Korean J Biol Sci 2: 341-349, 1998

The Effects of Surfactants on the Biosynthesis of Galactolipid and the Composition of Fatty Acids in Chloroplast Envelope and Thylakoid Membrane of *Chlorella ellipsoidea*

Eun A Choi, Kyung Suk Chung, and Chong Sam Lee*

Department of Biology, College of Natural Sciences, Sungshin Womens University, Seoul 136-742, Korea

Key Words:
Chlorella ellipsoidea
Surfactant
Galactolipid
Fatty acid

To analyze the effects of surfactants on the biosynthesis of galactolipid and the composition of fatty acids, the chloroplast envelope and thylakoid membrane were cultivated in medium treated with anionic surfactants, such as linear alkylbenzene sulfonate (0.002%, LAS), α-olefin sulfonate (0.01%, AOS), and sodium lauryl ether sulfate (0.08%, SLES), respectively. During the cultivation, the chloroplast envelope and thylakoid membrane were isolated from the cells collected at the early and middle phase of the culture and the contents of their fatty acid composition were compared with the control. When treated with surfactants, the contents of total lipid, MDGD methylesters, and DGDG methylesters decreased significantly when compared with the control. It was also confirmed that more unsaturated fatty acids were involved in the biosynthesis of galactolipid. The fatty acids utilized in the biosynthesis of MGDG were in the chloroplast envelope and in the control, and linoleic acid in LAS, linolenic acid and oleic acid in AOS, and linolenic acid and oleic acid in SLES. The fatty acids in the biosynthesis of DGDG were linolenic acid and oleic acid in the control, linolenic acid and stearic acid in LAS, oleic acid and linolenic acid in AOS, oleic acid and linolenic acid in SLES. In the thylakoid membrane, the major fatty acids in the biosynthesis of MGDG were linolenic acid and oleic acid in the control, oleic acid and linolenic acid in LAS, linolenic acid and linoleic acid in AOS, linolenic acid and palmitoleic acid in SLES. The fatty acids in the biosynthesis of DGDG were linolenic acid and oleic acid in the control, oleic acid and linolenic acid in LAS, linolenic acid and linoleic acid in AOS, palmitoleic acid and oleic acid in SLES.

Synthetic detergent has been used widely since it was first invented in Germany in 1930. However, it has some demerits as well; it produces foam, prevents the treatment of wastewater, and causes skin problems (Kang, 1983; Kim, 1986). Surfactants themselves cause BOD loading in the hydrosphere, suppress decomposition of organic compounds by microbes at a density of about 50 mg/l, and decrease the cohesion effect of organic compounds in excessive quantities, and as a consequence, prevent the treatment of wastewater (Park, 1978).

Surfactants are released directly into the natural environment after being used in households, so their hazard to the environment is becoming a major environmental concern.

If surfactants are present in the hydrosphere, the amount of oxygen that dissolve into water from the air decreases, the self-decomposition of surfactants by animals or plants in the river decreases, and consequently, the amount of dissolved oxygen decreases (Almeida et al., 1994).

Synthetic detergent dissolves the lipid and suppresses the phosphorylation of mitochondria and chloroplasts (Helenius et al., 1975). Outer membrane and plasma membrane of *E. coli* are differently sensitive to surfactants. Triton-X 100 dissolves only the cell membrane under the existence of Mg^{+2} (Cho et al., 1981). It was reported by Filip et al. (1973) that the outer membrane of *E. coli* has tolerance against the dissolution of sodium lauryl sarcosinate, an anionic surfactant.

Linear alkylbenzene sulfonate (LAS), α-olefin sulfonate (AOS) and sodium lauryl ether sulfate (SLES) are typical anionic surfactants most widely used as detergents (Moreno et al., 1994). In particular, LAS has been used since 1965 because it is a biologically soft type and the linear alkyl group is more easily decomposed by microbes than previously used AOS.

* To whom correspondence should be addressed.
Tel: 82-2-920-7172, Fax: 82-2-953-2091
E-mail: cslee@cc.sungshin.ac.kr

However, the material of the phenol family and the toxicity of this material is poisonous to fish and phytoplanktons (Kang, 1983; Kim, 1986). Besides, it has poisonous effects on the water by causing instability in the lipid bilayer membrane and hydrophobic bond, and so does not maintain the stability of many globular proteins (Womack et al., 1983).

The content of lipid per cell in plants accounts for 5-10% of dry weight, most of which exists in the membrane (Ohlorogge et al., 1995). The functions of the membrane lipid are enhanced permeability and fluidity of the membrane and enzyme activity (Moore, 1982; Yoshida and Vemura, 1986). Galactolipid constituting the cell membrane protects the cells from harmful conditions, such as low pH and hydrolytic enzyme and also protects the cells against the bacterial toxin (Alberts et al., 1994).

Various types of galactolipid are monogalactosyldiacylglyceride (MGDG), digalactosyldiacylglyceride (DGDG), trigalactosyldiacylglyceride (TGDG), sulphoquinovosyldiacylgylceride (SQDG). Galactolipid constituting the plant cell membrane has high contents of MGDG and DGDG. Fatty acids composing galactolipid have many double bonds in their structure and are highly unsaturated.

In the chloroplast of higher plants, the content of MGDG is high and there is a great amount of unsaturated fatty acids (Allen et al., 1971; Joyard et al., 1980). During their cell growth, the content of MGDG is higher than that of DGDG. This phenomenon is because MGDG serves to make more thylakoid layer (Roughan et al., 1972). If the composition of fatty acids in the membrane lipid is changed, the membrane formation may be broken (Gounaris et al., 1983).

The fatty acids in lipid is determined by genetic factors, nutrition, temperature, and other external environmental elements (Uemura et al., 1995). It was reported that the fatty acids composed of galactolipid give rise to changes in the growth phase (Koiwai et al., 1982).

The study on the effects of the various environmental elements on the lipid metabolism in the cells and the degradation of the surfactants by organisms have been done, but little attention has been given to the effects of surfactants on the biosynthesis of galactolipid in plant cells and the composition of fatty acids in plant cells. This study involved the effects of surfactants on the biosynthesis of galactolipid, their composition and contents of fatty acids as compared with the control.

Materials and Methods

Cultivation of Chlorella ellipsoidea

C. ellipsoidea was cultivated in M4N medium (Tamiya et al., 1953) treated with LAS (0.002%), AOS (0.01%), and SLES (0.08%) surfactants, respectively.

