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The Effects of Carbon Sources on the Biosynthesis of the Phospholipid and the Fatty Acid Composition of Mitochondria in *Chlorella ellipsoidea*

Seung Hee Yoon, Jae Seon Jang and Chong Sam Lee*

Department of Biology, College of Natural Sciences, Sungshin Women's University, Seoul 136-742, Korea

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Abstract: The biosynthesis of phospholipid and the composition of fatty acid were analyzed in mitochondria isolated from *Chlorella ellipsoidea* treated with carbon sources (glucose, sucrose, raffinose) during the culture. The growth of *Chlorella* and total lipid contents in mitochondria treated with various carbon sources was increased to compare with the control. When *Chlorella* mitochondria was treated with various carbon sources, four kinds of phospholipid were increased predominantly. The major fatty acids utilized for the biosynthesis of the phospholipid were analyzed linoleic acid (average 25.18%) and stearic acid (average 10.52%) in the control. But, it was shown that the major fatty acids in *Chlorella* mitochondria treated with glucose were stearic acid (average 30.93%), palmitic acid (average 17.47%) and stearic acid (average 20.31%), linoleic acid (average 16.68%) in sucrose treatment and oleic acid (average 17.17%), palmitic acid (average 15.64%) in raffinose treatment.

Key words: carbon sources, *Chlorella ellipsoidea*, fatty acid, phospholipid.

Phospholipid, which composed the major ingredients of the membrane, increased the capacity of the permeability of ions and small molecules through the membrane (Arron *et al.*, 1979), and was utilized for structural materials of mitochondria and chloroplast in plant cells.

The contents of lipid per cell were determined according to the type of cell and the composition of the medium. Total lipid was composed of 10% dry quantities in plant leaf, whereas algal cells contained more quantities of lipid than in the case of these plants (Kates, 1970; Valtersson *et al.*, 1986).

The phospholipids among total lipids occupied 20%. The major phospholipids composed of cells were phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidylinositol (PI) (Cobon *et al.*, 1974; Matsuzake *et al.*, 1983). But, cardiolipin in the mitochondria of rat liver cell was the only phospholipid that existed (Johnson *et al.*, 1975).

The contents and composition of phospholipid, sterol, and fatty acids were affected byvarious environmental conditions (e.g. temperature, pH and medium composition).

Polyunsaturated fatty acid in plant tissue cells were 80% at 12℃, 51% at 20℃ and 30% at 30℃ (Browse and Slack, 1983). Therefore, the content of unsaturated fatty acid in low temperature was increased, whereas in high temperature, the content of saturated fatty acid was increased. Also, when *S. uvarum* was grown in a medium with limited sucrose concentration, the formation of saturated fatty acid declined and the content of unsaturated fatty acid, such as oleic acid, increased (Knivett and Cullen, 1965).

And now, carbon sources were the major energy source of the cell and were absorbed into cell in order to be utilized as respiration substrates. Such sources were accelerated cell growth by increased enzyme activity which operated physiological metabolism and formed acetyl CoA as the precursor of protein and fatty acid synthesis.

When *Bacillus subtilis* variants were grown in medium that contained carbon sources of high concentration, spore formation was induced by the imbalance of nutritional conditions (Takahashi and Mackenzic, 1981). *Sulfolobus solfataricus* utilized glucose as a single carbon source, offered to energy occurring by ATP-dependent phosphorylation and NAD^+-dependent dehydrogenation (De Rosa *et al.*, 1984).

The *Chlorella* cell wall was composed of alkali-soluble hemicellulose and alkali-insoluble materials. The absorption rate of glucose increased as the cells grew with glucose treatment because glucose was largely utilized for the composition of a solid cell wall in the

*To whom correspondence should be addressed.
Tel: 82-2-920-7172, Fax: 82-2-953-2091.

daughter cell as the *Chlorella* cell divided (Takeda and Hirokawa, 1983). Also, in inorganic nutrition medium which contained 0.2% glucose, *Chlorella* formed large quantities of chlorophyll and carotenoid. But, *Saccharomyces carlsbergensis* synthesized a lot of triacylglycerol, whereas phospholipid biosynthesis was inhibited (Paltauf and Johntson, 1970; Azcon-Bieto, 1983). Sucrose transfered to a higher plant prior to other sugar was utilized for biosynthesis of protein and carbohydrates, and the carbon skeleton of hexose (Giaquinta, 1979; Thomas *et al.*, 1983). In particular, the mechanism of photosynthesis was affected according to the volume of sucrose contained in leguminous plant cytoplasm (Sawada *et al.*, 1987).

In recent years, increased emphasis has been given to the study on the effect of cellular metabolism in organisms under various environmental conditions.

The present paper is a comparative analysis of the biosynthesis of phospholipid and fatty acid composition in mitochondria isolated from *Chlorella* treated with glucose, sucrose, and raffinose during cultivation.

Materials and Methods

Chlorella ellipsoidea culture

Chlorella ellipsoidea was cultured in M4N medium (Tamiya *et al.*, 1953) treated with glucose (5 mM), sucrose (10 mM) and raffinose (30 mM), respectively. The cultures were incubated at 25℃ under an atmosphere that involved 5% CO_2 gas and were irradiated with 2 KLux light continously. Cell growth was measured by packed cell volume using a haematocrit.

Separation of mitochondria

The mitochondria of *Chlorella* was obtained in the beginning and middle of cultivation (Fig. 1), isolated according to the method described by Lee and Chin (1964).

