Sulfolipid Localization in Lamellar Lipoprotein[1, 2]

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The plant sulfolipid, 6-sulfo-α-D-quinovopyranosyl-(1 → 1′)-2′-,3′-di-O-acyl-D-glycerol, has been found in high concentrations in all and only in photosynthetic O_2-producing organisms yet investigated (5, 6). Shibuya and Hase (21) recently demonstrated the concerted changes in chloroplast development and sulfolipid content in a green alga, *Chlorella protothecoides*. It is increasingly apparent that these surfactant lipids play an important role in chloroplast lipoprotein structure. For an intrinsic study of biochemistry of the sulfolipids, the localization of the compounds in the organelle is of obvious interest. The intracellular distribution of the sulfolipid has been studied (29, 30) but the difficulty of quantitatively isolating chloroplasts from photosynthetic tissues precluded assignment of their distribution within the cell on the basis of radioactivity measurements.

In the present study, a radiochemical investigation on the distribution of various components was first conducted with the chloroplasts isolated from C^{14}-labeled *Lemna perpusilla*. Since the lamellar fraction was found to represent practically whole chloroplasts, as far as the glycolipid contents were concerned, it seemed desirable to develop a procedure for the quantitative isolation of such chloroplast fragments from whole plant tissues. A radiochromatographic investigation of an S^{35}-labeled preparation thus obtained has demonstrated the specific localization of the sulfolipids in the lamellar fragments, a particulate fraction from chloroplasts first described by Park and Pon (19, 20) as containing pigments and part of the electron transport systems for photosynthesis.

This paper reports the details of these experiments as well as of the chemical characterization of a spinach lamellar preparation, and discusses significance of the present findings.

Material and Methods

Lemna perpusilla Uniformly Labeled with C^{14} or S^{35}. *Lemna perpusilla* 6746, original strain supplied by Professor S. Tamura, University of Tokyo, was grown under photosynthetic conditions in a synthetic medium (12). Uniform labeling of this water plant with radiocarbon was achieved by growing it in $C^{14}O_2$ for 1 week during which the number of plants increased approximately 10-fold (16). *Lemna* uniformly labeled with S^{35} was prepared by culture in a low-sulfur medium, in which most of the sulfate in the synthetic medium (12) was replaced by nitrate, containing $S^{35}O_4^=$ (700 μc/μatom S) under photosynthetic conditions for 4 days. The plants increased in number 3-fold during the culture.

Preparation of Chloroplasts and Their Fragments from Lemna-C^{14}. Chloroplasts and chloroplast fragments were prepared from *Lemna perpusilla* uniformly labeled with C^{14} using a modification of the Park and Pon (19) procedure. *Lemna*-C^{14} was harvested by filtration and washed with a sucrose-phosphate buffer, pH 7.4, which contained 0.5 M sucrose, 0.1 M potassium phosphate and 10^{-2} M EDTA. Approximately 15 *Lemna* plants were ground in a chilled mortar with 2 ml of the sucrose-phosphate buffer and transferred to a centrifuge tube with 3 ml of the same buffer. The homogenate was centrifuged at 200 *g* for 5 minutes and the precipitate was again ground with 5 ml of the sucrose-phosphate buffer, and recentrifuged. The green precipitate was designated as 0.2 P. The combined supernatant liquid was centrifuged at 600 *g* for 12 minutes. The precipitate was once washed by resuspending it in 2 ml of 0.5 M sucrose and centrifuging at 600 *g* for 12 minutes. The precipitated chloroplasts were suspended in 4 ml of 10^{-3} M potassium phosphate buffer, pH 7.4, and sonically ruptured at 9 kc for 90 seconds. The sonicate was transferred to a centrifuge tube with additional 3 ml of the 10^{-3} M buffer and centrifuged at 14,000 *g* for 10 minutes. The green supernatant was centrifuged at 110,000 *g* for 10 minutes. The precipitate was designated as 110 P. The supernatant liquid was again centrifuged at 145,000 *g* for 30 minutes. The supernatant and the precipitate were called 145 S and 145 P, respectively. 145 P was dark green and 145 S was completely colorless. All operations were carried out at or near 0°.

