14′C–H), 6.18 (d, J=11.6 Hz, 1H, 3-vinyl), 6.12 (d, J=11.6 Hz, 1H, 10′C–H), 6.05 (s, 1H, 8′C–H), 5.38 (m, 1H, 3′C–H), 5.27 (d, J=19.6 Hz, 1H, 13^2-H), 5.11 (d, J=20.1 Hz, 1H, 13^2-H), 4.81 (m, 1H, 3C–H), 4.50 (m, 1H, 18-H), 4.31 (m, 1H, 17-H), 3.71 (q, J=7.6 Hz, 2H, 8-ethyl), 3.68 (s, 3H, 12-Me), 3.60 (d, J=18.3 Hz, 1H, 7C–H), 3.42 (s, 3H, 2-Me), 3.26 (s, 3H, 7-Me), 2.69 (m, 1H, 17-CH_2CH_2), 2.55 (d, J=18.3 Hz, 1H, 7C–H), 2.49, 2.30 (m, 1H, 2H, 17-CH_2CH_2), 2.28 (m, 1H, 4′C–H_{eq}), 2.24 (m, 1H, 4C–H_{eq}), 2.04 (s, 3H, 3′C–acetyl), 2.00 (m, 1H, 2′C–H_{eq}), 1.98 (s, 3H, 20C–H), 1.97 (s, 3H, 20′C–H), 1.89 (s, 3H, 19C–H), 1.81 (s, 3H, 19′C–H), 1.81 (d, 3H, 18-Me), 1.73 (m, 1H, 4C–H_{ax}), 1.71 (t, J=7.6 Hz, 3H, 8-ethyl), 1.52 (m, 1H, 4′C–H_{ax}), 1.42 (m, 1H, 2′C–H_{ax}), 1.38 (s, 3H, 16′C–H), 1.38 (m, 1H, 2C–H_{eq}), 1.35 (s, 3H, 18′C–H), 1.21 (m, 1H, 2C–H_{ax}), 1.14 (s, 3H, 18C–H), 1.07 (s, 3H, 17′C–H), 0.98 (s, 3H, 16C–H), 0.83 (s, 3H, 17C–H), −1.7 (br s, NH). HRMS (FAB) Found : m/z 1174.684. Calcd for $C_{75}H_{90}N_4O_8$: M, 1174.676 (M^+).

Dyad 3d was prepared as described in preparation of **3c**, using 7.3 mg (14 μmol) of pyropheophorbide-*a* methyl ester and 14.0 mg (21 μmol) of zeaxanthin. **3d**; (3.9 mg, 26%). Mp 140—144 °C. ^{1}H NMR ($CDCl_3$) δ=9.53 (s, 1H, 10-H), 9.41 (s, 1H, 5-H), 8.57 (s, 1H, 20-H), 8.02 (dd, J=11.5 Hz, J=17.8 Hz, 1H, 3-vinyl), ca. 6.64 (m, 1H, 11′C–H), ca. 6.62 (m, 1H, 11C–H), ca. 6.62 (m, 1H+ 1H, 15C–H+ 15′C–H), 6.36 (d, J=14.6 Hz, 1H, 12′C-H), 6.34 (d, J=14.7 Hz, 1H, 12C–H), 6.30 (dd, J=18.1 Hz, J=1.4 Hz, 1H, 3-vinyl), ca. 6.24 (m, 2H, 14C–H+ 14′C–H), 6.18 (dd, J=11.5 Hz, J=1.2 Hz, 1H, 3-vinyl), 6.15 (d, J=12.9 Hz, 1H, 10′C–H), 6.12 (d, J=13.4 Hz, 1H, 10C–H), 6.2—6.1 (2H, 7′C–H+ 8′C–H), 6.1—6.0 (2H, 7C–H+ 8C–H), 5.29 (d, J=19.5 Hz, 1H, 13^2-H), 5.12 (d, J=20.0 Hz, 1H, 13^2-H), 4.99 (m, 1H, 3C–H), 4.52 (m, 1H, 18-H), 4.33 (m, 1H, 17-H), 3.99 (m, 1H, 3′C–H), 3.71 (q, J=7.8 Hz, 2H, 8-ethyl), 3.69 (s, 3H, 12-Me), 3.42 (s, 3H, 2-Me), 3.26 (s, 3H, 7-Me), 2.70, 2.53 (m, 1H, 1H, 17-CH_2CH_2), 2.39 (m, 1H, 4C′–H_{eq}), 2.32 (m, 1H, 4C–H_{eq}), 