## RESEARCH NOTE

# EVIDENCE FOR THE FORMATION OF A CHLOROPHYLL a/ZEAXANTHIN COMPLEX IN LECITHIN LIPOSOMES FROM FLUORESCENCE DECAY KINETICS

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Abstract—The interaction of Chl a with zeaxanthin (Zea), which is an analogue of lutein, has been studied in soya bean lecithin liposomes using the fluorescence of Chl as monitor. The fluorescence emission spectrum at 4.2 K of Chl a showed characteristic changes in the presence of Zea: the emission maximum shifted from 688 nm to 680 nm, and a peak at 731 nm appeared. The fluorescence decay kinetics of Chl a alone could be described by the sum of two exponential components ( $\tau_1 \approx 0.8$  ns,  $\tau_2 \approx 2.5$  ns). In the presence of Zea a component with a long lifetime,  $\tau \approx 5$  ns, appeared with a large relative amplitude (40%). This indicated the formation of a Chl a/Zea complex, in which Chl a/Chl a interaction is negligible, presumably because of strong interaction between Chl a and Zea. The fluorescence anisotropy decay kinetics supported the hypothesis of the formation of a large Chl a containing complex in the presence of Zea. A rotational correlation time,  $\phi \approx 14$  ns at  $4^{\circ}$ C and  $\phi \approx 21$  ns at  $30^{\circ}$ C, was found, which is distinctly larger than for samples containing Chl a only. We interpret these results as further evidence for a strong interaction between Chl a and Zea in the hydrophobic environment of the lecithin liposomes. This interaction may also occur in the Chl-proteins of the Chl a/b light-harvesting complex of plant photosynthesis.

## INTRODUCTION

Chlorophyll (Chl)† a is the most important pigment in plant photosynthesis. It occurs in the light-harvesting antenna complexes, and is also an essential component of the photosynthetic reaction centre complexes. Thornber  $et\ al$ . (1987) have given a review of the types of complexes in plants, and their nomenclature. In the antenna its function is to absorb light and transfer singlet excitation to the reaction centre, where primary charge separation takes place. The Chl a is associated with other pigments (Chl b and carotenoids) in the Chl-protein complexes, and the interaction between these constituents determines to a large extent how the complexes function in photosynthesis.

The carotenoids can be divided into unsubstituted carotenes and xanthophylls.  $\beta$ -Carotene functions as the main accessory light-harvesting pigment in the Chl a-containing reaction centre antenna complexes transferring its excitation energy to Chl a by an exchange mechanism (Naqvi, 1980). It also protects against photo-inactivation of the Chl by accepting triplet excitation energy. In the reaction centre  $\beta$ -carotene has been suggested to play an

indirect role in electron transfer (Searle and Wessels, 1978). The xanthophylls—lutein, neoxanthin, and violaxanthin—also play a role in plant photosynthesis. They are more abundant in the Chl a/b light-harvesting complexes than in the reaction centre antenna complexes. Of these xanthophylls lutein is by far the most important (Wessels, 1968; Braumann et al., 1982). Lutein has been shown to transfer excitation energy to Chl a (Siefermann-Harms and Ninneman, 1982), and could also act together with \beta-carotene to deactivate Chl triplet states. This suggests a strong interaction between lutein and Chl, but the character of this interaction is still unclear. It could be primarily due to the proteins holding the two pigments at the required distance and orientation, or to a direct interaction, such as H-bonding, between them. The only active groups in lutein which could play a role in specific bond formation are the two hydroxyls, present one on each of the rings. It is also possible that the long unsaturated backbone of lutein interacts hydrophobically with the phytyl side-chain of Chl. The interaction takes place within the hydrophobic interior of the proteins, and this is presumably an important factor. Therefore if we wish to model these interactions it is essential to use a model hydrophobic environment.

Studies of light-induced electron transfer in bilayer systems containing a variety of carotenoids and Chl have shown that the presence of zeaxanthin

<sup>\*</sup>To whom correspondence should be addressed.  $†Abbreviations: A_0$ , initial anisotropy; Chl, chlorophyll; Zea, zeaxanthin.

