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On the influence of the lipid matrix on energy transfer processes in self-assembling chlorophyll-lipid systems

M. van Gurp, G. van Ginkel and Y.K. Levine

Department of Molecular Biophysics, Physics Laboratory, University of Utrecht, Utrecht (The Netherlands)

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Life-time measurements for chlorophyll *a* and pheophytin *a* in lipid vesicles of digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) at different pigment-lipid ratios are presented. On increasing the pigment concentration weakly fluorescent dimers are formed, to which the excited state energy is transferred, thus causing a shortening of the average lifetime. This quenching behaviour is less efficient for chlorophyll *a* in sulfoquinovosyldiacylglycerol than in digalactosyldiacylglycerol, but no differences are found for pheophytin *a* in sulfoquinovosyldiacylglycerol and digalactosyldiacylglycerol. It is suggested that the negatively charged headgroup of sulfoquinovosyldiacylglycerol bonds with the Mg^{2+} of chlorophyll *a*, thus holding the chlorophylls apart and preventing them from forming aggregates. Large differences exist between the concentration quenching curves of chlorophyll *a* and pheophytin *a* . The significant lower quenching efficiency of the pheophytin *a*-lipid system is partly due to a smaller dimerization of pheophytin *a* molecules, but mainly to the mutual orientation of the monomers and dimers which leads to low energy transfer rates.

Introduction

In recent years our knowledge about the primary processes of photosynthesis in higher plants has increased considerably. A great deal of attention has been paid to the molecular structure and organization of light harvesting antenna complexes and reaction centres in thylakoid membranes [1,2]. The various protein complexes together with the membrane lipids form a highly structured but asymmetric system, in which protein lipid interactions play an important role. It is now generally accepted that the photosynthetic pigments responsible for light energy collection and charge separation occur only bound to different polypeptides. Chlorophylls are thought to be tightly bound boundary lipids of the pigment-protein complexes having their phytyl tails in the hydrocarbon phase of the acyl lipid bilayer [3]. Monogalactosyldiacylglycerol (MGDG), which is the major lipid in thylakoid membranes, does not form a lamellar structure in an aqueous system, and it is suggested that it may be involved in protein packaging [4]. Bilayer-forming lipids, like digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG), may very well be responsible for the formation of the bilayer which serves as a stable matrix in which the proteins are embedded. It is generally recognized

Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol.

Correspondence: M. van Gurp, Department of Molecular Biophysics, Physics Laboratory, University of Utrecht, P.O. Box 80000, 3508 TA Utrecht, The Netherlands.

that the various thylakoid membrane lipids play an important role in the primary processes of photosynthesis. They are important not only because they contribute to a supramolecular organization in the thylakoids, but also because they influence various processes through specific interactions with proteins, pigments and other lipids [1]. It is still not known if and how e.g. lipid–pigment interactions affect the organization and energy transfer between different pigment-protein complexes. To obtain information on such specific interactions it is necessary to simplify the system under study so that it can be described by as few parameters as possible and yet remains realistic. Although the study of intact chloroplasts or isolated pigment-protein complexes yields useful information about the in vivo system, valuable additional information can be derived from much simpler model systems [5].

In this study we focus on the influence that the lipid headgroup polarity and the presence of Mg^{2+} in chlorophyll exert on energy transfer in self-assembling pigment-lipid systems. We present lifetime measurements of chlorophyll *a* and pheophytin *a* in lipid vesicles of DGDG and SQDG, two thylakoid lipids that form a lamellar phase in an aqueous environment. It is known that when the concentration in such systems is increased, energy transfer takes place between pigment monomers, and from monomers to dimers that are formed [6,7]. There are some interesting similarities between this model system and the in vivo situation. Slow inductive resonance energy transfer [8] between the pigments occurs, the pigments are oriented with respect to each other (which influences the energy transfer efficiency), and at increased concentration the chlorophylls come closer to each other, possibly leading to the formation of dimers or other associates that trap the excited state energy. We do not expect this to happen in vivo because the pigments are bonded to polypeptides, but it will be interesting to see if dimer formation is influenced by the lipid environment.

