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, 1968*a*), and changes in metabolism, especially a decrease in the capacity of oxidative phosphorylation (Günther & Mariss, 1968*b*). 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 (Fiil & 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, 1968*a*) 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* A324-1 (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 tetrphosphate, however, which can regulate phospholipid synthesis (Merlie & Pizer, 1973), probably does not always participate. The increase of guanosine tetrphosphate is small after addition of colicin or during phosphate starvation (Lazzarini, Cashel & Gallant, 1971).

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

	Lipid phosphate			
	Normal*		Mg-deficient†	
	$\mu\text{mol/g dry wt}$	% total	$\mu\text{mol/g dry wt}$	% total
Total lipid phosphate	105.7 $\pm$ 2.5	100	148.3 $\pm$ 8.3	100
Phosphatidylethanolamine	82.9 $\pm$ 2.6	78.4 $\pm$ 2.5	107.5 $\pm$ 5.9	72.5 $\pm$ 4.0
Cardiolipin	11.5 $\pm$ 1.0	10.9 $\pm$ 0.9	23.6 $\pm$ 2.4	15.9 $\pm$ 1.6
Phosphatidylglycerol	9.0 $\pm$ 1.0	8.5 $\pm$ 0.9	8.9 $\pm$ 1.8	6.0 $\pm$ 1.2
Phosphatidylserine	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1	3.3 $\pm$ 2.0	2.2 $\pm$ 1.3

* Mean of 7 experiments, $\pm$ S.D.

† Mean of 10 experiments, $\pm$ 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 (Günther & 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-CH₃OH-H₂O (70:25:4, by vol.) and solvent system 2 of CHCl₃-CH₃OH-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

- BARTLETT, G. R. (1959). Phosphorus assay in column chromatography. *Journal of Biological Chemistry* **234**, 466-468.
- CAVARD, D., RAMPINI, C., BARBER, E. & POLONOWSKI, J. (1968). Activité phospholipasique et autres modifications du métabolisme des phospholipides consécutives à l'activation des colicines sur *E. coli*. *Bulletin de la Société de Chimie Biologique* **50**, 1455-1471.
- CRONAN, J. E. JUN. & VAGELOS, P. R. (1972). Metabolism and function of the membrane phospholipids of *Escherichia coli*. *Biochimica et biophysica acta* **265**, 25-60.
- FIL, A. & BRANTON, D. (1969). Changes in the plasma membrane of *Escherichia coli* during magnesium starvation. *Journal of Bacteriology* **98**, 1320-1327.
- FOLCH, J., LEES, M. & SLOANE STANLEY, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497-509.
- GÜNTHER, T. & DORN, F. (1966). Über die ATPase von *E. coli* B 163 und seiner K⁺-Mangelmutter B 525. *Zeitschrift für Naturforschung* **21b**, 1076-1081.
- GÜNTHER, T. & DORN, F. (1969). Über die intrazelluläre Mg-Ionenaktivität von *E. coli*-Zellen. *Zeitschrift für Naturforschung* **24 b**, 713-717.
- GÜNTHER, T. & MARISS, P. (1968a). Über den K⁺-Transport bei Mg-arm gewachsenen Zellen von *E. coli*. *Zeitschrift für Naturforschung* **23 b**, 334-338.
- GÜNTHER, T. & MARISS, P. (1968b). Über den Stoffwechsel magnesiumarm gewachsener Zellen von *E. coli*. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **349**, 623-631.
- HORIUCHI, T., HORIUCHI, S. & MIZUNO, D. (1959). Degradation of ribonucleic acid in *Escherichia coli* in phosphorus-deficient culture. *Biochimica et biophysica acta* **31**, 570-572.
- KANEMASA, Y., AKAMATSU, Y. & NOJIMA, S. (1967). Composition and turnover of the phospholipids in *Escherichia coli*. *Biochimica et biophysica acta* **144**, 382-390.
- KENNEL, D. & KOTOULAS, A. (1967). Magnesium starvation of *Aerobacter aerogenes*. II. Rates of nucleic acid synthesis and methods for their measurement. *Journal of Bacteriology* **83**, 345-356.
- LAZZARINI, R. A., CASHEL, M. & GALLANT, J. (1971). On the regulation of guanosine tetraphosphate levels in stringent and relaxed strains of *Escherichia coli*. *Journal of Biological Chemistry* **246**, 4381-4385.

- LUSK, J. E. & KENNEDY, E. P. (1972). Altered phospholipid metabolism in a sodium-sensitive mutant of *Escherichia coli*. *Journal of Bacteriology* **109**, 1034-1046.
- LUTSCH, G. & VENKER, P. (1969). Intracytoplasmatische Membranen in *E. coli* nach Magnesium- bzw. Phosphat-Mangel. *Naturwissenschaften* **56**, 568.
- MARCHESI, S. L. & KENNEL, D. (1967). Magnesium starvation of *Aerobacter aerogenes* III. Protein metabolism. *Journal of Bacteriology* **93**, 357-366.
- MCCARTHY, B. J. (1962). The effects of magnesium starvation on the ribosome content of *Escherichia coli*. *Biochimica et biophysica acta* **55**, 880-888.
- MERLIE, J. P. & PIZER, L. I. (1973). Regulation of phospholipid synthesis in *Escherichia coli* by guanosine tetraphosphate. *Journal of Bacteriology* **116**, 355-366.
- MORGAN, C., ROSENKRANZ, H. S. & ROSE, H. M. (1966). Electron microscopy of magnesium-depleted bacteria. *Journal of Bacteriology* **91**, 891-895.
- STÁRKA, J. & MORAVOVÁ, J. (1970). Phospholipids and cellular division of *Escherichia coli*. *Journal of General Microbiology* **60**, 251-257.