The cell was centrifuged at 600×*g* for 4 min and suspended in 0.005 M Tris buffer (pH 7.4) solution containing 0.25 M sucrose and 0.005 M EDTA, and was smashed with a sonicator (Sonics & Ins. Model VC 250B).

The smashed materials were centrifuged at 300×*g* for 3 min and the supernatant obtained was centrifuged at 6,000×*g* for 10 min again. The supernatant was then centrifuged at 15,000×*g* for 20 min and washed 2 times with 0.005 M Tris buffer containing 0.25 M sucrose. The sedimented mitochondria was then used in this study.

Extraction of total lipid

The total lipid in the mitochondria of *Chlorella* was

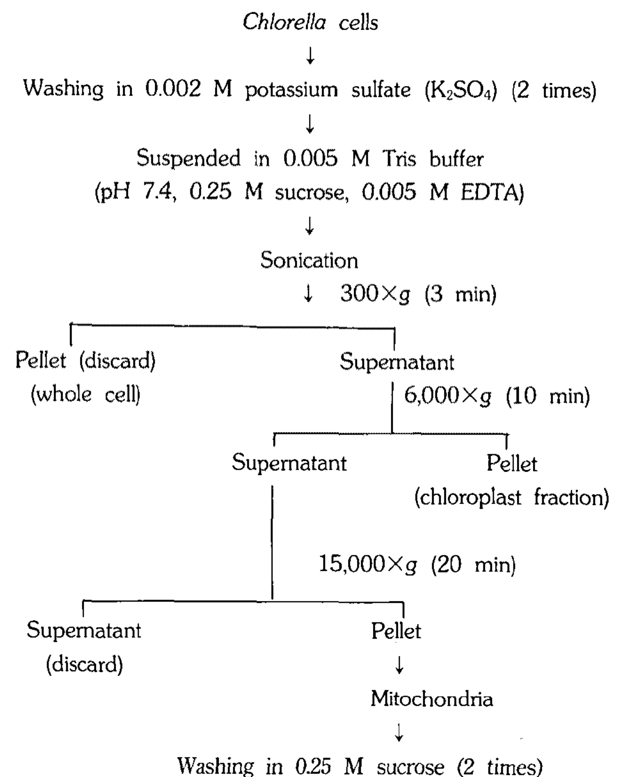

Fig. 1. Isolation of mitochondria in *Chlorella ellipsoidea*

extracted according to a modified method described by Bligh and Dyer (1959). After separation, chloroform was added to the mitochondria : methanol (1 : 2 v/v), shaken for 30 min, was added to the same quantity of distilled water, separated after leaving, and the total lipid extracted by filtering the separated chloroform layer using Whatmann No. 1 filter paper. After the methanol layer, the upper layer, the added and mixed chloroform, and the total lipid were re-extracted in a separated chloroform layer by filtration using the same filter paper. After being extracted the total lipid was dried in 45~50℃, and the dry weight was measured.

Separation and identification of phospholipid

The major phospholipid, such as phosphatidycholine (PC), phosphatidyethanolamine (PE), phosphatidyglycerol (PG) and phosphatidyinositol (PI) in extracted total lipid were isolated by thin layer chromatography (TLC, Desaga) according to the procedure of Turner and Rouser (1970). A glass plate (20×20 cm) used in TLC was precoated with a 0.25 mm layer of silicagel (Merck), dried in room temperature and activated in a 110~120℃ dry oven for 60 min. The solvent, containing chloroform-methanol-28% ammonia water (65 : 25 : 2 v/v/v) for the first expansion and chloroform-acetone-methanol-acetic acid-distilled water (3 : 4 : 1 : 1 : 0.5 v/v/v/v/v) for the second expansion, was used accord-

ing to a two-one dimension method. The phospholipids separated from the total lipid were identified and compared with the standard material (Sigma, St. Louis, USA). Developing reagents used 0.2% ninhydrin in saturated butanolin PE, dragendroff reagents in PC, periodata-schiff's reagents in PI, and sulfuric acid with 20% ethanol in PG (Skipski and Ballay, 1969).

Methyl esterification of fatty acid

In order to analyze the composition and contents of fatty acid composed of PC, PE, PG and PI were methyl esterified by the Allen and Good method (1971). To the separated phospholipid in each plate was added 5 ml of methanol with 5% sulfuric acid and heptadecanoic acid as an internal standard, cooled after heating for 120 min in a 68~70℃ dry oven, and then the same quantity of distilled water was added and shaken. To the homogenate was added 2 ml of hexane and the separated hexane layer after strong shaking. This separation procedure was repeated 3 times. To the separated hexane layer was added 5 ml of saturated sodium bicarbonate, and the separated hexane layer was shaken. The contents of fatty acid methyl ester in each phospholipid were measured after the separated hexane layer was dried.

Assay of fatty acid

The composition and contents of fatty acids, of which each phospholipid was composed, were analyzed by gas chromatograpy(GC, Varian 3300). The identification of each fatty acid was resloved by comparision with standard material (Sigma), such as lauric acid (12:0), myristic acid(14:0), palmitic acid(16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). The used column, such as the stainless steel column (3 mm×3 m), used 15% DEGS (diethylglycol succinate) as packing material and the H_2-flame ionization detector (FID) as a GC detector. Analysis conditions are described as follows:

Injection Port Temperature : 230℃
Column Temperature : 170℃
Detector Oven Temperature : 240℃
Carrier Gas : N_2 (30 ml/min)

Results

Growth

Changes in the growth of *Chlorella* treated with various types of sugar during the culture are represented in Fig. 2.