It is very difficult to give a theoretical description of energy transfer and quenching in the system under study, because it is two-dimensional and highly ordered. In energy transfer it is often assumed that the distribution of the molecules involved is isotropic and the influence of the orientation on the energy transfer efficiency is neglected [9,10]. Recently an attempt was made to describe the energy transfer in and between bilayers [11] taking a simple anisotropy into account. It was concluded that the orientation greatly influences the energy transfer. At the moment it is still not possible to describe the energy transfer between monomers and between monomers and traps in a two-dimensional system in which the molecules have a three-dimensional anisotropy. The application of any existing theory would yield unrealistic and unphysical results and amount to little more than intricate curve-fitting. We therefore confine ourselves to a qualitative analysis.

Materials and Methods

Chlorophyll *a* was extracted from fresh spinach according to Terpstra and Lambers [12] and purified using thin-layer chromatography. It was treated with a weakly acid solution to produce pheophytin a. The purity of these pigments was checked by comparing the absorption spectra of dilute acetone solutions with known spectra. Digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) were obtained from Lipid Products. The purity of the lipids obtained was checked regularly using high performance thin-layer chromatography as described in previous papers [44,45]. The lipids were found to be without any traceable impurities.

A constant amount of 2.5 μg pigment in solution was added to various amounts of lipid in solution to give pigment-lipid ratios ranging from 1 : 2.8 to 1 : 5000. The solvent was evaporated by a flow of nitrogen and the dried samples were kept for 18 h under vacuum. 3 ml of a 0.02 M Tris-EDTA buffer with pH 8.0 was then added, and the samples were sonicated for 15 min so that a clear vesicle suspension was obtained. The samples were kept strictly under N_2 gas, which was sufficient to prevent oxidation of the lipids. This can easily be checked by measuring their absorption spectrum in the 200–300 nm range [27,45]. Freeze-fracture measurements showed that we had obtained unilamellar vesicles with a diameter of 40–80 nm. Some of the DGDG samples with the largest amounts of lipid did not become totally

clear, even if the sonication time was extended to 30 min. The vesicle suspensions were stored under N_2 in the dark at 4°C before the measurements were done. Because SQDG is slightly acid it may possibly cause pheophytinization of the chlorophyll *a*. From absorption spectra we calculated that this happened only to a very limited extent: less than 5% of the chlorophyll *a* was turned into pheophytin *a*. We found this to be a negligible quantity.

Time-resolved fluorescence measurements were carried out in Daresbury (U.K.), using synchrotron radiation as a tunable light source with high repetition frequency [13]. Light of the required wavelength (bandwidth of 2 nm) was selected with a monochromator. The light was passed through a lens to give parallel light and then through a Glan Thompson prism to give vertically polarized light. The light was incident on a cuvette containing the vesicle suspension: the fluorescence was detected at 90° to the incident beam through an analyzer set at the magic angle. Interference filters were used on the excitation side, and cut-off filters in the emission beam to get rid of stray light. Lamp profiles were obtained at the fluorescence wavelength using Ludox as a scatterer. Detection was performed with a cooled Philips XP2233B photomultiplier tube, and data were collected using the single photon counting technique with a TAC (Ortec) and MCA (Techno) and stored on a DEC minicomputer. The decay curves were deconvoluted and fitted to a two-exponential decay using a non-linear least-squares Marquardt procedure. Chlorophyll *a* was excited with 435 nm light and pheophytin *a* with 408 nm light. In both cases fluorescence was detected at 680 nm.

Results and Discussion

(a) Analysis of the decay curves

A typical example of a decay curve together with residuals and autocorrelation is shown in Fig. 1. It is known that when quenching occurs one finds no exponential decay. For small trap concentrations (but direct trapping) and an isotropic distribution the Förster theory predicts an $\exp(-t/\tau + kt^{-d/6})$ decay, where d is the dimension of the system [14]. Although similar decay curves have been found for plant systems [15] it

