Flash Photolysis of Liposomes Containing Chlorophyll and Zeaxanthin, as a Function of Temperature (2°–34 °C)

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The transfer of triplet excitation from chlorophyll to zeaxanthin in liposomes is a function of temperature and pigment concentration.

At 525 nm both chlorophyll and zeaxanthin triplet states are observed. The result is a biphasic increase in absorption. The rise time of absorption by the chlorophyll triplet is much faster, than by the zeaxanthin triplet. With increasing temperature the contribution of absorption by zeaxanthin (relative to that of chlorophyll) at 525 nm increases, and its rise time gets faster. At high ratios of zeaxanthin to chlorophyll, temperature has less effect on both the rise time and absorption by the zeaxanthin triplet state.

The chlorophyll triplet is measured at 780 nm. It decays faster with increasing temperature and or increasing ratio of zeaxanthin to chlorophyll.

The results are interpreted in terms of: increasing fluidity of the lipid liposome with temperature, formation of zeaxanthin-chlorophyll complexes at high ratios of zeaxanthin and chlorophyll, presence of different lipid phases in the liposome bilayer.

Introduction

From studies of photoconductivity of lipid bilayers containing chlorophyll and various carotenoids, Rich and Brody [1] concluded that there is interaction between the pigments. The largest interaction is between chlorophyll *a* and zeaxanthin (zea). They showed that the dihydrocarotenes (*e.g.* zea) give rise to the highest photocurrents. The interaction between chlorophyll and the various carotenoids, in liposomes, can be modified by temperature [2].

The interaction between pigments in liposomes as a function of temperature and pigment concentration is the subject of this paper. Using flash photolysis the interaction is evaluated by measuring the formation and decay of the absorption band of the carotene triplet at 525 nm and the chlorophyll triplet at 760–790 nm (Mathis and Setif, [3]). Triplet-triplet energy transfer from chlorophyll *a* to carotenoids was reported by many workers (*e.g.* Kramer and Mathis, [4]).

Methods and Materials

The techniques and apparatus used to measure the spectral changes are described by Schenck and Mathis [5]. The sample was irradiated with a 20 ns laser flash (wavelength 600 nm). Preparation of liposomes, using a sonication technique, was described by Brody [6]. The liposomes were suspended in 50 mM phosphate buffer, pH 7.6. Soy bean lecithin was obtained from Sigma Chem. Co. (St. Louis, Mo.); zeaxanthin (zea) was obtained from Ciba (Basel, Swiss); both were used without further purification. Chlorophyll *a* (Chl) was isolated and purified as described by Aghion *et al.* [7].

Argon gas was bubbled through the liposome suspension to remove air. (Out-gassing using the technique of freezing and thawing under vacuum, seems to modify the liposomes; results obtained with such liposomes are not reproducible.)

The kinetics of the spectral change at 525 nm are divided into two phases; a fast rise (less than 50 ns) of amplitude I_f, a slow rise of amplitude I_s having a half time of T_s. The biphasic rise is due to the overlapping spectra of the triplet states of the Chl and carotene (at 525 nm). Pure absorption by the Chl triplet is at 780 nm and decays with a half time of T_d.

Results

The contribution of the slowly rising component, I_s (at 525 nm) increases (*i.e.* the ratio I_s/I_f increases) as temperature increases (Fig. 1). The increase in the ratio, with temperature, is most pronounced when the ratio of zea to Chl is low. As the con-

Abbreviations: Chl, chlorophyll *a*; zea, zeaxanthin; I_s, amplitude of the slow absorption component at 525 nm; I_f, amplitude of the fast absorption component at 525 nm; T_s, rise time (half life) of the slow component at 525 nm; T_d, decay time (half life) of chlorophyll triplet at 780 nm.

