Turnover of Phospholipids in *Bacillus cereus* under Various Growth Conditions

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Since Kanfer and Kennedy¹⁾ reported the turnover of phospholipids in growing *Escherichia coli*, many papers have described the correlations between physiological conditions of growth and the turnover of phospholipids.²⁻⁴⁾ However, the relation between turnover rates of phospholipids and growth rates is not yet clear. In *Bacillus cereus*, it has been found that the membranes were easily autolyzed and phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) were degraded by intracellular phospholipase C in the process.^{5,6)} Since the both phospholipids were known to be main components of membrane phospholipid of *B. cereus* and the turnover of phospholipids takes place in growing cells, it is of interest to see which kind of phospholipid mainly turns over in the cells. Therefore, experiments were undertaken to estimate turnover rate of each phospholipid class under several different growth conditions.

Bacillus cereus IAM 1656 was used throughout this study. The following three kinds of growth medium were used: medium A described previously as GYC medium;⁵⁾ medium BL containing 10 g lactate and 4 g casamino acid; and medium ES containing 10 g succinate, 2 g glutamic acid, 0.8 g leucine, 0.3 g valine, 0.17 g threonine, 0.07 g methionine and 0.07 g histidine. All the ingredients of each medium were dissolved in 1 liter of mineral salts solution reported previously⁵⁾ except that phosphate concentration was reduced as described below and 0.05 M Tris-HCl pH 7.2 was added. Cells were labeled for more than three generations at 36°C either in medium A, BL or ES with ³²P_i (Japan Radioisotope Association, Tokyo) (20 μ Ci/ μ mole/ml), harvested at log-phase, washed and resuspended in each of fresh medium without ³²P_i (3 μ moles phosphate/ml). During reincubation duplicate samples (9ml) were taken at intervals and lipids were extracted according to the method of Bligh and Dyer⁷⁾ from cells precipitated with added carrier cells (about 4 mg) by 5% trichloroacetic acid at 0°C. After separation of lipids on thin-layer chromatography with a solvent of chloroform-methanol-acetic acid (65:25:8, by volume), each spot was scraped off and its radioactivity counted in toluene-based scintillator.

TABLE I. TURNOVER RATES OF PHOSPHOLIPIDS IN DIFFERENT GROWTH RATES

Medium	Doubling time (a)	PG		PE	
		Half life (b)	b/a	Half life (c)	c/a
	(min)	(min)		(min)	
A	23	15	0.65	142	6.2
BL	46	31	0.67	354	7.7
ES	111	85	0.77	529	4.8

Table I shows the doubling time of cells in each medium and half lives of PG and PE. Since incorporation of ³²P into phospholipids from intracellular pool may continue for some time after resuspension in isotope-free medium, turnover rate should be compared only in the period of exponential decrease in radioactivity. During log phase of growth radioactivity of PG decreased exponentially in any medium, while PE was much more stable, i.e., half life (the time required for reduction to half value of preexisting radioactivity) of the phospholipid was 6 to 10 times longer in any growth rate than that of PG was. These facts are almost consistent with the results with *E. coli*.^{1,4)} but disappearance of prelabeled PG in one generation is greater in *B. cereus* (64%, see below) than in *E. coli* (40%⁴⁾). The half life of PG was shorter than doubling time of cells in any medium and the ratios of half life to doubling time were almost constant in all the media (Table I). This suggests that constant fraction of PG is decomposed in one generation even if growth rate varied in wide range and may be consistent with a notion that loss of radioactivity from the lipid takes place at a specific phase of cell division cycle suggested for *E. coli*.⁴⁾ The ratio of 0.67 means that 64% of the label is lost in one generation. As reported in the previous paper,⁶⁾ both PG and PE were non-specifically degraded by intracellular phospholipase C during autolysis in non-growing state but active degradation was specific for PG in growing state (turnover) and that was well controlled. Thus, the two degradative phenomena of phospholipid in growing and non-growing conditions are different.

Next, I have examined the turnover rate of the two phospholipids after the growth medium was changed during the growth. When culture was shifted down from medium A to ES growth was stopped for 6 hr and during the first 2 hr of the lag time the turnover of PG continued with half life of 103 min and then ceased (Fig. 1a). In the case of shift down from medium A to BL (Fig. 1b), after 1.5 hr of lag the growth was re-initiated and turnover of PG was found both in lag (97 min of half life) and in regrowing phase. On the other hand, shift up experiment from medium ES to A showed that both PG and PE turned over with faster rates (12 min and 26 min of half life for PG and PE,

FIG. 1. Turnover of ^{32}P -Phosphate in Phospholipids after Shift Down and Up of Growth Medium. Cells labeled with $^{32}\text{P}_i$ were washed and resuspended with other than first medium. Arrow indicates the time at which incubation in second medium was started. The time needed for centrifugation is not indicated. a, from medium A to ES; b, from medium A to BL; c, from medium ES to A. ●, optical density at 660nm of culture; ○ and △, radioactivities of PG and PE, respectively.

respectively) immediately after the growth shift than that found in medium A (Fig. 1c). It is not known why the radioactivities of phospholipids increased after the period of fast turnover. It is, thus, concluded that turnover rate of phospholipid during the lag period after growth shift is not simply correlated with growth rate although as a rule turnover of PG is slower after shift down and is faster after shift up than that in balanced growth.

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