Quantitative Isolation of Lamellar Membrane Fraction from Lemna-S^{35}. *Lemna perpusilla*-S^{35}, repeatedly washed with water and 10^{-3} M potassium phosphate buffer, pH 7.4, was well ground in a mortar with a small amount of the same buffer, and then disintegrated by sonic oscillation at 9 kc for 3 minutes. The sonicate was centrifuged at 14,000 *g* for

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10 minutes, and the precipitate was resuspended in the same buffer and centrifuged at 14,000 g for 10 minutes. This resuspension of the precipitate and centrifugation were further repeated 4 times until the precipitate (14 P) became completely colorless. The combined supernatant liquid was centrifuged at 145,000 g for 30 minutes. The dark green precipitate was washed once with water and collected by recentrifugation (145 P). The supernatant liquid (145 S) was completely colorless. All operations were carried out at or near 0°.

Radiochromatographic Analyses of Lemna Components. Cellular components of *Lemna*-C^{14} were separated into macromolecules, water-soluble low-molecular weight compounds and individual lipids by an ion-exchange resin-paper chromatography on Amberlite WB-2 (Rohm and Haas Company, Philadelphia) with the Marinetti solvent (13), diisobutyl ketone-acetic acid-water (8:5:1, v/v/v). S^{35}-Labeled components were chromatographed first with phenol-water (500:130, w/w) and second with butanol-propionic acid-water (142:71:100, v/v/v)(1). The solution or the suspension to be analyzed was directly applied on the origin of the chromatograms without previous extraction in order to avoid possible fractionation and oxidation of lipids. Quantitative extraction of all soluble components was effected with the first developing solvents as chromatography proceeded.

Identification of the compounds separated by chromatography was carried out by means of co-chromatography and coelectrophoresis on paper of the eluted materials or their deacylation or hydrolysis products with authentic compounds. Radioactivity of the spots on chromatograms was measured directly with 5-cm diameter end-window G.-M. tube (Type 5006, Nihon Musen Company, Tokyo).

Preparation and Chemical Analysis of Spinach Lamellar Fragments. Fresh spinach leaves obtained from local markets were washed with cold water and blotted dry. The leaves (180 g) were homogenized in 180 ml of 0.1 M potassium phosphate buffer, pH 7.4, in a blendor operated at full speed for 2 minutes. The homogenate was subjected to sonic rupture at 9 kc/sec for 3 minutes. The sonicate was filtered through 8 layers of cheesecloth. The filtrate was centrifuged at 14,000 g for 10 minutes. The supernatant liquid was then centrifuged at 145,000 g for 1 hour. The precipitate was resuspended in 30 ml of water with the aid of a Potter-Elvehjem homogenized, and again centrifuged at 145,000 g for 30 minutes to give approximately 200 mg (dry wt) of the dark green lamellar particulate fraction.

The final precipitate was fractionated into 3 major groups: lipids, nucleic acids and proteins-polysaccharides, by successive extraction with ethanol, chloroform-methanol (2:1, v/v), ethanol and acetone for lipids, and then 5 % hot perchloric acid for nucleic acids. Lipid content was estimated by drying a portion of the lipid fraction under reduced pressure and weighing it. Nucleic acid content was determined from the OD at 260 nm of the fraction assuming the OD $_{260}$ nm was 33 for 1 mg/ml solution. The protein and polysaccharide fraction, i.e. the residue of a hot perchloric acid extraction was dried at 110° for 3 hours and weighed. Protein and polysaccharide contents in this fraction were assumed to be the amount recovered as amino acids after acid hydrolysis and the residue thereof, respectively. Chlorophylls in the lipid fraction were estimated spectrophotometrically by measuring the OD at 663, 644 and 624 nm according to the equations described by Huzisige (11). The protein and polysaccharide fraction was hydrolyzed with 6 N hydrochloric acid at 110° for 22 hours, and its amino acid composition was determined essentially by the method of Spackman et al. (24) with an amino acid analyzer (Type II, Mitamura Riken Kogyo Inc., Tokyo).