During the cultivation, air containing 5% CO_2 was bubbled in at 25℃ and the light of 2,000 lux was constantly illuminated for 7 days. The growth of the cells during cultivation were measured in the packed cell volume by using a hematocrit.

Separation of chloroplasts

A slightly modified Lyttleton's method (1962) was used in separating chloroplasts.

The harvested cells were suspended in 0.5 M phosphate buffer (pH 7.5, containing 0.4 M NaCl) and mashed. These mashed cells were centrifuged (500 × g, 4 min), and the supernatant centrifuged (1,100 × g, 4 min) and again at 1,200 × g, 10 min. The separated chloroplasts were verified under the microscope in 0.4 M saline solution.

Separation of chloroplast envelope and thylakoid membrane

A slightly modified Poincelot's method (1973) was used to separate the chloroplast envelope and thylakoid membrane from the separated chloroplasts.

Twice the amount of swelling medium (10 mM Tricine-NaOH buffer, pH 7.6, containing 4 mM $MgCl_2$) was added to the harvested chloroplasts and after 30 min, shaken for 10 min then centrifuged (1,500 × g, 10 min). The precipitated pellet was taken as thylakoid membrane and the supernatant was recentrifuged (1,700 × g, 60 min) and the remaining pellet obtained. This pellet was resuspended in 0.5 M phosphate buffer (pH 7.5, containing 0.4 M NaCl and 30% sucrose) and centrifuged (73,000 × g, 60 min). The yellow layer was collected from this gradient and centrifuged (47,000 × g, 60 min).

Extraction of total lipid

A slightly modified Bligh and Dyer's method (1959) was used to extract total lipid from each membrane fraction. Chloroform : methanol (1 : 2, V/V) was added to the separated membrane. After shaking for 30 min, the same amount of distilled water was added and blended. The lipid extracted through the chloroform layer was filtered through filter paper (Whatmann no. 1).

After chloroform was added to the methanol layer and mixed, the chloroform layer was filtered through filter paper and the total lipid was reextracted. The extracted total lipid was dried in a dry oven at 40-50℃ and its content was measured.

Separation and identification of galactolipid

Galactolipid in the extracted total lipid was separated into MGDG and DGDG by thin layer chromatography (TLC, Desaga) as described by Turner and Rouse (1970). TLC glass plates (20 × 20 cm) used in TLC were coated with a silica gel 0.25 min thick (Merck, 60G), dried at room temperature and activated in an

oven at 110℃ for 60 min.

According to Chapmen and Barber (1987), the following method was used to separate galactolipid from total lipid. A mixture of chloroform : methanol : water (65 : 24 : 4, v/v) was used as the first expensive solvent, and a mixture of chloroform : acetone : methanol : acetic acid : water (100 : 40 : 20 : 20 : 10, v/v) was used as the second expensive solvent.

The separated galactolipid was identified to compare with the standard chemicals (Sigma), and 1-napthol was used as a developing reagent (Roughan, 1987).

Methyl esterification of fatty acids

To analyze the composition and the quantitative change of fatty acids composing galactolipid (GC, Varian 3400), MGDG and DGDG were methylated according to Allen and Good (1971). Four milliters of a trans-esterification mixture of methanol : sulfuric acid : benzene (100 : 5 : 5, v/v) was added to galactolipid from the TLC plate. The sample was dried in a dry oven at 70 ℃ for 60 min, cooled and shaken with 5 ml of distilled water. After 2 ml of hexane was added, it was shaken and the separated hexane layer was taken. The combined hexane layer was dried and the fatty acid methyl esters contained in each galactolipid were measured (Chapman and Barber, 1987).

Analysis of fatty acids

The kinds of fatty acids composing galactolipid in each membrane were analyzed by gas chromatography (GC, Varian 3300). The identification of each fatty acid was compared with standard chemicals (Sigma) such as lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitoleie acid (16:1), heptadecanoic acid (17:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), behenic acid (22:0), ervcic acid (22:1), and lignoceric acid (24:0).

The detector used was the FIDH2 flame ionization detector and the column, RT×2330 Megabore. GC conditions were as follows : column temperature (220 ℃), injector port temperature (230℃), detector oven temperature (250℃) and carrier gas (N_2, 80 ml/min).

Results

Growth of C. ellipsoidea cells

The growth of *C. ellipsoidea* treated with surfactants in the culture is shown in Fig. 1. The inhibiting effect of the growth was clear in cells treated with surfactants in the culture. The inhibiting effect was shown to have an average of 68.83% in LAS, 67.02% in AOS, and 57.73% in SLES as compared with the control. These results, show that the inhibiting effect of the growth of *C. ellipsoidea* was the strongest in LAS and AOS.

Total lipid content in the chloroplast envelope and thylakoid membrane

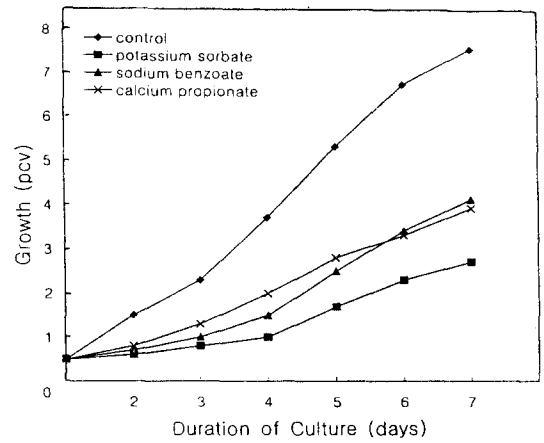

Fig. 1. Growth of *Chlorella ellipsoidea* treated with various surfactants.

Chloroplast envelope: The changes in the content of total lipid in chloroplast envelope is as shown in Fig. 2. For the control, the content of total lipid increased at a rate of 141.24% on the 3rd day, 56.92% on the 5th day, and 53.90% on the 7th day, thus 84.02% on average.

As shown in Fig. 2, the content of total lipid in LAS decreased at a rate of 44.69% on the 3rd day, 67.76% on the 5th day, and 69.03% on the 7th day compared with the control and thus 60.49% on average. Total lipid in AOS, decreased at a rate of 46.75% on the 3rd day, 67.97% on the 5th day, and 74.26% on the 7th day as compared with the control, thus 62.99% on average. In SLES, it decreased at a rate of 90.28% on the 3rd day, 69.81% on the 5th day, and 54.01% on the 7th day, thus 71.37% on average. Although there were no great differences among LAS, AOS, SLES in the declining rate of total lipid content in the chloroplast envelope, the decrease rate was the highest in SLES.