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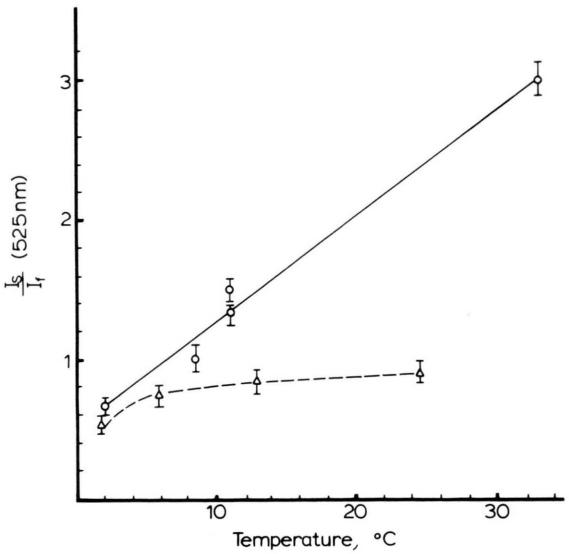

Fig. 1. Ratio of slow increase in absorption, I_s, to the fast increase in absorption, I_f, at 525 nm, as a function of temperature. For liposomes containing a ratio of zeaxanthin to chlorophyll of 3:2, the data are shown by open circles and a solid line; for a ratio of 4:1, the data are shown by open triangles and a broken line. Liposomes were suspended in 50 mM phosphate buffer, pH 7.6.

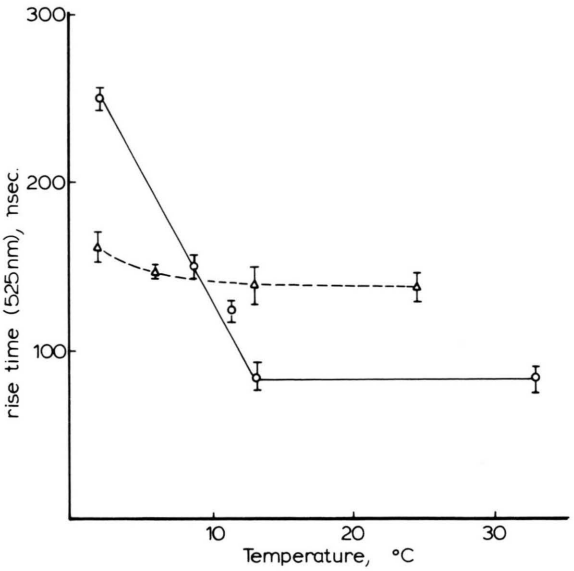

Fig. 2. Rise time (half time) of the slow increase in absorption at 525 nm as a function of temperature. For liposomes containing a ratio of zeaxanthin to chlorophyll of 3:2, the data shown by open circles and a solid line; for a ratio of 4:1 the data are shown by open triangles and a broken line.

centration of zea to Chl increases, temperature has less effect on the ratio I_s/I_f. When the ratio of zea to Chl is 4:1 there is only a small increase of I_s/I_f with temperature (Fig. 1).

At a low concentration of zea to Chl (1.5:1), T_s of the slow component, I_s, at 525 nm is very temperature dependent. The value of T_s gets shorter as the temperature increases (circles in Fig. 2). A minimal T_s is obtained at temperatures above about 13 °C.

At high concentration of zea to Chl (4:1), T_s is fast and almost independent of temperature (crosses in Fig. 2).

Liposomes containing only Chl (no zea) exhibit only a very fast rise in absorbance at 525 nm (shorter than 50 ns). There is no evidence of a biphasic response, between 2° and 25 °C.

In the case of 4:1, zea to Chl, there is a minimal change in both the ratio I_s/I_f and T_s with increasing temperature (Figs. 1 and 2). At lower concentration of zea there is a large increase of I_s/I_f and a decrease of T_s with increasing temperature.

The decay of the Chl triplet, T_d, is faster as the relative concentration of zea increases (Fig. 3). The relative concentration is calculated from (zea)/(Chl)/

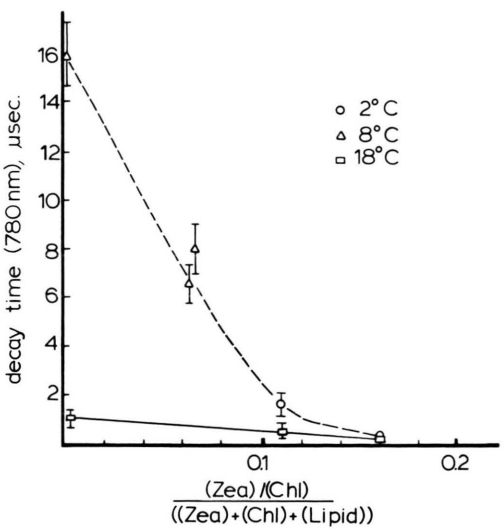

Fig. 3. Decay time (half time) of absorption at 780 nm of the chlorophyll triplet as a function of pigment concentration in the lipid liposome, given as zeaxanthin/chlorophyll/(zeaxanthin + chlorophyll + lipid). The decay at 2 °C is shown by circles, at 8 °C by triangles, and at 18 °C by squares.

