Changes in the composition of membrane phospholipids during the cell cycle of *Escherichia coli*

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Phospholipid concentrations have been estimated throughout the successive cell cycle in synchronously growing culture of *E. coli* B/r. Total phospholipid phosphorus was shown to be doubled in the period of time between two cell divisions, whereas during the division itself it did not change. Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) exhibit a stepwise increase during the cell cycle. It should be noted that the phase of accumulation of these lipids could shift depending on the duration of the cell cycle. The fall in level of PE was followed by a short-term increase (5–10 min). At the same time the level of cardiolipin was observed to be significantly increased.

Escherichia coli *Phospholipid* *Cell division*

1. INTRODUCTION

The importance of membrane lipids in the growth and proliferation of cells has been recognized for about 15 years [1–3]. It was shown that one of the most convenient objects in studying the role of membrane lipids in regulation of the cell cycle is *E. coli* which is deprived of intracellular membranes, and whose pool of lipid precursors is extremely small [4]. Various strains of *E. coli* were utilized in attempts to understand the turnover and mode of phospholipid synthesis by using radioactive labelling [5–13]. However, the studies were noticed to be both ambiguous and contradictory [13]. Most of the works mentioned were devoted to the rate of synthesis of total phospholipids in *E. coli*. Individual phospholipids, PE and PG, have been studied recently [14]. However, radioactive labelling permits only estimation of the rate of phospholipid synthesis, and the lack of precise information about the changes in the composition of individual phospholipids in the membrane of *E. coli* during the cell cycle is obvious.

Abbreviations: PE, phosphatidylethanolamine; CL, cardiolipin; PG, phosphatidylglycerol

This work is devoted to studying the concentrations of 3 major phospholipids, PE, PG and CL, in *E. coli* B/r during the division cycle.

2. MATERIALS AND METHODS

2.1. *Strains and culture conditions*

E. coli B/r CSH was maintained on agar-agar slopes prepared with beef-extract broth and grown in M9 minimal medium containing (per l distilled water): 0.4% (w/v) glucose as carbon source, 7 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 10 g NH_4Cl, 0.2 g $MgSO_4 \cdot 7H_2O$. Cells were grown in a rotatory shaker at 37°C.

2.2. *Synchronization procedure*

Bacteria were synchronized by the method of cell size selection [15,16]. Under our experimental conditions the duration of the cell cycle was 40 min at 37°C. The temperature variation from 38 to 33°C enabled us to obtain cells with a 30–55 min cycle.

2.3. *Cell counting*

The cells fixed with 0.1 HCl were counted in a Goriaev chamber. The synchrony index F was

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calculated from the formula presented in [7]. Absorbance of the bacterial samples was estimated at 540 nm.

2.4. *Cell sampling and fixation*

For phospholipid analysis, 15 ml cell suspension was placed into tightly stoppered centrifuge tubes containing 0.75 ml of 40% formaldehyde [6]. Sampling was carried out at 5-min intervals. The contents of each tube were thoroughly stirred to achieve better fixation of cells by formaldehyde. After 5 min centrifugation at 2500 × *g*, the supernatant was decanted and the lipids extracted from the suspended cells in the same tubes.

2.5. *Extraction procedure and analysis of phospholipids*

Lipid extraction was performed as in [18]. Determination of CL, PE and PG was carried out according to the one-dimensional TLC procedure of Mozharov [19]. For quantitative analysis, each individual phospholipid on the chromatograms was measured densitometrically with a Joyce Loebl 200-201, and calibration curves were constructed. The accuracy of the method was $\leqslant 10\%$.

2.6. *Phospholipid phosphorus*

Phospholipid phosphorus was determined by the method of Raheja et al. [20].

3. RESULTS

3.1. *Total phospholipid phosphorus content during synchronous growth*

Fig.1 illustrates the changes in absorbance, cell number and total phospholipid phosphorus content during the cell divisions of synchronously growing *E. coli* culture. The changes in absorbance are evidence for the exponential growth of the biomass. In contrast, the number of cells increases in stepwise fashion. The duration of the second cycle was 50 min, the third one lasting 40 min. The synchrony index was 0.85 and 0.68 for the two cycles under observation. As is observed, phospholipid phosphorus and, therefore, phospholipids exhibit step increments during the cell cycle. The content of phospholipid phosphorus remains practically unchanged during the cell division (5–15 min). In contrast, the amount of the biomass increases. Doubling of the phospholipid phosphorus

