

The Effects of Antibiotics on the Biosynthesis of the Phospholipid and the Fatty Acid Composition of *Chlorella ellipsoidea* Mitochondria

Seung Hee Yoon, Kwang Seok Seo and Chong Sam Lee

Department of Biology, College of Natural Sciences, Sungshin Women's Uni., Seoul, Korea

Chlorella ellipsoidea mitochondria의 인지질 생합성과 지방산 대사에 미치는 항생제의 효과

尹承姬 · 徐光錫 · 李鍾三

성신여자대학교 자연과학대학 생물학과

국문요약

Cycloheximide와 nalidixic acid를 처리한 배지에 *Chlorella ellipsoidea*를 배양하였을 때 mitochondria의 인지질 생합성과 그의 지방산 조성에 어떤 영향을 미치는 가를 분석하였다. 생장율과 total lipid 함량은 항생제 처리구에서는 대조구보다 낮게 나타났다. PC와 PI의 합성은 nalidixic acid 처리구에서는 억제되었고, PC, PE, PG 그리고 PI의 함량은 cycloheximide 처리구에서 억제되었다. 항생제 처리구에서 여러가지 인지질 형성에 이용된 주요 지방산은 배양 말기에 stearic acid, myristic acid, palmitic acid, oleic acid, linoleic acid 및 linolenic acid를 인 것이 분석되었다.

Keywords : Phospholipid, Fatty acid, Antibiotics, *Chlorella ellipsoidea*

I. Introduction

Phospholipids involved in the biomembrane were enhanced the capacity of the permeability an ion and the small molecule through membrane¹⁾, and utilized for the structural materials of mitochondria and chloroplast in plant cells.

Phospholipids contained in endoplasmic reticulum and mitochondria were synthesized independently. Phospholipids, which consist in the mitochondrial membrane within a castor-oil plant cell, were formed in the outer membrane and inner membrane. PE(phosphatidylethanolamine), PI(phosphatidylinositol) and PC(phosphatidylcholine) were produced in the inner membrane, whereas PG(phosphatidylglycerol) was formed in the outer membrane.²⁾ However, PC was produced in microsome and transferred into mitochondria by the transfer protein and the lipid carrier using as transfer were maintained the balance toward the participation of

phospholipid between the membranes. The contents and composition of phospholipid, sterol and fatty acid were affected by the various environmental conditions(e.g., temperature, pH and medium composition).

Polyunsaturated fatty acids in the plant tissue cell were formed 80% at 12°C, 51% at 20°C and 30% at 30°C.³⁾ Therefore, the unsaturated fatty acids were increased at the low temperature, whereas in the high temperature, the saturated fatty acids were increased. Antibiotics permeated in cells have effected on the synthesis of phospholipid, protein and nucleic acid, as well as the structure of membrane. Especially, antibiotics of the polyene family coupled on the phospholipid of membrane, formed the membraneous pore in the outer membrane and so caused the biochemical, physiological change.⁴⁾

Not only cycloheximide decreased the RNA synthesis^{5,6)}, but also the protein synthesis of cy-

toplasm ribosome in *Euglena gracillis* inhibited and the cell division pause owing to decreasing the enzyme activity was decreased when the nuclear DNA was synthesized.⁷⁾ In the wild type of *Physarum polycephalum*, not only the synthesis of DNA was decreased, but also the protein formation was inhibited because of cycloheximide blocked the phosphorylation reaction from thymidine to thymidine triphosphate.⁸⁾ In the *Saccharomyces cerevisiae*, cycloheximide inhibits the fermentation of glucose and fructose.⁹⁾

Due to nalidixic acid, which inhibits the DNA synthesis in bacteria, as the antibacterial agent, hinders the absorption of oxygen, the ATP synthesis in mitochondria of the mouse liver cell by the inhibition of the phosphorylation reaction was decreased.¹⁰⁾ Nalidixic acid (50 µg/ml) caused 100% the white peril in *Euglena gracillis* owing to the decrease of chloroplast formation by the inhibition of DNA synthesis in chloroplast.¹¹⁾ Such antibiotics more permeated the mitochondrial envelope than the nuclear envelope in *Saccharomyces cerevisiae*, inhibit the mitochondrial DNA synthesis.¹²⁾ Also, nalidixic acid inhibited the activity of DNA polymerase, ATP dependent deoxyribonuclease, and DNA gyrase and so remarkably affected to DNA synthesis owing to the formation of relaxation complex analogues. On the other hand, when *E. coli* 15ATU, precultured in the medium treated with succinate, were cultured in medium added with glucose after cultured in the nalidixic acid treated medium, the content of DNA was increased 77%.¹³⁾

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

This present paper analyzed the biosynthesis of phospholipid and the composition of fatty acid in mitochondria isolated from *C. ellipsoidea* treated with cycloheximide and nalidixic acid.

