Phospholipids of *Escherichia coli* in Magnesium Deficiency

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Growing bacteria in a magnesium-deficient medium causes loss of ribosomes (McCarthy, 1962), inhibition of nucleic acid (Kennell & Kotoulas, 1967) and protein synthesis (Marchesi & Kennell, 1967), inhibition of growth (McCarthy, 1962), inhibition of intracellular K⁺ accumulation (Günther & Mariss, 1968a), and changes in metabolism, especially a decrease in the capacity of oxidative phosphorylation (Günther & Mariss, 1968b). Moreover, infoldings of the plasma membrane near the end of the cell and intracytoplasmic membranes are shown in Mg-starved *Escherichia coli* by electron microscopy (Füll & Branton, 1969; Lutsch & Venker, 1969; Morgan, Rosenkranz & Rose, 1966). For comparison with these morphological results we examined the content and composition of phospholipids in *E. coli* which had been Mg-starved under comparable conditions. Total phospholipids increased on Mg starvation (Table 1); this corresponds to the increase of membranes. Phosphate starvation also causes a reduction of ribosomes and growth rate (Horiuchi, Horiuchi & Mizuno, 1959) and formation of intracytoplasmic membranes (Lutsch & Venker, 1969). Thus certain nutrient deficiencies produce a complex alteration in the regulation of metabolism.

The phospholipid pattern changed during Mg deficiency, because the increase in phospholipid content was limited to phosphatidylethanolamine and cardiolipin. Expressed relative to total extracted phospholipids, there was more cardiolipin and less phosphatidylglycerol and phosphatidylethanolamine. An altered regulation of metabolism could again be the reason. This alteration could have been produced by differences in the Mg-dependence of the enzymes involved in the phospholipid turnover, but as the average intracellular Mg ionic activity (assuming there is no compartmentation) is essentially undiminished in Mg-deficient bacteria (Günther & Dorn, 1969), this is unlikely. In Mg deficiency the concentrations of extracellular Mg and intracellular K⁺ are reduced (Günther & Mariss, 1968a) so these ions may be involved. An effect of extracellular Mg ions on phospholipid metabolism has been shown in the Na⁺-sensitive mutant *E. coli* ~324−I (Lusk & Kennedy, 1972). Sodium ions inhibited growth of this strain and caused less synthesis of phosphatidylethanolamine and more of cardiolipin. High Mg, Ca or Sr concentrations normalized growth and phospholipid metabolism without altering the intracellular Na⁺ or Mg contents.

Relatively less phosphatidylglycerol and more cardiolipin were also observed after the transition from exponential to stationary phase (Cronan & Vagelos, 1972) and after the addition of dinitrophenol, colicin K (Cavard, Rampini, Barber & Polonowski, 1968) or penicillin (Stárka & Moravová, 1970). According to Cronan & Vagelos (1972), ‘These manipulations, although very diverse, have one character in common, they all result in decreased cellular phosphorylating ability’. Guanosine tetraphosphate, however, which can regulate phospholipid synthesis (Merlie & Pizer, 1973), probably does not always participate. The increase of guanosine tetraphosphate is small after addition of colicin or during phosphate starvation (Lazzarini, Cashel & Gallant, 1971).
Short communication

Table 1. Phospholipids of normal and Mg-deficient Escherichia coli

<table>
<thead>
<tr>
<th>Lipid phosphate</th>
<th>Normal*</th>
<th>Mg-deficient†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/g dry wt</td>
<td>% total</td>
</tr>
<tr>
<td>Total lipid phosphate</td>
<td>105.7±2.5</td>
<td>100</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>82.9±2.6</td>
<td>78.4±2.5</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>11.5±1.0</td>
<td>10.9±0.9</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>9.0±1.0</td>
<td>8.5±0.9</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>1.1±0.1</td>
<td>1.0±0.1</td>
</tr>
</tbody>
</table>

* Mean of 7 experiments, ± S.D.
† Mean of 10 experiments, ± S.D.

*Escherichia coli* B163 was grown aerobically at 37 °C in glucose (0.75 %)-minimal medium in Erlenmeyer flasks by gassing with 95 % O₂–5 % CO₂ (Gunther & Dorn, 1966). For Mg-deficient bacteria the concentration of Mg in the growth medium was 3 μM; for normal bacteria and Mg-deficient bacteria, 1 and 5 l medium were used respectively. (Under these conditions, after 16 h the normal and Mg-deficient bacteria had reached an absorbance at 405 nm of 0.7 and 0.1 respectively, corresponding to 0.4 and 0.06 g protein/l, and thereafter continued in the stationary phase.) Overnight cultures (20 h) were washed twice in double distilled water, frozen in liquid N₂, and freeze-dried. The lipids were extracted with chloroform–methanol (2:1, v/v). The extract was washed with 0.04 % CaCl₂ (Folch, Lees & Sloane Stanley, 1957). The phospholipids were chromatographed in two dimensions on silica gel thin-layer plates (Kieselgel 60 HR reinst, Merck, Darmstadt, Germany). Solvent system 1 was composed of CHCl₃–CH₂OH–H₂O (70:25:4, by vol.) and solvent system 2 of CHCl₃–CH₂OH–7M NH₄OH (60:35:5, by vol.) (Kanemasa, Akamatsu & Nojima, 1967). The plates were developed in iodine vapour. The spots were scraped off and their phosphate contents determined according to Bartlett (1959). Reference compounds were obtained from Applied Science Laboratories, Inc., State College, Pennsylvania.

REFERENCES