((zea) + (Chl) + (lipid)). (Similar curves are obtained when T_d is plotted as a function of the ratio (zea)/(Chl). This effect is most pronounced at lower temperature (2 °C) (circles in Fig. 3). At higher temperature (18 °C) the decay time is much faster (1 μs or less); increasing concentration has only a small effect on the decay time. The time response at 780 nm is of the order of 0.5 μs, much longer than at 525 nm (perhaps because of a strong fluorescence artifact).

Admitting air to the liposome system results in the triplet absorption, at 780 nm, decaying almost ten times faster (at 2 °C). Mathis and Setif [3] reported that the decay was more than ten times faster after admitting air, in a system containing Chl in SDS micelles at 21 °C. On the other hand, the presence or absence of oxygen has no significant effect on the kinetics of absorption observed at 525 nm.

Discussion

The rise time, T_s, at 525 nm is interpreted as the time required for a molecule of Chl in the triplet state to encounter and transfer its excitation to a molecule of zea, within the lifetime of the excited triplet state of Chl. In the solid lipid phase, T_s is slow because low fluidity of the lipid limits diffusion of the molecules of zea and Chl. The fluidity of the bilayer increases (viscosity decreases) as temperature increases. For any given concentration of pigments, T_s is inversely related to the viscosity in the lipid bilayer forming the liposome. As the temperature is raised, T_s becomes faster (Fig. 2). As the fluidity increases the excited Chl molecule may migrate over a greater area, thus increasing its rate of encounter with molecules of zea.

The mechanisms operating in the liposome may be summarized as follows. The Chl triplet is formed by the following series of reactions: $\mathrm{Chl} + h\nu \rightarrow \mathrm{Chl}^* \rightarrow \mathrm{Chl}^T$. The Chl triplet may return to the ground state by several possible pathways involving zea. At low concentrations of zea to Chl triplet transfer may occur by diffusion controlled encounters between the Chl triplet and zea (reaction (a)).

$$\mathrm{Chl}^T + \mathrm{zea} \rightarrow \mathrm{Chl} + \mathrm{zea}^T . \qquad \text{(a)}$$

At higher concentrations, Chl and zea may form complexes (reaction (b)), which would markedly increase the rate of triplet-triplet transfer and make the process relatively temperature independent.

$$\mathrm{Chl} + \mathrm{zea} \rightarrow \mathrm{Chl}\cdot\mathrm{zea} \rightarrow \mathrm{Chl}^T\cdot\mathrm{zea} \rightarrow \mathrm{Chl}\cdot\mathrm{zea}^T . \qquad \text{(b)}$$

Alternatively, the Chl triplet may be quenched by a number of different processes. At high concentrations of zea or Chl there may be formation of oligomers (reaction (c)), as well as complexes (reaction (b)). Interaction between the oligomers and the Chl triplet may result in quenching of the triplet (reactions (d) and (e)).

$$n^{\cdot}\mathrm{zea} \rightarrow \mathrm{zea}_n ; \quad n^{\cdot}\mathrm{Chl} \rightarrow \mathrm{Chl}_n \qquad \text{(c)}$$

$$\mathrm{Chl}^T + \mathrm{Chl}_n \rightarrow \mathrm{Chl} + \mathrm{Chl}_n ; \qquad \text{(d)}$$

$$\mathrm{Chl}^T + \mathrm{zea}_n \rightarrow \mathrm{Chl} + \mathrm{zea}_n . \qquad \text{(e)}$$

If every transfer of energy from Chl triplet resulted in formation of a zea triplet, then one would expect the decay time at 780 nm to equal the rise time at 525 nm. However, this is not observed experimentally with the liposome systems. Consequently, the Chl^T is not in an ideal, homogeneous population. The rise time at 525 nm is of the order of 0.2 μs or less (Fig. 2), while the decay time at 780 nm is, of the order of, 2 to 20 μs (Fig. 3). This discrepancy indicates that the surface of the liposome is divided into two, or probably more, lipid domains. Such domains can readily arise from the heterogeneous composition of fatty acids present in the soy bean lecithin used to form the liposomes. The lipid phase of lecithin is determined primarily by its fatty residues and to a certain extent by materials dissolved in the lipid phase. Because of the wide range of fatty acids, the lipid phase change in the various domains occurs over a wide temperature range.