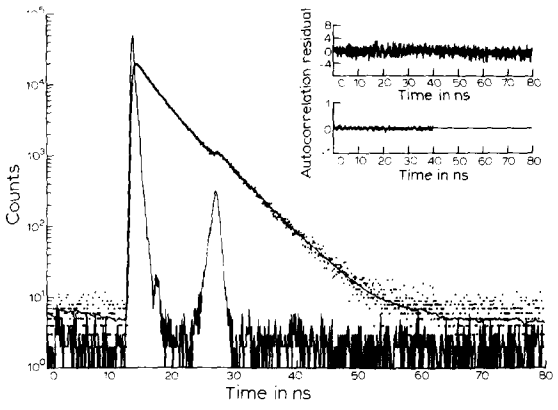

Fig. 1. Decay curve of the fluorescence of chlorophyll *a* in vesicles of DGDG. Pigment-lipid ratio is 1:5000. Average lifetime is 4.09 ns. The experimental results of fluorescence and lamp profile are shown, together with the two-exponential fit. The residuals and their autocorrelation function are given in the inset.

has been pointed out that the Förster theory is not generally applicable to such highly organized and complex systems [16]. We fitted our data to a two-exponential decay; a three-exponential fit produced only negligible improvement. The average lifetime was calculated from the two lifetimes τ_1 and τ_2 and pre-factors a_1 and a_2:

$$\langle t \rangle = (a_1\tau_1^2 + a_2\tau_2^2)/(a_1\tau_1 + a_2\tau_2)$$

At low concentrations the average lifetime was found to be 4.1 ns for chlorophyll *a* and 6.7 ns for pheophytin *a*, both in DGDG and SQDG. Beddard et al. [7] reported a lifetime of 5.7 ns for chlorophyll *a* in phosphatidylcholine (PC) vesicles. In PC monolayers the lifetime of chlorophyll *a* was 5.5 ns according to Agrawal et al. [17] and 4.3 ns according to Picard et al. [18]. Differences could of course be due to the lipid environment, but could also be caused by the presence of molecular oxygen [19]. As far as is known to us pheophytin *a* lifetimes in lipid environment have not been presented before.

(b) Concentration quenching

Figs. 2 and 3 show the average lifetime of chlorophyll *a* and pheophytin *a* in DGDG and SQDG as a function of the concentration (logarithm of the pigment-lipid ratio). Similar con-

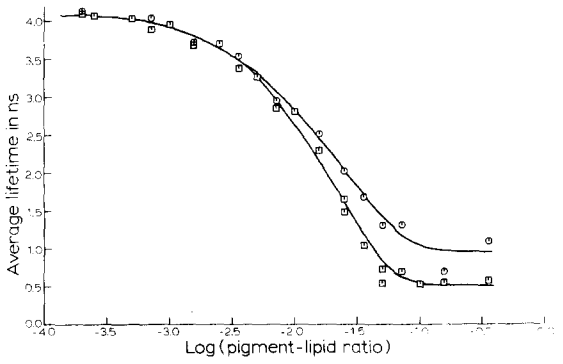

Fig. 2. Average lifetime of chlorophyll *a* in lipid vesicles as a function of the logarithm of the pigment-lipid ratio; □, DGDG; ○, SQDG.

centration quenching curves, but generally of the quantum yield, have been published extensively [6,7,20–23]. Self-assembling pigment-lipid systems are often used as model systems, but the energy transfer mechanism and trapping formation are not yet fully understood. At increased concentrations quenching species occur; it has been suggested that these are aggregates [6], excimers [24] or ionpairs produced by electron transfer [25]. Excitation energy of a monomer can be transferred to such a trap, resulting in a shortening of the measured average lifetime. Excitation energy of a monomer can also be transferred to another monomer and at higher concentration (as is to be expected in highly ordered systems) to many different monomers before being trapped. Such a hopping mechanism is thought to exist in the photosynthetic organisms [26]. It can be shown that even in isotropic systems at intermediate densities at least 10 transfers between monomers occur before the exciton is trapped (Knoester, J. (1987) personal communication).