II. Materials and Methods

1. *C. ellipsoidea* culture

C. ellipsoidea was grown in M4N medium¹⁴⁾ treated with cycloheximide (10 µg/ml, Sigma) and nali-

dixic acid (20 µg/ml, Sigma), respectively. The cultures were incubated at 25°C under atmosphere involved 5% CO₂ gas and irradiated 2 Klux light continuously. Cell growth was measured by packed cell volume using haematocrit.

2. Isolation of mitochondria

Mitochondria in *Chlorella* collected in the beginning phase and the middle phase of the cultivation, isolated according to the method¹⁵⁾ (Fig. 1).

The cells were centrifuged at 600×g for 4 min. and suspended 0.005M Tris buffer (pH 7.4) solution containing 0.25M sucrose and 0.005M EDTA, smashed with the sonicator (Sonics & Ins. model VC 250B).

The smashed cells were centrifuged at 300×g for 3 min. and the supernatant obtained was centrifuged at 6,000×g for 10 min. again and then, this supernatant was centrifuged at 15,000×g for 20 min. and washed 2 times with 0.25M sucrose and these sedimented mitochondria used to this experiment.

3. Extraction of total lipid

Total lipid in *Chlorella* mitochondria extracted according to the modified method.¹⁶⁾ Separated mitochondrias added chloroform : methanol (1 : 2, v/v), and shaken for 30 min., and added, mixed the same quantity of the distilled water and the separated chloroform layer filtered by using Whatmann No. 1 filter paper and extracted the total lipid. After the methanol layer, upper layer, added and mixed with chloroform again, total lipid re-extracted by filtration using the same filter paper in the separated chloroform layer. And after the extracted total lipid dried in 45-50°C, dry weight measured.

4. Separation and identification of phospholipid

The major phospholipids, such as PC, PE, PG and PI, extracted from total lipid by the thin layer chromatography (TLC, Desaga). Glass plates (20×20 cm) used in TLC were coated with a 0.25 mm thick of silica gel (Merck), dried in room temperature and activated in 110-120°C dry oven for 60 min. before the use. Chloroform-methanol-28% ammonia water (65 : 25 : 2, v/v/v)