One group of domains could contain both Chl and zea, while another group might contain primarily Chl and little if any zea that may accept triplet excitation. In the first group the Chl and zea population would be in a position for efficient triplet-triplet transfer, giving rise to absorption by the triplet state of zea (Fig. 2). In another lipid domain, containing primarily Chl, the slower decay of Chl triplet would be observed.

The slowly rising component of the absorption change at 525 nm, I_s, is ascribed primarily to the zea triplet. This component probably reflects a diffusion controlled, temperature dependent, reaction (at a zea:Chl ratio of 3:2). When the triplet

state of zea is formed from the Chl triplet the absorbance increases, since the absorption coefficient of the triplet state of zea is much greater than that of the Chl triplet. The molar absorption coefficients of Chl and carotene, at 525 nm, are 15 000 and 100 000, respectively [8, 9].

As the lipid goes into the gel phase (higher fluidity), T_s becomes faster (Fig. 2) and the value of the ratio I_s/I_f increases with temperature (Fig. 1). In the gel phase more interactions between Chl and zea are possible within the lifetime of the Chl triplet state due to the greater diffusion that is possible at the lower viscosity. At lower temperature the fluidity is lower, so there is a lower rate of diffusion controlled interaction between zea and Chl triplets.

At low concentrations of zea, formation of oligomers is of minor importance, the major factor governing T_s being the fluidity of the lipid bilayer. At a low zea to Chl ratio (3:2) the contribution of I_s is larger than at higher ratios of zea to Chl (4:1) (Fig. 1). Temperature effects both the decay of Chl 780 nm (Fig. 3) (reactions (d) and (e)) and rise of zea 525 nm (Fig. 2) (reaction (a)). Both are apparently diffusion controlled reactions.

At a high zea to Chl ratio (4:1) reactions (b) and (c) should prevail, *i.e.* there should be high populations of oligomers and Chl-zea complexes. Only a small number of Chl triplets are free to diffuse, in order to encounter a molecule of zea in the lipid bilayer. From the fast rise time of absorption by zea triplets (Fig. 2) it is apparent that Chl (not complexed with zea) doesn't have far to migrate to encounter a molecule of zea. In addition, there are fewer uncomplexed Chl's to excite the uncomplexed zea, by a temperature dependent process. This results in a decrease in the contribution of I_s (the ratio I_s/I_f is lowered) and relatively temperature independent (Fig. 1).

At a high zea to Chl ratio the population of zea oligomers (reaction (c)) increases so the rate of triplet quenching is greater (reaction (e)). The result is a further decrease in the number of successful transfers, consequently there is a lower value for the ratio I_s/I_f at high ratios of zea to Chl (4:1) than at lower ratios (3:2) (Fig. 1).

As the ratio of zea to Chl increases the concentration of pigment complexes likewise increases. In the zea-Chl complex the rate of triplet-triplet transfer is very fast so that fluidity of the lipid has little effect on interaction between Chl^T and zea (reaction (b)). In addition, since transfer is very fast the rise of absorption by zea^T cannot be experimentally separated from the fast rising component of absorption, I_f, the result is a low value of I_s/I_f. When the population of complex is high, both T_s (Fig. 2) and I_s/I_f (Fig. 1) are rather independent of temperature.

One of the results (shown in Fig. 2) is not readily interpreted without additional *ad hoc* assumptions. Specifically, the origin for the difference in T_s at elevated temperature, between high and low ratios of Chl to zea, is not apparent (Fig. 2). The difference in T_s might be related to different rate processes operating at high and low ratios of zea to Chl in different lipid domains.

Some of the temperature dependent reactions of photosynthesis and other biological phenomena, occurring at or within biomembranes may involve processes similar to those touched on in this paper.

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