(Zea) results in the largest photocurrents (Rich and Brody, 1982). Studies of the kinetics of absorption changes induced by 610 nm light have shown that Chl a transfers triplet energy to zeaxanthin when they were present together in lecithin liposomes at room temperature (Brody, 1984; Brody et al., 1986). Zeaxanthin is an analogue of lutein, differing only in the position of the double bond in one of the rings, and it is a physiologically important plant carotenoid (Demmig et al., 1988; Demmig-Adams et al., 1989; Bilger et al., 1989). The ratio of Zea/ Chl a used in the liposome model system (Brody, 1984; Brody et al., 1986) is higher than that found in vivo (Wessels, 1968; Braumann et al., 1982). However, the overall in vivo ratio can be misleading, as specific interactions and locally high concentrations of Zea within the Chl-proteins are likely. Two components could be observed in the absorption kinetics, attributed to the decay of <sup>3</sup>Chl and <sup>3</sup>Zea respectively (Brody, 1984). Light of wavelength 610 nm excites Chl a only, as Zea has no absorption in the red. Therefore <sup>3</sup>Zea must be formed indirectly, probably via intersystem crossing from excited singlet Chl a, followed by triplet excitation energy transfer to Zea. <sup>3</sup>Zea could in principle also be formed by electron transfer from Zea to Chl a (Beddard et al., 1977), followed by spin dephasing and recombination to the Zea molecular triplet state, although there is no direct evidence for this process. In both cases a very close association betwen Chl a and Zea would be required, which therefore implies the formation of a complex between them. This takes place in the absence of the ordering induced by the proteins of the naturally occurring systems, illustrating the strength of the interaction between Chl a and Zea.

The triplet energy transfer was found to be a function of temperature and pigment concentration (Brody, 1984). The results at low Zea/Chl a ratios were interpreted in terms of a greater fluidity of the liposome at higher temperature, which allowed an increased probability of interaction between freely migrating Chl a and Zea. At higher Zea/Chl a ratios, on the other hand, triplet energy transfer was independent of temperature, and a Chl a/Zea complex appeared to be formed. The dihydroxy-carotenes, zeaxanthin and lutein, showed the largest temperature-induced shifts in carotenoid absorption at 520 nm, of the carotenoids tested, when present in liposomes together with Chl a (Brody et al., 1986).

The purpose of the present work is to investigate, by the use of time-resolved fluorescence emission and anisotropy techniques, the interaction of Zea with Chl a and the state of aggregation of the Chl a in a hydrophobic environment at high ratios of Zea/Chl a. Fluorescence emission decay kinetics have been used widely to study the state of aggregation of Chl a in lipid systems (e.g. Beddard et al., 1976), and fluorescence anisotropy decay kinetics can give

information on the relative molecular sizes of Chl a-containing complexes.

The structure and conformation of the carotenoid can be expected to be very critical in its interaction with Chl a so that extrapolation of our results to the major chloroplast carotenoids,  $\beta$ -carotene and lutein, should perhaps be done with caution. A systematic study of the effect of known changes in carotenoid structure and conformation on Chl a/carotenoid interaction should be the subject of future research.

#### MATERIALS AND METHODS

The samples were prepared from soya bean lecithin, Chl a and Zea as previously described (Brody, 1984). Soya bean lecithin was obtained from Sigma Chemical Company (St Louis, MO). Chl a was isolated and purified as described by Aghion et al. (1969). Zea was obtained from Hoffmann-La Roche Ltd (Basle, Switzerland). The dried mixture of pigments and lipid was resuspended in 50 mM phosphate buffer, pH 7.6 using a Branson B15 sonifier for 5 min running on power 3 and 40% duty cycle, the sample being cooled with ice and maintained in a N<sub>2</sub> atmosphere to prevent pigment degradation.

The pigment composition of the samples relative to 24 parts of lecithin was: I, 1 Chl a and 2 Zea; II, 1 Chl a; III 3 Chl a. Control samples contained either no pigments, or 2 Zea.

Steady state fluorescence spectra were measured at 4.2 K using a laboratory-built set-up described by Van der Bent *et al.* (1975). The reproducibility of the wavelength scale of the monochromator (Spex 0.25 m) was  $\pm 1$  nm.

