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Abstract

By inoculating E. coli B into the semisynthetic medium we conducted shaking culture, and observed alterations of the total phospholipid contents and the amounts of individual phospholipid components in various stages of growth. The results are briefly summarized as follows. 1. The total phospholipid content has been found to be greater during early culture period, while it decreases as the growth age advances. 2. Phosphatidyl ethanolamine gradually increase as the culture period approaches the stationary phase. 3. Phosphatidic acid and phosphatidyl glycerol decrease precipitously as growth age advances. 4. Cardiolipin shows the maximum content in the middle log phase when the growth rate is most speedy.

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ALTERATION OF PHOSPHOLIPID AT VARIOUS GROWTH PHASES OF *ESCHERICHIA COLI*

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The lipid is an object of attention because the membrane delimiting the cell, and presumably conferring to it its characteristic of impermeability, is supposed to be constituted largely of lipid. As such, recently much interest is being focussed on the analysis and dynamic aspects of bacterial phospholipids, principal constituents of the cell membrane. In previous investigations of the composition of the phospholipids from *E. coli B*, the authors demonstrated the presence of cardiolipin, in addition to phosphatidyl ethanolamine and phosphatidyl glycerol in the phospholipid fraction (1).

Phospholipids, especially cardiolipin and phosphatidyl glycerol localized exclusively in the cytoplasmic membrane, might reflect direct function of the membrane in the division process. We, therefore, think it useful to see whether any change in phospholipids occurs along with various culture conditions (2, 3, 4). In the present study alteration of phospholipid composition was investigated at various stages of growth. As a result it has been found that growth phase clearly gives considerable effects on the phospholipid composition and must be strictly controlled in investigation of phospholipids.

MATERIAL AND METHODS

Microorganism:

E. coli B was grown in the semisynthetic medium at 37°C with gentle shaking (1). In order to check the composition of phospholipid by the content of ^{32}P in the individual phospholipids, 20 μc-30 μc [^{32}P] labeled H_3PO_4 per ml was added from the start of incubation. Turbidity was measured periodically and sampled aliquot volume from medium at various stages. The cells were harvested by centrifugation at 4°C.

Extraction and fractionation of phospholipids:

The precipitates were placed in 40 ml of chloroform-methanol (2 : 1, v/v) and allowed to stand at room temperature for several hours. The extract was separa-

ted by filtration through paper filter. The residues were once more extracted in the same manner overnight. The extracts were evaporated to dryness and the carrier phospholipid from *E. coli* was added to the ^{32}P-labeled phospholipid when occasion demanded. The extract thus obtained was purified by Folch's partition dialysis procedures (5). Fractionation into the individual phospholipids was carried out with silica gel G thin-layer chromatography. Phospholipids were located by iodine vapor, and the spot corresponding to each component was scraped from the plate.

^{32}P estimation:

Incorporation of ^{32}P into the total phospholipid was estimated by radioactive measurement of aliquot volume from the total lipid fraction. The relative amount of individual phospholipids was determined by measurements of scraped radioactive spots.

RESULTS

Characterization of individual components of phospholipid:

Since the total lipid fraction contains only 4 % of neutral lipid and the rest being all phospholipid, the total lipid fraction is used without any treatment as phospholipid fraction. Major components of phospholipid are phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG) and cardiolipin (CL), while minor components are phosphatidic acid (PA) and an unknown substance (assumed to be bis-phosphatidic acid, UN), as reported previously (6). Fig. 1 gives a radioautogram of the thin-layer chromatograph of phospholipid from cells at the late log phase.

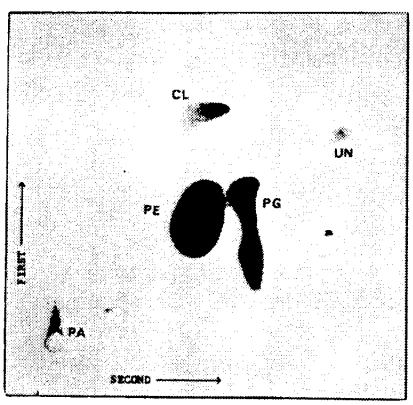

Fig. 1 Autoradiogram of the phospholipid of *E. coli* at the late logarithmic phase. The lipid was extracted from cells grown with $H_3{}^{32}PO_4$ containing semisynthetic medium. Two-dimensional chromatography was performed with solvent of chloroform-methanol-water (70: 25: 4, v/v, first development) and chloroform-methanol-ammonia (60: 25 : 4, v/v, second development).

