Biochimica et Biophysica Acta, 599 (1980) 63—72

BBA 78788

THE FLUIDITY OF CHLOROPLAST THYLAKOID MEMBRANES AND THEIR CONSTITUENT LIPIDS

A COMPARATIVE STUDY BY ESR

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(Received October 1st, 1979)

Key words: Chloroplast; Thylakoid membrane; Membrane lipid; Spin labeling; (Hordeum vulgare)

Summary

Comparative measurements were made of the fluidity of chloroplast thylakoids, total membrane lipids and polar lipids utilizing the order parameter and motion of spin labels.

No significant differences were found in the fluidity of membranes or total membrane lipids from a wild type and a mutant barley (*Hordeum vulgare* chlorina f_2 mutant) which lacks chlorophyll *b* and a 25 000 dalton thylakoid polypeptide. Redistribution of intrinsic, exoplasmic face (EF) membrane particles by unstacking thylakoid membranes in low salt medium also had no effect on membrane fluidity. However, heating of isolated thylakoids decreased membrane fluidity.

The fluidity of vesicles composed of membrane lipids is much greater than that of the corresponding membranes. Fluidity of the membranes, however, increased during greening indicating that the rigidity of the membranes, compared with that of total membrane lipids, is not caused by chlorophyll or its associated peptides. It is concluded that the restriction of motion in the acyl chains in the thylakoids is not caused by chlorophyll or the major intrinsic polypeptide but by some other protein components.

Abbreviations: ESR, electron spin resonance; LHCP, light-harvesting chlorophyll protein; SDS, sodium dodecyl sulphate.

Introduction

Many biological membranes are currently interpreted in terms of the Singer-Nicolson model in which the proteins are globular structures within a fluid lipid bilayer [1]. In this model, only the boundary lipids, those lipids immediately adjacent to intrinsic proteins, would be expected to show a greatly restricted fluidity of their acyl chains [2,3]. Providing such boundary lipids form a small percentage of the total lipid in the membrane, their contribution to the bulk lipid fluidity, measured by spin labelling and electron spin resonance spectroscopy (ESR), will be small because the reporter molecule will preferentially partition into the most fluid region of the heterogeneous membrane environment [4]. In vesicles formed from the cell-envelope of *Halobacterium cutirubrum* [5] the motion of nitroxide labelled fatty acids is however very much restricted compared to that in vesicles of the extracted lipids alone. This has been interpreted as a special case in which the membrane consists entirely of a protein matrix and hydrophobically bound, ordered lipids and results from the high ratio (5 : 1) of protein to lipid. Similar results have also been reported using Sindbis virus membranes for which the protein lipid ratio is 2 : 1 [6] (cf. also Ref. 7). In cytochrome oxidase preparations which were reconstituted with known amounts of lipids, NMR studies indicated that at protein to lipid ratios greater than 1 : 1 the acyl chains of the lipid were disordered and their motion was considerably restricted [7]. Similar conclusions have been reached with reconstituted rhodopsin preparations [8].

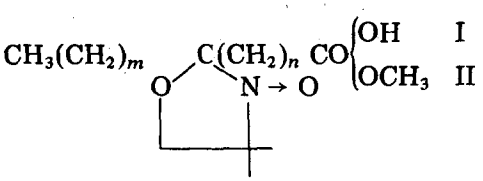
We were interested in examining a membrane system in which the protein lipid ratio is approximately 1 : 1, and where there is no a priori reason to believe that intrinsic protein will affect the fluidity of the bulk of lipids within the bilayer. Chloroplast thylakoid membranes were chosen because the motion of the acyl chains of isolated lipids was less restricted than the lipids of the membranes [9]. In addition the chloroplast has the advantage that it can be manipulated by genetic, experimental and environmental means to vary both the peptide composition and the structure of the membranes as visualised by freeze-fracture electron microscopy [10,11]. The results reported provide clear evidence that in thylakoid membranes the 'order' of the bulk lipids, measured by spin labelling, is increased by protein. This increase in order is not however due to the most abundant thylakoid proteins, the apoproteins of the chlorophyll protein complexes. These results have been previously reported in preliminary form [12].

