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Effect of Growth Temperature on Lipid and Fatty Acid Compositions in Photosynthetic Bacterium, *Rhodopseudomonas sphaeroides S*

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光合成細菌 *Rhodopseudomonas sphaeroides S* の脂質および脂肪酸組成に与える生育温度の影響

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Introdution

It is well known that many organisms adjust the lipid and fatty acid composition in response to growth temperature.[1.2] When the temperature of growth is lowered, the proportion of unsaturated fatty acids increase. The data suggest that the temperature acclimatization involves the reshuffling of physical characteristics in lipids by changing fatty acid components and lipids.[3] According to recent study,[4] *Anacystis nidulans* changes lipid and fatty acid composition, the fatty acid varying in chain length. In *Anacystis variabli*, however, the lipid composition remaines constant and only fatty acid components vary in degree of unsaturation with the growth temperature.

In the present study, we investigated the lipid and fatty acid compositions in photosynthetic bacterium, *Rhodopseudomonas sphaeroides S* grown at 15°, 30° and 40°C, and variations in lipid and fatty acid after temperature shift. Effect of the growth temperature on the cell structure was also observed.

Materials and Methods

Rps. sphaeroides S was obtained from Dr. Kitamura, Tokyo Metropolitan University, and was grown at 30°C in Lascelles' Medium MS[5] under semi-anerobic conditions, illuminated with incandescent light at about 3,000 lux (standard culture condition). Cells in the middle-logarithmic phase were used for experiments. Growth of cells was determined by measuring the optical density at 660 nm of the culture.

Cells were harvested by centrifugation at 15,000×g for 10 min, and washed with distilled water by centrifugation. The total lipids in the cells were extracted with acetone-methanol (3 : 2, v/v) and then n-butanol by sonication and centrifugation. The extracted lipids were fractionated on silica gel column by successive elution with chloroform (for pigments and non-polar lipids), acetone (for glycolipids), and chloroform-methanol-water (5 : 5 : 1, v/v) (for phospholipids).

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Lipid components were identified on TLC by several spray reagents and comparison with R_f values obtained with lipid standards.[6] IR analysis and GLC of the hydrolysis products from glycolipids were also carried out for the identification. Lipid contents in the cells and lipid compositions were determined by TLC-FID (TLC with flame-ionization detector).[7] The solvent systems used were chloroform/ethyl acetate/acetone/methanol/acetic acid/water (60 : 12 : 16 : 2 : 3, v/v) for glycolipids and benzene/diethyl ether/ethyl acetate/methanol/formic acid/water (20 : 20 : 8 : 10 : 2 : 1, v/v) for phospholipids. Fatty acid analysis was carried out by GLC on a Hitachi model 063 with H_2 flame-ionization detector, using 2 m×3 mm stainless column packed with 10% ethylene glycol succinate polyester on Celite 545. Protein was determined by Sensitive Biuret Method reported by Koh and Putnam.[8]

For electron microscopy, the bacterial cells were centrifuged, and the pellets were fixed with 3% glutaraldehyde in 50 mM phosphate buffer (pH 7.5) containing 0.5 M sucrose and 10 mM KCl, and postfixed with 1% OsO_4. The samples were then dehydrated and embeded in Epon 812. Thin sections were examined with a Hitach HU-12A.

Results

Temperature-dependence of growth in Rps. sphaeroides S

Fig. 1 shows the growth curves of the bacterium at different temperatures. The normal growth was observed at 30°C and also at 40°C although the cell mass at the stationary

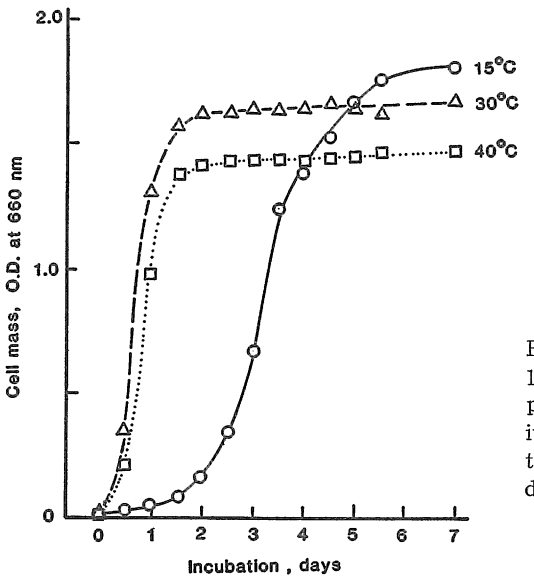

Fig. 1. Growth curves of *Rps. sphaeroides S at* 15°, 30° and 40°c. The cells inthe middle-logarithmic phase, which were grown under the standard conditions, were inoculated and incubaed at the temperature ndicated under the same conditionsas the standard except the temparature.

phase was slightly lower. The growth at 15°C deviated from those at 30° and 40°C, showing a slow growth with the stationary phase at around 7 days but with a higher cell mass than others.

