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The enzymic synthesis of a ribitol 5-phosphate polymer

The work of BADDILEY and co-workers has demonstrated the existence of a large variety of ribitol phosphate polymers (ribitol teichoic acids) as cell-wall components of Gram-positive bacteria[1]. The common feature of these polymers is a chain of ribitol phosphate units linked by phosphodiester linkages. The polymers is 'ated from different bacteria differ in their chain length and in the nature and linkage of various hexoses to the ribitol units. They all contain D-alanine esterified to one of the ribitol hydroxyl groups. In a previous communication, BURGER AND GLASER[2] have presented evidence for the enzymic synthesis of a glycerolphosphate polymer by a particulate enzyme from *Bacillus licheniformis*. We have now examined a similar enzyme preparation from *Lactobacillus plantarum* (ATCC 8014) which incorporates ribitol phosphate from CDP-ribitol into a polymer. The ribitol teichoic acid from this organism has been isolated by ARCHIBALD *et al.*[3] and shown to contain glucose and D-alanine as substituents on the ribitol phosphate polymer.

Lyophilized cells of *Lactobacillus plantarum* harvested during the logarithmic growth phase were prepared as described previously[4]. 1 g of dried cells was suspended in 8 ml of 0.05 M Tris–chloride, 0.01 M $MgCl_2$, 0.001 M EDTA (pH 8.0) and shaken with 8 g of glass beads in a Nossal shaker for 3 20-sec periods. The supernatant fluid after centrifugation at 12000 × *g* for 15 min was centrifuged at 105000 × *g* for 2 h. The particulate fraction was washed by centrifugation with the same buffer and used as an enzyme source in the experiments indicated below.

As shown in Table I, the particulate enzyme will synthesize from CDP-ribitol a radioactive material which precipitated with the protein on acidification. The reaction appears to be specific since neither D-ribitol 5-phosphate nor CDP-glycerol are active as substrates for the enzyme. Unincubated controls showed no activity.

The enzymic synthesis of the ribitol phosphate polymer is dependent on the addition of Mg^{2+} and shows a pH optimum between 7 and 8. The K_m for CDP-ribitol is approximately 0.1 mM.

TABLE I

ENZYMIC SYNTHESIS OF POLYRIBITOLPHOSPHATE

The reaction mixtures contained 20 μmoles of Tris–chloride, 4 μmoles of $MgCl_2$, 0.4 μmole of EDTA, 0.03 μmole of the substrates indicated and enzyme in a final volume of 0.6 ml (pH 8.0). At the end of the incubation the reaction was stopped by the addition of 0.08 ml of 3 N $HClO_4$. The precipitate was washed three times by centrifugation with 1 ml of 0.15 N $HClO_4$ and once with 1 ml of water. It was dissolved in 1 ml of 1% of NH_4OH and a suitable aliquot counted in a low background counter. The specific activity of CDP-ribitol and ribitol-5-*P* was 30000 counts/min/μmole. CDP-glycerol had 15000 counts/min/μmole. Different enzymes were used in Expts. A, B, and C.

Exp.	*Additions*	*Time (h)*	*mμmoles incorporated into polymer*
A	CDP-ribitol	0	0
	CDP-ribitol	1.5	1.8
	Ribitol-5-P	1.5	0
B	CDP-ribitol	3.0	2.34
	CDP-glycerol	3.0	0
C	CDP-ribitol	0	0
	CDP-ribitol	0.5	1.04
	CDP-ribitol	1.0	2.38
	CDP-ribitol	1.5	3.05

The identification of the product of the reaction as a ribitol phosphate polymer is based on the following observations. The synthesized material can be freed of protein by phenol treatment[2]. The aqueous phase after the phenol treatment was extracted with chloroform and dialyzed overnight against a large volume of distilled water without loss of radioactivity. The radioactive material in 0.01 M Tris–chloride buffer (pH 8.0) is completely excluded from Sephadex G-50.

After alkaline hydrolysis (1 N KOH, 100°, 3 h) the radioactive material is converted to a mixture of ribitol diphosphates, ribitol monophosphates and ribitol in a ratio approaching 1:2:1*.

The radioactive material is not hydrolyzed by ribonuclease, deoxyribonuclease or by *Escherichia coli* alkaline phosphatase.

After partial alkaline hydrolysis (0.1 N KOH, 100°, 10 min), a compound(s)* with mobility relative to ribitol 5-phosphate of 0.67 in the neutral ethanol ammonium acetate solvent[5] could be isolated. On further alkaline hydrolysis it gave rise to ribitol diphosphate(s), ribitol monophosphate(s) and ribitol. After treatment with *E. coli* alkaline phosphatase a compound(s) with $R_{ribitol\text{-}5\text{-}P}$ 1.5 was formed. This second compound on further alkaline hydrolysis gave rise to ribitol monophosphate and ribitol, but no ribitol diphosphate was formed. On the basis of these observations, the compound isolated from the partial hydrolysate appears to be one or more of the isomers of ribitol-phosphate-ribitol-phosphate which by treatment with phosphatase is converted to ribitol-phosphate-ribitol.

The products from the exhaustive alkaline hydrolyses, as well as the "dimer" isolated from the partial alkaline hydrolyses, are the products expected from a linear chain of ribitol units linked by phosphodiester linkages. By analogy with the observations of ARCHIBALD *et al.*[3], the phosphate linkage can be assumed to be between C-1 and C-5 of successive ribitol units. It is not known whether the ribitol phosphate polymer synthesized by the enzyme is free or linked to some other material present in the particulate fraction.

It is known that this strain of *L. Plantarum*[6] produces a glycerol teichoic acid as well as a ribitol teichoic acid. We have so far been unable to synthesize a glycerol phosphate polymer with enzyme preparations from this organism.

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* Alkaline hydrolysis will give a mixture of D-ribitol 5-phosphate and D-ribitol 4-phosphate. The ribitol diphosphate will consist of a similar mixture of isomers. The chromatographic methods used will not distinguish these compounds.