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Phospholipid Turnover in a Conditional Polyamine Auxotroph of *Escherichia coli*

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Polyamines are required for rapid phospholipid turnover in *Escherichia coli* growing in media of low osmolarity.

The major polyamines of *Escherichia coli* are putrescine and spermidine; spermidine has been implicated in many aspects of cell metabolism including the synthesis of ribonucleic acid (RNA) and protein (1, 2, 8, 9). Several lines of evidence suggest that putrescine may play a role in the process of adaptation to the external osmolarity. Wild-type strains, permanently adapted to media of low osmotic strength, contain large quantities of putrescine which are rapidly lost to the medium after a sudden increase in external osmolarity (7). Polyamine-depleted cultures of several conditional polyamine auxotrophs grow especially slowly in media of low osmolarity; the rate of growth is fully restored by addition of exogenous putrescine and stimulated to a lesser extent by spermidine (5). Total phospholipids, phosphatidylethanolamine (PE), and phosphatidylglycerol (PG) turn over at increased rates in cultures adapted to media of low osmolarity (6). The present experiments were designed to ask whether a high cellular polyamine content, and especially a high putrescine content, is required to maintain normal rates of phospholipid turnover.

All studies were carried out with *E. coli* MA-159, a strain in which putrescine synthesis can be blocked by addition of arginine to the medium (3, 4). Media used were low-osmolarity minimal medium (LOMM; approximately 90 mOsm) and LOMM containing 0.2 or 0.3 M glucose (290 and 390 mOsm, respectively). LOMM medium containing 100 μg of L-arginine per ml is abbreviated as LOMMA (5).

Phospholipid turnover was substantially reduced by polyamine depletion (Fig. 1). Addition of putrescine to depleted cultures significantly accelerated the rate of turnover, and spermidine actually returned the turnover rate to that of a nondepleted culture. In a medium of higher osmolarity, polyamine depletion had no effect on the rate of phospholipid turnover; the slightly slower turnover rate for the culture containing exogenous putrescine was not observed in several other experiments.

These turnover rates were not simply a reflection of the growth rates of the cultures. In LOMMA media, the culture containing putrescine doubled more rapidly and yet turned over [^{32}P]phospholipid more slowly than the culture containing spermidine (cf. Table 1 and Fig. 1). In media of higher osmolarity, all cultures lost radioactivity at similar rates, even though the growth rates differed substantially.

In media of low osmolarity, polyamine depletion and exogenous polyamines affected PE turnover in a manner similar to the turnover of total phospholipid (Fig. 2B). PG also turned over more slowly in polyamine-depleted cultures (Fig. 2A). Both putrescine and spermidine stimulated PG turnover in depleted cultures; however, these data are not sufficiently precise to distinguish between the two polyamines. In cultures of higher osmolarity (LOMM with 0.3 M glucose), polyamine depletion and addition of exogenous polyamines had no significant effect on catabolism of PE and PG.

Polyamine depletion had no measurable effect on phospholipid composition of cultures in media of either higher or low osmolarity (data not shown). Thus, the observed differences in turnover rates for total phospholipid were not caused by a mjaor alteration in the relative amounts of PG and PE present.

Addition of putrescine or spermidine to polyamine-depleted cultures stimulated incorporation of [^{32}P]orthophosphate into phospholipid; however, these increases in both cases paralleled the growth rates of the cultures (Table 1). For the culture containing putrescine, the slightly higher rate of synthesis and

