Interaction of Triton X-100 with the pigment–protein complexes of photosynthetic membranes

Denis J. MURPHY*† and Roslyn T. PRINSLEY†

**Department of Environmental Biology, Research School of Biological Sciences, Australian National University, Canberra, A.C.T. 2601, Australia, and †Research Institute for Photosynthesis, University of Sheffield, Sheffield S10 2TN, U.K.*

(Received 12 October 1984/8 February 1985; accepted 14 March 1985)

The interaction of the non-ionic detergent Triton X-100 with photosynthetic membrane components of *Pisum sativum* (pea) is described. The detergent affected both the wavelength and the intensity of the 77K fluorescence-emission peaks of both Photosystem I and Photosystem II preparations, in addition to the effects on whole thylakoids recently described by Murphy & Woodrow [(1984) *Biochem. J.* **224**, 989–993]. Below its critical micellar concentration, Triton X-100 had no effect on 77K fluorescence emissions even after prolonged incubations of up to 30 min. Above the critical micellar concentration of about $0.16 \text{ mg} \cdot \text{ml}^{-1}$, Triton X-100 caused a dramatic increase in the intensity of the 680 nm emission. The intensity of the 680 nm fluorescence emission continued to increase as more Triton X-100 was added, until limiting concentrations of detergent were reached. These limiting concentrations were proportional to the amount of membrane present and generally occurred at Triton X-100/chlorophyll (w/w) ratios of 100–200:1. In all cases the detergent effect was seen within 10 min, and is often considerably faster, with longer detergent treatments causing no further effects. The data are discussed in terms of a three-stage mechanism for detergent solubilization of membrane components.

Non-ionic surfactants, such as Triton X-100, have been used extensively in a wide variety of biological systems for both preparative and functional studies of membrane proteins. The interaction of such micelle-forming amphiphiles with biological membranes is a highly complex process. It involves the initial insertion of individual surfactant molecules into the lipid bilayer, followed by the formation of mixed lipid–detergent micelles and even large lipoprotein bilayer discs stabilized by a pseudo-micellar annulus of detergent (Dunahay *et al.*, 1984). The process culminates in the incorporation of all of the membrane components into detergent micelles (Helenius & Simons, 1975; Tanford & Reynolds, 1976; Lichtenberg *et al.*, 1983). In principle, all of these processes are reversible on detergent removal, although, in

the case of membrane proteins, the proviso must be added that irreversible denaturation must not have occurred.

In studies of photosynthetic membranes, Triton X-100 has been employed for the preparation of a variety of fractions ranging from pure LHC-I (Haworth *et al.*, 1983; Anderson *et al.*, 1983) or LHC-II pigment–protein complexes (Mullet *et al.*, 1980) to fractions containing relatively large fragments of appressed thylakoid membranes (Dunahay *et al.*, 1984). In general, it has been found that the structural integrity of protein complexes purified in the presence of Triton X-100 was very good (Mullet *et al.*, 1980; Anderson *et al.*, 1983). In contrast, the effects of the detergent on thylakoid membrane function, and their possible reversibility, which are of great interest for reconstitution studies, largely remain uncharacterized.

The effects of Triton X-100 on energy transfer and electron transport in photosynthetic membranes, and the subsequent reversal of these effects after membrane reconstitution, have been examined in a series of recent studies (Murphy, 1984; Murphy & Woodrow, 1984; Murphy *et al.*, 1984). In the present paper, the effects of Triton X-100 on

Abbreviations used: LHC-I and LHC-II, light-harvesting complexes I and II; PSI and PSII, Photosystems I and II.

† To whom correspondence and reprint requests should be addressed at the following permanent address: Department of Botany, University of Durham, South Road, Durham DH1 3LE, U.K.

a series of functional properties of thylakoid membranes, or subfractions thereof, have been studied. It is concluded that Triton X-100 manifests its effects on different membrane functions at detergent concentrations that may vary more than 500-fold from the most labile function (PSII oxygen evolution) to the most resistant function (LHC-II fluorescence emission at 680 nm). A mechanism is proposed for the sequential interaction of Triton X-100 with photosynthetic membrane components.