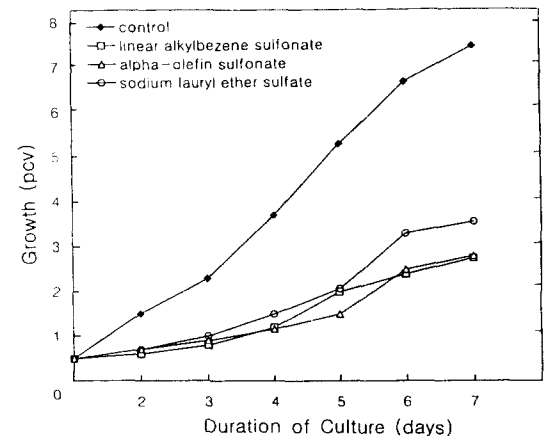

Fig. 2. Changes in contents of total lipids in *Chlorella ellipsoidea* chloroplast envelope treated with surfactants.

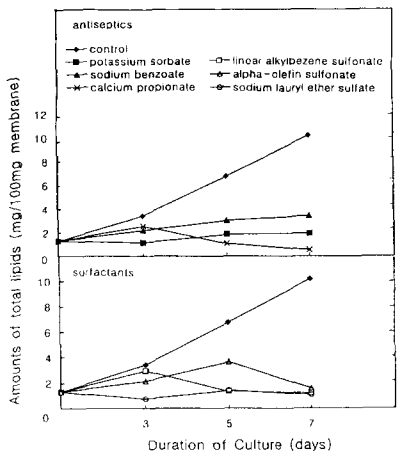

Fig. 3. Changes in contents of total lipids in *Chlorella ellipsoidea* thylakoid membrane treated with surfactants.

Thylakoid membrane: The changes in the contents of total lipid in the thylakoid membrane are shown in Fig. 3. For the control, the content of total lipid increased at a rate of 44.31% on the 3rd day, 15.09% on the 5th day, and 70.92% on the 7th day, thus 43.44% on average.

With the control, the content of total lipid in LAS decreased at a rate of 62.65% on the 3rd day, 69.5% on the 5th day, and 68.41% on the 7th day, thus 66.85% on average. It decreased at a rate of 59.07% on the 3rd day, 21.64% on the 5th day, and 40.50% on the 7th day, thus 40.40% on average for AOS. In SLES, it decreased at a rate of 42.39% on the 3rd day, 44.40% on the 5th day, and 49.35% on the 7th day, thus 45.38% on the average. These results confirmed that three surfactants of LAS had the strongest inhibiting effect on total lipid content in the thylakoid membrane.

MGDG and DGDG methyl esters

Chloroplast envelope: The changes in the contents of galactolipid in the chloroplast envelope were as shown in Fig. 4. The content of MGDG methyl esters and in the control on average were 26.88% and 9.15% of total lipid, respectively. As shown in the composition of galactolipid of the chloroplast envelope, the content of MGDG was higher than that of DGDG. The content of MGDG in the control increased at a rate of 14.15% on the 3rd day, 39.59% on the 5th day, 45.18% on the 7th day, thus 32.97% on the average. The content of DGDG for the control increased at a rate of 7.21% on the 3rd day, remarkably 122.10% on the 5th day, 20.79% on the 7th day, thus 50.03% on the average.

The content of MGDG in the chloroplast envelope in LAS decreased at a rate of 18.52% on the 3rd day, 8.57% on the 5th day, 5.44% on the 7th day, thus 10.84% on the average as compared with the control. DGDG decreased at a rate of 72.87% on the 3rd day,

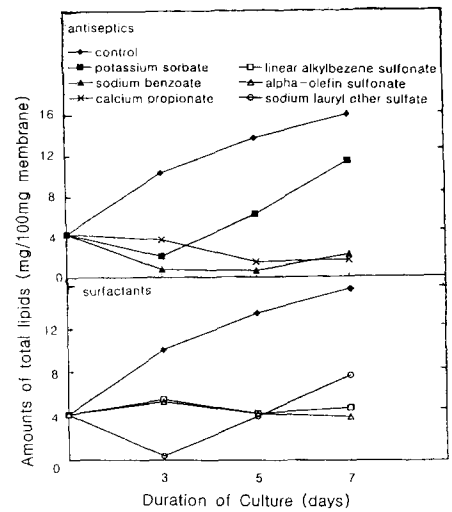

Fig. 4. Changes in contents of monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) methyl esters of *Chlorella ellipsoidea* chloroplast envelopes treated with surfactants.

85.11% on the 5th day, 65.34% on the 7th day, thus 74.44% on average. These results showed that the biosynthesis of galactolipid in LAS has a much stronger inhibiting effect on DGDG than on MGDG. In AOS, the content of MGDG decreased at a rate of 35.98% on the 3rd day, 64.90% on the 5th day, 74.59% on the 7th day, thus 58.49% on average. Also that of DGDG decreased at a rate of 47.50% on the 3rd day, 42.73% on the 5th day, 57.24% on the 7th day, thus 49.19% on average. In AOS, the content of MGDG

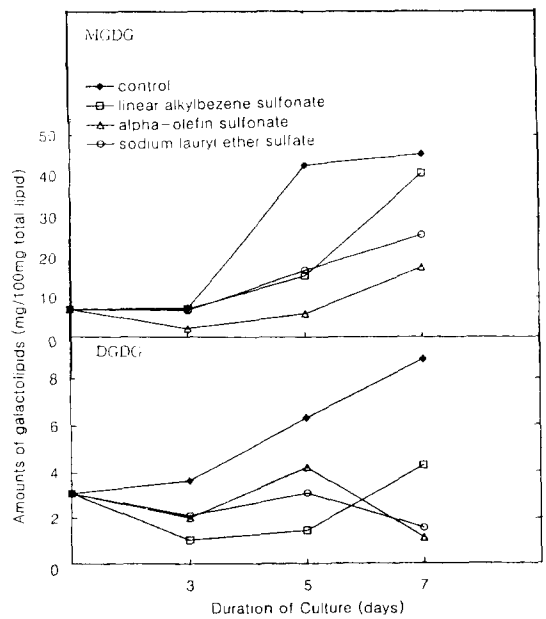

Fig. 5. Changes in contents of MGDG and DGDG methyl esters in *Chlorella ellipsoidea* thylakoid membrane treated with surfactants.

and DGDG increased until the 5th day and then decreased to some extent at the last stage of cultivation. It was also shown that there was no great differences in rate decreases between MGDG and DGDG. In SLES, the content of MGDG decreased at a rate of 84.21% on the 3rd day, 54.33% on the 5th day, 50.71% on the 7th day, thus 63.08% on average. That of DGDG decreased at a rate of 47.50% on the 3rd day, 79.54% on the 5th day, 27.46% on the 7th day, thus 51.50% on average.