Materials and Methods

Barley plants (*Hordeum vulgare* L. cv. Abyssinian and chlorina f_2 mutant [13]) were grown in a growth room in continuous light at 25°C (or as described in the text). Spinach plants (*Spinacea oleracea* var. Dominant) were grown in the field during the months of July to October. Chloroplasts were routinely isolated by blending the leaves in 0.05 M phosphate buffer, pH 7.2 containing 0.01 M KCl and 0.3 M sucrose as previously described [14]. Isolated chloroplasts were washed twice in 0.05 M phosphate buffer containing 0.01 M KCl, pH 7.2, to give thylakoids substantially free of stroma protein. Destacking and

restacking of chloroplast grana was performed by the isolation and resuspension techniques described by Staehlin [15]. Total lipids (including chlorophyll) were removed from the thylakoid membranes by extracting with chloroform/methanol, (2 : 1, v/v). The lipids were dried and fractionated on acid washed Florisil as described by Nolan and Bishop [9].

For ESR studies, chloroplast thylakoid membranes were suspended at a final chlorophyll concentration of 1 to 2 mg/ml. Lipids were dispersed, by gentle sonication, in 0.1 M Tris acetate buffer, pH 7.2, containing 0.001 M EDTA and $2 \cdot 10^{-4}$ M *N*-ethylmaleimide to give a concentration of about 10 mg/ml. The suspension consisted of multilayered liposomes. Membrane lipids and liposomes were labelled with nitroxide derivatives of either stearic acid (I) or methyl stearate (II) of the general formula.



The spin labels used in the present work were $I_{(m,n)}$ and $II_{(m,n)}$ where (m,n) = (11,4), (8,7), (5,10) and (1,14).

Nitroxide spin labels were incorporated into the preparations by drying the labels onto the side of a small glass vial to which was added the thylakoid membranes or sonicated lipids and then agitating on a Vortex mixer for 2×10 s. The ratio of lipid to spin label was always greater than 100 : 1. There was no evidence of line broadening of spectra due to spin-spin interaction as judged by varying the ratio of lipid to spin label by a factor of five. Spectra were recorded using a Varian E-4 ESR spectrometer fitted with a temperature-controlled cell housing. Each spectrum was recorded three times to permit accurate calculation of the motion and order parameters. Quantitative assessment of the amplitude of motion characterising the flexibility gradient of the acyl fatty acid chains of membrane lipids was determined from the order parameter S_n where n designates the position of the nitroxide group measured by the number of methylenes from the polar head group. S_n was calculated from the separation of the inner and outer extrema ($2T_1$ and $2T_{11}$) as described by Esser and Lanyi [5]. Where the value of $2T_{11}$ could not be determined with confidence (as with $I_{(5,10)}$ in some lipid preparations) S_n was calculated from $2T_1$ alone using a value for a' (the hyperfine splitting constant) of 14.35.

For labels undergoing relatively rapid isotropic motion, S_n can not be determined but the rate of tumbling, expressed as the rotational correlation time τ_0, is proportional to the viscosity of the host system incorporating the label [16]. In this study we calculated τ_0, the empirical motion parameter, as described by Henry and Keith [17]. To a first approximation τ_0 is a measure of molecular tumbling and between the limits 10^{-10} and 10^{-8} s can be used as a comparative measure of membrane fluidity.