Spinach lamellar particles, quantasomes, prepared by the procedure of Park and Pon were extracted with hot 80 % ethanol and hot ethanol-toluene (3:1). The combined extracts were concentrated in vacuo and taken up in chloroform. The lipids were freed of sugars and salts by passing through a Sephadex G-50 column by the method of Wells and Dittmer (28). The chloroform was replaced with ethanol and the lipids deacylated during 20 minutes at 37° with 0.1 M sodium methylate in methanol. Two-dimensional chromatograms of the products were sprayed with the periodate-SO_2-Schiffs reagents to reveal glycosides and glycerophosphoryl esters.

Results

The result of the Amberlite WB-2 chromatography of the 5 subcellular fractions from *Lemna perpusilla*-C^{14} chloroplasts is shown in table I. Since the plant was uniformly labeled with C^{14} by culture in $C^{14}O_2$ the radioactivity of each compound represented the relative molar concentration of its constituting carbons. The 0.2 P fraction contained large unruptured fragments and only its 80 % ethanol extract could be analyzed. Since 14 P was not washed, it had a dense green color, indicating contamination by 110 P and 145 P in this fraction. The total radioactivity of 14 P, 110–145 P and 145 S was 41, 52 and 7 % of the total chloroplast activity, respectively. The C^{14} percentages of the major glycolipids, sulfolipid, galactosyl diglyceride, and digalactosyl diglyceride (3, 9), in chloroplasts were 2, 3, and 5 respectively. Of these activities, 86, 92 and 85 % were found in 110 P and 145 P. Both were equivalent to the lamellar lipoprotein (8, 19); in later experiments both 110 P and 145 P were designated simply as 145 P. Although this fraction contained fragments of other membranous structures, the lipid composition reflected that of isolated chloroplasts.

The 14 P fraction from S^{35}-labeled *Lemna* was cold-aged (Shibuya and Maruo, to be published) by freezing a suspension (15 mg dry wt/ml) at −30° for 2 days. It was thawed slowly and centrifuged at 1000 × g for 10 minutes. The precipitate was resuspended and recentrifuged to obtain microsome-

Table I. *Distribution of Radioactivity in Subcellular Fractions of* Lemna perpusilla *Uniformly Labeled with* C^{14}

Fraction** / Compound	0.2 P Ethanol extract	14 P	110 P	145 P	145 S
	cpm	cpm	cpm	cpm	cpm
Macromolecules	...	38,900	26,200	4100	3390
Water-soluble low M. W. compounds	7500	3020	3390	1380	3320
Sulfolipid	6770	237	1660	364	84
Digalactosyl diglyceride	17,900	600	4100	877	290
Galactosyl diglyceride	10,500	240	2210	555	139
Other lipids*	58,800	2240	11,200	2080	493
Total	...	45,200	48,800	9360	7820

* Other lipids included chlorophylls, triglycerides, lysosulfolipid and phospholipids.
** Data are for particles from isolated chloroplasts except for fraction 0.2 P.

