THE LOCATION OF THE D-ALANYL ESTER IN THE RIBITOL TEICHOIC ACID OF STAPHYLOCOCCUS AUREUS

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SUMMARY

Periodate oxidation of the ribitol teichoic acid in cell walls of Staphylococcus aureus 52A2, a bacteriophage-resistant mutant which lacks N-acetylglucosamine on the teichoic acid, demonstrated that D-alanine is esterified to the 2-hydroxyl groups of D-ribitol 5-phosphate residues.

INTRODUCTION

The ribitol teichoic acid of Staphylococcus aureus accounts for about half the weight of the cell walls and consists of a poly(ribitol phosphate) chain substituted with N-acetylglucosamine and D-alanine (1). N-Acetylglucosamine is linked to the 4-hydroxyl group of each D-ribitol 5-phosphate residue and D-alanine is in ester linkage to a hydroxyl group in some ribitol residues (2). The presence of D-alanine protected ribitol residues from periodate oxidation but since its removal by mild alkali hydrolysis rendered all ribitol residues susceptible to periodate oxidation, Baddiley et al. (2) were able to conclude that D-alanine must be linked either to the 2- or 3-hydroxyl group of ribitol. A suggestion that alanine may be linked to glucosamine in S. aureus Copenhagen was based on measurements of periodate consumption and formaldehyde production rather than analysis for ribitol remaining after periodate treatment (3).

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An opportunity to determine which ribitol hydroxyl group is linked to alanine was provided by a mutant of S. aureus H, selected for its resistance to bacteriophage. The mutant, strain 52A2, lacks N-acetylglucosaminyl residues on the teichoic acid (4), and it is presumed to be a one step mutant since phage-sensitive revertants have been obtained containing normal levels of N-acetylglucosamine. The mutant lacks UDP - N -acetylglucosamine-poly-(ribitol phosphate) N-acetylglucosaminyltransferase, whereas the parent strain and revertants contain normal levels of the enzyme (5). D-Alanine was present in the teichoic acid of the mutant (52A2) to the same extent as in that of the parent strain H (4). Using the mutant strain, the exact location of D-alanine in teichoic acid can be determined for the following reason. If the alanine were linked to the 3-hydroxyl group, the ribitols to which alanine is attached would be protected from periodate oxidation, but if the linkages were to the 2-hydroxyl, all ribitol residues would be susceptible to oxidation.

METHODS & MATERIALS

S. aureus H and S. aureus 52A2 (obtained from Dr. A. N. Chatterjee) were grown as described previously (4). Cell walls were prepared according to Sharon and Jeanloz (6) except that the trypsin step was omitted and the pH did not rise above 6.8.

The ester-linked D-alanine was cleaved from teichoic acid by suspending cell walls in 0.1N NaOH for 20 min at 37° (4). The reaction mixture was then neutralized with 1N HCl.

Periodate oxidation of teichoic acid was carried out in 0.1M sodium meta-periodate buffered with 0.02M sodium acetate, pH 4.6. The reaction mixture was kept in the dark at room temperature for 2 hr, after which unreacted periodate was destroyed by adding a ten-fold excess of ethylene glycol.

For the determination of ribitol, teichoic acid was hydrolyzed in 2.0N

NaOH in a stoppered polypropylene tube for 3 hr at 100°. The hydrolysate was deionized with Dowex 50-H^+ and the insoluble material as well as the resin removed by filtering.

The dephosphorylation of ribitol phosphates obtained by alkali hydrolysis was accomplished by incubating the sample overnight at room temperature in 1 ml of 0.2M sodium carbonate buffer, pH 9.6, with 50 μg of E. coli alkaline phosphatase (Sigma) per 2 mg sample of original wall.

For paper chromatography, glucosaminyl ribitol present in phosphatase treated alkali hydrolysates was hydrolyzed in 2N HCl for 2 hr at 100°, giving a mixture of glucosamine, ribitol, and anhydroribitol (7). HCl was removed by evaporation in vacuo over NaOH pellets. The presence of ribitol and anhydroribitol was demonstrated by descending paper chromatography on Whatman No. 1 paper with n-butanol/ethanol/water/conc. ammonia (40:10:49:1 by volume) (organic phase) (8). Ribitol and anhydroribitol were detected by a periodate-benzidine dip (9).

Total and inorganic phosphate were determined by the method of Chen et al. (10).