Results

The most abundant thylakoid protein is a 25 000 dalton polypeptide which forms part of the apoprotein of the light-harvesting chlorophyll protein com-

plex (LHCP) [10,11]. The effect of this protein on the fluidity of membrane lipid was investigated by comparing the motion of the nitroxide spin labels intercalated into thylakoid membranes from normal and mutant barley. The mutant of barley used was one which lacks chlorophyll *b*, LHCP, a major 25 000 dalton lamellar polypeptide [18] and has reduced diameter particles on the EFs fracture face [10,11]. Table I shows the order parameters obtained for the nitroxide spin labels $I_{(11,4)}$, $I_{(8,7)}$ and $I_{(5,10)}$ in chloroplast fragments of mutant and wild type barley. There was no difference between the S_n values for wild type and mutant barley chloroplasts nor in the S_n values for total lipid, determined by $I_{(11,4)}$. The S_n value for total lipid was however significantly lower than that of the corresponding thylakoid membranes of both normal and mutant barley.

Unstacking of thylakoid membranes under conditions of low cation concentration results in a redistribution of the large EFs fracture face particles [15]. These particles, normally located in the grana regions, become evenly distributed into grana and stromal regions during unstacking [15]. The S_n values for $I_{(11,4)}$ obtained with spinach chloroplasts following experimental unstacking and restacking are shown in Table II. No significant difference was observed in the S_n values after this treatment. This indicates that the redistribution of large particles and associated LHCP does not significantly alter lipid ordering, in agreement with the results reported above for wild type and mutant barley. Failure to detect differences in either of the above experiments might have resulted from preferential incorporation of the spin label into the ectoplasmic half of the lipid bilayer. This is unlikely as chloroplast fragments were used and similar S_n values were obtained when thylakoids were sonicated in the presence of spin label, conditions which would ensure labelling both halves of the bilayer.

During the greening of etiolated leaves, synthesis of chlorophyll is accompanied by marked qualitative and quantitative changes in the constituent peptides of the thylakoids [19]. Amongst these changes, the most prominent are increases in the apoproteins of chlorophyll protein complex I and LHCP. The fluidity of thylakoid membranes during chloroplast development was monitored with a variety of spin labels to see if the relative rigidity of the membrane resulted from insertion of chlorophyll. Results for $I_{(8,7)}$ are presented in Table III. At all stages the lipids in the membranes are relatively immobile and

TABLE I

A COMPARISON OF THE FLUIDITY OF CHLOROPLAST THYLAKOID MEMBRANES AND TOTAL LIPIDS OF WILD TYPE AND A MUTANT OF BARLEY LACKING CHLOROPHYLL *b*

Data are presented as S_n values determined with $I_{(11,4)}$, $I_{(8,7)}$ and $I_{(5,10)}$ at 22°C.

Label	Chloroplasts		Total lipid	
	Wild type	Mutant	Wild type	Mutant
$I_{(11,4)}$	0.767 ± 0.006	0.765 ± 0.008	0.726 ± 0.006 *	0.716 ± 0.005 *
$I_{(8,7)}$	0.751 ± 0.008	0.749 ± 0.011	—	—
$I_{(5,10)}$	0.687 ± 0.014	0.688 ± 0.012	—	—

* Lipid values significantly different from corresponding chloroplast values $P < 0.01$.

TABLE II

THE INFLUENCE OF MEMBRANE STACKING ON THE S_n OF SPINACH CHLOROPLAST THYLAKOID MEMBRANES AS MONITORED BY $I_{(11,4)}$ AT 22°C

Conditions	Stacked	Unstacked	Experimentally restacked
S_n	0.734 ± 0.008	0.745 ± 0.007	0.745 ± 0.001

the tendency is for their fluidity to increase as chlorophyll and its associated proteins are synthesised. We also observed (results not shown) an increase in membrane lipid fluidity in chloroplasts after 36 h greening compared to that of the original etioplast membranes as monitored by $I_{(11,4)}$, $I_{(5,10)}$, $I_{(1,14)}$, $II_{(1,14)}$.