Thylakoid membrane: The changes in the contents of the two kinds of galactolipid in the thylakoid membrane by surfactants is shown in Fig. 5. For the control, the content of MGDG was 25.55% and that of DGDG was 5.45%. The content change of MGDG methyl esters in total lipid increased at a rate of 7.96% on the 3rd day, remarkably 506.21% on the 5th day, 40.88% on the 7th day, thus 184.93% on average. The content of DGDG increased rate of 61.46% on average.

In LAS, the content of MGDG increased predominantly during cultivation, showing no great inhibitional effect by surfactants as compared with the control. The level of MGDG decreased at a rate of 4.49% on the 3rd day, 78.63% on the 5th day, 11.10% on the 7th day, thus 31.49% on average. It was decreased to some extent on the 5th day and increased remarkably on the 7th day. The content of DGDG decreased at a rate of 71.95% on the 3rd day, 77.25% on the 5th day, 51.02% on the 7th day, thus 66.74% on average. Therefore, it was shown that LAS had a stronger inhibiting effect on DGDG than on MGDG during the biosynthesis of galactolipid in the thylakoid membrane. In AOS, the content of MGDG increased constantly during the cultivation, increasing at a rate of 71.03% on the 3rd day, 86.16% on the 5th day, 61.17% on the 7th day, thus 72.79% on average. The content of DGDG increased until the 5th day and decreased predominantly on the 7th day, decreased at a rate of 44.99% on the 3rd day, 34.49% on the 5th day, 86.17% on the 7th day, thus 55.22% on average. In SLES, the content of MGDG decreased at a rate of 10.13% on the 3rd day, 60.23% on the 5th day, 42.62% on the 7th day, thus 37.66% on average. DGDG content decreased at a rate of 42.24% on the 3rd day, 51.34% on the 5th day, remarkably 82.24% on the 7th day, thus 58.64% on average. From these results it was confirmed that LAS and SLES had a great effect on DGDG, while AOS on MGDG.

Fatty acid

The changes in the composition of fatty acids in the formation of galactolipid, such as MGDG and DGDG were analyzed during the cultivation of *C. ellipsoidea* in the presence of surfactants. The fatty acids analyzed were lauric acid, myristic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, behenic acid, erucic acid, and lignoceric acid.

Chloroplast envelope: The changes in the composition of fatty acids in the biosynthesis of MGDG in the chloroplast envelope is shown in Table 1. For the control, oleic acid (14.45%), and linolenic acid (12.83%) were shown at the beginning of the culture, oleic acid (15.48%), linolenic acid (16.09%) on the 3rd day, palmitoleic acid (15.00%), linolenic acid (15.30%) on the 7th day. From these results, it was observed that palmitoleic acid, oleic acid, and linolenic acid were in the biosynthesis of MGDG in the control.

Myristic acid (7.62%) and linolenic acid (35.09%) were utilized on the 3rd day, linoleic acid (10.23%) and linolenic acid (15.15%) on the 5th day, stearic acid (8.53%), and linolenic acid (38.25%) on the 7th day.

Table 1. Changes in fatty acid methyl esters contents expressed as a percentage (%) of MGDG in *Chlollera ellipsoidea* chloroplast envelope treated with various surfactants during cultivation

Fatty acid (%)	Duration of culture (days)												
	0	3				5				7			
	cont[1]	cont	LAS[2]	AOS[3]	SLES[4]	cont	LAS	AOS	SLES	cont	LAS	AOS	SLES
Lauric	-	-	2.46	0.37	0.32	-	3.41	0.33	0.59	-	-	-	0.58
Myristic	3.72	0.76	7.62	1.79	0.98	2.15	7.83	2.18	1.31	1.67	5.03	1.63	1.27
Palmitic	4.57	5.29	6.11	6.10	2.47	3.53	5.18	4.21	2.88	8.26	6.04	3.99	2.83
Palmitoleic	12.45	14.10	-	1.64	1.89	20.66	3.27	13.37	2.18	15.00	-	17.64	1.40
Heptadecanoic	7.72	7.63	-	6.69	5.51	6.72	3.14	6.61	5.64	6.23	-	6.76	5.55
Stearic	3.76	3.91	7.24	7.56	4.60	4.51	6.28	5.14	30.73	4.20	8.53	6.77	4.07
Oleic	14.45	15.48	-	17.41	11.44	16.02	3.11	12.11	12.32	14.42	-	14.15	13.08
Linoleic	3.69	2.37	7.03	10.42	8.24	2.03	9.23	6.05	8.41	5.75	7.58	6.98	7.05
Linolenic	12.82	16.09	35.09	21.12	12.36	3.93	15.15	11.69	12.21	15.03	38.25	17.20	13.59
Arachidic	1.71	0.58	4.64	7.59	3.09	2.24	5.98	4.41	4.38	1.36	3.57	4.06	3.51
Behenic	3.49	3.99	3.27	3.04	-	4.17	5.12	0.50	1.85	2.63	2.45	1.66	1.75
Erucic	4.31	4.93	-	5.37	6.84	3.50	4.30	3.16	3.19	2.19	-	5.04	0.46
Lignoceric	2.54	2.23	1.64	0.53	2.47	1.76	3.74	0.24	1.89	1.09	1.76	1.42	0.43
Unknown	24.77	22.64	24.63	10.34	39.79	28.78	24.26	29.55	39.42	21.90	21.75	32.95	44.43
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]control, [2]linear alkylbenzene sulfonate, [3]α-olefin sulfonate, [4]sodium lauryl ether sulfate.