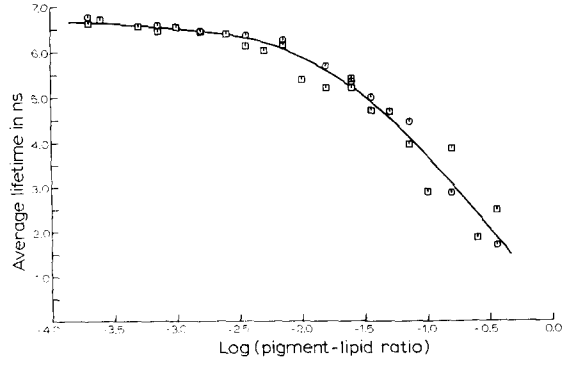

Fig. 3. Average lifetime of pheophytin *a* in lipid vesicles as a function of the logarithm of the pigment-lipid ratio; □, DGDG; ○, SQDG.

From the curves one can obtain a so-called half quenching concentration, which is the concentration at which the lifetime (or quantum yield, or fluorescence anisotropy) is reduced to half of its original value. This half quenching concentration is often related to the critical distance R_0, the distance between two molecules at which the energy transfer rate is equal to the inverse of the lifetime. R_0 appears in Förster's theory and originates from Perrin [28]. However, it should be realized that in mixed systems of monomers and quenchers both can act as donors as well as acceptors, and two or more critical distances will then have to be considered. In this case a theoretical description is necessary, in which the anisotropy is incorporated. We found as half quenching concentrations (pigment-lipid ratios) about 1 : 65 for chlorophyll *a* in DGDG, 1 : 45 for chlorophyll *a* in SQDG and 1 : 8 for pheophytin *a* in both DGDG and SQDG.

(c) Influence of Mg^{2+}

On increasing the chlorophyll *a* concentration in the lipid membrane its absorption spectrum does not change appreciatively. It is therefore difficult to make comparisons with spectroscopic properties of chlorophyll *a* in different organic solvents. We found a red shift of the Q-band from 670 nm at low concentrations to 676 nm at high concentrations; this was accompanied by a broadening of the bandwidth from 24 to 36 nm, both in DGDG and SQDG. A similar effect was found by Lee [6] for chlorophyll *a* in vesicles of dioleoylPC and dipalmitoylPC. He concluded that aggregates were formed having a 680 nm absorption peak, and that these were similar to the ones found in concentrated hexane solutions [29]. We measured the excitation spectrum of a 1 : 1 chlorophyll *a*-lipid dispersion and found that it resembled the absorption spectrum at low concentrations, except for a small shoulder at 680 nm (Fig. 4). This seems to support Lee's finding that a dimer is formed with a 680 nm absorption peak. This dimer is very weakly fluorescent, and we concluded that even at

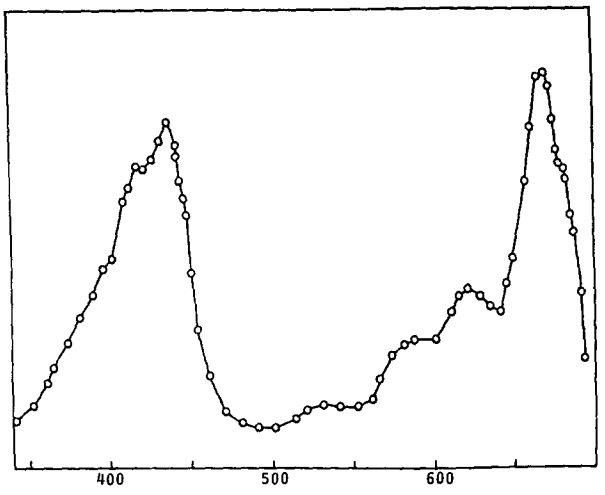

Fig. 4. Excitation spectrum of a 1:1 chlorophyll *a* - DGDG dispersion in Tris-EDTA buffer.

high concentrations the fluorescence is predominantly monomer fluorescence. Fragata [30] found a very small additional absorption peak at 740 nm for chlorophyll *a* in PC and identified this as a chlorophyll hydrate, as was proposed by Fong and Koester [31]. According to Beddard and Porter [24] no dimer is formed; instead, a statistical pair is formed in which one of the chlorophylls is excited and both chlorophylls are kept apart by some complexing with the lipid. A similar small change in the absorption behaviour of chlorophyll *a* was found in ethanol-water mixtures, when the water content was increased [32,33]. It was concluded that a dimer is formed using one molecule of water and with the two porphyrin planes 4.2 Å apart.