In all experiments below the cells at the late-logarithmic phase were harvested and used for lipid analysis.

Identification of lipids

The total lipid extracts from the cells grown at 40°C were fractionated into 3 fractions of non-polar lipids (containing pigments) glycolipids, and phospholipids on silica gel column. TLC analysis of the phospholipid fraction showed that phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidylcholine (PC) were present as the major components with diphosphatidylglycerol (DPG) as a minor component.

The glycolipid fraction was separated into 3 components on TLC. One of them was identified as sulfoquinovosyl diacyl glycerol (SQDG) by cochromatography with the standard and α-naphthol-H_2SO_4 color reagent and by IR analysis. The same analysis and GLC of the hydrolysis products indicated that the other two components were monoglucosyldiacylglcerol (MGDG) and diglucosyldiacylglycerol (DGDG).

TABLE I LIPID CONTENTS AND COMPOSITIONS OF *RPS. SHPHAEROIDES S* GROWN AT 15°, 30° AND 40°C

	15°C cells		30°C cells		40°C cells	
	Amounts*	%	Amounts*	%	Amounts*	%
Total lipid extracts	21.4	100	19.9	100	16.4	100
Pigments and non-polar lipids	3.64	17.0	3.56	17.9	3.41	20.8
Polar lipids	17.78	83.0	16.30	82.1	12.98	79.2
glcolipids	0.85	4.0	1.02	5.1	0.87	5.3
MGDG	—	—	0.08	0.4	0.10	0.6
DGDG	—	—	0.20	1.0	0.25	1.5
SQDG	0.85	4.0	0.74	3.7	0.52	3.2
phospholipids	16.93	79.0	15.32	77.0	12.11	73.9
DPG	—	—	1.41	7.1	0.85	5.2
PE	3.92	18.3	6.27	31.5	4.52	27.6
PG	5.51	25.7	2.47	12.4	4.17	25.4
PC	7.50	35.0	5.17	26.0	2.57	15.7

* mg per 100 mg protein

Comparison of lipid and fatty acid compositions among the cells grown at different temperatures

Table I shows lipid contents and compositions in the cells grown at 15°, 30° and 40 °C, as in Fig. 1. The lipid content decreased with an increase of the growth temperature. Remarkable changes with the growth temperature were found in glycolipid components. There was one component of glycolipid, SQDG, in the 15°C cells, while two additional components of MGDG and DGDG occurred in the 30°C and 40°C cells replacing a part of SQDG. Phospholipids account for about 80% of the total lipid extacts. In the cells grown at higher temperature, the phospholipid content decreased. The most rapid decrease was found in PC, which was in the highest content in the 15°C cells. PE rather increased in the 30°C and 40°C cells. DPG was found in the cells grown at 30° and 40°C,

TABLE II FATTY ACID COMPOSITION OF TOTAL LIPIDS FROM *RPS. SPHAEROIDES S* GROWN AT 15°, 30° AND 40°C

Fatty acids	15°C cells	30°C cells	40°C cells
		wt, %	
16 : 0	2	8	16
16 : 1	1	2	2
18 : 0	3	11	13
18 : 1	94	79	69
saturated	5	19	29
unsaturated	95	81	71
C_{16} acids	3	10	18
C_{18} acids	97	90	82

but it was lacking in the 15°C cells.

Table II shows fatty acid composition in the total lipid extracts from the cells grown at 15°, 30° and 40°C. When the growth temperature shifted up, the amount of 18 : 1 acid decreased while saturated acids of 18 : 0 and 16 : 0 increased. These changes resulted in a decrease of average numbers of double bonds and average carbon numbers of chain length in the fatty acid molecules with an increase of the growth temperature.

Changes in the lipid and fatty acid compositions after temperature shift

Fig. 2 shows changes in the amount of lipid components after temperature shift from 40° to 15°C. On lowering the temperature, a steady increase of total phospholipids was observed. The component most responsible for the increase was PC. The amounts of PG and PE showed rather unstable changes. A minor component of DPG completely disappeared in the early stage after the temperature drop. In the glycolipids, SQDG increased in parallel with decreases of MGDG and DGDG, the total amount of glycolipids remained almost constant. The results show a rapid and smooth changing to the characteristic lipid composition in the cells grown at 15°C (Table I).