Table 2. Changes in fatty acid methyl ester content expressed as a percentage (%) of DGDG in *Chlorella ellipsoidea* chloroplast envelope treated with various surfactants during cultivation

Fatty acid (%)	Duration of culture (days)												
	0	3				5				7			
	cont[1]	cont	LAS[2]	AOS[3]	SLES[4]	cont	LAS	AOS	SLES	cont	LAS	AOS	SLES
Lauric	-	-	3.97	-	2.09	-	-	-	2.19	-	-	0.51	0.38
Myristic	1.66	-	5.10	0.7	8.65	6.32	1.84	0.72	0.59	-	3.11	0.85	1.09
Palmitic	5.19	2.94	2.71	3.15	2.93	2.23	4.11	3.37	2.89	2.05	5.52	2.60	2.85
Palmitoleic	7.27	15.72	-	0.79	-	12.99	-	19.47	0.75	6.72	-	18.54	2.20
Heptadecanoic	6.68	7.12	-	6.78	7.58	8.02	-	8.34	7.69	5.12	-	6.18	5.65
Stearic	5.13	2.65	5.59	4.75	3.78	3.63	8.01	4.25	4.52	1.63	10.34	5.11	5.42
Oleic	12.15	20.28	-	15.46	13.73	14.71	-	18.04	15.91	15.87	-	17.93	13.56
Linoleic	11.81	3.92	4.09	8.99	6.94	5.07	7.93	5.13	7.59	16.68	11.73	7.96	6.73
Linolenic	16.16	17.54	42.55	18.84	11.49	16.35	40.77	13.23	13.98	20.89	30.49	17.65	15.02
Arachidic	-	1.28	3.33	4.03	2.58	1.77	4.72	2.37	3.74	-	5.88	3.75	5.05
Behenic	5.61	4.59	1.41	1.65	0.54	0.38	3.34	0.47	-	6.21	3.03	0.26	2.22
Erucic	9.00	4.57	-	2.37	3.56	3.26	-	1.53	3.63	8.98	-	1.63	1.42
Lignoceric	5.49	2.25	2.61	1.42	0.61	1.60	2.75	2.73	0.47	4.12	2.45	2.30	2.43
Unknown	14.27	17.05	28.64	31.07	29.62	23.67	26.53	29.79	36.05	11.73	27.45	13.73	35.98
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]control, [2]linear alkylbenzene sulfonate, [3]α-olefin sulfonate, [4]sodium lauryl ether sulfate.

Oleic acid (17.41%) and linolenic acid (21.12%) were used on the 3rd day, palmitoleic acid (13.37%), oleic acid (12.11%) on the 5th day, palmitoleic acid (17.64%), and linolenic acid (17.20%) on the 7th day. In SLES, oleic acid (12.44%) and linolenic acid (13.36%) were utilized on the 7th day. The results for AOS were somewhat similar to those of the control.

Oleic acid (12.44%, 12.32%, 13.08%) and linolenic acid (13.36%, 12.21%, 13.51%) were utilized on the 3rd, 5th, and 7th day.

The changes in the composition of fatty acids in the biosynthesis of DGDG in the chloroplast envelope are as shown in Table 2. Stearic acid (5.59%, 8.01%), linolenic acid (42.55%, 40.77%) were used on the 3rd and 5th day, respectively. On the 7th day, linoleic acid (11.73%) and linolenic acid (30.49%) were utilized. In contrast to the control, the saturated fatty acid, stearic acid, was utilized in the biosynthesis of galactolipid in the LAS and AOS treatment groups. Oleic acid (15.46%) and linolenic acid (18.84%) were utilized on the 3rd day, and on the 5th and 7rh day, palmitoleic acid (19.47%, 18.54%) and oleic acid (18.04%, 17.93%) were used, respectively. Oleic acid and linolenic acid were for the biosynthesis of galactolipid in the culture. Thylakoid membrane: The changes in the composition of fatty acids in the biosynthesis of MGDG in the thylakoid membrane were as shown in Table 3.

Fatty acids composing MGDG of the thylakoid membrane were oleic acid (13.94%, 14.03%), and linolenic acid (18.26%, 18.45%) in the beginning and on the 3rd day in the control. On the other hand, palmitic acid (15.46%) and oleic acid (12.05%) were utilized on the 5th day, palmitoleic acid (16.50%) and oleic acid (11.90%) on the 7th day.

Oleic acid and linolenic acid were utilized in the cultivation. Linoleic acid (14.73%) and linolenic acid (23.37%) on the 3rd day, stearic acid (9.74%) and linolenic acid (21.33%) on the 5th day, linoleic acid (13.37%) and linolenic acid (20.41%) on the 7th day. In SLES, oleic acid (10.36%, 12.81%) and linolenic acid (13.49%) were utilized on the 3rd and 5th day. On the 7th day, palmitoleic acid (13.81%) and linolenic

Table 3. Changes in fatty acid methyl ester content expressed as a percentage (%) of MGDG in *Chlorella ellipsoidea* thylakoid membrane treated with various surfactants during cultivation

Fatty acid (%)	Duration of culture (days)												
	0	3				5				7			
	cont	cont	LAS	AOS	SLES	cont	LAS	AOS	SLES	cont	LAS	AOS	SLES
Lauric	-	-	-	0.52	0.52	-	-	0.53	-	2.06	-	0.72	0.38
Myristic	0.38	0.76	1.79	8.65	1.05	1.98	1.96	7.05	0.45	1.79	0.74	1.76	11.94
Palmitic	3.33	7.16	5.00	6.57	2.49	15.46	4.43	1.93	2.56	7.99	3.15	3.42	3.16
Palmitoleic	5.09	10.26	3.35	1.65	10.60	10.31	1.73	2.90	12.80	16.50	0.66	3.15	13.81
Heptadecanoic	6.89	6.80	6.72	-	5.74	6.80	7.04	1.21	6.92	8.20	6.76	5.05	6.16
Stearic	2.04	1.12	6.37	8.49	3.52	3.06	5.77	9.74	3.93	3.43	4.39	9.22	3.18
Oleic	13.94	14.03	15.92	2.19	10.76	12.05	12.91	3.37	12.81	11.90	12.72	2.92	12.80
Linoleic	4.71	1.62	8.06	14.73	5.11	4.05	8.56	8.61	6.20	3.38	6.74	13.37	6.43
Linolenic	18.26	18.45	7.62	23.37	13.49	12.02	13.24	21.23	12.81	10.32	14.02	20.41	14.71
Arachidic	2.36	-	7.71	-	3.11	1.44	5.94	1.55	3.08	1.93	4.18	1.59	3.21
Behenic	6.01	5.24	2.15	2.06	1.58	2.45	2.90	3.53	0.70	2.58	1.59	3.07	0.78
Erucic	9.09	6.21	2.79	8.68	3.28	3.39	3.63	8.17	1.69	2.94	2.46	6.87	2.52
Lignoceric	4.59	3.33	0.83	1.23	1.30	1.61	1.62	3.29	0.81	2.38	0.42	2.57	1.59
Unknown	22.86	24.97	27.69	14.87	37.45	25.38	25.27	22.32	35.24	24.06	42.17	35.10	19.33
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 4. Changes in fatty acid methyl ester content expressed as a percentage (%) of DGDG in *Chlorella ellipsoidea* thylakoid membrane treated with various surfactants during cultivation