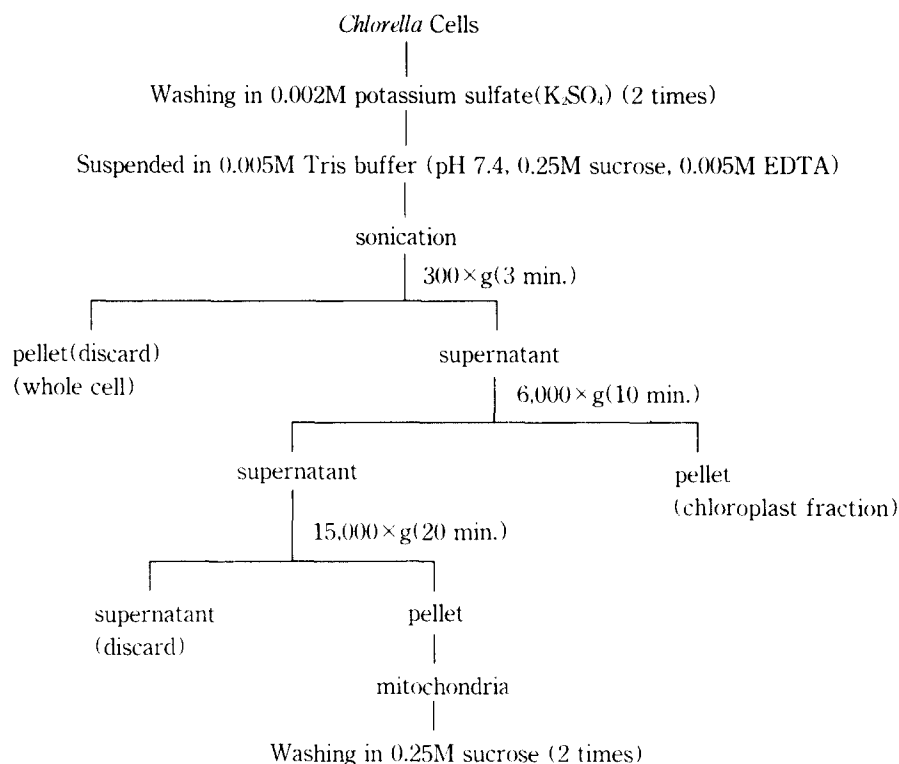


Fig. 1. Isolation of mitochondria in *Chlorella ellipsoidea*

for the first expansive solvent and chloroform-acetone-methanol-acetic acid-distilled water(3:4:1:1:0.5, v/v/v/v/v) for the second expansive solvent were used according to the two-one dimension method.¹⁷⁾ The phospholipids separated from total lipid by TLC were identified to compared with the standard chemicals(Sigma). The developer used the saturated butanol mixed with 0.2% ninhydrin for PE, the dragendroff reagent for PC, the periodata-schiff's reagent for PI and the sulfuric acid mixed with 20% ethanol for PG, respectively.¹⁸⁾

5. Methylesterification of fatty acids

The separated phospholipids were methylated by the method¹⁹⁾ in order to the analysis of the fatty acid composition and content. Phospholipids separated from each plate were added 5ml methanol contained 5% sulfuric acid and heptadecanoic acid as the internal standard, cooled after dried for 120 min. in 68-70°C dry oven and

then, the same quantity of distilled water was added and shaken. The homogenates added 2 ml hexane and separated the hexane layers after powerfully shaken. This separation procedures repeated 3 times. The separated hexane layers were added 5 ml the saturated sodium bicarbonated and separated the hexane layer after shaken. The contents of fatty acid methyl ester involved in each phospholipids measured after the separated hexane layers dried.

6. Assay of fatty acid

The composition and the contents of fatty acids composed of each phospholipid analyzed by gas chromatography(GC, Varian 3300). The identification of fatty acids, 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) was resolved by comparing the standard chemicals(Sigma). The used column, such as stainless steel column (3 mm×3

m), used 15% DEGS(diethylglycol succinate) as packing material and H_2 -flame ionization detector (FID) as GC detector. Analysis conditions were described as following:

Injection Port Temperature : 230°C

Column Temperature : 170°C

Detector Oven Temperature : 240°C

Carrier Gas : N_2 (30 ml/min.)

III. Results

1. Growth

From Fig. 2, as compared with the control, growth in the cycloheximide treatment was decreased 22.00%, and also in the nalidixic acid treatment, the inhibition rate was showed 11.11%.

2. Total lipid

The content of total lipid was decreased 25.00% at 3rd day of the culture, whereas increased 90.00%, 165.00% at 5th and 7th day of the culture, respectively. Otherwise, the contents of total lipid in mitochondria in the cycloheximide treatment were decreased 40.00%, 60.53% and 39.62% at 3rd, 5th and 7th day of the culture to compare with the control. Inhibition rates in nalidixic acid treatment were 13.33%, 15.79% and 43.40% at 3rd, 5th and 7th day of the culture, respectively (Fig. 3).

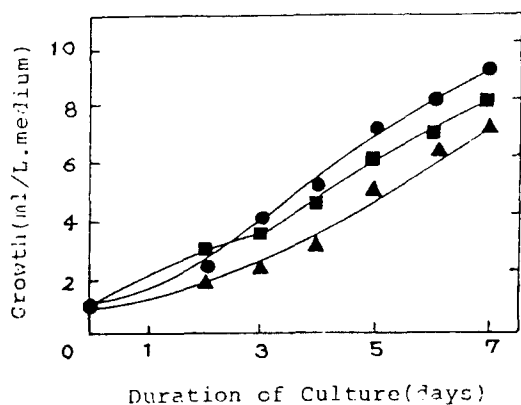


Fig. 2. Growth of *C. ellipsoidea* treated with antibiotics during the cultivation.

● : Control, ▲ : Cycloheximide, ■ : Nalidixic acid

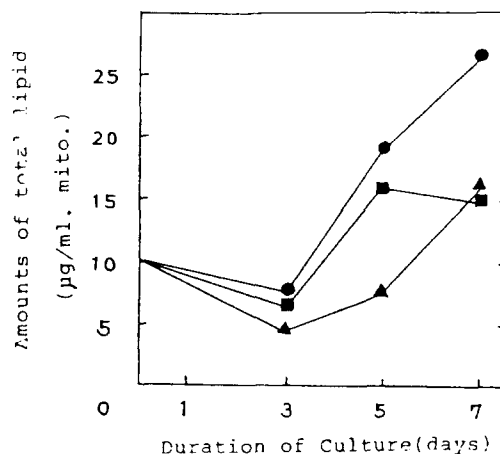


Fig. 3. Changes in contents of total lipids in *Chlorella* mitochondria treated with antibiotics during the cultivation.