Pheophytin *a*, on the other hand, shows a marked red shift of its Q band when the concentration is increased. At low concentrations the Q-band peak is at 672 nm; at high concentrations a new peak appears at 696 nm. Similar absorption changes were found in ethanol/glycerol mixtures [32]. Here it was concluded that one sort of aggregate was present, presumably a dimer.

Figs. 2 and 3 show that there is a striking difference between the quenching behaviour of chlorophyll *a* and pheophytin *a* in the lipid systems. In the pheophytin *a* system the quenching is much less efficient; its half quenching concentration is 5–8-times higher than in chlorophyll *a*. This is in marked contrast to the finding of Yuen et al. [34]. They reported that the dimerization constant of pheophytin *a* is about 3-times higher than the one for chlorophyll *a* in pyridine. These authors, however, conclude from their measurements on covalently-linked pairs [35] that pheophytin *a* is a less efficient quencher than chlorophyll *a*. In aqueous systems like our lipid vesicles association of chlorophyll *a* and pheophytin *a* will not be the same as in pyridine [5,35]. Kelly and Porter [21] reported that the self-quenching of pheophytin *a* in phosphatidylcholine was less efficient than that of chlorophyll *a*, but only by a factor of less than two. They concluded that this is due to a smaller overlap integral for absorption and fluorescence of pheophytin *a*. We, on the other hand, found a much larger overlap for pheophytin *a* than for chlorophyll *a*, as reported earlier by Gurinovitch et al. [32,33] for the absorption spectra of chlorophyll *a* and pheophytin *a* in ethanol/water mixtures. Upon increasing the water content in such mixtures chlorophyll and pheophytin dimers are formed, causing a quenching of the fluorescence signal. In this case, however, the pheophytin fluorescence is quenched more efficiently than that of chlorophyll (Fig. 5) as is expected from the larger overlap integral for pheophytin. The difference in quenching behaviour in the isotropic ethanol-water and the anisotropic lipid-water systems can be explained by an orientation of the pheophytin

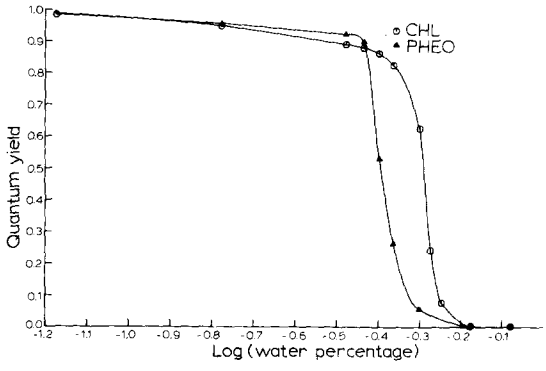

Fig. 5. Concentration quenching curves of chlorophyll *a* and pheophytin *a* in water/ethanol mixtures. The pigment concentration was kept constant ($5 \cdot 10^{-6}$ M), while the water-ethanol ratio was varied. Quantum yields are given relative to the yield of a $5 \cdot 10^{-6}$ M pigment-ethanol solution.

dimer in the membrane, which is unfavourable for an efficient energy transfer from monomer to dimer.

Linear dichroism measurements on macroscopically ordered membranes (for a description see e.g. Ref. 36) yield the order parameter of the absorption moment $S = 1/2(3\langle\cos^2\theta\rangle - 1)$, where θ is the angle between the absorption moment and the normal to the membrane. We found that S of the Q-band of chlorophyll *a* remained constant ($S = 0.30 \pm 0.03$) when the concentration was increased whereas S of the pheophytin dimer Q band (696 nm, $S = 0.13 \pm 0.01$) was markedly lower than for the monomer Q band (672 nm, $S = 0.27 \pm 0.05$). From this we can conclude that the pheophytin *a* dimer Q band is oriented unfavourably for efficient energy transfer. As is known, the dipole-dipole energy transfer rate depends among other things on the relative orientation of the emission moment $\bar{v}$ of the donor and the absorption moment $\bar{\mu}$ of the acceptor. This dependence is given by the so-called orientation factor.

$$k^2 = [(\bar{\mu}\cdot\bar{v}) - 3(\bar{\mu}\cdot\bar{r})(\bar{v}\cdot\bar{r})]^2$$

where $\bar{r}$ is the separation vector between the two dipoles. From the expression for k^2 it can easily be seen that when the two dipoles are side by side (as is the case in bilayers) parallel orientation yields $k^2 = 1$, whereas perpendicular orientation yields $k^2 = 0$. Although these are extreme situations, it is clear that in ordered systems there may be very large differences in the efficiency of energy transfer to differently oriented acceptors.