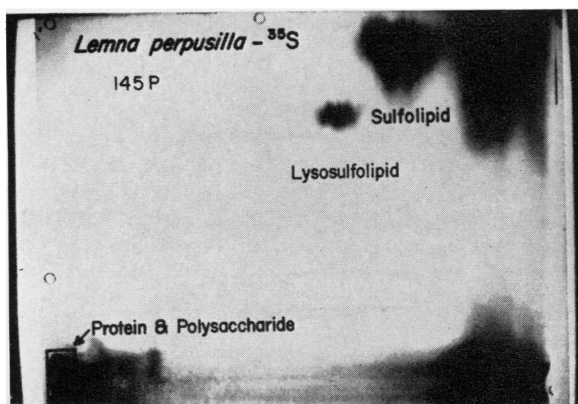

FIG. 1. Radiogram of lamellar particles quantitatively isolated from *Lemna perpusilla* uniformly labeled with S^{35}. Chromatographed in x-direction first, with phenowater and y-direction second, with butanol-propionic acid-water.

free lamellar fragments. The supernatant, which would contain S^{35}-labeled sulfolipid of microsomal or other particulate structures, contained no detectable radioactivity.

Figure 1 represents a typical autoradiogram of the lamellar particulate fraction isolated from *Lemna*-S^{35}. Major S^{35}-spots found were those of the sulfolipid, the lysosulfolipid and macromolecules which remained on the origin, as well as that of an unidentified compound with R_F values in both directions almost equal to those of the green pigments. The sulfolipid and the lysosulfolipid did not appear in the autoradiograms of the 14 P and 145 S fractions of *Lemna*-S^{35} but a minor radioactivity was observed when the corresponding areas of the chromatograms were carefully measured with a G.M. counter. Only the origin was significantly active in the chromatogram of 14 P, whereas many water-soluble compounds were present, such as glutathione, sulfur-containing amino acids and sulfotrioses (23) besides protein and polysaccharide of the origin in the chromatogram of 145 S. The result of the 2-dimensional radiochromatography of these 3 fractions of *Lemna*-S^{35} is shown in table II. Since S^{35}-labeling of the plant was uniform the observed radioactivities represented relative molar concentrations. The sulfolipid and the lysosulfolipid were found concentrated in the lamellar particulate fraction of the plant.

Fractionation and chemical analyses of the lamellar particulate fraction isolated directly from spinach leaves were carried out as described for *Lemna*. The results are summarized in table III. Amino

Table II. *Distribution of the Sulfolipids in* Lemna perpusilla-S^{35}

Fraction*		14 P	145 S	145 P
Sulfolipid	S^{35} cpm	570	1980	28800
	%	2	6	92
Lysosulfolipid	S^{35} cpm	0	335	4890
	%	0	6	94

* Particulate fractions were isolated directly from the homogenate.

Table III. *Characterization of Spinach Lamellar Fragments**

	Weight %
Lipid**	52.1
Chlorophylls***	10.1
Protein†	46.1
Polysaccharide	17
Nucleic acid††	1.8

* Purified by cold-aging (freeze-reaggregation technique) to separate from microsomal particles.
** Extracted by methanol and chloroform-methanol and dried at reduced pressure under nitrogen.
*** Calculated from absorbancy at 578 nm according to Warburg.
† Residue after extraction of lipid and nucleic acid. N = 15.32 %, C = 48.49 %, H = 6.62 %, S = 0.
†† Calculated from absorbancy at 260 nm in hot perchloric acid extract.

acid composition of the protein moiety of the preparation is shown in table IV.

Deacylation of spinach lamellar lipids revealed 4 main products. The most predominant were mono- and digalactosyl glycerol. Approximately equal amounts of diglycerophosphate (GPG) and sulfoquinovosyl glycerol (GQS) were observed. Although the method is sensitive to glycerophosphoryl choline (GPC), none (i.e. less than 5 % of total lipids) was observed. A small amount of diglycerophosphoryl glycerol (GPGPG) was detected. The lipids of lamellae, then, consist largely (ca. 70 %) of equal amounts of the galactosyl dilinolenins and of equal but lesser amounts of sulfolipid and phosphatidyl glycerol. The marked absence of lecithin in quantasomal lipids and its generally high (30–50 % of phospholipids) concentration in total leaf lipids suggests that it is involved in membrane structures other than the lamellae.