● : Control, ▲ : Cycloheximide, ■ : Nalidixic acid

3. Phospholipid

As shown in Fig. 4, the PC content in the control was increased 14.97% at 3rd day, markedly 165.24% at 5th day and 255.61% at 7th day of the culture. The PC content in the cycloheximide treatment was inhibited 42.33%, 61.09% and 30.88% at 3rd, 5th and 7th day of the culture to compare with the control, respectively. The PC content was increased 66.51% at 3rd day in nalidixic acid treatment, whereas decreased 24.19% at 5th day and 53.68% at 7th of the culture. So, the predominant inhibitory effect for the PC synthesis was more showed in the cycloheximide treatment than the nalidixic acid treatment.

The changes in the PE content in the control were decreased 25.51% at 3rd day to compare with the beginning of the culture, whereas increased 80.00%, 170.83% at 5th, 7th day of the culture. Compared to the control, the PE content in the cycloheximide treatment was inhibited 44.02%, 53.47% and 56.92% at 3rd, 5th and 7th day of the culture, respectively. The PE content in the nalidixic acid treatment was rapidly increased 96.74% at 3rd day, 3.70% at 5th day, whereas markedly decreased 47.08% at 7th day of the culture. The change in content of PG in the control

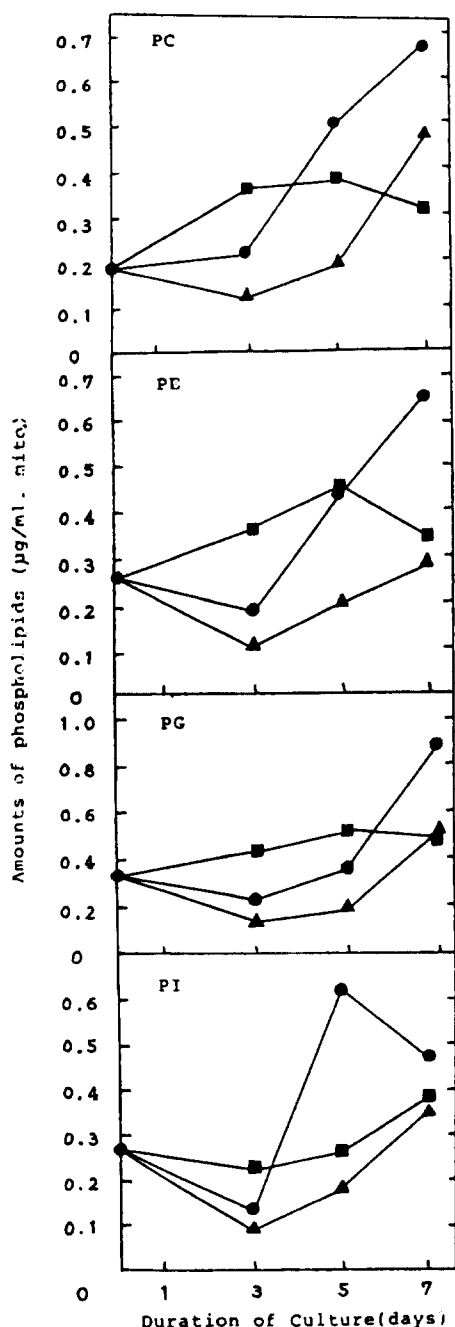


Fig. 4. Changes in contents of phospholipids in *Chlorella* mitochondria treated with antibiotics during the cultivation.

● : Control, ▲ : Cycloheximide, ■ : Nalidixic acid

Abbre. PC : phosphatidylcholine

PE : phosphatidylethanolamine

PG : phosphatidylglycerol

PI : phosphatidylinositol

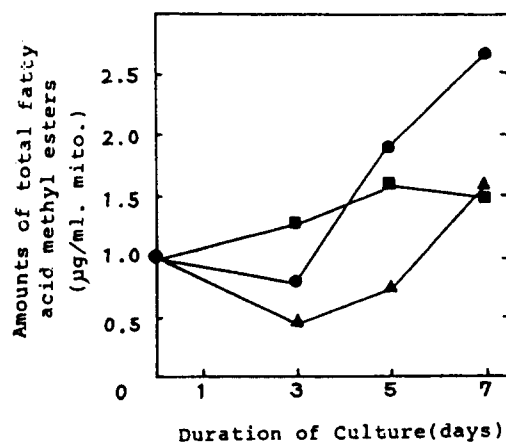


Fig. 5. Changes in contents of total fatty acid methyl ester in *Chlorella* mitochondria treated with antibiotics during the cultivation.