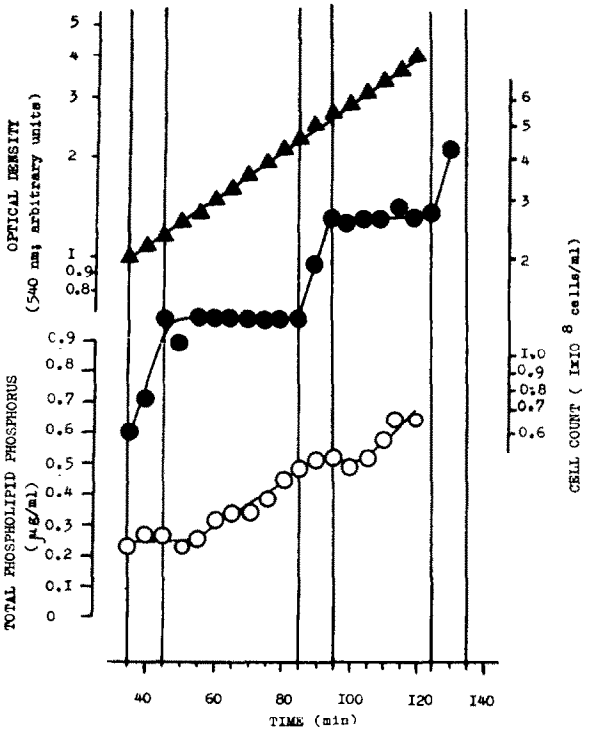

Fig.1. Changes in absorbance, cell number and total phospholipid phosphorus content during the cell cycle of *E. coli*. (▲) Absorbance at 540 nm, (●) cell number in 1 ml of each of the samples, (○) total phospholipid phosphorus in 1 ml of each of the samples. The time just after synchronization is on the abscissa. The narrow vertical columns indicate the time of cell divisions.

content is observed in the interval between two cell divisions. Our results are consistent with those on the stepwise increase in the rate of phospholipid synthesis in the interval between the divisions [8,11–14].

3.2. *Content of individual phospholipids during the cell cycle*

For phospholipid analysis one-dimensional TLC was utilized (fig.2, see section 2). The results of the quantitative estimation of the 3 major phospholipids are presented in fig.3. It can be seen that no significant increase in the contents of PE, PG and CL was observed during the cell divisions. However, their levels were increased in the interval between the divisions, which is consistent with the results obtained on the total phospholipid phosphorus content. In the case of the 40 min cycle, the time interval between the starting point of

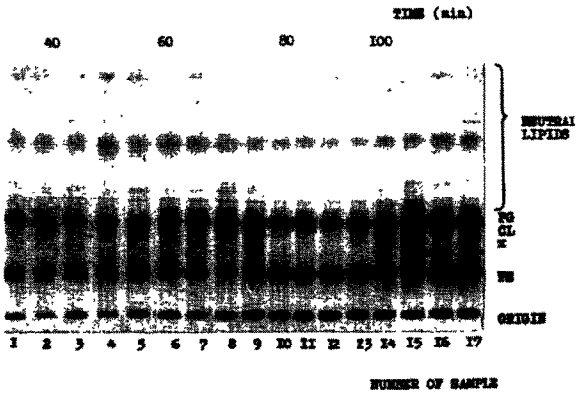

Fig.2. Chromatogram of the membrane phospholipids of synchronously growing *E. coli*. Sampling was carried out at 5-min intervals. The time of sampling of each sample is indicated at the top of the figure.

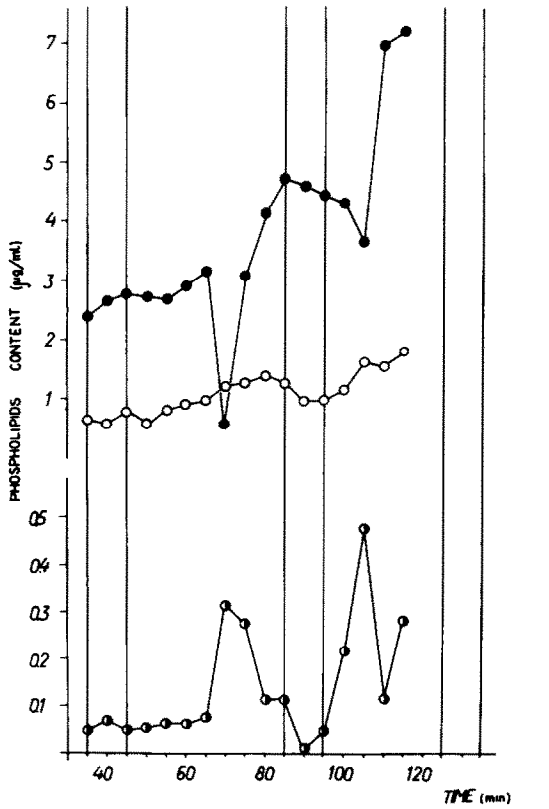

Fig.3. Changes in the content of individual phospholipids in synchronously growing culture of *E. coli*. (●) Level of PE; (○) level of PG; (◑) level of CL. The time just after synchronization is on the abscissa. Phospholipid content is in 1 ml of each of the samples. The narrow vertical columns indicate the time of cell divisions.