Fatty acid (%)	Duration of culture (days)												
	0	3				5				7			
	cont[1]	cont	LAS[2]	AOS[3]	SLES[4]	cont	LAS	AOS	SLES	cont	LAS	AOS	SLES
Lauric	-	-	0.43	2.12	0.13	-	-	2.80	-	-	-	0.48	-
Myristic	-	0.57	1.49	5.43	0.85	-	0.71	5.31	0.87	-	0.73	1.14	1.34
Palmitic	2.19	1.23	4.87	4.97	2.91	6.19	4.49	1.18	3.34	2.71	3.28	3.69	5.68
Palmitoleic	4.52	5.07	2.60	9.95	12.69	18.12	12.02	1.41	16.02	4.76	0.61	0.70	10.93
Heptadecanoic	6.77	9.27	6.73	9.12	5.63	7.22	6.72	6.09	8.94	5.20	6.14	7.25	7.53
Stearic	1.87	1.45	5.83	7.24	4.33	6.16	4.27	6.93	3.82	4.47	4.47	9.98	4.38
Oleic	12.56	11.19	14.97	3.03	13.79	26.56	13.60	2.53	15.18	11.81	12.98	3.26	10.73
Linoleic	4.12	13.52	9.03	10.75	8.85	3.82	5.46	11.46	5.49	11.38	6.96	14.14	8.12
Linolenic	19.91	18.79	11.68	18.86	0.75	15.17	12.84	21.50	13.64	18.17	15.24	22.17	11.31
Arachidic	-	-	6.71	-	4.15	-	4.29	-	2.92	-	4.50	1.81	5.10
Behenic	7.88	6.73	1.86	2.67	2.25	-	0.93	19.06	-	6.99	1.82	3.17	1.37
Erucic	15.40	9.03	2.32	3.67	1.58	-	1.52	5.29	2.59	13.65	2.84	7.47	2.34
Lignoceric	8.49	4.22	0.87	2.06	3.84	-	0.70	1.06	2.34	7.58	1.05	2.88	2.17
Unknown	16.29	18.93	30.61	20.13	38.25	16.76	38.45	14.48	24.94	13.18	39.33	15.67	29.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]control, [2]linear alkylbenzene sulfonate, [3]α-olefin sulfonate, [4]sodium lauryl ether sulfate.

acid (14.71%) were used. Compared with the control, these results showed a slight difference in the composition and the content of fatty acids.

The changes in the composition of fatty acids in the biosynthesis of DGDG in the thylakoid membrane are shown in Table 4.

For the control, linolenic acid (19.91%) and erucic acid (15.00%) were utilized at the beginning of the culture, and linoleic acid (13.52%) and linolenic acid (18.79%) on the 3rd day, palmitoleic acid (18.12%) and oleic acid (26.56%) on the 5th day, linolenic acid (18.17%) and erucic acid (13.65%) on the 7th day. These results indicated that oleic acid, linolenic acid and erucic acid were mostly important in the biosynthesis of DGDG in the thylakoid membrane.

Oleic acid (14.97%, 13.60%, 12.98%) and linolenic acid (11.68%, 12.84%, 15.24%) were used on the 3rd, 5th and 7th day. Linoleic acid (10.75%) and linolenic acid (18.86%) were utilized on the 3rd day, linolenic acid (21.50%) and behenic acid (19.06%) on the 5th day, linoleic acid (14.14%) and linolenic acid (22.17%) on the 7th day. In SLES, palmitoleic acid (12.69%, 16.02%) and oleic acid (13.79%, 15.18%) were used on the 3rd day. On the 7th day, palmitoleic acid (10.93%) and linolenic acid (11.31%) were used.

These results indicate that food preservatives and surfactants caused the changes in the composition of fatty acids in the plasma membrane, the chloroplast envelope, and the thylakoid membrane of *C. ellipsoidea*.

Discussion

If surfactants exist in the hydrosphere, oxygen in the air cannot dissolve into water, the decomposition of surfactants by marine and fresh water animals and plants will be affected causing decreases in BOD levels (Almeida et al., 1994). Surfactants are divided into cationic, anionic, and nonionic surfactants, among which anionic surfactants are most used as detergent for domestic use. Cationic and anionic surfactants cause the inhibition of the bacterial growth, destroy the cell membrane of microbes, and result in the denaturation of enzyme proteins (Song, 1996). For these reasons, they are also used as disinfectants. LAS, AOS, and SLES are the most widely used anionic surfactants and they make the unstable double layer of lipid and hydrophobic bond in the lipid membrane.

This study clearly showed that the growth of *C. ellipsoidea* treated with surfactants in the culture was inhibited. This might be because LAS, AOS, and SLES affected cell formation and metabolism by dissolving the lipid membrane or destroying the cell membrane, denaturating enzyme protein, and inhibiting phosphorylation of mitochondria and chloroplasts.

The content of total lipid in the chloroplast envelope and thylakoid membrane treated with surfactants showed more than a 50% decrease on average compared with that of the control. This result may be because surfactants inhibited the metabolism and enzyme activation necessary for the synthesis of lipid, thus preventing normal lipid metabolism. Lipid is synthesized from keto acid which is generated by deamination of protein and from acetyl CoA which is formed by decomposition of carbohydrates. It was thought that this normal metabolic process was inhibited because surfactants might have changed the composition of the lipid in cell membrane, affected permeability, and inhibited the activation of enzyme necessary for lipid metabolism.