Experimental

Preparation of photosystem complexes

Dwarf pea (*Pisum sativum* L. var. Kelvedon Wonder) plants were grown in Vermiculite in a greenhouse for 2–3 weeks before harvesting. The leaves were homogenized for two bursts of 1 s in medium containing 0.33 M-glucose, 50 mM- $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffer, pH 6.5, 5 mM- MgCl_2 and 25 mM-NaCl. Chloroplast membranes were sedimented by centrifugation at 3000 *g* for 15 s. The chloroplasts were resuspended in a 'breaking' medium containing 5 mM- MgCl_2 and re-sedimented at 3000 *g* for 2 min. The thylakoid pellet was resuspended and left for 1.5 h in a 'stacking' medium of 0.33 M-sorbitol, 2 mM-Mes, pH 6.3, 5 mM- MgCl_2 and 15 mM-NaCl. For the preparation of PSI complexes, stacked thylakoids at 1 mg of chlorophyll $\cdot \text{ml}^{-1}$ were thrice passed through a Yeda press at 11 MPa (1600 lbf/in²). The pressed material was centrifuged at 40 000 *g* for 40 min and the supernatant from this was re-centrifuged at 100 000 *g* for 1 h to sediment the PSI fraction. This fraction was a highly purified stromal lamella preparation and was virtually free of contamination by LHC-II and PSII chlorophyll-protein complexes, as determined by PSII electron-transport rates, low-temperature fluorescence-emission spectra and polyacrylamide-gel electrophoresis. For preparation of PSII complexes, stacked thylakoids were incubated in the dark for 25 min at 2 mg of chlorophyll $\cdot \text{ml}^{-1}$ in stacking buffer containing 50 mg of Triton X-100 $\cdot \text{ml}^{-1}$. The PSII complexes were then sedimented at 40 000 *g* for 30 min. This PSII fraction contained only minimal LHC-I and PSI contamination. Both PSI and PSII complexes were resuspended in a medium, termed 'buffer A', which consisted of 0.33 M-sorbitol, 50 mM-Hepes, pH 7.6, 2 mM-EDTA, 5 mM- MgCl_2 and 1 mM- MgCl_2 . Unless specified otherwise, all procedures were carried out at 4°C. Chlorophyll concentrations were determined as described by Bruinsma (1961).

77 K fluorescence-emission spectra

Unless stated otherwise, fluorescence-emission

spectra were measured at 77 K with 35 μg of chlorophyll $\cdot \text{ml}^{-1}$ in a total sample volume of 100 μl . Fluorescence emissions were collected at an angle of 30° from the broad-band blue actinic light via a fibre-optic light-guide. Actinic-light intensity was 13 W $\cdot \text{m}^{-2}$. Emission wavelengths (± 1 nm) from 650 to 800 nm were detected by an E.M.I. 9558 photomultiplier tube and defined by a Corning 2-60 filter and an Applied Photophysics f3.4 grating monochromator. Scans were controlled and date-recorded, averaged (of five scans) and normalized on a BBC model B micro-computer. The sample vessel consisted of a brass-encased glass Dewar flask filled with liquid N_2 , into which glass tubes containing samples were immersed.

Results

The results presented in Fig. 1 show that, for both PSI and PSII complexes, the intensity of the major 77 K fluorescence emissions (at 730 and 685 nm respectively) was linear with respect to chlorophyll concentration over the range 0–50 $\mu\text{g} \cdot \text{ml}^{-1}$. In the case of the PSII emission spectrum, which exhibits peaks at 685 and 695 nm, the ratio of the intensity of these peaks also remained unchanged over the range 0–50 μg of chlorophyll $\cdot \text{ml}^{-1}$. This indicates that the spectra are not affected by artefacts due to self-absorption at these chlorophyll concentrations. In all subsequent fluorescence measurements, a chlorophyll concentration of 35 $\mu\text{g} \cdot \text{ml}^{-1}$ was used, since this was well within the linear portion of the intensity-versus-concentration curve, but was also sufficiently high to ensure a good signal-to-noise ratio under the normal operating conditions of the spectrofluorimeter.