● : Control, ▲ : Cycloheximide, ■ : Nalidixic acid

was decreased 30.62% at 3rd day, whereas increased 7.81% at 5th day, 17.94% at 7th day of the culture. Compared to the control, the PG content in the cycloheximide treatment was decreased 40.99%, 48.41% and 41.75% at 3rd, 5th and 7th day of the culture, respectively. Also, the PE content in cells treated with the nalidixic acid was increased 95.05% and 48.70% at 3rd and 5th day, whereas decreased 44.75% at 7th day of the culture, respectively.

The changes in content of PI in the control was decreased 51.14% at 3rd day, whereas increased 136.36%, 77.65% at 5th, 7th day of the culture, respectively. The PI content in the cycloheximide treatment was decreased 28.68%, 71.63% and 25.37% for total the culture periods to compare with the control. Also, the PI content in the nalidixic acid treatment was increased 68.22% at 3rd day, whereas decreased 57.82%, 21.32% at 5th, 7th day of the culture.

4. Total fatty acid methyl ester

The content of total fatty acid methyl ester in the control was decreased 21.00% at 3rd day of the culture compared to the beginning of the culture, whereas increased 89.70% at 5th day and 165.10% at 7th day of the culture. The inhibitory effect in the cycloheximide treatment was 43.04%, 60.57%

and 39.65% at 3rd, 5th and 7th day of the culture, whereas in the nalidixic acid treatment, increased 73.42% at 3rd day of the culture to comparison with the control, but decreased 15.60%, 43.42% at 5th, 7th day of the culture.

5. Fatty acid

The major fatty acids utilized the synthesis of phospholipids in *Chlorella* mitochondria were analyzed 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).

Table 1 was represented the composition changes of fatty acid composed of PC.

In case of the control, the use rate of linoleic acid and stearic acid was 57.48% and 22.24% in the beginning of the cultivation, and linoleic acid, lauric acid were 23.90%, 9.37% at 3rd day, and linoleic acid, stearic acid were 24.20%, 7.66% at 5th day of the culture. Linoleic acid and stearic acid were utilized 30.40%, 10.42% at 7th day of the culture for the PC formation. But, PC in the cycloheximide treatment was made use of linoleic acid(19.11%) and stearic acid(17.15%) at 3rd day of the culture. At 5th day of the cultivation, myristic acid and palmitic acid were utilized 21.58% and 15.80%, respectively. At 7th day of the culture, myristic acid and palmitic acid were utilized 20.90% and 14.45%, respectively. In the nalidixic acid treatment, myristic acid and pal-

mitic acid were utilized 24.51% and 18.58% at 3rd day, 20.42% and 15.47% at 5th day, 18.58% and 12.83% at 7th day of the culture.

The quantitative changes in fatty acids used the PC formation were shown in Table 2, respectively. In case of the control, stearic acid and linolenic acid were utilized 25.49%, 19.93% in the beginning of the culture, myristic acid(10.94%) and palmitic acid(6.94%) at 3rd day, linoleic acid(35.78%) and palmitic acid(15.25%) at 5th day, linoleic acid(38.70%) and palmitic acid(10.42%) at 7th day of the culture, respectively.

Linoleic acid and palmitic acid were utilized 6.73%, 4.84% at 3rd day in the cycloheximide treatment, and palmitic acid(13.99%), lauric acid (11.38%) at 5th day and myristic acid(8.52%), stearic acid(6.44%) at 7th day of the culture for the PE formation.

In the nalidixic acid treatment, myristic acid and palmitic acid were utilized 20.69% and 15.46% at 3rd day, 15.29% and 10.41% at 5th day, 20.45% and 14.54% at 7th day of the culture, respectively. A kind of fatty acids used the PE formation in the antibiotics treatment were variously showed.