(d) Lipid headgroup polarity

From Fig. 2 it can also be seen that the quenching of chlorophyll *a* is lipid dependent. It seems reasonable to assume that the same chlorophyll *a* dimers are formed in both lipids, but that the association occurs differently in different environments. Beddard et al. [7] found in similar experiments that chlorophyll *a* fluorescence was quenched more efficiently in PC than in a 3:1 MGDG-DGDG mixture. They concluded that it is the bulkier headgroups of the galactolipids and

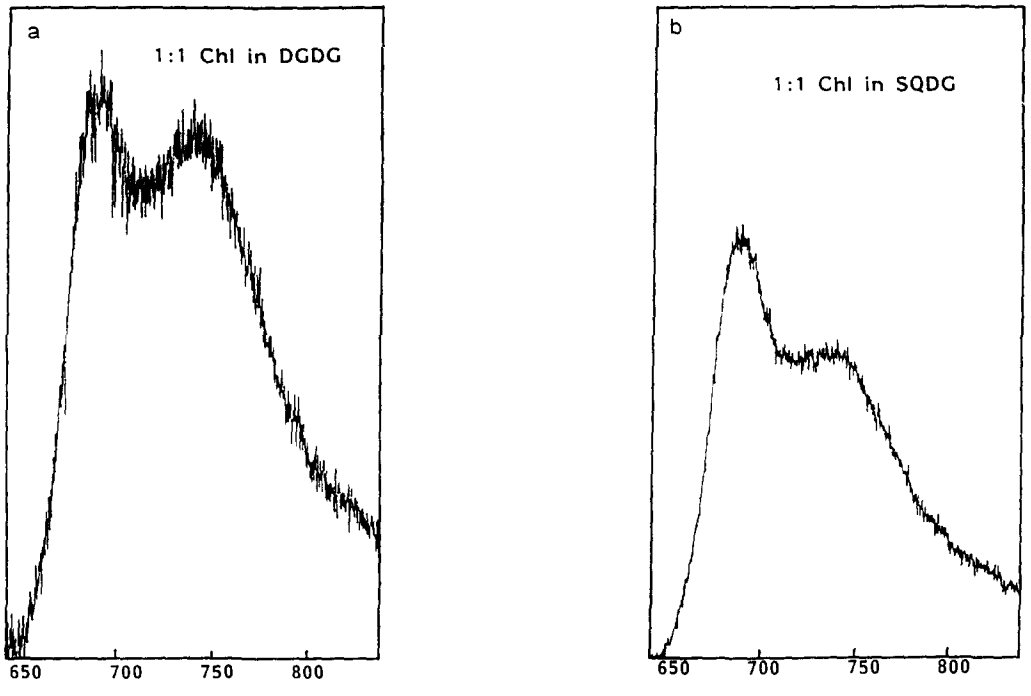

Fig. 6. Fluorescence spectra of a 1:1 chlorophyll *a* - lipid dispersion in Tris EDTA buffer. (a) DGDG; (b) SQDG.

possibly hydrogen bonding with the chlorophyll *a* which cause segregation of the chlorophylls, resulting in less efficient quenching. In our opinion a 3 : 1 molar ratio of MGDG and DGDG dispersed in an aqueous system does not form a purely lamellar system, but may very well form an inverted hexagonal H_{II} phase [37]. Their results cannot therefore be compared with ours. In addition we find that in DGDG, with the larger headgroup, fluorescence is quenched more efficiently than in SQDG. Measurements of the fluorescence spectrum at high concentrations show that the $Q_y(0-0)$ peak is reduced more in DGDG than in SQDG (Fig. 6). We conclude therefore that association of chlorophyll *a* occurs more readily in DGDG than in SQDG.