The fluorescence emission and anisotropy decay kinetics were measured using the single photon counting set-up described previously (Van Hoek et al., 1983). Briefly, the samples were excited with 610 nm light from a Rhodamine 6G dye laser, synchronously pumped by a mode-locked Ar ion laser. This excitation wavelength is absorbed exclusively by Chl a. The excitation pulse rate was reduced to 298 kHz using a laboratory-built set-up with an electooptical modulator (Van Hoek and Visser, 1981). The rate of detection of photons emitted from the sample was kept below a maximum of 15 kHz in order to prevent distortion of the detected kinetics due to pile-up. The time resolution of the data collection system was set at 23 ps/channel of the multi-channel analyser. Care was taken to correct for the background fluorescence from the lecithin and Zea. The data were analysed for up to three exponential components, the fitting algorithm minimising the weighted residuals ( $\chi^2$ ). The goodness of fit was also judged from Durbin-Watson values and the number of zero passages in the autocorrelation function (Ameloot and Hendrickx, 1982).

## RESULTS

The steady state fluorescence emission spectra of samples I, II, and III are shown in Fig. 1. These spectra were measured at low temperature (4.2 K) in order to increase the sharpness of the bands. Comparison of curve a with curve c clearly shows the effect of the presence of Zea in shifting the Chl a emission maximum to 680 nm from 688 nm (seen for Chl a alone at the same concentration). If liposomes containing Chl a and Zea separately were mixed, resonicated and left at room temperature for 1–2 h the Chl emission maximum shifted, but

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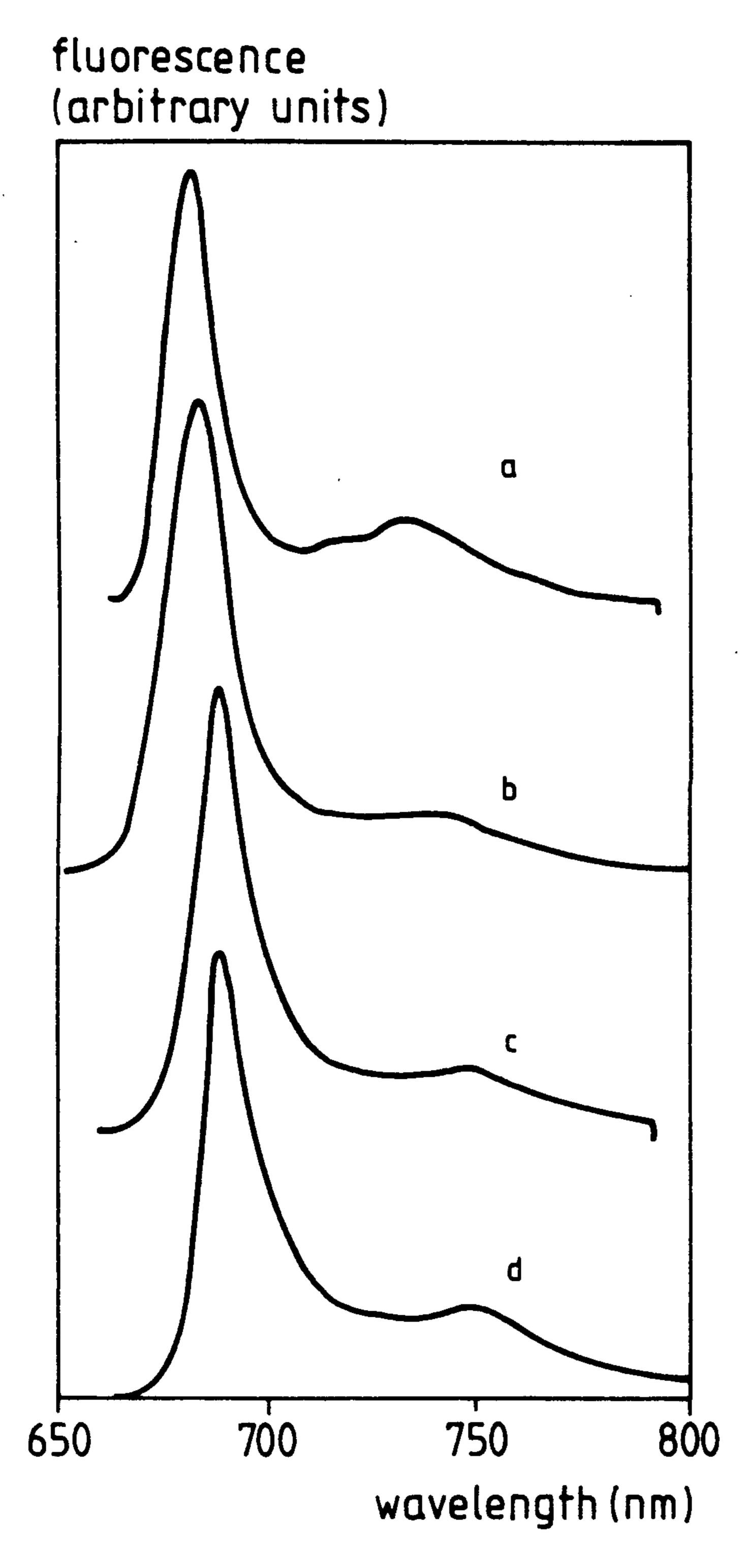