As shown in Fig. 2, it is noted that growth in various sugar types increased remarkably compared with the control. The increase rates in glucose and sucrose treatment were 33.33% and 44.46%, whereas in raffinose treatment, 55.56%. It is shown that the increase rate of *Chlorella* was accelerated in raffinose treatment.

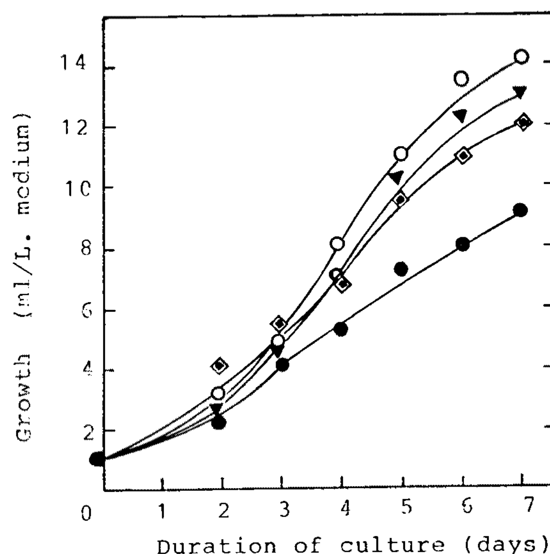

Fig. 2. Growth of *Chlorella ellipsoidea* treated with carbon sources during the cultivation. ◈: Glucose, ▼: Sucrose, ○: Raffinose, ●: Control.

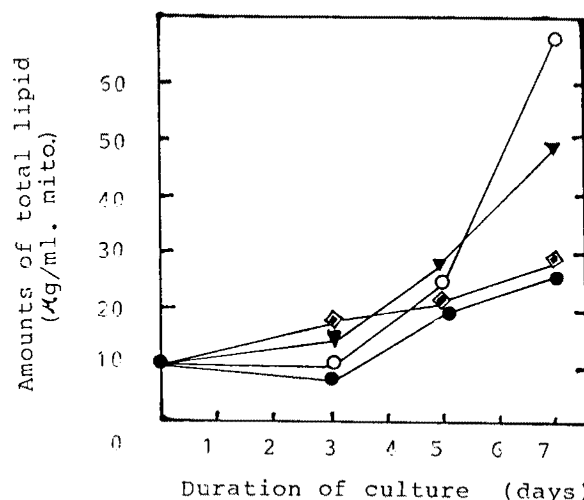

Fig. 3. Changes in contents of total lipids in *Chlorella* mitochondria treated with carbon sources during the cultivation. ●: Control, ◈: Glucose, ▼: Sucrose, ○: Raffinose.

Total lipid

Fig. 3 represents the total lipid content in *Chlorella* mitochondria treated with sugar, respectively.

It can be seen from Fig. 3 that the content of total lipid in the control decreased on the 3rd day of incubation compared with the beginning of the culture, whereas on the 5th and 7th day of the culture, it increased remarkably. The total lipid content decreased 25.00% on the 3rd day, whereas it increased 90.00%

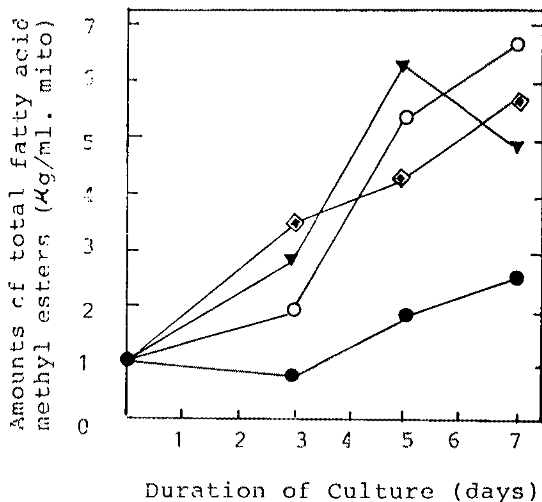

Fig. 4. Changes in contents of total fatty acid methyl esters in *Chlorella* mitochondria treated with carbon sources during the cultivation. ●: Control, ◈: Glucose, ▼: Sucrose, ○: Raffinose.

and 165.00% on the 5th and 7th day of the culture, respectively. The average increase rate was 76.67% for the culture periods. The content of total lipid in glucose treatment was predominantly increased 133.33% on the 3rd day of the culture compared with the control, 13.16% and 7.55% on the 5th and 7th day of the culture.

The average increase rate was 51.53% for total lipid synthesis during the culture. The content of total lipid in sucrose treatment remarkably increased in the beginning of the culture compared with the control, 93.33 %, 47.37% and 83.02% on the 3rd, 5th, and 7th day of the culture, respectively. For the cultivation period, the average increase rate was 74.57% for total lipid synthesis. The content of total lipid in raffinose treatment increased 33.33%, 42.11% and 152.83% on the 3rd, 5th, and 7th day of cultivation, respectively. During the culture, the average increase rate was 76.09% for total lipid synthesis.

Therefore, it was confirmed that lipid metabolism in raffinose treatment was remarkably accelerated.

Total fatty acid methyl ester

The quantitative changes of total fatty acid methyl ester in various types of sugar treatment are shown in Fig. 4.