Table 3 was represented the changes in the composition of fatty acids composed of PG. Linoleic acid and palmitic acid were used 20.10%, 10.21% in the beginning of the culture in the control. Also, stearic acid was utilized 16.20%, 17.24%

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

Duration of culture (days)	0				3			5			7		
Treatment	Cont.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.
Fatty acid	Cont.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.
Lauric acid(12:0)	-	9.37	5.43	15.50	0.60	11.28	8.46	10.20	12.70	5.41			
Myristic acid(14:0)	6.11	0.12	13.74	24.51	2.42	21.58	20.42	4.56	20.90	18.52			
Palmitic acid(16:0)	5.47	0.94	0.46	18.58	4.61	15.80	15.47	9.78	14.45	12.83			
Stearic acid(18:0)	22.24	0.48	17.15	0.97	7.66	10.08	0.57	10.42	5.61	0.47			
Oleic acid(18:1)	-	-	-	-	5.60	-	4.26	-	-	3.21			
Linoleic acid(18:2)	57.48	23.90	19.11	0.72	24.20	8.59	0.54	30.40	5.95	0.43			
Linolenic acid(18:3)	-	0.42	-	0.28	-	0.11	0.25	3.41	-	0.01			
Unknown	8.70	64.77	44.11	39.44	54.91	32.56	50.03	31.23	40.39	59.12			
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00			

<NOTE> Unit : %, Cont. : Control, Cyclo. : Cycloheximide, Nal. : Nalidixic acid

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

Duration of culture (days)	0	3			5			7		
Treatment	Cont.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.
Fatty acid	Cont.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.
Lauric acid(12:0)	0.43	5.84	0.64	12.58	5.46	11.38	10.41	6.21	3.12	13.48
Myristic acid(14:0)	4.23	10.94	3.28	20.69	3.23	6.61	15.29	4.12	8.52	20.45
Palmitic acid(16:0)	7.70	6.94	4.84	15.46	15.26	13.99	10.41	10.42	5.36	14.54
Stearic acid(18:0)	25.49	3.23	2.64	2.67	4.31	10.94	1.27	5.21	6.44	0.81
Oleic acid(18:1)	-	-	1.67	11.53	-	-	6.21	3.24	-	6.97
Linoleic acid(18:2)	17.40	4.07	6.37	1.58	0.46	8.49	0.54	1.46	3.85	-
Linolenic acid(18:3)	19.93	4.88	-	0.03	35.78	7.86	0.11	38.70	-	2.73
Unknown	24.82	64.10	80.56	35.46	35.50	40.73	55.76	30.64	72.71	41.02
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %. Cont. : Control. Cyclo. : Cycloheximide. Nal. : Nalidixic acid

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

Duration of culture (days)	0	3			5			7		
Treatment	Cont.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.
Fatty acid	Cont.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.
Lauric acid(12:0)	1.02	0.31	4.56	9.03	1.03	4.26	2.41	2.21	5.03	6.07
Myristic acid(14:0)	0.14	0.34	10.24	12.15	3.59	15.13	11.54	5.43	13.40	8.99
Palmitic acid(16:0)	10.21	-	12.74	1.48	7.01	17.66	1.02	6.46	14.64	20.82
Stearic acid(18:0)	5.40	1.46	-	3.12	16.07	16.23	2.46	17.24	1.78	11.40
Oleic acid(18:1)	1.20	-	4.63	18.70	2.12	-	16.40	6.48	-	10.74
Linoleic acid(18:2)	20.10	96.20	4.56	-	32.20	17.41	-	28.40	8.48	0.40
Linolenic acid(18:3)	3.44	-	-	-	4.57	12.74	2.41	5.70	-	-
Unknown	58.49	1.69	63.29	55.52	33.41	16.57	63.76	28.08	56.67	41.58
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %. Cont. : Control. Cyclo. : Cycloheximide. Nal. : Nalidixic acid

at 5th, 7th day of the culture. The major fatty acids utilized for the PC biosynthesis in the cycloheximide treatment were palmitic acid(12.74%), myristic acid(10.24%) at 3rd day of the culture and palmitic acid(17.66%), linoleic acid(17.41%) at 5th day, and palmitic acid(14.64%), myristic acid(13.40%) at 7th day of the culture. In the nalidixic acid treatment, the use rate of oleic acid and myristic acid were 18.70% and 16.40%, 12.15% and 11.54% at 3rd and 5th day of the culture, respectively. At 7th day of the culture, palmitic acid and stearic acid were used 20.82% and 11.40%, respectively.