According to the synthetic site of DAG or PA, which is the basic skeleton of galactolipid, it is divided into eukaryotic galactolipid which contains C-18 fatty acids at the first position of the glycerol backbone and prokaryotic galactolipid which contains C-16 fatty acids at the the second position of the glycerol backbone. The biosynthesis of eukaryotic galactolipid is as

follows. In ER, ER-acyltransferase converts into PA, and then PA induces DAG. DAG which has been formed in this way forms PC, PE, and PG. They form MGDG and DGDG by ER desaturase and at the same time, the reaction of desaturation occurs from oleic acid to linoleic acid. In this reaction the cytochrome b5 reductase system in ER which needs NADH as the electron donor ultimately participates. The biosynthesis of prokaryotic galactolipid occurs at the inner membrane of chloroplasts. PA is converted into DAG by PA-phosphatase and DAG synthesizes MGDG and DGDG. The reaction of desaturation from oleic acid into linolenic acid via linoleic acid and from palmitic acid into hexadecatrienoic acid occur by chloroplast desaturase.

It has been proven by a number of experiments that the composition of fatty acids changes according to environmental changes. As for the composition of total lipid involved in plant cells, unsaturated fatty acids also increase as the light is high (Hawke et al., 1974) and the content of linoleic acid at low temperature decreases (Sato and Murata, 1981; Chapman et al., 1983). In *Nicotiana tabacum* when its leaves are located on the higher site, the content of linolenic acid increases and other fatty acids decrease and linoleic acid increases, especially in its flowers and seeds (Matsuzake et al., 1983). The composition of phospholipid varies according to environmental elements.

The predominant fatty acids utilized in the biosynthesis of phospholipid in *Chlorella* treated with glucose in the culture were palmitic acid, stearic acid, linoleic acid, and linolenic acid, and also in sucrose treatment were palmitic acid, oleic acid, linoleic acid, linoleic acid (Kark and Lee, 1990). Therefore, it could be concluded that the composition and the content of fatty acids are sensitive to and highly affected by the environmental changes of the cell.

It was observed in this study that if the environmental conditions are changed, the contents of total lipid, MGDG, and DGDG and the composition of fatty acids in galactolipid are changed. Fatty acids involved in the biosynthesis of MGDG in the chloroplast envelope are as follows: linolenic acid and oleic acid in the control, linolenic acid and linoleic acid in LAS, linolenic acid and oleic acid in AOS, and linolenic acid and oleic acid in SLES. Fatty acids which were used in the biosynthesis of DGDG in the chloroplast envelope are as follows: linolenic acid and oleic acid in the control, and AOS linolenic acid and stearic acid in LAS, oleic acid and linolenic acid in AOS, oleic acid and linolenic acid in SLES. Fatty acids which were used in the biosynthesis of MGDG in the thylakoid membrane are as follows: linolenic acid and oleic acid in the control, oleic acid and linolenic acid in LAS, linolenic acid and linoleic acid in AOS, linolenic acid and palmitoleic acid in SLES. Fatty acids which were used in the biosynthesis of DGDG in the thylakoid membrane are as follows: linolenic acid in the control, oleic acid and linolenic acid in LAS, linolenic acid and linoleic acid in AOS, palmitoleic acid and oleic acid in SLES. Therefore, it was shown that fatty acids became shorter and the degree of desaturation became weaker according to the environmental changes.

Fatty acids composing the galactolipid of *Dunaliella salina* are characterized by a high degree of desaturation because the structure of those fatty acids contains many double bonds (Joyard and Douce, 1977). This corresponds to the results of the present study that fatty acids composing the chloroplast envelope and the thylakoid membrane are mostly unsaturated fatty acids. In addition, galactolipid in the chloroplast envelope is made up of MGDG and DGDG constituted of highly unsaturated fatty acids (mostly linolenic acid) (Williams, 1976). For the galactolipid in the thylakoid membrane, MGDG and DGDG contain highly unsaturated fatty acids (Quartacci et al., 1995). In the case of *C. vulgaris* MGDG, while it contains mostly fatty acids in the light, it contains about 20% of 18:3 fatty acid in the dark (Gurr and James, 1980). This corresponds to the results of the present study that when the composition of galactolipid in the thylakoid membrane of *Chlorella* was analyzed, the content of linolenic acid was high in MGDG and DGDG for the control and MGDG in LAS, AOS and SLES, and DGDG in LAS and AOS.

The composition of MGDG in the thylakoid membrane treated with surfactants was similar to that of control, but the composition of DGDG treated with surfactants showed weaker degrees of desaturation compared to that of the control, for LAS and SLES used 18:1 fatty acid (oleic acid). As shown above, during the growth of *C. ellipsoidea,* unsaturated fatty acids were mostly used in the biosynthesis of galactolipid in the chloroplast envelope and thylakoid membrane. This result corresponds to the results of other researchers'. The chain of fatty acids treated with surfactants became shorter and the degree of desaturation became weaker as compared with the control. The difference in the composition of fatty acids composing galactolipid has an effect on the function of each membrane (Ohlorogge and Browse, 1995).

In this experiment, unsaturated fatty acids were not changed into saturated acids as obviously as the above cases, but it was shown that the degree of desaturation became weaker compared to that of the control. In short, the change in the composition of fatty acids treated with surfactants resulted in a weaker degree of desaturation and shorter chains as compared with that of the control. In the biosynthesis of galactolipid in cells treated with surfactants, unsaturated fatty acids were mostly used, similar to the result of the control. But if treated with surfactants, shorter fatty acid chain were mostly used and the degree of desaturation became weaker as compared with the control. Therefore, it could lead to the conclusion that due to the change in the composition of galactolipid

involved in the biomembrane, the changes of the permeability of the membrane and the type of the substance to transport into the cells were given rise to and its contents were decreased, which consequently affects metabolism. The difference in growth could be enough evidence to support this conclusion.

References

Albert B, Bray D, Lewis J, Raff K, Roberts K, and Watson JD (1994) Molecular Biology of the Cell, 3rd ed. Garland Publishing Inc., New York, pp 477-485.

Allen CF and Good P (1971) Acyl lipids in photosynthetic system. *Methods Enzymol* 23: 523-547.

Allen CF, Trosper T, and Park RB (1972) Chlorophyll, glycerolipid and protein ratios in spinach chlotoplast grana and dtroma lamellae. *Biochem Biophy Res Commu* 48: 907-913.

