Preliminary Notes

PN 51002

On the accumulation of amino acid derivatives of phosphatidylglycerol in bacteria

During an investigation on the influence of nutritional environment on the phospholipids of some bacteria the addition of glucose was found to cause the most significant effects. When *Bacillus megatherium* was cultured in a broth containing glucose, a phospholipid was produced, which was not detected when the bacteria were grown in medium depleted of glucose. The compound appeared to belong to the class of amino acid esters of phosphatidylglycerol (PG)[1] and proved to be identical to PG ornithine[2] previously isolated from *B. cereus*[3]. Similar observations were made on *Staphylococcus aureus*, whose major phospholipids included a polyglycerol-phospholipid identical with or related to diphosphatidylglycerol[4], PG[4] and an amino acid derivative of PG[1]. The latter compound, PGL, appeared to contain the amino acid lysine. A similar observation had been made recently by MACFARLANE[5]. In the presence of glucose the amount of PGL produced by *S. aureus* was increased, the increase being balanced by a decrease of the relative amount of PG. Further studies demonstrated that the shift in the phospholipid distribution in this bacterium must be attributed to a lowering of the pH of the medium as a result of the fermentation of the added glucose.

The results of three somewhat different types of experiment, which support this conclusion, are presented in Fig. 1.

(A) Cultivation of *S. aureus* in a radioactive broth[6] enriched with glucose (5 g/l) changed the pH from 7.2 to 4.8 during 16 h incubation at 37°. Analysis of the phospholipids[6] at the end of this period revealed the presence of a high amount of PGL. However, when the pH of the medium was restored to the initial value (7.2) by the addition of NaOH, and the bacterial suspension was incubated another 3 h at this pH, a different phospholipid pattern resulted. A significant decrease of PGL accompanied by an increase of PG was observed.

(B) *S. aureus* grown without glucose in the medium exhibited PG as dominant phospholipid, whereas only a small amount of the amino acid ester derivative was present. The pH of the medium had changed from 7.2 to 7.4. When the pH was subsequently adjusted to 4.8 and the bacterial culture was kept for 3 h at 37°, a significant increase in the amount of PGL at the cost of PG was demonstrated.

(C) A crucial experiment was made by culturing *S. aureus* with and without glucose at pH 7.2 and 5.2 respectively. Under the first conditions, the pH of the medium decreased because glucose was present, but was prevented from falling below 6.2. The phospholipid fraction of the harvested bacteria contained PG as major compound, whereas according to expectation the amount of PGL was relatively small.

Abbreviations: PG, phosphatidylglycerol; PGL, phosphatidylglyceryllysine.

Since at pH 4.8 *S. aureus* does not grow adequately the bacteria were cultured at pH 5.2 without glucose and brought to pH 4.8 at the end of the logarithmic phase. Then the bacteria appeared to contain PGL as a major phospholipid, whereas PG itself was present in only low concentration.

If we conclude that the pH is of major importance for the phospholipid pattern of the bacteria studied, the question remains by what mechanism the acidic pH produces an accumulation of the amino acid ester of phosphatidylglycerol. Among many possibilities is the speculation that the shift in the proportions of PG and