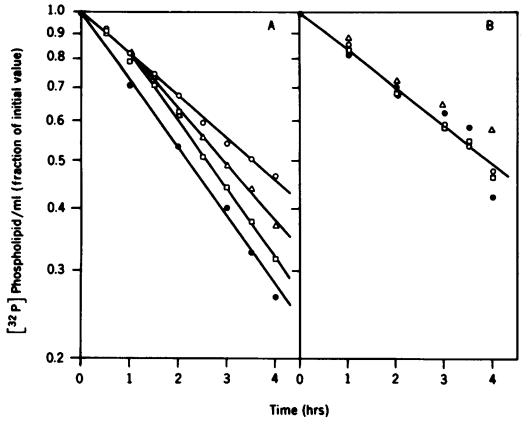

FIG. 1. *Effect of polyamines on turnover of total cellular phospholipid. For studies shown in part A, E. coli strain MA-159 was grown for 5 h in LOMMA medium. The culture was diluted with LOMMA medium to an optical density at 600 nm of 0.200, and 3.7 ml of the culture was incubated for an additional 2 h with 600 μCi of [32P]orthophosphate (New England Nuclear Corp.). The cells were then collected on membrane filters, washed with warm LOMMA medium, and resuspended in 30 ml of this medium. Samples of 10 ml were then pipetted into three flasks. At 7.4 h after arginine addition (zero time in the figure), putrescine (100 μM) was added to the first flask (△) and spermidine (100 μM) was added to the second (□). The third received no addition (○). Samples were removed and extracted as described previously (6). For comparison, an entirely separate culture was grown in LOMM medium (no polyamine depletion), labeled, and chased as described above (●). Studies in part B were similar, except that all media contained 0.2 M glucose.*

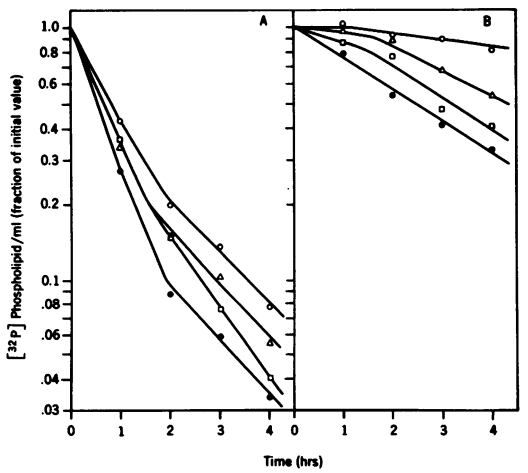

FIG. 2. *Effect of polyamines on turnover of PE and PG in media of low osmolarity. A culture of strain MA-159 was grown for 5 h in LOMMA medium, incubated with [32P]orthophosphate, and resuspended in fresh medium as described in the legend to Fig. 1. Samples were then pipetted into three flasks: the first received putrescine (100 μM, △); the second, spermidine (100 μM, □); and third, no addition (○). A separate culture in LOMM medium (no polyamine depletion) was similarly incubated with isotope and suspended in fresh medium (●). Samples were removed from all four cultures at later times; phospholipids were extracted and separated by thin-layer chromatography (6). Areas of radioactivity were located by autoradiography, scraped into scintillation vials, and counted. Part A, turnover of PG; part B, turnover of PE.*

TABLE 1. *Growth rates of E. coli MA-159 in minimal media*

Medium	Doubling time (min)
LOMM	76
LOMMA[a]	210
LOMMA with putrescine[b]	76
LOMMA with spermidine[b]	91
LOMM with 0.2 M glucose	75
LOMMA with 0.2 M glucose[a]	150
LOMMA with 0.2 M glucose and putrescine[b]	74
LOMMA with 0.2 M glucose and spermidine[b]	74

[a] Growth slows to the rates shown approximately 6 h after addition of arginine (100 μg/ml) and does not change for many hours thereafter.

[b] Cultures were depleted of polyamines by growth for 7 h in the presence of arginine. Exogenous putrescine (100 μM) stimulated growth to the rate shown after a lag of 50 min; the lag period with the same concentration of spermidine was 60 min. In media of higher osmolarity, cultures containing either polyamine began logarithmic growth after a lag time of 30 min.

lower rate of catabolism should produce a slight accumulation of phospholipid; however, no such accumulation was observed.

The rapid turnover of phospholipids in cultures of low osmolarity has been shown to require the presence of a full cellular polyamine complement. Rapid turnover is probably mediated by spermidine and not putrescine, because only spermidine is capable of fully restoring the turnover rate to that of a nondepleted culture.

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