Table IV. *Amino Acid Composition of the Protein Moiety of Spinach Lamellar Membrane Fragments*

	Mole %		Mole %
Glycine	9.02	Threonine	5.74
Alanine	7.99	Half cystine	5.95
Valine	6.95	Methionine	1.12
Leucine	8.80	Arginine	4.92
Isoleucine	5.61	Histidine	2.04
Proline	4.21	Lysine	4.67
Phenylalanine	4.75	Aspartic acid	8.81
Tyrosine	3.07	Glutamic acid	10.45
Serine	5.93	Ammonia	7.28

Discussion

Lemna perpusilla, a small water plant with 1 to 3 mm leaves, was selected for ease of labeling and tissue disruption. There have been several limitations in studying the lipid distribution in subcellular fractions of plants: a method to prepare chloroplasts and chloroplast-free tissues in quantitative yield has not been available; quantitative extraction and purification of lipids without loss or oxidation have been difficult; and methods for determination of individual lipids in small samples have not been established. By noting differences in concentration of the lipids in chloroplasts and whole plants, Wintermans reported that beet chloroplasts (29) contained 33 % of the sulfolipid; recently he found 73 % of spinach leaf sulfolipid in chloroplasts (30). The analytical methods he employed were subject to the above limitations and it seemed appropriate to proceed with a reinvestigation. In the present study, the last 2 limitations in quantitative analysis of lipids have been overcome by employing radiochromatographic procedures in which relative molar concentrations of the sulfolipids were accurately determined. The direct separation of cellular components by applying the whole preparation to the chromatographic origin has improved the reliability of the analyses.

The sulfolipid and the lysosulfolipid occur essentially only in the lamellar particulate fraction in *Lemna*. This result and other available data suggest that the sulfolipid is an integral part of the photosynthetic structure. It and the galactosyl dilinolenins (10) are among the first lipids to have their biological function associated with their intracellular location. The sulfonic acid groups of the lipids are strongly anionic under all conditions; the surfactants properties of these amphipathic molecules may be expected to stabilize active protein conformation and charge distribution in the quantum conversion apparatus. One can easily suspect that they thereby play a physical role in photosynthesis as well as being large reservoirs for reduced sulfur and carbohydrates (4, 7, 14, 22, 23). The remarkably different fatty acid composition of the sulfolipid (50 % linolenic acid, 18:3 and 50 % palmitic acid, 16:0) in alfalfa leaves (17) resembles that of typical phosphatides (lecithin) not present in lamellar lipoprotein. Spinach lamellar particles contained no detectable lecithin; this is a characteristic component of plant mitochondria and other membrane systems. Phosphatidyl glycerol is the major phospholipid of the lamellae. Its possible role in carbohydrate metabolism has been discussed (2).

Radiochemical analysis of the components in chloroplasts and in 3 chloroplast fractions from *Lemna*-C^{14} revealed that approximately 90 % of the glycolipids were associated with the 110 to 145 P fractions. Since the 14 P, the chloroplast membrane fraction, was contaminated by considerable amounts of 110 P and 145 P components, it was unreasonable to suspect all the glycolipids were present only in the 110 P and 145 P fractions. These fractions, later simply designated as 145 P, were presumed to be equivalent, in size and chemical nature to the lamellar particles described by Park and Pon.

The results in table I also reveal that the 2 galactolipids (cf. 10), galactosyl diglyceride and digalactosyl diglyceride, were concentrated in the quantasomes whereas phospholipids and triglycerides were distributed in other fractions. The lamellar particulate preparations described here could have been contaminated by nonchloroplast components. It is, however, very unlikely that such contaminating fragments contained appreciable sulfolipid since it was absent in isolated mitochondria and ribosomes (22).