It is interesting to note that from the results shown in Fig. 3 we cannot draw conclusions about differences in the quenching behaviour of pheophytin *a* in DGDG and SQDG. This strongly suggests that the negative headgroup of SQDG is bonded to the Mg^{2+} ion in chlorophyll, thus keeping chlorophyll *a* from aggregation. Although this cannot be seen from the concentration quenching curves, absorption spectra show that pheophytin *a* dimers occur at lower concentrations in SQDG than in DGDG. This suggests that, contrary to the finding for the chlorophyll system, SQDG influences the dimer formation of pheophytin *a* positively.

At this point it is interesting to consider the specific roles of DGDG and SQDG in thylakoid membranes. However, surprisingly little is known about their function although it is generally recognized [1,3,38] that lipids play an important role in the photosynthetic apparatus. Conformational studies [39,40] demonstrate that chlorophyll molecules can be incorporated into bilayers with the phytol chain inserted into the hydrophobic core so as to form a lock-and-key fit with the fatty acid chains. The motional state of the phytol chains of the chlorophyll even appears to be similar to that of the fatty acid chains [41]. A number of studies [42,43], however, suggest that a large portion of SQDG is closely associated with protein-pigment complexes rather than with the bulk lipid matrix. It has been proposed that the negative charge of SQDG plays a role in stabilizing the functional conformation of these complexes in the membrane and that the lipid molecules are involved in optimizing catalytic activity.

In accordance with these previous findings we suggest that SQDG binds to the protein-bound chlorophyll in the thylakoid membrane, thus helping to stabilize the protein-chlorophyll complexes and possibly preventing the formation of chlorophyll aggregates. We believe that studies of simple model systems will provide valuable insight into this problem. To this end studies of reconstituted systems of LHCII particles in lipid vesicles and macroscopically oriented samples are now in progress in our laboratory.

Conclusions

Negatively charged sulpholipids are associated with chlorophyll via its Mg^{2+} ion. They prevent chlorophylls from forming strongly quenching aggregates. By their binding to chlorophyll they may be involved in stabilizing pigment-protein complexes in the thylakoid membrane. Orientation plays an important role in the efficient transfer of excited state energy in ordered systems.

Acknowledgements

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References

1 Murphy, D.J. (1986) Biochim. Biophys. Acta 864, 33–94.
2 Ort, D.R. (1986) in Encyclopedia of Plant Physiology, Vol. 19: Photosynthesis III (Staehelin, L.A. and Arntzen, C.J., eds.), pp. 143–196, Springer Verlag, Berlin.
3 Anderson, J.M. (1975) Biochim. Biophys. Acta 416, 191–235.
4 Gounaris, K., Brain, A.P.R., Quinn, P.J. and Williams, W.P. (1983) FEBS Lett. 153, 47–52.

