The presence of ribitol phosphate in bacterial cell walls

The occurrence of the nucleotides cytidine diphosphate glycerol and cytidine diphosphate ribitol in *Lactobacillus arabinosus*[1], and the isolation from this organism of a trichloroacetic acid-soluble polymer containing glycerophosphate and ribitol phosphate residues[2] suggested to us that macro molecules containing polyol phosphate residues might occur in other cell fractions. Consequently, the cell walls of this and other bacteria have been examined by techniques originally developed during work on the nucleotides. We are grateful to Dr. M. R. J. SALTON for samples of cell walls which were prepared by methods previously described by him[3].

The walls of *L. arabinosus* 17-5 and of the vegetative form of *Bacillus subtilis* were hydrolysed in 2 *N*-HCl at 100° for 3 h and the products examined by paper chromatography in *n*-propyl alcohol–ammonia (*d*, 0.88)–water (6:3:1). When the paper was sprayed with the periodate-Schiff reagents for the detection of glycols[4] a strong spot corresponding to 1,4-anhydroribitol and a somewhat weaker spot of its phosphate were observed. Considerably weaker spots corresponding to isomeric ribitol phosphates were also detected. The colours and rate of development of these spots were characteristic of a hydrolysate of a substance containing ribitol phosphate residues[5]. The identification of hydrolysis products was confirmed by the use of spray reagents for phosphates. In addition to inorganic phosphate, anhydroribitol phosphate, and the traces of isomeric ribitol phosphates, an unidentified organic phosphate was detected. Although the nature of this compound is not yet established, it presumably arises by hydrolysis of an unidentified grouping in the cell wall.

Quantitative analysis of the hydrolysis products from a cell wall preparation from *L. arabinosus* was carried out by elution from a paper chromatogram and determination of the phosphorus content of the eluted material. The cell walls contained 3.4% phosphorus and it was found that 67–76% of this could be accounted for as degradation products of ribitol phosphate (*i.e.* as inorganic phosphate, anhydroribitol phosphate, and traces of ribitol phosphate isomers). The amount of anhydroribitol formed during hydrolysis was estimated by visual comparison on paper with standard amounts of authentic material, using the periodate-Schiff spray reagents. The values obtained supported the view that inorganic phosphate formed during hydrolysis of the cell walls was derived largely from ribitol phosphate residues. From these results it follows that about 20% of the cell wall consists of ribitol phosphate. The walls of *B. subtilis* contain 4.2% phosphorus and, although quantitative information is not yet available, paper chromatograms of hydrolysates were indistinguishable from those obtained from *L. arabinosus*. This suggests that *B. subtilis* cell walls may contain nearly 30% of ribitol phosphate.

We conclude that ribitol phosphate is an important component of the cell walls of *L. arabinosus* and *B. subtilis*. It has not yet been possible to examine many different bacteria but preliminary experiments have failed to demonstrate the presence of polyol phosphates in the walls of *Micrococcus lysodeikticus* and *Escherichia coli*. The sensitivity of the method, however, may not be sufficiently high to detect traces of ribitol phosphate in these organisms. It would seem that the presence of ribitol phosphate residues in the cell wall may be related to the metabolic state of an organism, since no trace of the compound was detected in spore walls of *B. subtilis*.

Although the above results give no indication of the way in which the ribitol phosphate residues are bound chemically with the other cell wall components, it is reasonable to assume a similarity between the part of the wall containing ribitol phosphate and the trichloroacetic acid-soluble polymer isolated earlier from *L. arabinosus*. It is interesting, however, that although the polymer contains more glycerophosphate than ribitol phosphate, we were unable to detect glycerol or its phosphates in hydrolysates of bacterial cell walls. On the other hand, if the trichloroacetic acid-soluble material is a mixture of a glycerophosphate polymer and a ribitol phosphate polymer, then a metabolic relationship between the latter and the cell wall substance would seem probable.

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