Figure 1. Fluorescence emission spectra of Chl a in lecithin liposomes. Temperature 4.2 K, excitation 610 nm, emission bandwidth 5 nm. (a) sample I, Chl a + Zea. (b) samples containing Chl a and Zea resonicated together. (c) sample II, Chl a. (d) sample III,  $3 \times concentrated$  Chl a.

only to 682 nm (curve b). The emission spectrum of the sample containing a higher concentration of Chl a (curve d) showed a maximum at 690 nm. The fluorescence emission spectra also show interesting differences in the 710–750 nm region. Sample I had bands at 713 and 731 nm, whilst Chl a alone showed a peak at 745 nm and a small second band at  $\approx$ 725 nm.

Chl a fluorescence kinetics in liposomes were measured at two temperatures (4°, 30°C) and at two wavelengths (679, 712 nm), and the results are given in Tables 1, 2 and 3.

The fluorescence decay in the absence of Zea (Table 1) could be adequately described by two

Table 1. Fluorescence decay kinetics of Chl a in liposomes (sample II)

	Condition	S		Compone	nt index		
		1		1			
T (°C)	λ <sub>em</sub> (nm)	φrel φf	α	7	α	7	
4	679	1.628	0.52	0.80	0.48	2.51	
4	712	1.674	0.47	0.74	0.53	2.51	
30	679	1.641	0.49	0.83	0.51	2.42	
30	712	1.742	0.45	0.89	0.55	2.45	

Lifetimes are in ns. Typical errors in the lifetimes  $(\tau_i)$  is  $\pm 5\%$ , and in the amplitudes  $(\alpha_i)$  is 8%.  $\phi_f^{rel}$  is the relative fluorescence yield:  $\Sigma \alpha_i \tau_i$ .

exponential components with lifetimes  $\tau_1 \approx 0.8$  and  $\tau_2 \approx 2.5$  ns, having approximately equal amplitudes. Some small variations were apparent:  $\tau_1$  increased and  $\tau_2$  decreased at the higher temperature; also  $\alpha_2$  was slightly larger at the higher temperature and at the longer emission wavelength.

The effect of increasing Chl a concentration on the fluorescence decay kinetics is shown in Table 2. The kinetics were described adequately by three components with lifetimes:  $\tau_1 \approx 0.25$  ns,  $\tau_2 \approx 0.8$  ns, and  $\tau_3 \approx 1.5$  ns.

The fluorescence decay kinetics are clearly different in the presence of Zea (Table 3), compared to sample II (Table 1). Three exponential components were required to describe adequately the kinetics, and the new component ( $\tau_3$ ) had a lifetime of  $\approx 5$  ns and a large amplitude (35–40%). The lifetimes of the faster components,  $\tau_1 \approx 0.3$  ns and  $\tau_2 \approx 2.0$  ns, were shorter than when Zea was absent ( $\tau_1 \approx 0.8$  ns and  $\tau_2 \approx 2.5$  ns).

The experimental data on the kinetics of fluorescence anisotropy decay are presented in Tables 4, 5 and 6.