Fig. 3 shows changes in lipids after temperature shift from 15° to 40°C. This shows a roughly reversed pattern of changes with that in Fig. 2. However, some deviations were observed in the phospholipid changing. When comparing with the cells grown at 40°C (Table I), there still was

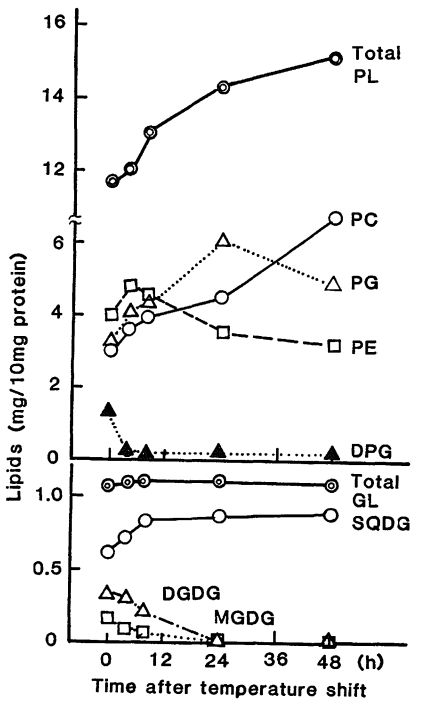

Fig.2. Changes in the amounts of lipid components after the temparature shift from 40° to 15°C. The cells in the late-logarithmic phase grown at 40°C were transferred into a 15°C bath, and incubated, The cells were harvested at the time indicated, and subjected to lipid analysis.

quite a difference in lipid composition even in the cell at 48 h after temperature shift.

Tables III and IV show the changes in total fatty acid composition after temperature shifts from 40° to 15°C and from 15° to 40°C, respectively. On lowering temperature, the proportion of 18 : 1 acid increased while those of 16 : 0 and 18 : 0 acids decreased. These indicated that a rapid alteration of fatty acid components occurred to adapt the cells to a low temperature. On raising temperature (Table IV), however, the decrease of 18 : 1 acid and increase of 16 : 0 acid proceeded but at a very slow rate, and most of the characteristic of the 15°C-grown cells in fatty acid compositions still remained even in the cells at 48 h after the temperature shift.

Effect of growth temperature on the cell structure

Fig. 4 shows thin sections of the cells grown at 15° and 40°C. It can be observed that the 40°C cells were larger in size than the 15°C cells. The sectioned profile of the 40°C cells varied from oblong to spindle, while that of the 15°C cells was from elliptical to oblong. A thin and smooth envelope was observed in the 40°C cells, but a thick and rough one was seen in the 15°C cells.

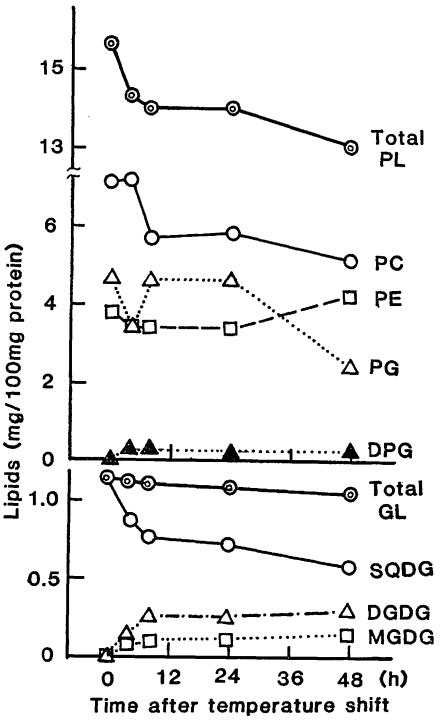

Fig. 3. Changes in the amounts of lipid components after the temparature shift from 15° to 40°C. The cells in the late-logarithmic phase grown at 15°C were transferred into a 40°C bath, and changes in lipid components were followed.

TABLE III CHANGES IN FATTY ACID COMPOSITION OF TOTAL LIPIDS AFTER TEMPERATURE SHIFT FROM 40° TO 15°C

Fatty acids	Time, h			
	0	12	24	48
			wt,%	
16 : 0	15	8	4	2
16 : 1	2	2	2	1
18 : 0	12	10	7	5
18 : 1	71	80	87	92

TABLE IV CHANGES IN FATTY ACID COMPOSITION OF TOTAL LIPIDS AFTER TEMPERATURE SHIFT FROM 15° TO 40°C

Fatty acids	Time, h			
	0	12	24	48
			wt,%	
16 : 0	1	3	3	5
16 : 1	1	1	1	1
18 : 0	3	3	5	4
18 : 1	94	93	91	90