The content of total fatty acid methyl ester in the control decreased 21.00% on the 3rd day compared with the beginning of the culture, whereas it increased 89.70% on the 5th day and 165.10% on the 7th day of the culture, remarkably. It was observed that the average increase rate was 77.93%.

From Fig. 4, it was shown that the content of total

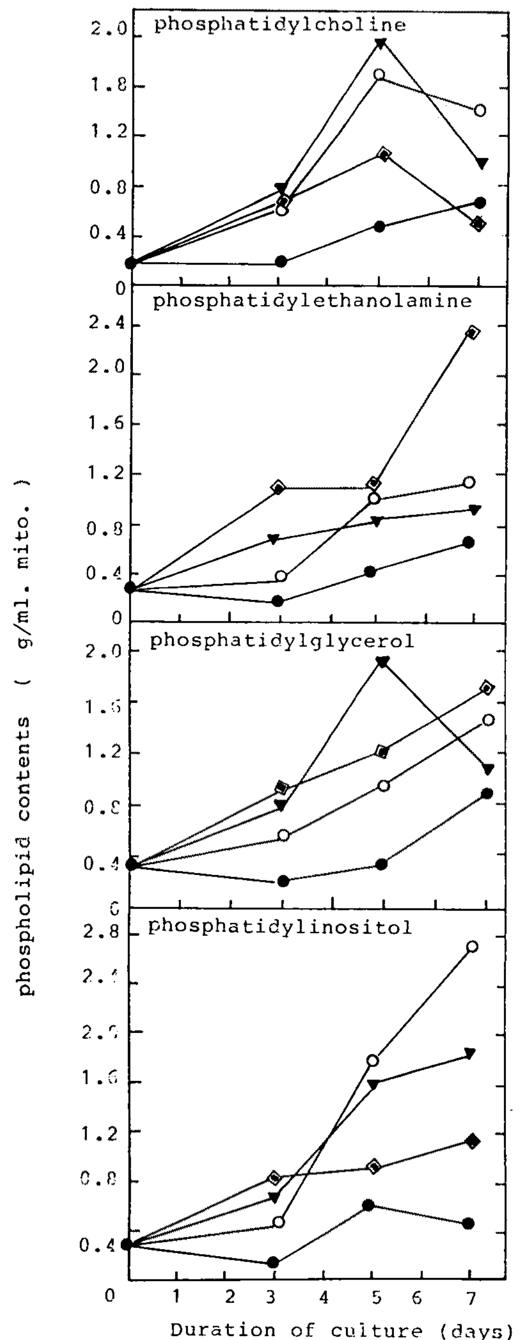

Fig. 5. Changes in contents of phospholipids in *Chlorella* mitochondria treated with carbon sources during the cultivation. ●: Control, ◈: Glucose, ▼: Sucrose, ○: Raffinose.

fatty acid methyl ester in glucose treatment remarkably increased 342.91% on the 3rd day, 126.52% on the at 5th day, and 115.01% on the 7th day of the culture compared with the control. The average increase rate was 194.81%. The content of total fatty acid methyl ester in sucrose treatment increased 267.09% on the 3rd day, 231.79% on the 5th day, and 84.87% on the 7th day of the culture compared with the control.

It was confirmed that the average increase rate of total fatty acid methyl ester quantities for the culture period increased 194.58%. Also, the content of total fatty acid methyl ester in raffinose treatment increased 153.16%, 184.66% and 152.70% on the 3rd, 5th, and 7th day of the culture compared with the control, respectively. It was shown that the average increase rate accelerated 163.51% during the growth period. The quantitative changes of total fatty acid methyl ester showed a growth effect in glucose treatment predominantly.

Phospholipid

The content changes of various phospholipids in mitochondria are shown in Fig. 5.

As pointed out in Fig. 5, the PC content in the control markedly increased 14.97% on the 3rd day, 165.24 % on the 5th day, and 255.61% on the 7th day compared with the beginning of the culture. So, the average content of PC accelerated 145.21% during the culture period. Compared with the control, the PC content in glucose treatment increased 211.63%, 111.69% on the 3rd and 5th day of the culture, whereas it decreased 27.67% on the 7th day of the culture. And so, the average increase rate of PC was 98.55%. The PC content in sucrose treatment increased 253.95%, 294.56%, and 51.88% on the 3rd, 5th, and 7th day of the culture compared with the control, respectively. The average increase rate of PC was 200.13%. The PC content in raffinose treatment increased 196.74%, 242.74% and 105.41% on the 3rd, 5th, and 7th day of the culture during the culture period, respectively. The average increase rate of PC was 181.63%. Therefore, the synthesis of PC in sucrose treatment was more accelerated than other carbon sources.

Compared to the beginning of the culture, the content of PE in the control decreased 25.51% on the 3rd day of the culture, whereas it increased 80.00% and 170.83% on the 5th and 7th day of the culture. The average increase rate of PE was 75.11%. The PE synthesis in sucrose treatment increased 274.46% on the 3rd day, and 96.06% and 43.08% on the 5th and 7th day of the culture compared with the control. The average growth rate of PE was 137.87%. The PE content in raffinose treatment increased 96.74% on the 3rd day, and 131.71% and 77.23% on the 5th and 7th day of the culture. The average increase rate of PE was 101.89%.

It was confirmed that glucose was largely utilized for PE biosynthesis.