Fig. 1. Effect of chlorophyll concentration on the intensity of 77 K fluorescence emission by (a) PSI complexes at 735 nm and (b) PSII complexes at 685 nm.

Complexes were isolated from pea thylakoids, and fluorescence emissions were measured as described in the Experimental section.

The kinetics of Triton X-100 interactions with PSI and PSII complexes were monitored by studying the time course of the increase in 680nm fluorescence emission at 77K after addition of the detergent to the complexes at $35\mu\text{g}$ of chlorophyll $\cdot\text{ml}^{-1}$. At Triton X-100 concentrations below $0.15\text{--}0.18\text{mg}\cdot\text{ml}^{-1}$ there was no increase in the 680nm emission, irrespective of incubation time or chlorophyll concentration. Even at very low chlorophyll concentrations, where the detergent/chlorophyll ratio reached 20:1, there was still no evidence of the onset of membrane solubilization as judged by an increase in the 680nm emission. At Triton X-100 concentrations above $0.15\text{--}0.18\text{mg}\cdot\text{ml}^{-1}$ there was a rapid increase in 680nm intensity. The half-time of this increase ($t_{1/2}$) was roughly proportional to the detergent/chlorophyll ratio up to limiting values where solubilization was already complete after only 10s. The detergent concentration at these limiting values of $t_{1/2}$, depended on chlorophyll concentration. These limiting values of $t_{1/2}$ occurred in the region of a 100:1 Triton X-100/chlorophyll ratio for PSI complexes and 30:1 ratio for PSII complexes at low chlorophyll concentrations, i.e. $5\text{--}50\mu\text{g}\cdot\text{ml}^{-1}$. Even at relatively low Triton X-100 concentrations, e.g. $0.35\text{mg}\cdot\text{ml}^{-1}$, where the Triton X-100/chlorophyll ratio was only 10:1, the $t_{1/2}$ of 680nm intensity was only of the order of 0.50.5min.

At much higher chlorophyll concentrations, i.e. above $100\mu\text{g}\cdot\text{ml}^{-1}$, the Triton X-100-induced rise in 680nm intensity was appreciably slower, even when high ratios of detergent to chlorophyll (40:60:1) were employed. However, in all cases, $t_{1/2}$ occurred within 4min of the addition of detergent and the rise in 680nm intensity was complete in less than 10min. These increases in 680nm intensity were accompanied, in the case of PSI complexes, by a simultaneous decrease in 735nm intensity. This change in the 735nm intensity was smaller than the change in 680nm intensity, but otherwise their kinetics were identical, albeit in opposite directions (results not shown).

It was thus demonstrated that the increases in 680nm intensity due to Triton X-100 solubilization reached a maximum within 2min at a chlorophyll concentration of $35\mu\text{g}\cdot\text{ml}^{-1}$. Similar results were obtained over the entire concentration range of $5\text{--}30\mu\text{g}$ of chlorophyll $\cdot\text{ml}^{-1}$. Therefore PSI or PSII complexes were incubated for 2min in the presence of a range of Triton X-100 concentrations at four different chlorophyll concentrations (Figs. 2 and 3 respectively). The aim of this experiment was to determine whether the onset of Triton X-100 solubilization, and its subsequent kinetics with respect to Triton X-100 concentration, were affected by chlorophyll concentration.

Fig. 2. Effect of Triton X-100 concentration on the intensity of F_1 fluorescence emission of PSI complexes at chlorophyll concentrations of (a) $10\mu\text{g}\cdot\text{ml}^{-1}$, (b) $20\mu\text{g}\cdot\text{ml}^{-1}$, (c) $35\mu\text{g}\cdot\text{ml}^{-1}$ and (d) $50\mu\text{g}\cdot\text{ml}^{-1}$.