the cycle and the point corresponding to the maximal rate of phospholipid content increase ($t_{v_{max}}$) was 20 min for PE and 15 min for PG. $t_{v_{max}}$ rises with increase in duration of the cell cycle. The stage of phospholipid accumulation in this case is shifted towards the end of the cycle (fig.4). The mode of changes in phospholipid content remains the same. It was also found that the rate of change of PE was 3×10^{-3} μg/ml per min during the cell division and 5×10^{-2} μg/ml per min in the interval between the divisions. Analysing the alterations in PE level during the cell cycle in detail, a dramatic decrease of PE was noticed at definite moments of synchronous growth. Fig.2 shows points 8 and 9 (for the second cycle) and point 15 (for the third cycle) to be indicative of these moments (cf. fig.3, where the decrease is observed at the 70th, 75th and 110th minutes after the beginning of cell synchronization). As a rule, the moment of the sharp decrease precedes the step increment in PE level and depends on the duration of the cell cycle (τ). Upon increasing τ from 30 to 50 min, the moment of the decrease is shifted from 0.2 to 0.7 units of the cycle time (fig.4). It should be noted that in all cases the decrease is observed 15–20 min before the successive divisions.

CL was found to be almost completely doubled (fig.3) at the end of the cycle. The many-fold increase in CL content is observed to occur

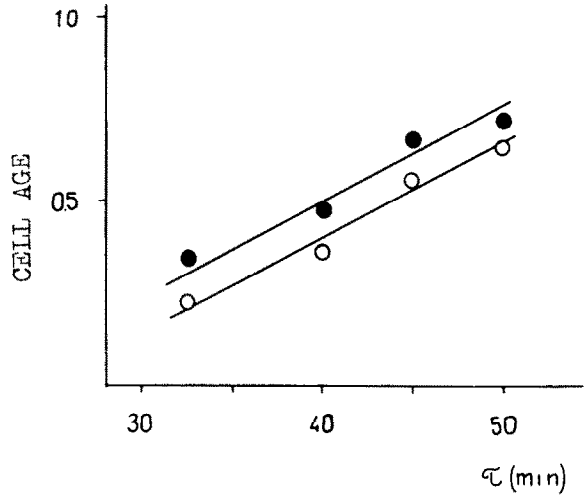

Fig.4. Dependence of PE fall and CL increase during the cell cycle of *E. coli* on the duration of the cycle (τ). (●) $t_{v_{max}}$, (○) fall in the level of PE and increase in CL content.

simultaneously with the fall in PE level, which results in a significant change in the proportion of these phospholipids during 5–10 min of the cycle.

4. DISCUSSION

The curve for total phospholipid phosphorus content described here shows the stepwise increase in the level of phospholipids during the cell cycle. Our results coincide well with those demonstrating the step increments in the rate of lipid synthesis during the cycle of *E. coli*[8,11,13,14]. However, in these works it has been noted that the rate of phospholipid synthesis remains constant during the division, which is expected to result in an increase in phospholipid content. Our results argue against this suggestion. Such a discrepancy may be due to the peculiarities of the *E. coli* strain used or to the possible activation of phospholipase during the division. It should be noted that our results on changing the phospholipid phosphorus content in *E. coli* are similar to those obtained for synchronously growing culture of *Saccharomyces cerevisiae* [21]. The changes in the contents of PE and PG during the cycles of both objects were also found to be similar.

A considerable decrease in the level of PE and simultaneous increase of cardiolipin content, which we have observed, provide evidence of a dramatic change in the ratio of membrane phospholipids at definite moments of the *E. coli* cell cycle. The physiological significance of this phenomenon is not clear, nor the reason for such changes in PE and CL levels. However, we have noticed that the dramatic decrease always occurs 15–20 min before the subsequent division. Also, the cycle of chromosome replication was found to be completed just at that moment [22]. All these facts allow for the possibility of a relationship between the phenomenon under consideration and the DNA termination process.

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