The content of PG in the control decreased 30.62% on the 3rd day, whereas it increased 7.81% on the 5th day, and 170.94% on the 7th day of the culture. The PG content in glucose treatment was 311.71%, 256.81%, and 97.23% on the 3rd, 5th, and 7th day of the culture, respectively. The average increase rate was 221.92%. The PG content in sucrose treatment increased 253.60% on the 3rd day and 453.33% on the 5th day of the culture, whereas it decreased 24.91 % on the 7th day of the culture. The average increase rate of PG was 243.95%. The PG content in raffinose treatment was 147.75% on the 3rd day, 170.14% on the 5th day, and 68.97% on the 7th day of the culture. The average increase rate was 128.95%. It was analyzed that sucrose was largely utilized for the synthesis of PG.

Compared to the beginning of the culture, the content change of PE in the control decreased 25.51% on the 3rd day of the culture, whereas it increased 80.00% and 170.83% on the 5th and 7th day of the culture. The average increase rate of PE was 75.11%. The PE synthesis in sucrose treatment increased 274. 46% on the 3rd day, and 96.06% and 43.08% on the 5th and 7th day of the culture compared with the control. The average increase rate of PE was 137.87%. The PE content in raffinose treatment increased 96.74 % on the 3rd day, and 131.71% and 77.23% on the 5th and 7th day of the culture. The average increase rate of PE was 101.89%. The results showed that glucose was largely utilized for the biosynthesis of PE.

The content change of PI in the control was decreased 51.14% on the 3rd day of the culture, whereas it increased 136.36% and 77.65% on the 5th and 7th day of the culture, respectively. The average content of PI increased 54.29%. Compared to the control, the content of PI in glucose treatment increased 548.06% on the 3rd day, 47.28% on the 5th day, and 143.09% on the 7th day of the culture. The average increase rate of PI was 246.14% during the culture period. The PI content in sucrose treatment increased 415.50% on the 3rd day, 145.81% on the 5th day, and 289.13% on the 7th day of the culture. The average increase rate of PI was 163.15%. Compared to the control, the PI content in raffinose treatment was 248.84%, 183.49 %, and 479.10% on the 3rd, 5th, and 7th day of the culture, respectively. The average increase rate was 303.81%. Raffinose was largely utilized for the biosynthesis of PI on the basis of these results.

Fatty acid

Lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) which utilized the synthesis of PC, PE, PG and PI in *Chlorella* mitochondria were analyzed.

The composition changes of fatty acid in various sugar treatments are shown in Tables 1, 2, 3 and 4.

Table 1. Changes in contents of fatty acid methyl esters of phosphatidylcholine in *Chlorella* mitochondria treated with various carbon sources during the cultivation

Duration of culture (days)	0	3				5				7			
Treatment / Fatty acid	Cont.	Cont.	Glu.	Suc.	Raff.	Cont.	Glu.	Suc.	Raff.	Cont.	Glu.	Suc.	Raff.
Lauric acid (12:0)	–	9.37	0.15	1.51	4.72	0.60	–	15.83	17.16	10.20	0.69	0.44	4.81
Myristic acid (14:0)	6.11	0.12	0.48	11.01	10.51	2.42	9.80	8.71	11.81	4.56	3.43	1.04	3.99
Palmitic acid (16:0)	5.47	0.93	40.91	13.72	24.64	4.61	20.63	19.95	15.25	9.78	5.79	46.84	14.26
Stearic acid (18:0)	22.24	0.48	–	8.05	0.53	7.66	3.57	19.00	5.47	10.42	59.40	12.00	–
Oleic acid (18:1)	–	–	7.73	–	24.87	5.60	10.49	–	–	–	–	17.90	20.54
Linoleic acid (18:2)	57.55	23.90	18.95	16.39	–	24.20	8.76	–	12.14	30.40	29.54	5.70	16.17
Linolenic acid (18:3)	–	0.42	24.16	–	–	–	25.60	–	0.17	3.41	–	–	1.40
Unknown	8.63	64.78	7.62	49.32	34.73	54.91	21.15	36.51	38.00	31.23	1.15	16.08	38.83
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

Unit : %, Cont. : Control, Glu. : Glucose, Suc. : Sucrose, Raff. : Raffinose.

Table 2. Changes in contents of fatty acid methyl esters of phosphatidylethanolamine in *Chlorella* mitochondria treated with various carbon sources during the cultivation

Duration of culture (days)	0	3				5				7			
Treatment / Fatty acid	Cont.	Cont.	Glu.	Suc.	Raff.	Cont.	Glu.	Suc.	Raff.	Cont.	Glu.	Suc.	Raff.
Lauric acid (12:0)	0.43	5.84	1.03	3.84	6.03	5.46	2.37	2.79	2.18	6.21	5.42	1.79	0.79
Myristic acid (14:0)	4.23	10.94	7.34	1.68	8.82	3.23	7.49	7.30	10.67	4.12	7.21	0.34	0.51
Palmitic acid (16:0)	7.70	6.94	12.89	5.25	6.37	15.26	25.74	18.04	10.32	10.42	28.60	12.90	4.98
Stearic acid (18:0)	25.49	3.23	21.10	21.13	18.92	4.31	60.47	10.38	10.17	5.21	40.30	59.02	68.12
Oleic acid (18:1)	–	–	4.91	16.73	2.14	–	–	–	–	3.24	2.10	–	9.24
Linoleic acid (18:2)	17.40	4.07	32.75	4.78	26.74	0.46	–	42.98	28.97	1.46	–	–	3.90
Linolenic acid (18:3)	19.93	4.88	5.71	2.51	0.45	35.78	–	7.85	–	38.70	8.10	6.25	0.18
Unknown	24.82	64.10	14.27	44.08	30.53	35.50	3.93	10.66	37.69	30.64	8.27	19.70	12.28
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

Unit : %, Cont. : Control, Glu. : Glucose, Suc. : Sucrose, Raff. : Raffinose.