It was previously shown (Table I) that the S_n value for $I_{(11,4)}$ in a total lipid preparation was significantly lower (i.e. lipids less ordered) than that of the original thylakoid membranes from which it was derived. This observation was extended with a range of spin labels and constituent lipid fractions to determine the relative contribution of particular lipids to the fluidity gradient. The results are presented in Table IV as S_n values for $I_{(11,4)}$, $I_{(8,7)}$ and $I_{(5,10)}$ and in Table V as the empirical motion parameter τ_0 for $II_{(8,7)}$, $II_{(5,10)}$, $II_{(1,14)}$ and $I_{(1,14)}$. With all labels and with thylakoid membranes of both barley and spinach, the fluidity of the total lipids was significantly greater than that of the corresponding thylakoids. Since this was evident with both $I_{(5,10)}$ and $I_{(1,14)}$ the restriction in acyl chain fluidity extends to the middle of the bilayer.

An S_n value determined with total lipid preparations which include chlorophyll, may be misleading because there is compelling evidence that the greater part of the chlorophyll in membranes is combined with protein rather than lipid [10,11]. We therefore determined the S_n value for a thylakoid phospholipid preparation with additions of various proportions of galactolipids and galactolipids plus pigments (Table VI) (cf. Ref. 9). The S_n values obtained for phospholipid preparations were in all instances less than those of the corresponding total lipids and were further reduced by the addition of glycolipids (principally monogalactosyldiacylglycerol and digalactosyldiacylglycerol).

Addition of chlorophyll to either the phospholipid preparations or phospholipid plus glycolipid preparations resulted in significant increase in S_n values indicating a reduction in fluidity (i.e. an increase in order). For $I_{(11,4)}$ and $I_{(8,7)}$, the chlorophyll addition restored the S_n value almost to that of lipisomes of the total lipids, but with no combinations of lipids did the restric-

TABLE III

CHANGES IN CHLOROPLAST MEMBRANE FLUIDITY DURING THE GREENING OF ETIOLATED BARLEY LEAVES AS MONITORED BY THE ORDER PARAMETER S_n OF $I_{(8,7)}$

	Time of illumination (h)							
	0	0.5	1	2	3	5	16	36
S_n	0.803	0.778	0.780	0.777	0.773	0.753	0.745	0.753

TABLE IV

A COMPARISON OF THE S_n VALUES FOR BARLEY AND SPINACH CHLOROPLASTS WITH THEIR CONSTITUENT LIPIDS AT 22°C

	Barley			Spinach	
	$I_{(11,4)}$	$I_{(8,7)}$	$I_{(5,10)}$	$I_{(8,7)}$	$I_{(5,10)}$
Chloroplasts	0.771 ± 0.004	0.711 ± 0.001	0.677 ± 0.012	0.712 ± 0.008	0.649 ± 0.005
Total lipid	0.726 ± 0.009	0.674 ± 0.010	0.589 ± 0.007	0.635 ± 0.006	0.522 ± 0.010
Phospholipids	0.690 ± 0.006	0.644 ± 0.007	0.565 ± 0.008		
Phospholipids + galactolipids (4:1, w/w)	0.675 ± 0.004	0.623 ± 0.012	0.509 ± 0.004		
Phospholipids + galactolipids (6:4, w/w)	0.656 ± 0.004	0.614 ± 0.006	0.502 ± 0.015		
Phospholipids + pigments (2:1, w/w)	0.718 ± 0.002	0.666 ± 0.007	0.567 ± 0.008		
Phospholipids + pigments + galactolipids (4:2:1, w/w)	0.710 ± 0.004	0.668 ± 0.003	0.543 ± 0.006		

tion of motion approach that of the original thylakoid membrane.

In many biological systems nitroxide spin labels accept electrons and lose their paramagnetism. When sulphydryl groups are responsible for the reducing effect, the addition of *N*-ethylmaleimide (at about $2 \cdot 10^{-4}$ M) to the membrane suspension prevents loss of signal [20]. Heating the suspension at 70°C for 5 min also prevents loss of signal. However, as shown in Table VI there is a significant increase in the S_n value of thylakoid membranes after this type of heat treatment. This result is interpreted in terms of further cross-linking between thylakoid proteins during heat denaturation resulting in restriction of acyl chain movement.