Two components were resolved for sample II, with  $\phi_1 \approx 0.3$  ns and  $\phi_2 \approx 5$  ns (Table 4).  $\phi_2$  showed a tendency to shorten at higher temperature as might be expected if it were associated with the motion of large aggregates of Chl a in the lipid

Table 2. Fluorescence decay of Chl a (concentrated) in liposomes (sample III)

	Conditions		Component index						
			1				3		
T (°C)	λ <sub>em</sub> (nm)	φrei	α	7	α	7	α	7	
4	679	0.552	0.57	0.25	0.35	0.81	0.09	1.52	
4	712	0.611	0.57	0.28	0.36	0.90	0.07	1.78	
30	679	0.568	0.49	0.22	0.40	0.76	0.11	1.45	
30	712	0.658	0.43	0.28	0.44	0.79	0.12	1.51	

Lifetimes are in ns. Typical errors in the lifetimes  $(\tau_i)$  is  $\pm 5\%$ , and in the amplitudes  $(\alpha_i)$  is 8%.  $\phi_i^{rel}$  is the relative fluorescence yield:  $\Sigma \alpha_i \tau_i$ .

bilayer. The amplitudes ( $\beta$ ) hardly varied, except that  $\beta_1$  was larger in 679 nm emission, resulting in the larger initial anisotropy ( $A_0$ ) at this wavelength.

The anisotropy decay of sample III (Table 5) could be fitted adequately by two components.  $A_0$  is lower than for sample II, and  $\phi_1$  showed extremely small values:  $\phi_1 \approx 20$  ps at 30°C.

Anisotropy measurements on sample I are presented in Table 6.  $A_0$  was distinctly higher than for sample II, and furthermore three components were

needed to describe adequately the anisotropy decay. The shortest component had a rotational correlation time,  $\phi_1 \approx 0.2$ –0.3 ns, similar to that for Chl a alone. The second component had  $\phi_2 \approx 1$ –2 ns with a relatively constant amplitude. The longest component is the most interesting as its correlation time,  $\phi_3 \approx 14$  ns at 4°C and  $\approx 21$  ns at 30°C, was well outside the range of values seen for Chl a alone (samples II and III). The value of  $\phi_3$  increased at 30°C, although the amplitude ( $\beta_3$ ) was then smaller.

Table 3. Fluorescence decay kinetics of Chl a/Zea in liposomes (sample I)

Conditions			Component index						
			1		2		3		
T (°C)	λ <sub>em</sub> (nm)	φrel	α	7	α		α	7	
4	679	2.731	0.38	0.32	0.22	2.20	0.39	5.37	
4	712	1.981	0.54	0.22	0.15	1.70	0.31	5.19	
<b>30</b>	679			0.41	0.25	2.07	0.44	5.01	
<b>30</b>	712	2.126	0.47	0.25	0.19	1.82	0.34	4.94	

Lifetimes are in ns. Typical errors in the lifetimes  $(\tau_i)$  is  $\pm 5\%$ , and in the amplitudes  $(\alpha_i)$  is 8%.  $\phi_i^{rel}$  is the relative fluorescence yield:  $\Sigma \alpha_i \tau_i$ .

Table 4. Fluorescence anisotropy of Chl a in liposomes (sample II)

	Conditions			Compone	nt index
T (°C)	λ <sub>em</sub> (nm)	$A_0$		1	2
4	679 .	0.097	β	$0.08 \pm 0.01$	$0.02 \pm 0.003$
4	712	0.072	φβ	$0.29 \pm 0.04$ $0.05 \pm 0.005$	$5.74 \pm 1.65$ $0.02 \pm 0.003$
30	679	0.106	φβ	$0.30 \pm 0.07$ $0.09 \pm 0.004$	$5.38 \pm 1.38$ $0.02 \pm 0.003$
30	712	0.078	φβ	$0.28 \pm 0.03$ $0.07 \pm 0.007$	$5.19 \pm 1.46$ $0.02 \pm 0.003$
			ф	$0.20 \pm 0.04$	$3.99 \pm 0.80$

 $<sup>\</sup>phi$  is the correlation time in ns, and  $\beta$  is the amplitude of the anisotropy component.

Table 5. Fluorescence anisotropy of Chl a (concentrated) in liposomes (sample III)

	Condition	S		Component index		
T (°C)	λ <sub>em</sub> (nm)	$A_0$		1	2	
4	679	0.053	β	$0.07 \pm 0.04$	$0.02 \pm 0.007$	
			ф	$0.06 \pm 0.08$	$0.94 \pm 0.41$	
4	712	0.034	β	$0.03 \pm 0.007$	$0.01 \pm 0.003$	
			ф	$0.14 \pm 0.09$	$3.50 \pm 2.32$	
30	679	0.044	β	$0.09 \pm 0.05$	$0.02 \pm 0.006$	
			ф	$0.02 \pm 0.08$	$0.99 \pm 0.40$	
30	712	0.044	β	$0.08 \pm 0.01$	$0.01 \pm 0.002$	
			ф	$0.02 \pm 0.04$	$4.28 \pm 2.05$	

 $<sup>\</sup>phi$  is the correlation time in ns, and  $\beta$  is the amplitude of the anisotropy component.