PSI complexes were incubated with Triton X-100 for 2min before freezing in liquid N_2 .

In Fig. 2 and subsequent Figures, the following symbols are used: F_1 , fluorescence peak arising from antenna chlorophyll molecules in intact membranes (about 685nm), or free LHCP-II or PSI centres in solubilized membranes (680nm); F_2 , fluorescence peak from PSII reaction centres (695nm); F_3 , fluorescence peak from PSI complexes, probably arising from a component of LHCP-I (735nm). From Fig. 2 it can be readily discerned that the onset of the F_1 increase resulting from Triton X-100 solubilization of PSI complexes occurred at $0.1\text{--}0.3\text{mg}$ of Triton X-100 $\cdot\text{ml}^{-1}$ at all but the highest chlorophyll concentrations. However, the magnitude of the increase in F_1 intensity continued to rise with increasing Triton X-100 concentrations until it reached limiting values. These values were clearly related to the chlorophyll concentration. Thus the F_1 maximum was reached at about 3, 10, 20 and 30mg of Triton X-100 $\cdot\text{ml}^{-1}$ at chlorophyll concentrations of 10, 20, 35 and $50\mu\text{g}\cdot\text{ml}^{-1}$ respectively.

The onset of the increase in F_1 intensity of PSII complexes also occurred at $0.1\text{--}0.3\text{mg}$ of Triton X-100 $\cdot\text{ml}^{-1}$ at 10, 20 and $35\mu\text{g}$ of chlorophyll $\cdot\text{ml}^{-1}$ (Fig. 3), in a similar manner to PSI complexes. PSII complexes also behaved very much like PSI complexes in that their F_1 intensities peaked at

Fig. 3. Effect of Triton X-100 concentration on the intensity of F_1 fluorescence emission of PSII complexes at chlorophyll concentrations of (a) $10 \mu\text{g}\cdot\text{ml}^{-1}$, (b) $20 \mu\text{g}\cdot\text{ml}^{-1}$, (c) $35 \mu\text{g}\cdot\text{ml}^{-1}$ and (d) $50 \mu\text{g}\cdot\text{ml}^{-1}$.

PSII complexes were incubated with Triton X-100 for 2 min before freezing in liquid N_2 .

higher Triton X-100 concentrations if the chlorophyll concentration was increased. One striking difference between PSI and PSII was the decline in F_1 intensity of PSII complexes at very high detergent concentrations. This decline was related to a broadening of the F_1 peak and the appearance of a new fluorescence peak at about 672 nm. A series of representative 77K fluorescence-emission spectra showing the effect of Triton X-100 solubilization on PSI and PSII complexes are shown in Fig. 4. The appearance of the 672 nm emission peak can be seen at the highest Triton X-100 concentrations only in PSII complexes. However, when a similar experiment was performed with whole thylakoids, which contain both PSI and PSII complexes, there was also a decrease in F_1 intensity and an appearance of a 672 nm peak at high (about $10 \text{ mg}\cdot\text{ml}^{-1}$) Triton X-100 concentrations (results not shown).

The increase in intensity of the F_1 emission at 680 nm can be seen after the progressive addition of Triton X-100 to PSI complexes (Fig. 4a). During this time, the intensity of the F_3 peak at about 735 nm was unaltered, although its wavelength was blue-shifted by 2–4 nm in the presence of the detergent. At the highest Triton X-100 concentra-

tions, the F_1 peak was also blue-shifted by about 2 nm (see also Fig. 5). In Fig. 5 the effect of Triton X-100 concentration on the wavelength of the F_1 emission peak of PSI and PSII complexes is shown. In a similar manner to its effect on fluorescence intensity, the detergent began to reduce the F_1 wavelength at 0.1–0.3 mg of Triton X-100 $\cdot\text{ml}^{-1}$. The magnitude of the subsequent wavelength reduction was related to the chlorophyll concentration, although, even at the highest detergent/chlorophyll ratios, there was still evidence of a continuing blue-shifting of the F_1 peak. This indicates that some membrane structures may not have been completely solubilized, even at detergent/chlorophyll ratios in excess of 5000:1.