Table 1 shows the change of fatty acid composed of PC.

In the case of the control, it was shown that the use rate of linoleic acid and stearic acid was 57.48% and 22.24% in the beginning of the culture, linoleic acid and lauric acid was 23.90% and 9.37% on the 3rd day, and linoleic acid and stearic acid was 24.20% and 7.66% on the 5th day of the culture. Linoleic acid, stearic acid and lauric acid were utilized for 30.40%, 10.42% and 10.20% on the 7th day of the culture for PC formation.

It was analyzed in glucose treatment that palmitic acid and linoleic acid were utilized for 40.91% and 24.16% on the 3rd day of the culture, 20.63% and 25.60% on the 5th day, and stearic acid and linoleic acid utilized 59.40% and 29.54% on the 7th day for phospholipid synthesis.

It was analyzed in sucrose treatment that linoleic acid and palmitic acid were utilized for 16.39% and 13.72% on the 3rd day, palmitic acid and stearic acid for 19.95 % and 19.00% on the 5th day, and palmitic acid and oleic acid utilized for 46.84% and 17.90% on the 7th day of the culture during the formation of PC. It was analyzed in raffinose treatment that palmitic acid and oleic acid were utilized for 26.64% and 24.87% on the 3rd day, lauric acid and palmitic acid for 17.16% and 15.25% on the 5th day, and oleic acid and linoleic acid for 20.54% and 16.17% on the 7th day of the culture. The composition of fatty acid utilized for the culture period was shown variously. It was analyzed that the fatty acids composed of PC in various sugar treatments were palmitic acid, stearic acid, and linoleic

Table 3. Changes in contents of fatty acid methyl esters of phosphatidylglycerol in *Chlorella* mitochondria treated with various carbon sources during the cultivation

Duration of culture (days)	0	3				5				7			
Treatment / Fatty acid	Cont.	Cont.	Glu.	Suc.	Raff.	Cont.	Glu.	Suc.	Raff.	Cont.	Glu.	Suc.	Raff.
Lauric acid (12:0)	1.02	0.31	0.69	0.30	2.78	1.03	2.40	–	–	2.21	5.78	–	14.70
Myristic acid (14:0)	0.14	0.34	0.89	0.76	1.93	3.59	1.26	–	16.08	5.43	2.57	0.26	5.38
Palmitic acid (16:0)	10.21	–	4.54	7.46	8.27	7.01	5.67	7.81	19.85	6.46	7.46	22.42	30.28
Stearic acid (18:0)	5.40	1.46	21.60	33.70	33.53	16.07	22.47	6.64	–	7.46	20.51	5.95	–
Oleic acid (18:1)	1.20	–	–	–	41.86	2.12	–	1.78	–	6.48	0.24	10.58	–
Linoleic acid (18:2)	20.10	96.20	–	42.43	–	31.20	5.78	7.79	53.46	28.40	–	6.10	–
Linolenic acid (18:3)	3.44	–	–	11.24	0.12	4.57	–	–	0.21	5.70	4.60	7.56	–
Unknown	58.49	1.69	72.28	4.11	11.51	34.41	62.42	75.98	10.40	37.86	58.84	47.13	49.64
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

Unit : %, Cont. : Control, Glu. : Glucose, Suc. : Sucrose, Raff. : Raffinose.

Table 4. Changes in contents of fatty acid methyl esters of phosphatidylinositol in *Chlorella* mitochondria treated with various carbon sources during the cultivation

Duration of culture (days)	0	3				5				7			
Treatment / Fatty acid	Cont.	Cont.	Glu.	Suc.	Raff.	Cont.	Glu.	Suc.	Raff.	Cont.	Glu.	Suc.	Raff.
Lauric acid (12:0)	5.43	2.15	7.36	6.86	3.41	2.71	9.76	8.83	20.35	4.78	10.78	3.47	0.28
Myristic acid (14:0)	16.05	2.46	1.36	13.91	13.28	3.30	8.54	14.37	1.86	8.56	6.83	6.82	0.37
Palmitic acid (16:0)	25.22	10.50	9.24	7.97	–	14.60	21.40	13.22	26.05	8.20	26.76	15.47	11.83
Steanc acid (18:0)	25.79	14.60	19.08	13.33	–	22.10	30.56	–	–	14.20	41.44	34.20	74.57
Oleic acid (18:1)	–	–	–	0.50	–	–	2.41	–	–	–	1.62	25.08	4.38
Linoleic acid (18:2)	–	30.50	52.56	11.89	24.43	56.43	–	11.56	9.59	56.20	1.14	–	5.19
Linolenic acid (18:3)	4.57	2.67	–	1.12	–	–	5.40	–	–	2.78	–	–	–
Unknown	22.94	37.12	10.40	44.42	58.88	0.86	21.93	42.02	42.15	5.28	11.43	14.96	3.38
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

Unit : %, Cont. : Control, Glu. : Glucose, Suc. : Sucrose, Raff. : Raffinose.

acid.