5 Seely, G. (1977) in Primary Processes of Photosynthesis (Barber, J., ed.), pp. 1–53, Elsevier, Amsterdam.
6 Lee, A.G. (1975) Biochemistry 14, 4397–4402.
7 Beddard, G.S., Carlin, S.E. and Porter, G. (1976) Chem. Phys. Lett. 43, 27–32.
8 Förster, T. (1949) Z. Naturforsch. 4a, 321–327.
9 Huber, D.L. (1979) Phys. Rev. B20, 2307–2314.
10 Wolber, P.K. and Hudson, B.S. (1979) Biophys. J. 28, 197–210.
11 Baumann, J. and Fayer, M.D. (1986) J. Chem. Phys. 85, 4087–4107.
12 Terpstra, W. and Lambers, J.W.J. (1983) Photobiochem. Photobiophys. 6, 93–100.
13 Munro, I.H. and Schwentner, N. (1983) Nucl. Instr. Meth. 208, 819–834.
14 Paillotin, G., Swenberg, C.E., Breton, J. and Gaecintov, N.E. (1979) Biophys. J. 25, 513–534.
15 Harris, L., Porter, G., Synowiec, J.A., Tredwell, C.J. and Barber, J. (1976) Biochim. Biophys. Acta 449, 329–339.
16 Knox, R.S. (1977) in Primary Processes of Photosynthesis (Barber, J., ed.), pp. 55–97, Elsevier, Amsterdam.
17 Agrawal, M.L., Chauvet, J.P. and Patterson, L.K. (1985) J. Phys. Chem. 89, 2979–2982.
18 Picard, G., Munger, G., Leblanc, R.M., Le Sage, R., Sharma, D., Siemiarczuk, A. and Bolton, J.R. (1986) Chem. Phys. Lett. 129, 41–47.
19 Connolly, J.S., Janzen, A.F. and Samuel, E.B. (1982) Photochem. Photobiol. 36, 559–563.
20 Trosper, T., Park, R.B. and Sauer, K. (1968) Photochem. Photobiol. 7, 451–469.
21 Kelly, A.R. and Porter, G. (1970) Proc. Roy. Soc. Lond. A315, 149–161.
22 Luisetti, J., Galla, H.J. and Möhwald, H. (1978) Ber. Bunsenges. Phys. Chem. 82, 911–916.
23 Chauvet, J.P., Agrawal, M.L., Hug, G.L. and Patterson, L.K. (1985) Thin Solid Films 133, 227–234.
24 Beddard, G.S. and Porter, G. (1976) Nature 260, 366–367.
25 Seely, G.R. (1978) Photochem. Photobiol. 27, 638–654.
26 Pearlstein, R.M. (1982) Photochem. Photobiol. 35, 835–844.
27 Klein, R.A. (1970) Biochim. Biophys. Acta 210, 486–489.
28 Perrin, F. (1932) Ann. Phys. 17, 283–314.
29 Cotton, T.M., Trifunac, A.D., Ballschmiter, K. and Katz, J.J. (1974) Biochim. Biophys. Acta 368, 181–198.
30 Fragata, M. (1977) Photosynthetica 11, 296–301.
31 Fong, F.K. and Koester, V.J. (1976) Biochim. Biophys. Acta 423, 52–64.
32 Gurinovitch, G.P. and Strelkova, T.I. (1968) Biofizika 13, 782–792.
33 Gurinovitch, G.P., Sevchenko, A.N. and Solov'ev, K.N. (1968) Spectroscopy of Chlorophyll and Related Compounds, Minsk.
34 Yuen, M.J., Shipman, L.L., Katz, J.J. and Hindman, J.C. (1980) Photochem. Photobiol. 32, 281–296.
35 Katz, J.J., Norris, J.R., Shipman, L.L., Thurnauer, M.C. and Wasielewski, M.R. (1978) Annu. Rev. Biophys. Bioeng. 7, 393–434.
36 Norden, B., Lindblom, G. and Jonas, I. (1977) J. Phys. Chem. 81, 2086–2093.
37 Brentel, I., Selstam, E. and Lindblom, G. (1985) Biochim. Biophys. Acta 812, 816–826.
38 Siegenthaler, P.A. and Rawyler, A. (1986) in Encyclopedia of Plant Physiology, Vol. 19: Photosynthesis III (Staehelin, L.A. and Arntzen, G.J., eds.), pp. 693–705, Springer Verlag, Berlin.
39 Brasseur, R., De Meutter, J. and Ruysschaert, J.-M. (1984) Biochim. Biophys. Acta 764, 295–300.
40 Rosenberg, A. (1967) Science 157, 1191–1196.
41 Eigenberg, K.E., Croasmun, W.R. and Chan, S.Y. (1981) Biochim. Biophys. Acta 642, 438–442.
42 Sakai, W.S., Yamamoto, H.Y., Miyazaki, T. and Ross, J.W. (1983) FEBS Lett. 158, 203–207.
43 Barber, J. and Gounaris, K. (1986) Photosynth. Res. 9, 239–249.
44 Van de Ven, M., Kattenberg, M., Van Ginkel, G. and Levine, Y.K. (1984) Biophys. J. 45, 1203–1210.
45 Koole, P., Dammers, A.J., Van Ginkel, G. and Levine, Y.K. (1984) Biochim. Biophys. Acta 777, 297–305.