D-alanine in the neutralized supernatants of mild alkali hydrolysates was determined by the method of Ghuysen et al. (11). Purified D-amino acid oxidase and catalase were from Worthington Biochemicals, Freehold, N.J., and lactic dehydrogenase was from Sigma Chemical Co., St. Louis, Mo.

Ribitol was determined enzymatically in a reaction mixture (0.3 ml.) containing 0.01M NAD, 0.33M sodium carbonate buffer, pH 9.5, and 0.5 units of Aerobacter aerogenes ribitol dehydrogenase (5).

To check on recoveries, standard solutions of alanine, ribitol, and D-ribitol 5-phosphate were treated under the same conditions of hydrolysis, periodate oxidation, etc. In the case of ribitol-phosphate there was a loss of

17% of the ribitol after strong alkali hydrolysis and phosphatase treatment, and ribitol values were corrected by this amount.

TABLE 1

RECOVERY OF TEICHOIC ACID COMPONENTS FROM S. AUREUS H AND S. AUREUS 52A2 CELL WALLS

Teichoic Acid Component	Cell Wall	
	S. aureus H	S. aureus 52A2
Phosphate	1135	985
Ester Linked D-Alanine	460	445
Ribitol	present[a]	780
Ribitol-after periodate oxidation	270[b]	<10[c]
Ribitol-after mild alkali hydrolysis and periodate oxidation	<10[c]	<10[c]

Results are in mμmoles/mg dry cell wall.

(a) In acid hydrolysates of non-oxidized walls, the presence of ribitol and anhydroribitol was confirmed by paper chromatography.

(b) The presence of anhydroribitol and ribitol was detected also by paper chromatography.

(c) No ribitol or anhydroribitol was detected in hydrolysates by paper chromatography.

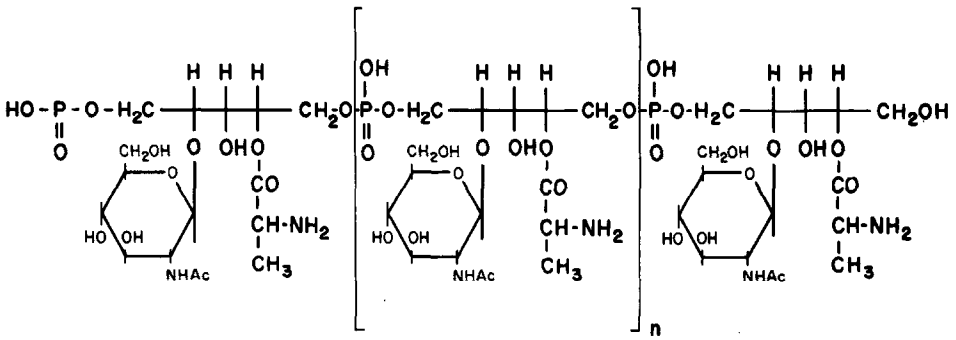
RESULTS AND DISCUSSION

The Table confirms and extends the work of Baddiley et al. (2) on periodate oxidation of S. aureus H teichoic acid. Ribitol residues bearing a D-alanyl ester substituent were protected from oxidation but all ribitol residues were destroyed by periodate after removing alanine with mild alkali hydrolysis.

The low recoveries of ribitol in strain H may be ascribed to the fact that

some fragments of oxidized N-acetylglucosamine still remained attached to the ribitol even after the strong alkali hydrolysis. Another possibility for this low recovery (60% of theoretical) may be that a small proportion of strain H ribitol residues lack N-acetylglucosamine substituents altogether.

The Table also shows that periodate oxidation destroyed all of the ribitol in the ribitol teichoic acid of strain 52A2 cell walls, irrespective of the presence or absence of ester alanine. Therefore, D-alanine is not esterified to the 3-hydroxyl groups of the ribitol residues. It seems unlikely that alanine is on the 4-hydroxyl groups since these are occupied by N-acetylglucosamine residues in the parent strain (2). The mutational change from strain H to strain 52A2 involves only the loss of N-acetylglucosaminyltransferase activity (5). Assuming that the specifity of alanine addition to teichoic acid in the absence of N-acetylglucosamine remains unchanged, it may be concluded that the D-alanyl ester residues are located on the 2-hydroxyl groups of D-ribitol 5-phosphage residues as shown in the accompanying structure.



It is interesting to note that both in strains H and 52A2 periodate oxidation of intact walls solubilized about 90% of the cell wall phosphate. This implies that ribitol residues adjacent to the murein do not possess ester-linked alanine and are susceptible to periodate oxidation and subsequent solubilization of the teichoic acid chains.

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