Discussion

The solubilization of photosynthetic membranes by a non-ionic detergent, such as Triton X-100, appears to proceed in at least three stages. This is true whether the membranes be whole thylakoids or vesicles enriched in certain thylakoid proteins. The first stage involves the incorporation of the monomeric form of Triton X-100 into the membranes. This will occur at Triton X-100 concentrations below its critical micellar concentration of about $0.16 \text{ mg}\cdot\text{ml}^{-1}$ (Kushner & Hubbard, 1954). The incorporation of cone-shaped molecules, such as Triton X-100, into a biological membrane will impose alterations in the packing of the lipid and protein components of the membrane. These perturbations in the membrane organization may lead to changes in its function. Both PSI and PSII electron-transport activities were sensitive to Triton X-100 below its critical micellar concentration (results not shown), although there was no effect whatsoever on 77K fluorescence emission at these low detergent concentrations. This was true even for prolonged exposure (i.e. 20–30 min) of the membrane fractions to such Triton X-100 concentrations. A similar lack of effect of a range of surfactants, at subsolubilizing concentrations, on the fluorescence yield of wheat thylakoids has recently been reported (Bartzatt *et al.*, 1983).

The second phase of membrane solubilization occurred at, or slightly above, the critical micellar concentration of Triton X-100. This phase was characterized by the onset of a rapid increase in both the PSI activity and the low-wavelength (F_1) component of the 77K fluorescence emission (Figs. 2–4). Both of these effects are probably due to membrane fragmentation and the formation of mixed micelles. It has recently been reported by Benesova *et al.* (1984) that low concentrations of Triton X-100 inhibited PSI activity in *Beta vulgaris* (beet) thylakoids, in a manner similar to that observed in the present study, although the

Fig. 4. 77K fluorescence-emission spectra of (ai) untreated PSI complexes, (aii) PSI complexes + 0.3 mg of Triton X-100 $\cdot$ ml $^{-1}$, (aiii) PSI complexes + 3.0 mg of Triton X-100 $\cdot$ ml $^{-1}$, (aiv) PSI complexes + 100 mg of Triton X-100 $\cdot$ ml $^{-1}$, (bi) untreated PSII complexes, (bi) PSI complexes + 0.3 mg of Triton X-100 $\cdot$ ml $^{-1}$, (biii) PSI complexes + 3.0 mg of Triton X-100 $\cdot$ ml $^{-1}$ and (biv) PSII complexes + 30 mg of Triton X-100 $\cdot$ ml $^{-1}$

All spectra were recorded at 35 μ g of chlorophyll $\cdot$ ml $^{-1}$. Scans were performed as described in the Experimental section.

subsequent enhancement of PSI activity at high Triton X-100 concentrations was much less marked than that demonstrated here for both whole thylakoids and PSI complexes. At these higher detergent concentrations there is also a physical dissociation of pigment-protein complexes, as recently demonstrated by Murphy (1984). This would cause a decrease in the amount of coupling between the LHCs and the reaction-centre complexes of both PSI and PSII. The result would be a reduction in the quenching of LHC fluorescence by the PSI and PSII reaction centres, which would in turn lead to a substantial increase in the low-wavelength (about 680 nm) fluorescence emission associated with free LHC-II (Anderson *et al.*, 1978) or decoupled LHC-I-PSI complexes (Murphy, 1984).