Albeida JLG, Dufaux M, Ben Taarit Y, and Naccache C (1994) Linear alkylbenzene. *Journal of American Oil Chemist's Society* 71: 675-694.

Bligh EQ and Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37: 911-917.

Chapman DJ and Barber J (1987) Chromatographic separation, identification, and analysis of phosphatides. *Methods Enzymol* 148: 294-319.

Chapman DJ, De-Felice J, and Barber J (1983) Growth temperature effects on thylakoid membrane lipid and protein content of pea chlotoplasts. *Plant physiol* 72: 225-228.

Cho YD, Kim TU, and Choi HG (1981) A study on the effect of ginseng saponin fraction on cell wall. *Korean J Ginseng Sci* 5: 65-72.

Freese E, Sheu CW, and Galliers E (1973) Function of lipophilic acids as antimicrobial food additives. *Nature* 241: 321-325.

Gounaris A, Mannock DA, Sen A, Brain APR, Williams WP, and Quinn PJ (1983) Polyunsaturated fatty acyl residues of galactolipids are involved in the control of bilayer/non-bilayer lipid transitions in higher plant chlotoplasts. *Biochim Biophys Acta* 732: 229-242.

Gurr MI and James AT (1980) Lipid Biochemistry: an Introduction. Chapman and Hall, London, 155-177.

Hand VC and Williams GK (1987) Structure-activity relationships for sorption of linear alkylbenzene sulfonates. *Environ Sci & Technol* 21: 370-373.

Hawke JC, Rumsby MG, and Leech RM (1974) Lipid biosynthesis in green leaves of developing maize. *Plant Physiol* 53: 555-561.

Helenius A and Simons K (1975) Solubilization of membranes by detergents. *Biochim Biophys Acta* 415: 29-79.

Joard J and Douce R (1977) Site of synthesis of phosphatidic acid and diacylglycerol in spinach chloroplast. *Biochim Biophys Acta* 486: 273-285.

Joard J, Siebertz HP, and Heinz E (1980) Distribution of radioactive lipids between envelopes and thylakoid from chloroplasts lanelled *in vivo*. *Eur J Biochem* 108: 171-176.

Kang YH (1983) Environmental Science, Synthetic Dertergent and Eutrophication, Hyeongseol Press, pp 404-405.

Kark HS and Lee CS (1990) Effects of carbon sources on the synthesis of phospholipids and fatty acid composition in chloroplast of *Chlorella ellipsoidea*. *Korean J Bot* 33: 49-54.

Kim HS (1986) Environmental Science, Synthetic Detergent and Phosphate. Donghwa Technol, Seoul, pp 238-240.

Koiwai A, Suzuki F, Matsuzaki T, and Kawashma N (1982) Changes in glycerolipids and their fatty acid composition during anaturation of tobacco seeds. *Phytochemistry* 21: 305-308.

Lyttleton JW (1962) Isolation of ribosome from spinach chloroplast. *Exp Cell Res* 26: 312-317.

Matsuzake T, Koiwai A, and Kawashima N (1983) Total fatty acid and polar lipid content in developing flower of *Nicotiana tabucum*. *Plant Cell Physiol* 24: 199-206.

Moore TS Jr (1982). Phospholipid biosynthesus. *Annu Rev Plant Physiol* 33: 235-296.

Moreno A, Ferrer J, Benvia FR, and Prats D (1994) Las monitoring in a treatment plant. *Water Res* 28: 2183-2189.

Ohlorogge J and Browse J (1995) Lipid biosynthesis. *Plant Cell* 7: 957-970.

Poincelot RP (1973) Isolation and lipid composition of spinach chloroplast envelope membrane. *Arch Biochem Biophys* 159: 134-142.

Park HS (1978) Surfactants and environmental pollution II. *J Korean Fib Engineer* 15: 32-39.

Quartacci MF, Pinzino C, Sgherri CLM, and Navari-Izzo F (1995) Lipid composition and protein dynamics in thylakoids of two wheat cultivars diffeerently sensitive to drought. *Plant physiol* 108: 191-197.

Roughan PG (1987) Plant acyl lipids-structure, distribution and analysis. *Methods Enzymol* 148: 327-337.

Roughan PG and Boardman NK (1972) Lipid composition of pea and bean leaves during chloroplast development. *Plant Physiol* 50: 31-34.

Sato N and Murata N (1981) Studies on the temperature shift-induced desaturation of fatty acids in monogalactosyl diglycerol in the blue green algae (cyanobacterium), *Anabaena variabilis*. *Plant Cell Physiol* 22: 1043-1050.

Song HI (1996) Increased uptake of cadmium by surfactants in a cadmium-tolerant yeast. *Korean J Environ Health Soc* 22: 104-113.

Tamiya H, Shibata K, Lwamura T, Sasa T, and Morimura Y (1953) Effects of oridernally intermittent illumination on the growth and some cellular characteristics of *Chlorella*. *Carnegie Inst. Wash Pub* 600: 76-81.

Traina SJ, Mcavoy DC, and Versteeg DJ (1996) Association of linear alkyl benzene sulfonates with dissolved humic substances and its effect on bioavailability. *Environ Sci & Technol* 30: 1300-1309.

Turner JD and Rouser G (1970) Precise quentitative determination of human lipids by thin-layer and triethylaminoethylcellulose column chromatography. Plasma lipids. *Anu Biochem* 38: 437-445.

Uemura MD, Raymond AJ, and Peter LS (1995) Cold accilimation of *Arabiodopsis thaliana*: effect on plasma membrane lipid composition and freeze-induced lesions. *Plant Physiol* 109: 15-50.

Williams JP, Watson GR, and Leung SPK (1976) Galactolipid synthesis in *vicia faba* leaves. Formation and desaturation of long chain fattu acid in phosphatidylcholine, phosphatidylglycerol, and the galactolipid. *Plant Physiol* 57: 179-184.

Womack MD, Kendall DA, and MacDonald RC (1983) Detergent effects on the enzyme activity and solubilization of lipid bilayer membranes. *Biochem Biophys Acta* 733: 210-215.

Yoshida S and Vemura M (1986) Lipid composition of plasma membrane and tonoplasts isolated from etiolated seedling of mung bean (*Vigna rasiate* L.). *Plant Physiol* 82: 807-812.

[Received May 26, 1998; accepted July 15, 1998]