An inspection of S_n values for barley thylakoids obtained with $I_{(8,7)}$ over some months showed that values ranged from 0.7 to 0.8 although duplicate preparations on any one day were in excellent agreement (S.D. of ±0.01). The barley plants used in this study were grown at high density together with other plants in a growth chamber for seven to eight days and light intensity was thus

TABLE V

COMPARISON OF THE MOTION OF SPIN LABELS WITH BARLEY AND SPINACH CHLOROPLAST THYLAKOIDS WITH THAT OF THEIR EXTRACTED LIPIDS

Data are presented as τ_0 (s) × 10^{10} determined with $II_{(8,7)}$, $II_{(5,10)}$, $II_{(1,14)}$ and $I_{(1,14)}$

Label	$II_{(8,7)}$		$II_{(5,10)}$		$II_{(1,14)}$		$I_{(1,14)}$	
Plant	Barley	Spinach	Barley	Spinach	Barley	Spinach	Barley	Spinach
Chloroplasts	182	178	58	59	18.7	12.6	21.9	19.0
Total lipid	42.4	33.1	25.3	18.1	9.9	9.4	13.0	12.0

TABLE VI

EFFECT OF HEATING TO 70°C FOR 5 min ON THE S_n OF BARLEY CHLOROPLAST THYLAKOIDS AS MONITORED BY $I_{(8,7)}$ AND $I_{(5,10)}$

Label	Control	Heated
$I_{(8,7)}$	0.711 ± 0.008	0.780 ± 0.009 *
$I_{(5,10)}$	0.677 ± 0.012	0.737 ± 0.010 *

* Significantly different from corresponding control $P < 0.01$.

considered a potential source of variability. This source of variation was examined directly by growing two groups of plants in the same growth room at markedly different light intensities. The results are presented in Table VII. Thylakoid membranes and total lipid preparations from the plants grown at the higher light intensities were more fluid than those from the lower light intensity. The peptide profiles (results not shown) determined by SDS-polyacrylamide gel electrophoresis were qualitatively very similar for both treatments and it was concluded that the differences in absolute S_n values are probably due to different lipid composition and/or ordering. Acyl chain mobility is however, in both cases, restricted by thylakoid proteins.

Discussion

The results presented in this paper demonstrate that nitroxide spin labels enter a lipid environment in chloroplast membranes which is much less fluid than that in liposomes composed of the membrane lipids. This reduction in fluidity is not caused by chlorophyll as shown by measurements made on thylakoid membranes from greening leaves. Nor is it likely to be due to binding of spin label to intrinsic membrane proteins. Such binding, to the extent of 60% of the added probe, has been reported for bacterial chromatophores [21]. However the protein/lipid ratio of chromatophores is 3 : 1 by weight whereas in chloroplast thylakoid membranes it is 1 : 1. In addition we observed no difference between thylakoids from wild type and mutant barley where the mutant lacks LHCP and a major 25 000 dalton polypeptide.

Absence of the 25 000 dalton polypeptide from the mutant barley is asso-

TABLE VII

EFFECT OF GROWTH CONDITIONS ON THE S_n VALUE FOR BARLEY CHLOROPLAST THYLAKOIDS AS MONITORED BY $I_{(8,7)}$ AT 22°C

Barley plants were grown for 8 days on a cycle of 24°C for 16 h light and 18°C for 8 h dark. The incident intensities were 10 and 2 $W \cdot m^{-2} \cdot s^{-1}$ for the high and low light growth conditions, respectively.

	High light growth		Low light growth	
	Chloroplasts	Total lipid	Chloroplasts	Total lipid
S_n	0.731 ± 0.008	0.648 ± 0.008	0.772 ± 0.003 *	0.684 ± 0.012 *

* Low light treatment significantly different from high light, $P < 0.01$.

ciated with a reduced size of the large particles on the EFs face. If the spaces between the large EFs particles were occupied by 'free' lipids, then it is unlikely that spin label techniques would detect any difference in fluidity between thylakoids of wild type and mutant barley, but neither should there be any difference between membranes and liposomes composed of membrane lipids. The lack of any difference between the fluidity of liposomes made of lipids from normal and mutant barley reflects the similarity of the lipid composition of their thylakoids [21].

