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Preliminary communication

Amino-sugar phosphates from the cell wall of *Micrococcus lysodeikticus**

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The presence of 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose 6-phosphate (muramic acid 6-phosphate) in the cell walls of *Micrococcus lysodeikticus*[1–3] and several other bacteria has been reported[4–13]. The occurrence, in *Micrococcus lysodeikticus*, of another amino-sugar phosphate, with an electrophoretic mobility similar to that of 2-amino-2-deoxy-D-glucose 6-phosphate (glucosamine 6-phosphate), has also been reported[14]. On removal of the phosphate group, this sugar liberated an amino sugar that did not correspond to any known amino sugar on paper chromatography[14]. We now report the isolation and characterization of glucosamine 6-phosphate, as well as of muramic acid 6-phosphate, from the lysozyme-resistant cell-walls of *Micrococcus lysodeikticus*.

The lysozyme-resistant material obtained from cell walls, prepared according to the procedure of Sharon and Jeanloz[15], was treated with 4M hydrochloric acid for 10 h at 80°. After evaporation, the acid-free residue in water was adsorbed on a column of Dowex 50 X-8 (H^+, 200–400 mesh) ion-exchange resin, and the column was eluted with water. Three fractions were obtained. The first mainly contained D-glucose. The second fraction showed a single component on thin-layer chromatography (t.l.c.) and reacted positively with ninhydrin, the Hanes–Isherwood[16] and Park–Johnson[17] reagents, and with the modified Elson–Morgan reagent[18] to give an absorption maximum at 510 nm, indicating the presence of a reducing sugar having an amino and a phosphate group. Finally, treatment of the sugar phosphate with alkaline phosphatase (calf mucosa, Sigma Chemical Company, St. Louis, MO) liberated muramic acid.

In t.l.c., the third fraction showed a single component that gave a positive stain

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TABLE I

HYDROCHLORIC ACID TREATMENT OF SYNTHETIC MURAMIC ACID 6-PHOSPHATE

Hydrochloric acid (molarity)	*Time (h)*	*Temp. (°)*	*Compounds formed*[a]		
			Muramic acid 6-phosphate	*Muramic acid*	*Glucosamine 6-phosphate*
2	16	100	++++	+	–
4	16	100	++	+++	trace
4	10	80	+++++	–	–
6	1	100	++++	+	–
6	2	110	++	+++	trace

[a]The products of hydrolysis were examined by t.l.c. in 4:1:5 (v/v, upper layer) 1-butanol–acetic acid–water, and 1:3:1 (v/v) methanol–chloroform–water.

with ninhydrin and the Hanes–Isherwood reagent[16]. The sugar was reducing[17], and gave a positive Elson–Morgan reaction with an absorption maximum at 530 nm. Treatment of the sugar phosphate with alkaline phosphatase released a sugar, identical with glucosamine (t.l.c.), which gave arabinose (t.l.c.) on degradation with ninhydrin[19]. Periodate treatment of *N*-acetylglucosamine phosphate degraded the sugar, and no release of formaldehyde was detected. The results strongly suggest the presence of a phosphate group at C-6 of glucosamine.

In order to further establish that glucosamine 6-phosphate is an original sugar component of the cell walls and does not arise as the de-etherification product of muramic acid 6-phosphate during acid hydrolysis, synthetic muramic acid 6-phosphate[3] was treated with various concentrations of hydrochloric acid for several time-intervals (see Table I). The products of hydrolysis clearly indicate that the ether bond in muramic acid 6-phosphate is stable to the acid conditions used and that these treatments removed only the phosphate group. During acid hydrolysis, D-glucosamine 6-phosphate might arise from D-glucosamine 4-phosphate[20]; however, this seems unlikely, as the D-glucosamine residues in cell walls are linked at C-4, and nonreducing terminal 2-acetamido-2-deoxy-D-glucose residues were shown[1], by methylation studies, to be free of substituents.

As no inorganic phosphate was released by treatment of the nondialyzable cell-wall with alkaline phosphatase[21], it is probable that the D-glucosamine 6-phosphate residues serve, like the muramic acid 6-phosphate residues, as a link between the antigenic polysaccharide chains and the peptidoglycan chain.

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