Fatty acids consisted in PI in the antibiotics

treatment were shown in Table 4. In case of the control, palmitic acid and stearic acid were closely utilized 25.22%, 25.79% at the beginning of the culture. For the total culture period, stearic acid was utilized 14.60%, 22.10%, 14.10% and linoleic acid was used 30.50%, 56.43%, 56.20% for the phospholipid biosynthesis.

Lauric acid and myristic acid were utilized 25.83%, 23.38% at 3rd day of the culture, stearic acid(14.10%) and myristic acid(12.50%) at 5th day, palmitic acid(7.16%) and stearic acid(5.29%) at 7th day in the cycloheximide treatment, respectively. In the nalidixic acid treatment, palmitic acid and myristic acid at 3rd day of the cul-

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

Duration of culture (days)	0				3			5			7		
Treatment	Cont.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.
Fatty acid	Cont.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.	Cont.	Cyclo.	Nal.
Lauric acid(12:0)	5.43	2.15	25.83	15.45	2.17	5.02	10.23	4.78	2.71	6.39			
Myristic acid(14:0)	16.05	2.46	23.38	16.47	3.30	12.05	12.31	8.56	5.31	11.87			
Palmitic acid(16:0)	25.22	10.50	14.71	16.86	14.60	-	4.56	8.20	7.16	5.36			
Stearic acid(18:0)	25.79	14.60	-	5.12	22.10	14.10	5.02	14.20	5.29	5.74			
Oleic acid(18:1)	-	-	-	11.58	-	2.65	-	-	-	15.04			
Linoleic acid(18:2)	-	30.50	3.70	0.02	56.43	10.50	0.01	56.20	4.92	0.71			
Linolenic acid(18:3)	4.57	2.67	-	0.09	-	8.01	0.14	2.78	0.04	14.23			
Unknown	22.94	37.12	32.38	34.41	1.40	47.67	67.73	5.28	74.57	40.67			
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00			

<NOTE> Unit : %, Cont. : Control, Cyclo. : Cycloheximide, Nal. : Nalidixic acid

ture were used 16.86%, 16.47%. At 5th day of the culture, myristic acid and lauric acid were used 12.31%, 10.23%. At 7th day of the culture, oleic acid and myristic acid were used 15.04%, 11.87%. Thus, the composition and the content of fatty acids consisted in each phospholipid were variously showed depending upon each treatment and the culture periods.

IV. Discussion

In course of the DNA replication, cycloheximide is known to inhibit the DNA formation and blocks the synthesis of DNA polymerase which have the function of the repair and the chain elongation.^{30,31} Mitochondrial DNA in *Saccharomyces cerevisiae* possessed single copy toward the mitochondrial rRNA and the twenties tRNA gene and have been the protein biosynthesis system unlike the cytoplasm by the ribosome size and the difference of sensitivity to the antibiotics. Especially, cycloheximide was inhibited the formation of the cytochrome c oxidase complex which composed of the three subunit proceeded by the mitochondria protein.³² Also, this antibiotics inhibits the bond of phenylalanine, lysine and leucine to the endogenous mRNA at the stage of coding occurred proteinbiosynthesis from RNA.³³ And then the biosynthesis of protein didn't occurred as inhibiting the transfer of amino acid from the amino acyl-RNA

to peptide.

Therefore, the cell division was blocked and the cell growth was stopped owing to inhibition of the biosynthesis of the organic compound in cell.³⁴

In *Bacillus subtilis*, the formation of the DNA double helix was inhibited as nalidixic acid(20 µg/ml) acted to the DNA packing and replication course.³⁵ The 38S single-helix DNA in *E. coli* KL 16 was inhibited to convert the high molecular DNA by nalidixic acid. Also, the DNA formation was decreased because of the inhibition of the DNA duplication and the RNA transcription as hindering the DNA supercoiling and cleavage by this antibiotics acting to DNA gyrase.³⁶ Nalidixic acid was inhibited the recombination of the nucleotide base arrangement such as Chi(5'-GCTGGTGG-3') as acting recBCD enzyme. *E. coli* formed DNA from the chromosome attached the membrane and the composition of protein consisted of the membrane was altered because the change of DNA took place by nalidixic acid.

Also in this experiments, the growth of *Chlorella* treated with antibiotics during the culture was inhibited. This reason is considered because of the blocking the cell division due to inhibit DNA, RNA and protein formation by cycloheximide and nalidixic acid. And so the cell division didn't occurred because nalidixic acid was affected on the cell metabolism and the growth was inhibited.