The differences observed in the motion of spin labels in membranes compared to that in vesicles of their extracted lipids might in part be rationalised by considering the freeze-fracture particles to be protein aggregates surrounded by boundary lipids. Marcelja [23] has proposed that the effect of a protein on the order parameter of the acyl chains in a lipid bilayer is negligible after the two nearest neighbours. We have calculated the area of each half of the bilayer which could contain free lipid using the above proposition. For the outer half of the bilayer with particles 80 Å in diameter (surrounded by two layers of boundary lipids with an area per molecule of 70 Å [24] making an effective diameter 118 Å) and a density of 4000 particles per μm^2, 57% of the area is potentially occupied by free lipid. For the inner half of the bilayer with particles 180 Å in diameter (with lipid, the effective diameter would be 218 Å) and a density of 1500 particles per μm^2, 44% is available for free lipid. Provided the particles do not extend through the thylakoid membrane, we would, on the above calculations, expect no restriction in the motion of spin label in the membrane compared to that in lipid vesicles.

We therefore conclude that our results are incompatible with a fluid mosaic model of the thylakoid membrane in which all the proteins have the dimensions shown in freeze-fracture electron micrographs. It is possible for other reasons, e.g. existence of a protein network, that areas of free lipid are very small. If this were the case the spin labels would concentrate in these areas resulting in a broadening of the inner hyperfine splittings due to spin interaction [25]. No broadening of the midfield line of the absorption spectrum was observed even when the ratio of spin label to lipid was increased 5-fold.

It is also possible that the predominantly negative charge on the head groups of thylakoid lipids would repel acid spin labels and induce interaction with positively charged intrinsic proteins. This is also unlikely because the correlation coefficient, τ_0, of methyl esters of spin labels also indicate a marked decrease in fluidity in membranes compared with membrane lipids. The relatively increased motion of the methyl stearate labels compared to that of the corresponding acid labels, in both membranes and liposomes, might be attributed to their deeper penetration into the lipid bilayer.

While the restricted motion of a spin label in a membrane is not explicable in an interpretation of the fluid mosaic model as outlined above, the restricted motion is compatible with a model in which both the large and the small membrane particles observed in the fracture faces of thylakoids traverse the bilayer as proposed by Miller [26] and Arntzen [27]. Based on this model our calculations indicate there is no area available for free lipid and this would account for the restriction of spin label motion in thylakoid membranes. However, the two boundary layers of lipid, assumed to surround each membrane particle of

the dimensions above, can account for only about 10% of the total lipid. This figure is based on the assumption that the molecular weights of membrane proteins are comparable to those of globular proteins of similar dimensions.

Chlorophyll incorporated into liposomes made of phospholipids or phospholipids plus glycolipids resulted in an increased S_n value for the nitroxide labels $I_{(11,4)}$, $I_{(8,7)}$ and $I_{(5,10)}$. In view of the saturated nature of the phytol part of the chlorophyll molecule together with the bulky nature of the side chain methyl groups this result is expected. However, chlorophyll incorporation into the thylakoid membranes during greening was not accompanied by any similar restriction in acyl chain mobility. This result is consistent with all chlorophyll being protein associated in the thylakoid, although the effect of a very small fraction of chlorophyll in the free lipid might not be detected. Increased fluidity of barley thylakoid membranes after 36 h greening compared to that of the etioplast is perhaps due to the synthesis and incorporation of glycolipids particularly monogalactosyl- and digalactosyldiacylglycerols which takes place after 5 h of greening [28]. These thylakoid lipids have predominantly 2 and 3 unsaturated acyl chains.

The S_n values obtained for thylakoid membranes or vesicles of their constituent lipids are high compared to hydrated multilayers of egg lecithin [29] even though the chloroplast lipids contain a higher proportion of unsaturated fatty acids. Addition of double bonds after the first does not, however, have an appreciable effect on the correlation time measured by NMR tecnniques [30].