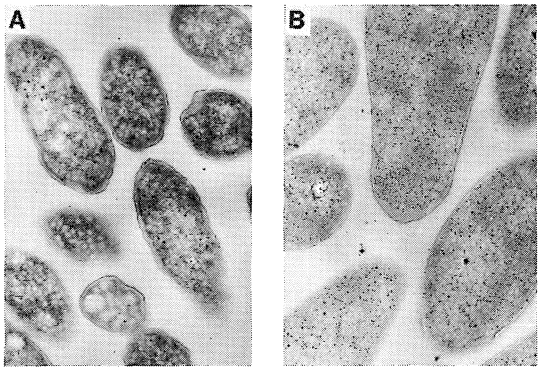

Fig. 4. Thin sections of *Rps. sphaeroides S* cells grown at 15°C (A) and 40°C (B). Magnification, × 40,000.

Discussion

In the present study we observed that photosynthetic bacterium, *Rps. sphaeroides S*, promotes reshuffling of lipid and fatty acid components for the temperature acclimatization. The characeristics in the cells grown at a lower temperature such as 15°C are (1) a higher content of phospholipids, (2) the largest amount of PC in lipid components, (3) the absence of DPG, MGDG, and DGDG, and (4) an increase of unsaturation degree and decrease of chain length. A reversed feature is found in the cells grown at higher temperatures such as 30° and 40°C. It is of interest that MGDG, DGDG, and DPG are produced only in the cells grown at higher temperature, suggesting that those lipid classes are required for keeping proper fluidity of the membranes at higher temperature. These temperature-dependent variation in lipid class is a new observation in the photosynthetic organisms. The results indicate that a wide range of variations in lipids with growth temperature is possible in *Rps. sphaeroides S* by altering lipid classes of phospho- and glycolipids and by changing fatty acids which vary in both unsaturation and chain length. The variations in lipids are similar to those in E. Coli, and are partly different from those in *A. nidulans* and *A. variabilis*.

The analysis of lipids in the cells after temperature shift proved that a rapid and smooth conversion of lipids takes place on the temperature drop (Fig. 2 and Table III), but on the temperature up the lipid conversion is rather slow and incomplete especially in the component fatty acids (Fig. 3 and Table IV). The reactions in variation of lipids with temperature involve both alteration and *de novo* synthesis of lipid and fatty acid molecules. When the temperature is lowered, the variation in fatty acid occurs in both desaturation and elongation because the amounts of 16 : 0 and 18 : 0 acids decrease with an increase of 18 : 1 acid. When the temperature is raised, however, the variation in fatty acid must be carried out mostly through de novo synthesis and degradation because 16 : 0 acid increases and 18 : 1 acid decreases. This would be one of the reasons for the slow conversion of lipids on the temperature up.

The differnce in the growth temperature induced a change in the cell structure (Fig. 4). This may be mostly attributed to the temperature-dependent variation of lipids. The effect of variation in lipids and cell structure on the cell functions is under study in our

laboratory.

Summary

The lipid and fatty acid compositions were affected by growth temperature in *Rhodopseudomonas sphaeroides S.* The cells grown at 15°C had lipids in higher content than those grown at 30° and 40°C. In the former cells the highest lipid class was phosphatidylcholine. In the latter cells, mono- and diglucosyldiacylglycerol and diphosphatidylglycerol were newly produced as minor components in addition to common major lipids. The growth temperature-dependent variations of fatty acids occurred in both unsaturation and chain length. On shifting down temperature a rapid conversion of lipid and fatty acid compositions took place, but on shifting up temperature the conversion was at a slower rate and incomplete. Electron microscopic observation indicated that a variation in the cell structure could be induced with the growth temperature.

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摘　　要

光合成細菌 *Rhodopseudomonas sphaeroides S* の脂質成分に与える培養温度の影響を調べた。15°, 30°, および40℃で培養した菌体を分析した結果，菌体脂質含量は培養温度の低下とともに増加した。15℃菌体では，リン脂質（主成分，レシチン），スルホ脂質が存在するが，30° および40℃菌体では，リン脂質（主成分，ホスファチジルエタノールアミン），スルホ脂質以外に少量のモノー，ジグルコシルジグリセリドおよびジホスファチジルグリセロールが新しく生成した。また，培養温度に依存して構成脂肪酸の不飽和度と鎖長とが変化した。培養温度を40℃から15℃へ下げると，脂質成分は急速に変化し，シフト後48時間で15℃菌体と同じ脂質組成パターンに変った。しかし，培養温度を15℃から40℃にシフトさせた場合は，菌体脂質の移行が比較的おそく，48時間では40℃菌体の脂質組成パターンを完全に獲得するに至らなかった。