Relation between the growth phase and the phospholipid present:
The radioactivity of the extractable lipid synthesized in the cells is found to increase closely resembling the growth curve of *E. coli B* during early, middle, late log and stationary phases, but more precisely it tends to decrease as the growth phase advances (Fig. 2). Moreover, in calculating ^{32}P per 1 mg of bacteria, it has been found that the phospholipid content at the logarithmic phase is greater than that at the stationary phase (Fig. 3).

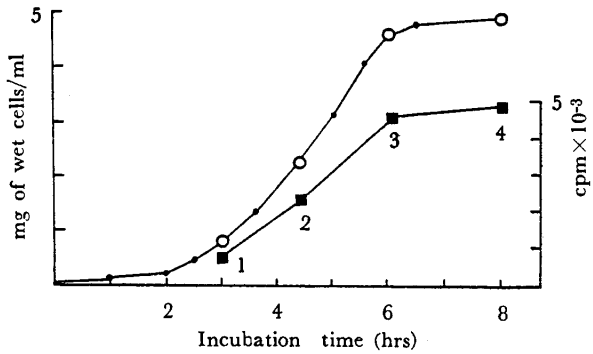

Fig. 2 Incorporation of ^{32}P into the total phospholipid of *E. coli B*.

Symbols: ○, Cell growth: ■, Incorporation of ^{32}P into the total phospholipid fraction from cells in 1 ml at various growth phases. 1, 2, 3, and 4 indicate the sampling points individually at eary, middle, and late logarithmic and stationary phases.

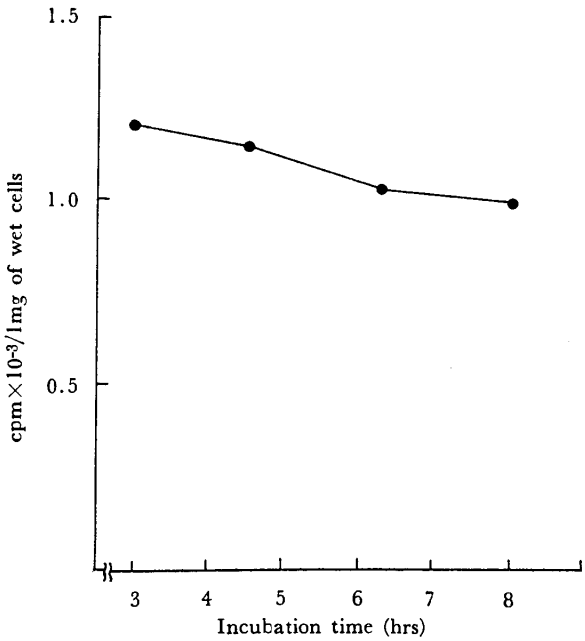

Fig. 3 Changes in the amount of synthesized total phospholipid from 1 mg of cells (wet weight) during growth.
Data were calculated from the ^{32}P incorporation into the total phospholipid and growth curve.

Changes in the amount of individual phospholipid components at various growth phases:

Taking ^{32}P-incorporation into phospholipid as the criterion, phospholipid contained in the cells as each growth phase was fractionated into individual components, and the composition of these components was compared. Table shows relative amounts of individual components at each phase. As a result it has been demonstrated that PE increases as the bacteria grow

TABLE PERCENTAGE OF THE INDIVIDUAL COMPONENTS OF THE PHOSPHOLIPID OF F. COLI B AT VARIOUS GROWTH PHASES

Phase	Early log	Middle log	Late log	Stationary
PE	62.9	66.5	77.6	82.1
PG	20.1	18.3	12.3	7.0
CL	3.6	4.5	4.0	2.9
PA	5.0	2.7	1.0	0.4
UN	2.1	3.2	3.5	2.0