Fatty acids which used PC composition are shown in Table 2.

It was observed that stearic acid and linolenic acid in the control were utilized for 25.49%, 19.93% for phospholipid composition, 10.94% for myristic acid and 6.94% for palmitic acid on the 3rd day of the culture, 35.78% and 15.25% for linoleic acid and palmitic acid on the 5th day, and 38.70% and 10.42% for linoleic acid and palmitic acid on the 7th day, respectively. Linoleic acid and stearic acid in glucose treatment were utilized for 32.75% and 21.10% on the 3rd day, and stearic acid and palmitic acid for 60.47% and 25.74%, and 40.30% and 28.60% on the 5th and 7th day of the culture for the formation of PC. Stearic acid and oleic acid in sucrose treatment were utilized for 21.13% and 28.60% on the 3rd day, linoleic acid and palmitic acid for 42.98% and 18.04% on the 5th day, and stearic acid and palmitic acid for 59.02% and 12.90% on the 7th day of the culture. Stearic acid in raffinose treatment was surveyed for 18.92%, 10.17% and 68.12 % on the 3rd, 5th, and 7th day of the culture, and showed 26.74% for linoleic acid on the 3rd day, and 10.67% for myrisic acid and 28.97% for linoleic acid on the 5th day of the culture during PE biosynthesis.

From Table 2, the fatty acids which utilized PE formation in carbon source treatments were palmitic acid, stearic acid and linoleic acid.

Table 3 represents the composition change of fatty acids composed of PG.

It shows that linoleic acid and palmitic acid were surveyed for 20.10% and 10.21% in the beginning of

the culture in the control, and linoleic acid for 96.20%, 31.10% and 28.40% on the 3rd, 5th, and 7th day of the culture, respectively. Therefore, the use rate of linoleic acid decreased according to the culture period. In addition, it was analyzed that stearic acid was utilized for 16.07% and 17.42% on the 5th and 7th day of the culture. Stearic acid and palmitic acid in glucose treatment were utilized for 21.60% and 4.54% on the 3rd day, stearic acid and palmitic acid for 22.47% and 5.78% on the 5th day, and stearic acid and lauric acid for 20.51% and 5.78% on the 7th day of the culture. Linoleic acid and stearic acid in sucrose treatment were composed of 42.43% and 33.70% on the 3rd day, palmitic acid and linoleic acid for 7.81% and 7.79% on the 5th day, and palmitic acid and oleic acid for 22.42% and 10.58% on the 7th day of the culture. Stearic acid and oleic acid in raffinose treatment were utilized for 33.53% and 41.68% on the 3rd day, linoleic acid and palmitic acid for 53.46% and 19.58% on the 5th day, and palmitic acid and lauric acid for 30.28% and 14.70% on the 7th day. It was shown that fatty acids composed of PG vary with the various sugar treatments.

Table 4 records the analysis of fatty acids composed of PI.

In the case of the control, it is shown that palmitic acid and stearic acid were surveyed for 25.22% and 25.79% in the beginning of the culture, stearic acid for 14.60%, 22.10% and 14.10% during the culture period, and linoleic acid was utilized for 30.50%, 56.43 % and 56.20% for phospholipid synthesis. Linoleic acid and stearic acid in glucose treatment were utilized for 52.56% and 19.08% on the 3rd day of the culture, stearic acid and palmitic acid for 30.56% and 21.40% on the 5th day, and stearic acid and palmitic acid for 41.44% and 26.76% on the 7th day. Myristic acid and stearic acid in sucrose treatment were utilized for 13.91 % and 13.33% on the 3rd day of the culture, myristic acid and palmitic acid for 14.37% and 13.22% on the 5th day, and stearic acid and oleic acid for 34.20% and 25.08% on the 7th day. Linoleic acid and myristic acid in raffinose treatment were utilized for 24.43% and 13.28% on the 3rd day, lauric acid and palmitic acid for 20.35% and 26.05% on the 5th day, and stearic acid and palmitic acid for 74.57% and 11.83% on the 7th day. It was confirmed that the fatty acids consisting of PI were palmitic acid and stearic acid.

Therefore, the composition and content of fatty acids composed of each phospholipid were shown variously according to the culture period and each treatment.

Discussion

The CO_2 release rate for respiration in wheat leaves was closely correlated with the CO_2 assimilation rate through carbohydrate anabolism. Because of the acceleration of oxygen absorption according to the increase of sugar content in wheat, respiration activity was increased by substrate supply to mitochondria (Azcon-Bieto *et al.*, 1983).

Therefore, it was shown in this paper that the growth of *Chlorella* treated with sugar was accelerated. But, when glucose was added to *Euglena gracilis* strain Z in an inorganic nutrition medium and cultured in the dark, a rudimentary pigment formed due to the inhibition of chloroplast formation, and inhibited enzyme biosynthesis participated in porphyrin synthesis and the end stage of chlorophyl formation (Schwelitz *et al.*, 1978).

The thylakoids of chloroplast showed cryoprotection action with the same concentration of glucose, sucrose and raffinose (Lineberger and Steponkus, 1980).