The wavelength maximum of the F_1 fluores-

cence peak in native thylakoid membranes is normally at 685 nm and is believed to be due to antenna-chlorophyll emission (Rijgersberg *et al.*, 1979; Breton, 1982). These antenna chlorophyll molecules are associated with the PSII reaction-centre complex, but their fluorescence emission is due mainly to the excitation energy transferred from chlorophyll molecules in the LHC-II complex. After the addition of increasing amounts of Triton X-100 to PSI or PSII complexes, there was no change in the F_1 wavelength until the critical micellar concentration of the detergent was reached. A progressive decrease in the F_1 wavelength maximum was then observed until, at 1–3 mg of Triton X-100 $\cdot$ ml $^{-1}$, a steady value of 680 nm was reached. These results may be interpreted as reflecting the gradual cessation of excitation-energy transfer from LHC-II to the PSII

Fig. 5. Effect of Triton X-100 concentration on the wavelengths of (a) PSI and (b) PSII F_1 fluorescence emission maxima at 77K

Chlorophyll concentrations ($\mu\text{g/ml}$) were: ■, 10; ▲, 20; ●, 35; ◆, 50.

antenna chlorophyll molecules as the protein complexes dissociate in the presence of the detergent.

The third phase of membrane solubilization was seen when Triton X-100 was considerably in excess of its critical micellar concentration. Most of the changes in membrane function associated with this phase began to occur at the critical micellar concentration. However, their magnitude increased with the detergent concentration until limiting values were reached. The limiting values of different membrane functions were reached at very similar Triton X-100 concentrations, providing these experiments were performed at similar chlorophyll concentrations. For example, at $10\mu\text{g}$ of chlorophyll $\cdot\text{ml}^{-1}$, both PSI activity and PSI F_1 fluorescence emission reached maximum values at 1 mg of Triton X-100 $\cdot\text{ml}^{-1}$, whereas that of PSII F_1 fluorescence emission was reached at 3 mg of Triton X-100 $\cdot\text{ml}^{-1}$. At $50\mu\text{g}$ of chlorophyll $\cdot\text{ml}^{-1}$, all of these maxima were reached at 10 mg of Triton X-100 $\cdot\text{ml}^{-1}$.

It may be deduced from these data that, although the onset of Triton X-100 solubilization occurred in the vicinity of its critical micellar concentration irrespective of the amount of membrane present, the kinetics of subsequent functional changes were strongly affected by the ratio of detergent to membrane. This is because the

critical micellar concentration of free (i.e. non-membrane-incorporated) Triton X-100 is independent of the amount of membrane present, and formation of mixed detergent-lipid micelles, with an associated perturbation of membrane organization, will occur above this concentration. However, for complete solubilization, in which all the lipid and protein is in mixed micelles, the minimum amount of detergent necessary will be proportional to the amount of membrane present (Lichtenberg *et al.*, 1983). In the present system, complete solubilization was achieved at relatively high detergent/chlorophyll ratios, i.e. 100–200:1. It is important to stress that, at lower detergent/chlorophyll ratios, e.g. 20:1, only partial membrane solubilization occurred *irrespective of the time of detergent incubation*. In all cases, no more solubilization occurred after 10 min of detergent incubation of membranes. Therefore the use of sub-saturating detergent concentrations leads to the formation of a mixture of solubilized and intact membranes.

The electrostatic and/or van der Waal's forces involved in protein-protein interactions are significantly stronger than the qualitatively similar forces involved in detergent-lipid or detergent-protein interactions. This means that, at sub-saturating detergent concentrations, membrane regions of significant protein-protein interaction, e.g. appressed thylakoids, would be left relatively intact, whereas other regions would undergo solubilization. This feature of Triton X-100 interactions with thylakoid membranes has been exploited in the preparation of so-called 'Photosystem II preparations' (Berthold *et al.*, 1981; Ford & Evans, 1983).