References

1 Singer, S.J. (1974) Annu. Rev. Biochem. 43, 805—833
2 Jost, P.C., Griffith, O.H., Capaldi, R.A. and Vanderkooi, G. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 480—484
3 Hesketh, T.R., Smith, G.A., Houslay, M.D., McGill, K.A., Birdsall, N.J.M., Metcalfe, J.C. and Warren, G.B. (1976) Biochemistry 15, 4145—4151
4 Oldfield, E. and Chapman, D. (1972) FEBS Lett. 23, 285—297
5 Esser, E.F. and Lanyi, J.K. (1973) Biochemistry 12, 1933—1939
6 Sefton, B.M. and Gaffney, B.J. (1974) J. Mol. Biol. 90, 343—358
7 Longmuir, K.J., Capaldi, R.A. and Dalquist, F.W. (1977) Biochemistry 16, 5746—5755
8 Hong, K. and Hubbel, W.L. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 2617—2621
9 Nolan, W.G. and Bishop, D.G. (1978) Arch. Biochem. Biophys. 190, 473—482
10 Anderson, J.W. (1975) Biochim. Biophys. Acta 416, 191—235
11 Thornber, J.P. (1975) Annu. Rev. Plant Physiol. 26, 127—158
12 Hiller, R.G. and Raison, J.R. (1978) Proc. Aust. Biochem. Soc. 11, 108
13 Highkin, H.R. (1950) Plant Physiol. 25, 294—306
14 Genge, S., Pilger, D. and Hiller, R.G. (1974) Biochim. Biophys. Acta 347, 22—30
15 Staehlin, L.A. (1976) J. Cell Biol. 71, 136—158
16 Sinensky, M. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 522—5●●
17 Henry, S.A. and Keith, A.D. (1971) Chem. Phys. Lipids 7, 245—265
18 Machold, O., Meister, A., Sagromsky, H., Hoyer-Hansen, G. and von Wettstein, D. (1977) Photosynthetica 11, 200—206
19 Hoyer-Hansen, G., Machold, A. and Kahn, A. (1976) Carlsberg. Res. Commun. 41, 349—357
20 Giotta, G.J. and Wang, H.H. (1972) Biochem. Biophys. Res. Commun. 46, 1576—1580
21 Birrell, G.B., Sistrom, W.R. and Griffith, O.H. (1978) Biochemistry 17, 3768—3773
22 Bolton, P., Wharfe, J. and Harwood, J.L. (1978) Biochem. J. 174, 67—72
23 Marcelja, S. (1976) Biochim. Biophys. Acta 455, 1—7
24 Lee, A.G. (1975) in Progress in Biophysics and Molecular Biology (Butler, J.A.Y. and Noble, D., eds.), Vol. 29, pp. 5—56, Pergamon Press, Oxford
25 Gordon, L.M., Sauerheber, R.D. and Esgate, J.A. (1978) J. Supramol. Struct. 9, 299—326
26 Miller, K.R. (1978) in Chloroplast Development (Akoyunoglou, G. and Argyroudi-Akoyunoglu, J.H., eds.), pp. 17—30, Elsevier-North/Holland Biomedical Press, Amsterdam

27 Arntzen, C.J. (1978) in Current Topics in Bioenergetics (Sanadi, D.R., ed.), Vol. 8, pp. 111—160, Academic Press, New York
28 Roughan, P.G. and Boardman, N.K. (1972) Plant Physiol. 50, 31—34
29 McConnell, H.H. and McFarland, B.G. (1972) Annals N.Y. Acad. Sci. 195, 207—217
30 Bishop, D.G., Kenrick, J.R., Bayston, J.H., McPherson, A.S., Johns, S.R., Coddington, J. and Willing, R.I. (1979) in Low Temperature Stress in Crop Plants: The Role of the Membrane (Lyons, J.M., Graham, D.G. and Raison, J.K., eds.), Academic Press, New York, in the press