Radiochromatography of S^{35}-*Lemna perpusilla* has revealed several other interesting features of this water plant. Sulfoquinovosyl glycerol which has been found in high concentrations (22, 23) and shown unique metabolism (21) in green algae has not been detected in *Lemna*. An extremely high radioactivity, which was easily adsorbed on paper as seen in figure 1, was found in an unidentified spot with R_F values similar to those of green pigments. Although this lipid-like compound has also been de-

tected in S^{35}-labeled green algae (21, 23), its cellular concentration has usually been much less than that of the sulfolipid. This compound was recently demonstrated to be associated with chloroplast development in *Chlorella protothecoides* and is now shown to be concentrated in the chloroplasts in *Lemna*. It may play an important role in sulfur metabolism and *Lemna* appears useful for the study of this compound.

The analyses shown in table III indicate the typical lipoprotein-like nature of the lamellar particulate fraction from spinach leaves. A minor amount of nucleic acids found in the preparation indicates a minimal ribosomal content of chloroplast fragments (18). Both DNA and RNA should be present. Amino acid composition of the protein moiety shown in table IV was not essentially different from those of average plant proteins, although somewhat higher contents in neutral amino acids and lower in basic amino acids could be pointed out. The result is similar to those reported by Menke (15) and Weber (25, 26).

These facts are in accord with the proposed hydrophobic association model of the lamellar lipoprotein (7, 27). They contraindicate a classical bimolecular lipid leaflet model for the lamellae which would require a protein of high basic amino acid content for stability of ionically bound lipid membranes. The striking difference between the fatty acid compositions of the 4 major surfactant lipids precludes nonspecific exchange of their hydrophilic moieties. The fatty acid complement of lipid hydrocarbon chains of the diglycerides probably decrees the site of their hydrophobic association within the lamellar lipoproteins, and thereby the location of the charged and hydrophilic groups at their exterior surfaces.

Summary

Radiochromatographic analysis of C^{14}-labeled *Lemna perpusilla* revealed specific concentration of major glycolipids in the particulate fraction of sonically ruptured chloroplasts. Development of a procedure for quantitative isolation of the quantasomes directly from uniformly S^{35}-labeled *Lemna* demonstrated that the sulfolipid and the lysosulfolipid are specifically localized in lamellar fragments or lipoprotein subunits of similar size. Lipid and amino acid composition of the spinach lamellar particulate fraction was examined. Their 4 major lipids are mono- and digalactosyl dilinolenin, phosphatidyl glycerol, and sulfoquinovosyl diglyceride. This association in lamellar lipoprotein particles is discussed.