2.27 (m, 2H, 17-CH_2CH_2), 2.02 (m, 1H, 4′C–H_{ax}), 1.97 (s, 3H+ 3H+ 3H, 19′C–H+ 20C–H+ 20′C–H), 1.95 (m, 1H, 4C–H_{ax}), 1.93 (s, 3H, 19C–H), 1.82 (d, J=6.8 Hz, 3H, 18-Me), 1.77 (m, 1H, 2′C–H_{eq}), 1.73 (s, 3H, 18′C–H), 1.71 (t, J=7.8 Hz, 3H, 8-ethyl), 1.65 (s, 3H, 18C–H), 1.60 (m, 1H, 2C–H_{eq}), 1.46 (m, 1H, 2′C–H_{ax}), 1.40 (m, 1H, 2C–H_{ax}), 1.07 (s, 3H+ 3H, 16′C–H+ 17′C–H), 1.02, 0.96 (s, 3H, 3H, 16C–H+ 17C–H), −1.7 (br s, NH). HRMS (FAB) Found: m/z 1084.677. Calcd for $C_{73}H_{88}N_4O_4$: M, 1084.681 (M^+).

Dyad 4c was prepared as described in preparation of **3c**, using 10.7 mg (19 μmol) of 3-devinyl-3-carboxy-pyropheophorbide-*a* methyl ester and 11.9 mg (18 μmol) of fucoxanthin. **4c**; (3.4 mg, 15%). Mp 166—170 °C. ^{1}H NMR ($CDCl_3$) δ=10.46, 9.63 (s, 1H, 1H, 5-H+ 10-H), 8.81 (s, 1H, 20-H), 7.25 (d, 1H, 10C–H), 6.77 (m, 1H, 15′C–H), 6.72 (d, 1H, 12C–H), 6.62 (m, 1H, 11C–H), 6.62 (m, 1H, 11′C–H), 6.45 (d, J=10.7 Hz, 1H, 14C–H), 6.36 (d, J=15.1 Hz, 1H, 12′C–H), 6.29 (d, J=11.7 Hz, 1H, 14′C–H), 6.14 (d, J=10.7 Hz, 1H, 10′C–H), 6.06 (s, 1H, 8′C-H), 5.66 (m, 1H, 3C–H), 5.40 (m, 1H, 3′C–H), 5.34 (d, J=19.5 Hz, 1H, 13^2-H), 5.18 (d, J=20.0 Hz, 1H, 13^2-H), 4.56 (m, 1H, 18-H), 4.37 (m, 1H, 17-H), 3.81 (d, J=18.6 Hz, 1H, 7C–H), 3.76 (q, J=7.3 Hz, 2H, 8-ethyl), 3.73, 3.71 (s, 3H, 3H, 2-Me+ 12-Me), 3.61 (s, 3H, 17-CO_2Me), 3.34 (s, 3H, 7-Me), 2.96 (m, 1H, 4C–H_{eq}), 2.75 (d, J=18.6 Hz, 1H, 7C–H), 2.73, 2.60 (m, 1H, 1H, 17-CH_2CH_2), 2.44 (dd, J=9.8 Hz, J=13.7 Hz, 1H, 4C–H_{ax}), 2.32 (m, 2H, 17-CH_2CH_2), 2.29 (m, 1H, 4′C–H_{eq}), 2.14 (m, 1H, 2C–H_{eq}), 2.04 (s, 3H, 3C-acetyl), 2.02 (s, 3H, 20C–H), 2.01 (s, 3H, 19C–H), 2.01 (s, 3H, 20′C–H), 2.00 (m, 1H, 2′C–H_{eq}), 1.98 (m, 1H, 2C–H_{ax}), 1.84 (d, J=7.3 Hz, 3H, 18-Me), 1.82 (s, 3H, 19′C–H), 1.73 (t, J=7.5 Hz, 3H, 8-ethyl), 1.52 (m, 1H, 4′C-H_{ax}), 1.42 (m, 1H, 2′C–H_{ax}), 1.41 (s, 3H, 18C–H), 1.39 (s, 3H, 16′C–H), 1.36 (s, 3H, 18′C–H), 1.33 (s, 3H, 16C–H), 1.15 (s, 3H, 17C–H), 1.08 (s, 3H, 17′C–H), −2.04 (br s, NH). HRMS (FAB) Found: m/z 1206.668. Calcd for $C_{75}H_{90}N_4O_{10}$: M, 1206.666 (M^+).