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Table 6. Fluorescence anisotropy of Chl a/Zea in liposomes (sample I)

	Conditions			Component index			
T (°C)	λ <sub>em</sub> (nm)	$A_0$		1	2	3	
4	679	0.156	β	$0.10 \pm 0.21$	$0.05 \pm 0.02$	$0.06 \pm 0.01$	
4	712	0.109	φβ	$0.20 \pm 0.46$ $0.004 \pm 0.025$	$1.09 \pm 0.67$ $0.04 \pm 0.01$	$13.3 \pm 2.4$ $0.05 \pm 0.005$	
30	679	0.176	φβ	$0.23 \pm 0.97$ $0.09 \pm 0.01$	$1.23 \pm 0.55$ $0.05 \pm 0.01$	$14.5 \pm 2.5$ $0.03 \pm 0.02$	
30	712	0.113	φβ	$0.36 \pm 0.13$ $0.008 \pm 0.025$	$2.53 \pm 1.30$ $0.06 \pm 0.01$	$20.4 \pm 17.0$ $0.03 \pm 0.01$	
			ф	$0.19 \pm 0.74$	$1.64 \pm 0.39$	$21.8 \pm 9.3$	

 $\phi$  is the correlation time in ns, and  $\beta$  is the amplitude of the anisotropy component.

We postulate that the appearance of the longest component,  $\tau_3 \approx 5$  ns, in the emission decay kinetics, and of  $\phi_3 \approx 14-21$  ns in the anisotropy decay kinetics, is the result of the formation of a Chl a/Zea complex when Zea is present.

#### **DISCUSSION**

# Sample homogeneity

It is almost certain that Chl a in sample I, which contains both Chl a and Zea, is heterogeneous, and that we have to consider the presence of several discrete emitting species. The known inhomogeneity of the lipid composition of soya bean lecithin is a likely cause of this. This lecithin was used, however, because it was found that stable, reproducible liposomes were then produced. Bilayers and liposomes formed with pure phospholipids and Zea were observed to be difficult to form and were moreover unstable (Rich and Brody, 1982). There are probably aggregates of Chl a of various sizes, together with the postulated Chl a/Zea complex. The distribution of Chl a between these species is expected to be dependent on pigment concentration and also on temperature. The fluorescence decay kinetics therefore contains components from different physical species. Although it is unlikely that the exchange of pigment between these species is fast enough to affect the fluorescence kinetics, the relative amplitudes of the components attributed to emission from the discrete species will vary with the temperature and detection wavelength.

Another important practical consideration is the homogeneity of the pigments used. It is known that available preparations of Zea are in fact a mixture of isomers, which are difficult to separate. These isomers could interact in different ways with the Chl a. Although the Chl a used was a purified sample, isomerisation reactions or formation of traces of pheophytin during the measurements or during the preparation also cannot be strictly ruled out.

It is important therefore to keep in mind the possibility that these heterogeneities may complicate the interpretation of the fluorescence decay kinetics.

## Fluorescence emission decay kinetics

The ratio of Chl a to Zea used in the present experiments is in the range that has been found by absorption measurements to suggest the formation of a complex (Brody, 1984).

It is not justified to assign the exponential components in the decay of samples II and III to aggregates of Chl a with definite aggregation numbers. There is probably a range of aggregate sizes, and the fluorescence decay would probably be better described by a continuous distribution of lifetimes rather than by the sum of two or three exponentials. It is clear from a comparison of the fluorescence decay kinetics of dilute and concentrated solutions of Chl a in liposomes (Tables 1 and 2) that the distribution of lifetimes is shifted towards smaller values for concentrated Chl a, where the average size of the Chl a aggregates is expected to be larger. However, for our present purposes the precise nature of these aggregates is not important.