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Literature Cited

1. Benson, A. A., J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas, and W. Stepka. 1950. The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. J. Am. Chem. Soc. 72: 1710–18.
2. Benson, A. A. 1958. Dynamic interrelationships of enzyme systems in hexose synthesis in vivo. Proc. Intern. Symp. Enz. Chem., Tokyo and Kyoto, 1957. K. Ichihara, ed. Maruzen Press, Tokyo. p 189–91.
3. Benson, A. A., R. Wiser, R. A. Ferrari, and J. A. Miller. 1958. Photosynthesis of galactolipids. J. Am. Chem. Soc. 80: 4740.
4. Benson, A. A. and I. Shibuya. 1961. Sulfocarbohydrate metabolism. Federation Proc. 20: 79.
5. Benson, A. A. and I. Shibuya. 1962. Surfactant lipids. In: Physiology and Biochemistry of Algae. R. A. Lewin, ed. Academic Press, New York. p 371–83.
6. Benson, A. A. 1963. The plant sulfolipid. Advan. Lipid Res. 1: 387–94.
7. Benson, A. A. 1964. Plant membrane lipids. Ann. Rev. Plant Physiol. 15: 1–16.
8. Calvin, M. 1962. The path of carbon in photosynthesis. Science 135: 879–89.
9. Carter, H. E., R. A. Hendry, and N. Z. Stanacev. 1961. Wheat flour lipids. III. Structure of the mono- and digalactosylglycerol lipids. J. Lipid Res. 2: 223–27.
10. Erwin, J. and D. Bloch. 1964. Biosynthesis of unsaturated fatty acids in microorganisms. Science 143: 1006–12.
11. Huzisige, H. 1957. Pigment systems. In: Koso Kenkyuho (Methods in Enzymology), Vol. 3. Asakura Shoten, Tokyo. p 448–56.
12. Hillman, W. S. 1961. Experimental control of flowering in *Lemna*. III. Am. J. Botany 48: 413–19.
13. Marinetti, G. V., J. Erbland, and J. Kochen. 1957. Quantitative chromatography of phosphatides. Federation Proc. 16: 837–44.
14. Martelli, H. L. and A. A. Benson. 1964. Sulfocarbohydrate metabolism. I. Bacterial production and utilization of sulfoacetate. Biochim. Biophys. Acta 93: 169–71.
15. Menke, W. and E. Jordan. 1959. Uber das lamellare struktur proteid der chloroplasten von *Allium porrum*. Z. Naturforsch. 14b: 234–40.
16. Miyano, M., C. S. Chen, and B. Maruo. 1965. Continuous culture of *Lemna perpusilla* in $C^{14}O_2$. Plant Cell Physiol. In press.
17. O'Brien, J. S. and A. A. Benson. 1964. Isolation and fatty acid composition of the plant sulfolipid and galactolipids. J. Lipid Res. 5: 432–36.
18. Odintsova, M. S., E. V. Golubeva, and N. M. Sissakian. 1964. Chloroplast ribosomes. Nature 204: 1090–91.
19. Park, R. B. and N. G. Pon. 1961. Correlation of structure with function in *Spinacea oleracea* chloroplasts. J. Mol. Biol. 3: 1–10.
20. Park, R. B. and N. G. Pon. 1963. Chemical composition and substructure of lamellae isolated from *Spinacia oleracea* chloroplasts. J. Mol. Biol. 6: 105–14.
21. Shibuya, I. and E. Hase. 1965. Degradation and formation of sulfolipid occurring concurrently with

de- and re-generation of chloroplasts in the cells of *Chlorella protothecoides*. Plant Cell Physiol. 6: 267–83.

22. Shibuya, I. and B. Maruo. 1965. Surfactant lipids of plant quantasomes. Plant Cell Physiol. In press.
23. Shibuya, I., T. Yagi, and A. A. Benson. 1963. Sulfonic acids in algae. Studies on microalgae and photosynthetic bacteria. Univ. Tokyo Press. Tokyo, Japan. p 627–36.
24. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190–1206.
25. Weber, P. 1959. Artspecifische Unterschiede zwischen lamellaren Strukturproteide aus Chloroplasten. Z. Naturforsch. 14b: 691–92.
26. Weber, P. 1962. Über Strukturproteide aus Chloroplasten verschiedener Pflanzen. Z. Naturforsch. 17б: 683–88.
27. Weier, T. E., A. Engelbrecht, A. Harrison, and E. B. Bailey. 1965. The globular structure of the membranes of chloroplasts of *Phaseolus vulgaris, Pisum sativum*, and *Aspidistra*. Am. J. Botany In press.
28. Wells, M. A. and J. C. Dittmer. 1963. The use of Sephadex for removal of nonlipid contaminants from lipid extracts. Biochemistry 2: 1259–63.
29. Wintermans, J. F. G. M. 1960. Concentrations of phospholipids and glycolipids in leaves and chloroplasts. Biochim. Biophys. Acta 44: 49–54.
30. Wintermans, J. F. G. M. 1963. Phosphatides and glycolipids of the photosynthetic apparatus. In: La Photosynthèse. Colloq. Intern. Centre Natl Rech. Sci. No. 119. Paris. p 381–85.
31. Yagi, T. and A. A. Benson. 1962. Plant sulfolipid. V. Lysosulfolipid formation. Biochim. Biophys. Acta 57: 601–03.