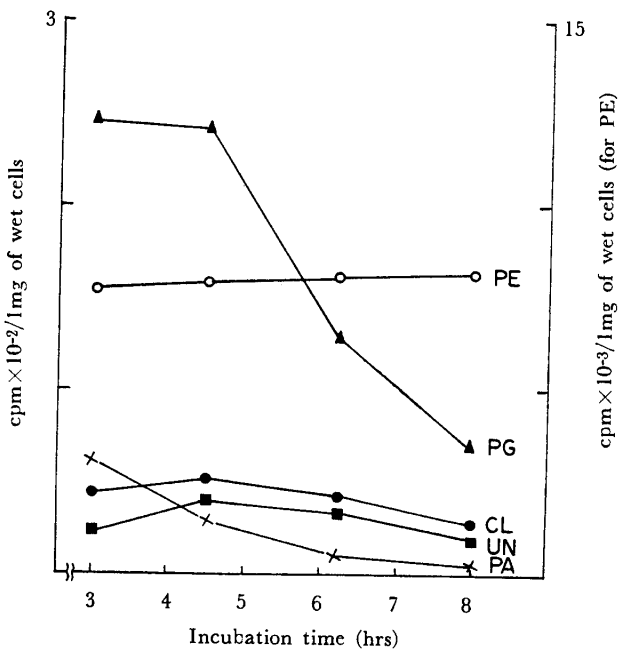

Fig. 4 Changes in the absolute amount of the individual phospholipid from 1 mg of cells (wet weight) during growth.

Data were calculated from the ^{32}P incorporation into each phospholipid. PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; CL, cardiolipin; UN, unknown phospholipid; PA, phosphatidic acid.

older, while PG decreases precipitously. CL and UN increase at the stages when the cells undergo the most rapid fission, *i. e.* at the middle and late logarithmic phases. In Fig. 4 is shown the comparison of absolute amou nts of each component per 1 mg cell at a given growth phase. In this instance, changes in the absolute amount of individual components tend to be similar to the changes in relative amounts, but such a tendency was more marked. PE increases at the stationary phase, while PG and PA show high values in the early log phase, and later as the bacteria grow older, these values decrease precipitously. CL and UN show the peak values in the middle to the late log phase.

DISCUSSION

Changes in lipid composition of various bacteria have been reported by many investigators to occur during the transition from the exponential growth phase to the stationary (2-5).

CRONAN has reported that total phospholipid content of *E. coli* does not change much in the exponential phase or in the stationary phase (4). However, the results of our analysis of phospholipids with *E. coli* cultured in semisynthetic medium have revealed that the absolute content of phospholipid is greater during the period from early log to middle log phase but decreases as it enters the stationary phase. Since most of phospholipid is contained in the cell membrane, it is understandable that a greater amount of phospholipid is found in the younger bacteria undergoing vigorous fission.

By the assays of individual phospholipids, it has been demonstrated that as the culture age advances, PE increases gradually, but PG and PA decrease precipitously. As PE is the structural component and its turnover rate is slow, it would be accumulated. On the other hand, the turnover of PA and PG is rapid and they seem to be precursors of various phospholipids, so that when their growing speed slackens, the demand for their pool will diminish, which is reflected by the decrease in their content. The turnover of CL is speedy as already reported by KANEMASA (1), and in the present experiment the content of CL is found to be greatest in the middle log phase when the growing rate is greatest, suggesting an important role played by CL, *e. g.* playing a role intermediate of cell wall synthesis or a role in active transport.

Summarily, the alteration of the individual phospholipid composition may be thought to take place in conformance with the adaptation of the bacteria to ever-changing environmental conditions in the course of

growth.

SUMMARY

By inoculating *E. coli B* into the semisynthetic medium we conducted shaking culture, and observed alterations of the total phospholipid contents and the amounts of individual phospholipid components in various stages of growth. The results are briefly summarized as follows.

1. The total phospholipid content has been found to be greater during early culture period, while it decreases as the growth age advances.
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