An interesting observation is, that when Zea was added (Table 3) a component with the lifetime expected for truly monomeric Chl a ( $\tau \approx 5$  ns) was observed, and with a large relative amplitude. We propose that this Chl a is closely associated with Zea in the form of a specific complex, in such a way that the Chl a is shielded from interaction with other Chl a molecules. The dependence of the amplitude of this component on temperature and emission wavelength indicates that the complex is more abundant at 30°C and that it emits more at shorter wavelengths. The remaining 60% of Chl a would be present in the form of aggregates, and strangely enough their average lifetime is shorter than that in sample II, indicating an average aggregation number higher than in the absence of Zea. A possible explanation is that the presence of the large Chl a/Zea complex causes a change in the distribution of the lipids within the lipid bilayer, and that the Chl a aggregates respond to this by increasing their size. The increase in relative Chl fluorescence yield ( $\phi_f^{rel}$ ) in the presence of Zea (see Tables 1 and 3) can be attributed to the formation of the complex. The Chl a not present in the Chl a/Zea complex shows a lower fluorescence yield in the presence of Zea (Table 3) than in its absence (Table 1).

The relative fluorescence yield shows a large decrease from  $\approx 1.7$  in sample II to  $\approx 0.6$  in sample III. This concentration quenching has been reported previously (Beddard *et al.*, 1976), and is due to an increased rate of radiationless transition from the excited singlet state in the Chl *a* aggregates. In the presence of Zea (sample I) the yield increases compared to sample II, the increase being most pronounced for 679 nm emission ( $\phi_f^{\text{rel}} \approx 2.8$ ). These yield changes are in agreement with the hypothesis of the presence of monomeric Chl *a* in sample I, which emits at shorter wavelength than the Chl *a* aggregates.

# Fluorescence anisotropy decay kinetics

The decay kinetics of fluorescence emission and anisotropy reflect different physical processes. The intensity of Chl a emission excited by a laser pulse decays with time due to the deactivation of the excited singlet state by fluorescence and radiationless decay processes, which include intersystem crossing to the triplet state and possibly also electron transfer. The fluorescence anisotropy decays from its initial value,  $A_0$ , due to the rotation of the excited Chl a molecule during the fluorescence lifetime. The rotational correlation time of liposomes as a whole is much longer than the timescale of our experiments and can be neglected, but the rotational motions of the Chl a within the lipid bilayer can be of the order of 1–10 ns. The fluorescence anisotropy would be expected to decay with up to three components for a completely anisotropic motion, although not all three are necessarily resolved. If the Chl a is present in aggregates of different sizes, each with a different mobility, then the anisotropy decay will best be described by a continuous distribution of rotational correlation times.

The transfer of singlet excitation between Chl a molecules via the Förster resonance mechanism could also lead to a decrease in anisotropy. This is a rapid ( $\leq 0.1$  ns) process, and it would be expected to give rise to very fast components in the anisotropy decay (Tanaka and Mataga, 1979). However, in our samples of Chl a in liposomes Förster energy transfer is unlikely to be an important process.

The time dependence of the anisotropy has been resolved (Tables 4, 5 and 6) into 2–3 exponential components, each characterised by a correlation time  $(\phi_i)$  and an amplitude  $(\beta_i)$ . The sum of the amplitudes would be expected to equal  $A_0$ , and this

is indeed found within the experimental error.  $A_0$  reflects the degree of ordering of the Chl a within the lipid bilayer.

The appearance of an anisotropy component with a correlation time of  $\phi \approx 14$  ns at 4°C in the presence of Zea is consistent with the formation of a Chl'a containing complex with a large, > 10 kD, molecular weight. This complex is suggested to be between Chl a and Zea, because higher Chl a aggregates do not show this long component in their anisotropy decay (Table 5).

It is seen that  $A_0$  decreases from  $\approx 0.09$  (sample II) to  $\approx 0.04$  (sample III) as the Chl a concentration is increased. In the presence of Zea (sample I) the value is larger ( $A_0 \approx 0.14$ ) indicating a higher degree of ordering of the Chl a in the Chl a/Zea complex. For both samples I and II there is a clear variation of  $A_0$  with temperature and emission wavelength,  $A_0$  being distinctly larger at higher temperature and at shorter emission wavelength.

The analysis of fluorescence anisotropy from heterogeneous systems can be extremely complex even leading to negative amplitudes for some components (Ludescher *et al.*, 1987), however it appears that our results may be understood using the simple approach outlined above.

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