Dyad 4d was prepared as described in preparation of **3c**, using 5.6 mg (9.9 μmol) of 3-devinyl-3-carboxy-pyropheophorbide-*a* methyl ester and 10.3 mg (18 μmol) of zeaxanthin. **4d**; (2.5 mg, 23%). Mp 151—155 °C. ^{1}H NMR ($CDCl_3$) δ=10.49, 9.63 (s, 1H, 1H, 5-H+ 10-H), 8.81 (s, 1H, 20-H), 6.69 (m, 1H, 11C–H), ca. 6.66 (m, 2H, 15C–H+ 15′C–H), 6.65 (m, 1H, 11′C–H), 6.42 (d, J=15.1 Hz, 1H, 12C–H), 6.37 (d, J=14.7 Hz, 1H, 12′C–H), ca. 6.26 (d, 2H, 11C–H+ 11′C–H), 6.27, 6.23 (d, 1H+ 1H, 7C–H+ 8C–H), 6.25 (d, 1H, 10C–H), 6.17 (d, J=11.2 Hz, 1H, 10′C–H), 6.14, 6.10 (d, 1H+ 1H, 7′C–H+ 8′C–H), 5.85 (m, 1H, 3C–H), 5.34 (d, J=20.0 Hz, 1H, 13^2-H), 5.19 (d, J=20.0 Hz, 1H, 13^2-H), 4.57 (m, 1H, 18-H), 4.37 (m, 1H, 17-H), 4.00 (m, 1H, 3′C–H), 3.76 (q, J=7.5 Hz, 2H, 8-ethyl), 3.73 (s, 3H+ 3H, 2-Me+12-Me), 3.61 (s, 3H, 17-CO_2Me), 3.34 (s, 3H, 7-Me), 3.02 (dd, J=16.6 Hz, J=5.4 Hz, 1H, 4C–H_{eq}), 2.74 (m, 1H, 17-CH_2CH_2), 2.71 (m, 1H, 4C–H_{ax}), 2.60 (m, 1H, 17-CH_2CH_2), 2.38 (m, 1H, 4′C–H_{eq}), 2.36 (m, 1H, 2C–H_{eq}), 2.32 (m, 2H, 17-CH_2CH_2), 2.16 (dd, J=12.5 Hz, J=12.5 Hz, 1H, 2C–H_{ax}), 2.05 (m, 1H, 4′C–H_{ax}), 2.05 (s, 3H, 19C–H), 2.01 (s, 3H, 20C–H), 1.99 (s, 3H, 20′C–H), 1.98 (s, 3H, 19′C–H), 1.92 (s, 3H, 18C–H), 1.84 (d, J=6.8 Hz, 3H, 18-Me), 1.77 (m, 1H, 2′C–H_{eq}), 1.74 (s, 3H, 18′C–H), 1.73 (t, J=7.8 Hz, 3H, 8-ethyl), 1.48 (dd, J=11.9 Hz, J=11.9 Hz, 1H, 2′C–H_{ax}), 1.37, 1.27 (s, 3H, 3H, 16C–H+ 17C–H), 1.08 (s, 3H+ 3H, 16′C–H+ 17′C–H), −2.04 (br s, NH). HRMS (FAB) Found: m/z 1116.667. Calcd for $C_{73}H_{88}N_4O_6$: M, 1116. 671.

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