In this experiment, and the growth acceleration effects in sugar treatment were shown in the order of raffinose, sucrose, and glucose in *Chlorella* compared with the control. This result showed a close resemblance to that of Kim (1992). In particular, growth was accelerated because the glucose content separated by hydrolysis after raffinose absorption increased and so respiration was enhanced. The total lipid in sugar treatment increased from the beginning of the culture compared with the control and raffinose was highly utilized for the synthesis of total lipid. It was probably caused by conversion to acetyl CoA, the precursor of lipid synthesis, from pyruvate formed through the glycolytic pathway due to the supply of glucose formed by the hydrolysis of raffinose.

Also, Kark (1990) reported that the activity of NADP-malate dehydrogenase and citrate cleavage enzyme as lipogenic enzyme in *Chlorella* chloroplast was accelerated by glucose and so increased the total lipid content.

In this study, total lipid synthesis in *Chlorella* mitochondria increased in the order of raffinose, sucrose, and glucose. It was probably caused by activation of the lipogenic enzyme so far as to convert raffinose and sucrose into glucose. Various phospholipids, such as PC, PE, PG and PI, are major phospholipids in plant and animal cells. In the case of *E. coli*, PE and PG were composed of 70~80% and 5~15% of phospholipids, in addition to PS and cardiolipin (Cronan and Vagelos, 1972). In *Nicotiana tabacum*, phospholipid composition was surveyed variously according to the developmental stage and organs of the cell, such as the ovary, petal and pistil, etc. (Koiwai *et al.*, 1982).

In this study, phospholipids, when *Chlorella* mitochondria were treated with various types of sugar during the culture, were increased compared with the control. But, the increase rate varied according to carbon sources.

These results were analyzed mainly owing to the utilization of glucose to PE and PG formation, sucrose to PC and PG formation, and raffinose to PC and PI formation. But, Kim (1992) reported that the contents of all phospholipids were increased by sucrose. On the other hand, in the case of chloroplasts, glucose remarkably accelerated the formation of PI, PC and PE, and sucrose was a PE and PI synthesis, whereas raffinose was utilized in a few quantities for three kinds of phospholipids (Kark, 1990).

These two results were found to be quite similar to those facts stating that a kind of carbon source utilized phospholipid biosynthesis according to the cell organelle. Otherwise, under the condition that the carbon supply was limited, PI and PG synthesis in yeast decreased because the DNA did not sufficiently supply the carbon needed for synthesis of the enzyme related phospholipid formation (Cobon *et al.*, 1974).

But, in this study, it was analyzed that carbon sourcese increased phospholipid biosynthesis owing to the supply of carbon sources for DNA formation. Fatty acid composition as well as phospholipid biosynthesis was affected by various environmental conditions.

Acetyl CoA using the precursor of fatty acid synthesis was formed by deamination of the protein and decarboxylation of the carbohydrate. In chloroplast, as [HCO_3^-] increased, acetyl CoA was largely synthesized and also also increased fatty acid formation (Roughan *et al.*, 1979). Blue-green algae, such as *Anacystis nidulans*, was composed of fatty acid which was situated in carbon number 14, 16, and 18. Single unsaturated fatty acid and saturated fatty acid were esterified in the first and second carbon number. Such an unsaturated degree and the chain length of fatty acid were affected according to an increase in temperature (Sato *et al.*, 1979). The level of unsaturated fatty acid increased if the temperature of the medium decreased because of the change of oxygen solubility, depending upon water temperature.

In blue flag, saturated fatty acid, such as stearic aicd, converted unsaturated fatty acid according to temperature (Browse and Slack, 1983). Also, the major fatty acids composed of chloroplast envelope were linoleic acid and linolenic acid. These fatty acids increased the degree of unsaturation by light (Ohnishi and Yamada, 1983). When the quantity of ammonium salt was limited in *E. coli*, the content of saturated fatty acid increased, whereas in limited glucose, unsaturated fatty acid increased (Marr and Ingraham, 1962).

The microsomal membrane in liver cells formed a fluid bilayer at a physiological temperature. In a such structure, the membrane component of microsome is capable of movement between membranes. Such a property affected the distribution of saturated fatty acid and unsaturated fatty acid of phospholipids composed of the membrane, and so the liquidity of the membrane decreased.

In this study, saturated fatty acid was utilized more than unsaturated fatty acid. The major fatty acid composed of phospholipids in *Chlorella* mitochondria was various according to the kind of phospholipid. Stearic acid, linoleic acid, palmitic acid, and lauric aicd in the control mainly utilized the biosynthesis of phospholipids. The fatty acids which utilized phoshpolipid synthesis were palmitic acid, linoleic acid, and stearic acid in glucose treatment, palmitic acid, stearic acid, linoleic acid, and oleic acid in sucrose treatment, and palmitic acid, oleic acid, stearic acid, and linoleic acid in raffinose treatment. Glucose showed a close resemblance to that of the control, but, oleic acid formation was inhibited in the control. It was surveyed that sucrose and raffinose were utilized for oleic acid formation. Fatty acids composed of mitochondria membrane of *Tetrahymena pyriformis* were stearic acid, linoleic acid, and linolenic acid (Gleason, 1976). The results showed a close resemblance to the fatty acid composition of *Chlorella* mitochondria. And so, fatty acids composed of cells were changed in composition by temperature, medium, and the site of the plant, as well as by carbon sources.

In this study, saturated fatty acids were utilized more than unsaturated fatty acids in carbon source treatment. It was analyzed that carbon sources accelerated the formation of fatty acid, lipid, and cell growth by means of supply of acetyl CoA, which used fatty acid biosynthesis via a glycolytic pathway and lots of energy in cell.

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