Compared to the control, the contents of total lipids in *Chlorella* mitochondria treated with antibiotics were decreased. This results were accord with the result that the contents of total lipid in *E. coli* were decreased by antibiotics.²⁷⁾

Lipid involved in cell was synthesized from the precursor of keto acid produced by the deamination of protein and acetyl CoA formed by the carbohydrate degradation. However, antibiotics inhibited the chloroplast duplication in the plant¹¹⁾, and failed the supply of acetyl CoA depending upon the inhibition of the carbohydrate synthesis, because photosynthesis didn't took place normally. Also, the contents of total lipid were decreased because the activity of enzyme participated in photosynthesis and the cell metabolism was not occurred by antibiotics normally. Additionally the contents of total lipid were decreased owing to inhibition of the synthesis of acetyl CoA carboxylase, which may be the major enzyme for the biosynthesis of fatty acid.²⁸⁾ Major phospholipids involved in the plant and the animal cells are PC, PE, PG and PI. In *E. coli*, PE and PG were composed of 70-80%, 5-15% among the phospholipid, and PS, cardiolipin, beside.²⁹⁾ In *Nicotiana tabacum*, the compositions of phospholipids variously surveyed according to the development stage and organ of cell, such as ovary, petal, and pistil, etc.³⁰⁾

It was showed that the contents of phospholipids in *Chlorella* mitochondria increased, whereas the contents of PC, PE, PG and PI in the cycloheximide treatment decreased. In the yeast mitochondria, PE was produced by the decarboxylation from PS formed after CDP-1,2-diacylglycerol and serine reacted and PC was produced by the methylation of PE by the action of the PE transferase^{31,32)}, or CDP-choline was produced by the action of 1,2-diacylglycerol.³³⁾ As this results, because cycloheximide inhibited the biosynthesis of phosphorylcholine-glyceride transferase using the PC synthesis, the contents of PC and PE was decreased.²⁸⁾ And also, it was analyzed that the synthesis of PC and PE were inhibited by the blocking the methylation and the decarboxylation reaction.

PG was formed from CDP-diglyceride and gly-

cerol-P in the castor-oil plant, that is PG was synthesized by the dephosphorylation after phosphatidylglycerol phosphate was formed by the catalyst of sn-glycerol-3-P; CMP-phosphatidyltransferase.³⁴⁾ But, the synthesis of PG was decreased as the decline of this enzyme activity by antibiotics. PI was formed via the various reaction steps from glycerol-P in mitochondria. Then, PI was decreased owing to declining the activity of COD-diglyceride-inositol transferase by cycloheximide.²⁾ In the nalidixic acid treatment, the inhibitory effects of the synthesis of PE and PG were not showed, whereas the PC and the PI synthesis were inhibited. The reduction of the PC synthesis was showed owing to blocking methyl supply by this antibiotics when PE was converted to PC. Inhibitory effect of antibiotics was not occurred because PI supplied the other phospholipid, which not produced by antibiotics.³⁵⁾ But, in this experiment, the PI synthesis was decreased. These results were not offered phosphate needed for the synthesis of phospholipid because of the inhibition of the ATP synthesis as blocking the absorption of oxygen.¹⁰⁾

In this study, the use rate of the saturated fatty acid was more high surveyed than those of the unsaturated fatty acid. The major fatty acids composed of phospholipid in *Chlorella* mitochondria were variously showed stearic acid, linoleic acid, palmitic acid, and lauric acid in the control were mainly utilized and myristic acid, palmitic acid, stearic acid in the cycloheximide treatment, and myristic acid, palmitic acid, oleic acid in the nalidixic acid treatment were used during the biosynthesis of phospholipid.

Summary

The biosynthesis of phospholipid and the composition of fatty acid in *C. ellipsoidea* mitochondria treated with antibiotics(cycloheximide, nalidixic acid) during the culture analyzed. The growth of *Chlorella* and the contents of total lipid in mitochondria treated with antibiotics were lower than those of the control. The synthesis of PC (phosphatidylcholine) and PI(phosphatidylinositol) were inhibited in the nalidixic acid treatment and

also the contents of PC(phosphatidylcholine), PE (phosphatidylethanolamine), PG(phosphatidylglycerol) and PI(phosphatidylinositol) in the cycloheximide treatment were also inhibited. The major fatty acids utilized for the various phospholipids formation in each antibiotics treatment were analyzed stearic acid, myristic acid, palmitic acid, oleic acid, linoleic acid and linolenic acid at the late phase of the culture.

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