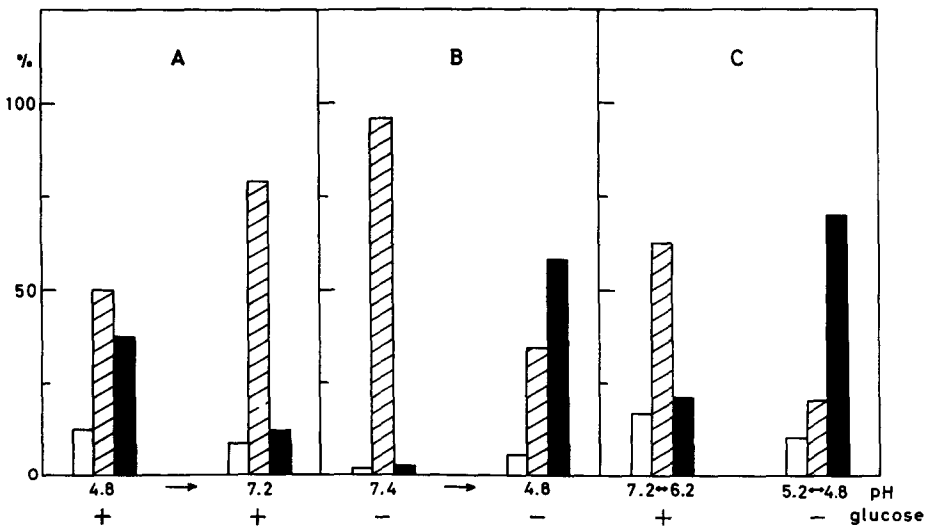

Fig. 1. The influence of the pH of the medium on the proportions of major phospholipids from *S. aureus*. A. Left hand columns represent the phospholipid distribution in a 16-h culture (37°) grown in the presence of glucose (initial pH 7.2). At the time of harvesting the pH of the medium was 4.8. Right hand columns give the phospholipid distribution of the same culture, which after adjusting the pH to 7.2 was incubated again for 3 h. B. Left hand columns indicate the situation in a 16-h culture (37°) grown without glucose (initial pH 7.2). The pH at the end of the incubation was 7.4. Right hand columns represent the composition of the phospholipids after the 16-h culture was brought to pH 4.8 and incubated for a further 3 h. C. Left hand columns represent the phospholipid pattern of a 12-h culture, grown with glucose. The pH was kept within the limits indicated. Right hand columns concern a 12-h culture grown at pH 5.2 without glucose. 1 h before harvesting the pH was adjusted to 4.8. Open bars, unidentified compound (chromatographically related to diphosphatidylglycerol); shaded bars: PG, solid bars, PGL.

PGL involves the pH optimum of one of the enzymes responsible for mutual conversions of both phospholipids *e.g.*, PG + lysine ⇋ PGL, or other biosynthetic and catabolic reactions. In recent experiments indications were obtained for the conversion of PG into PGL. The effects of the pH on this process as well as on the enzymic hydrolysis of PGL are the subject of current investigations. As regards both the physiological significance of PGL and its accumulation at pH 4.8, it could be argued that the phospholipid may play a part in the supply of lysine for the biosynthesis of cell wall. A blockade of the latter process at this acidic pH might be consistent with the observed accumulation of PGL. An alternative explanation, which we favour, attributes to this positively charged phospholipid the function of

balancing the charge of the lipid layer(s) of the membrane. As a response to the acidity of the surrounding medium a membraneous phospholipid containing a basic amino acid would be very suitable indeed. Whether the amino acid concerned can be released into the medium also needs investigation. With respect to the latter speculations about the function of amino acid esters of PG in the membrane it is worth noting that in a bacterium which grows at a low pH, namely *Lactobacillus acidophilus*, phospholipids with several basic groups were detected which, however, are probably different in chemical structure. Other bacteria which do not produce substantial amounts of amino acid esters of PG, when cultured at a low pH were found to increase the content of other phospholipid classes that contain a basic group *e.g.* phosphatidyl ethanolamine. Perhaps these observations bear some relation to the results recently reported by KANFER AND KENNEDY[7] on the variation of the phospholipid composition of *E. coli* during different growth phases.

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Department of Biochemistry,
State University Utrecht,
Croesestraat 79,
Utrecht (The Netherlands)

U. M. T. HOUTSMULLER
L. L. M. VAN DEENEN

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Inositol phosphates obtained from a phosphatido–peptide fraction of kidney cortex

FOLCH[1] has reported that a phosphatido–peptide fraction could be obtained by extraction of a trypsin-resistant protein-residue of beef brain with acidified chloroform–methanol. More recently, HUGGINS[2–4] has shown that a similar fraction could be obtained from kidney and secretory organs such as pancreas and submaxillary glands. According to these investigators, this fraction appears to contain inositol, phosphorus, glycerol, fatty acids and amino acids, presumably in peptide linkages.

This report is concerned (a) with experiments designed to determine the presence of inositol phosphates in the phosphatido–peptide fraction of kidney cortex and