These preparations are now known to be appressed thylakoid regions from which the non-appressed regions have been removed by solubilization (Dunahay *et al.*, 1984). When the detergent concentration in PSII preparations or thylakoids was raised even further, there were dramatic effects on F_1 fluorescence emission (see Fig. 3). These began at Triton X-100/chlorophyll ratios of 300:1 and reached limiting values at 1000:1. They involved the decrease in 680 nm fluorescence emission and the appearance of a peak at 672 nm. These effects are probably caused by the eventual solubilization of the appressed thylakoid regions. The removal of acyl lipid by the detergent probably results in destabilization of the protein-protein interactions and, in the limiting case, the complete dissociation of the protein complexes from one another. It may be that the 672 nm fluorescence emission is characteristic of fully dissociated (from other protein complexes) LHC-II complexes, where each LHC-II complex would be incorporated into a detergent micelle. This conclusion has

been strengthened by the recent finding that these changes were substantially reversible after detergent removal and the reincorporation of the protein complexes into liposomes (D. J. Murphy, unpublished work). We have also found that, at very high detergent/chlorophyll ratios, a substantial proportion of the LHC-II of thylakoid membranes became completely dissociated and would not aggregate even in the presence of Mg^{2+} . The effect of Triton X-100 on electron transport in plant mitochondria also exhibits three phases, occurring below, at, and substantially above, the critical micellar concentration of the detergent (Dizengremel, 1983a,b). It is thus likely that the mechanism of detergent-membrane interactions described herein is applicable to other biological systems.

This work was funded by a grant from the Agriculture and Food Research Council, U.K. D.J.M. is the recipient of a Royal Society Anglo-Australian Fellowship. We thank Professor U.-P. Shyminder, Dr. I. E. Woodrow and Dr. B. B. Pelzufer for their helpful comments and encouragement.

References

- Anderson, J. M., Waldron, J. C. & Thorne, S. W. (1978) *FEBS Lett.* **92**, 227–233
- Anderson, J. M., Brown, J. S., Lam, E. & Malkin, R. (1983) *Photochem. Photobiol.* **38**, 205–210
- Bartzatt, R. A., Young, C.-M. & Markwell, J. P. (1983) *Biochim. Biophys. Acta* **725**, 341–348
- Benesova, H., Hladic, J., Kaplanova, M. & Sofrova, D. (1984) *Photobiochem. Photobiophys.* **7**, 15–24
- Berthold, D. A., Babcock, G. T. & Yocum, C. F. (1981) *FEBS Lett.* **134**, 231–234
- Breton, J. (1982) *FEBS Lett.* **147**, 16–20
- Bruinsma, J. (1961) *Biochim. Biophys. Acta* **52**, 576–578
- Dizengremel, P. (1983a) *Physiol. Vég.* **21**, 731–741
- Dizengremel, P. (1983b) *Physiol. Vég.* **21**, 743–752
- Dunahay, T. G., Staehelin, L. A., Scibert, M., Ogilvie, P. D. & Berg, S. P. (1984) *Biochim. Biophys. Acta* **764**, 179–193
- Ford, R. C. & Evans, M. (1983) *FEBS Lett.* **160**, 159–164
- Haworth, P., Watson, J. L. & Artzen, C. J. (1983) *Biochim. Biophys. Acta* **724**, 151–158
- Helenius, A. & Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29–79
- Kushner, L. M. & Hubbard, W. D. (1954) *J. Phys. Chem.* **58**, 1163–1167
- Lichtenberg, D., Robson, R. J. & Dennis, E. A. (1983) *Biochim. Biophys. Acta* **737**, 285–304
- Mullet, J. E., Burke, J. J. & Arntzen, C. J. (1980) *Plant Physiol.* **65**, 814–822
- Murphy, D. J. (1984) in *Structure, Function and Metabolism of Plant Lipids* (Siegenthaler, P. A., ed.), pp. 465–467, Elsevier/North-Holland, Amsterdam
- Murphy D. J. & Woodrow, I. E. (1984) *Biochem. J.* **224**, 989–993
- Murphy, D. J., Crowther, D. & Woodrow, I. E. (1984) *FEBS Lett.* **165**, 151–155
- Rijgersberg, C. P., Amesz, J., Thielen, A. P. G. M. & Swager, J. A. (1979) *Biochim. Biophys. Acta* **545**, 473–482
- Tanford, C. & Reynolds, J. A. (1976) *Biochim. Biophys. Acta* **457**, 29–79