CHEMICAL AND IMMUNOCHEMICAL STRUCTURE OF TEICHOIC ACID FROM STAPHYLOCOCCUS AUREUS (COPENHAGEN)

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Teichoic acids are polymers of ribitol phosphate or glycerophosphate found in the cell walls of some gram-positive bacteria (1). The ribitol phosphate polymers contain glycosidically linked sugars and esterified D-alanine. That from Bacillus subtilis has been chemically identified as a polymer of 4-O-β-glucosyl-D-ribitol-5-phosphate in which the ribitol units are joined by 1,5-phosphodiester linkages; the position of the esterified D-alanine was not established (2). It has also been observed that a ribitol phosphate polymer from S. aureus H contains both N-acetylglucosamine and D-alanine (1).

In the course of studies of the structure and biosynthesis of the cell wall of S. aureus (Copenhagen), it became of interest to know the structure of the teichoic acid in this strain and its relationship to the other constituents of the wall. The polymer, which accounts for about 40% of the weight of the wall in this strain, was extracted by treatment of purified cell walls with cold trichloroacetic acid, and precipitated with ethanol-acetone. This material (I) contained ratios of ribitol phosphate: acetylglucosamine: D-alanine of 1:0.99:0.49. Extremely little free alanine was liberated during the extraction, and this quantity of D-alanine is the maximum which can be extracted with the polymer. The D-alanine was removed

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from I by treatment with LiOH at pH 11.5 at room temperature for 4 hours. The alanine-free teichoic acid (II) was separated from D-alanine by a column of Sephadex G-25. Treatment of I with 1N NaOH at 100° for 3 hours converted it to glucosaminyl-ribitol phosphates which were separated from other minor products of alkaline hydrolysis by chromatography on Dowex 1-chloride using gradient elution. The monomers were selectively N-acetylated with acetic anhydride in bicarbonate buffer to yield N-acetylglucosaminyl-ribitol phosphates (III), or they were dephosphorylated with semen phosphomonoesterase and then N-acetylated to yield N-acetylglucosaminyl-ribitol (IV). Periodate oxidations of I, II, and IV with measurements of periodate consumption and formaldehyde formation (Table I) have established that the polymer is analagous to that found in B. subtilis, being a polymer predominantly (cf. below) of 4(2)-O-β-acetylglucosaminyl-D-ribitol-5-phosphate in which the phosphodiester bridge is 1,5[1]. The β-linkage of the acetylglucosamine was established by hydrolysis of III and IV and, at a slower rate, of I and II by a purified β-acetylglucosaminidase from pig epididymis (4) (cf. also below). If the assumption is made that the D-alanine ester is stable under the conditions of periodate oxidation at pH 4.5 and 0°, these data also suggest that the D-alanine residues are esterified on the 6-position of statistically every other acetylglucosamine residue, since periodate consumptions of I and II were identical in rate and extent and both ribitol and acetylglucosamine were destroyed during periodate oxidation of II.

Concurrently with these studies of the structure of the polymer, investigation of the immunologically determinant groups in the cell wall of this organism had been undertaken (5). Rabbit antisera were prepared which agglutinated cell walls of this strain (but not those of another strain of S. aureus in use in the laboratory). The agglutination was specifically inhibited by I, II, III or IV, but not by ribitol phosphate or by any other

[1] A similar conclusion regarding the structure of the teichoic acid from another strain, S. aureus H, has been reached independently (3).

Table I

Periodate Oxidation of Various Products

Data are expressed as moles per mole of phosphate or amino sugar (in a repeating unit in the case of polymers)

Compound	Periodate Consumed		HCHO formed	
	Exper.	Theor.	Exper.	Theor.
Teichoic acid (I)	1.93	2.06*	0.06	**
Alanine-free teichoic acid (II)	2.00	2.06*	0.06	**
II after hydrolysis with β-acetylglucosaminidase (V)	2.26	2.27*	0.13	**
Acetylglucosaminyl-ribitol (IV)	3.00	3.00	1.1	1.0

* These values are greater than 2.0 because of an extra contribution from end-group ribitol units.

** Formaldehyde is only formed from the ribitol units on the non-phosphorylated end of the polymer. In the case of I and II the reciprocal of the amount formed, is, therefore, an estimate of the number of repeating units in the polymer.

component or degradation product of the cell wall. Acetylglucosamine and α-phenyl-acetylglucosamine (but not acetylgalactosamine, β-phenyl-acetylglucosaminide or β-1,4-acetylglucosaminyl-acetylglucosamine) would also specifically inhibit the agglutination. The immunochemical data, therefore, suggested that the acetylglucosamine linkages in the teichoic acid antigen were α, when enzymatic experiments had suggested they were β(5).

This paradox has been resolved by the following experiments. Careful measurement of hydrolysis of II by β-acetylglucosaminidase indicated that the reaction stopped at liberation of about 85% of the acetylglucosamine. The resultant polymer (V) was separated on a column of Sephadex G-25. It contained a ratio of ribitol phosphate: acetylglucosamine of 1:0.12. Treatment of V with a large amount of β-acetylglucosaminidase from pig epididymis liberated no further acetylamino sugar, but on treatment with an extract of rat epididymis (which contains an α- as well as a β-acetyl-

glucosaminidase (6)) acetylglucosamine was slowly liberated.[2] V was, furthermore, far more active than I, II, III, IV or any other compound as a specific inhibitor of agglutination (Table II).[3]

It appears, therefore, that the immunologically determinant group in the cell wall of this strain of S. aureus is an α-N-acetylglucosaminyl-ribitol in the ribitol phosphate polymer, possibly located at the non-phosphorylated end of the polymer. The β-acetylglucosaminyl substituents also found in this polymer appear to be immunologically inactive, at least in rabbits. The teichoic acids represent a new class of bacterial antigens and examination of the possibility that they are important immunological determinants in other serologically distinct strains of S. aureus, as well in other bacterial species,[4] will be of great interest.

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[2] Strong alkaline hydrolysis of teichoic acid (I) yields glucosaminyl-ribitol from the non-phosphorylated end of the chain and glucosaminyl-ribitol phosphates from the remainder of the polymer. These are readily separated with Dowex 1. When III (the N-acetylation product of the glucosaminyl-ribitol phosphates obtained in this manner from I) was treated with β-acetylglucosaminidase, 93-95% of the acetylglucosamine was removed. The monomer (III), therefore, contained about 1 α- to 14-20 β-linked N-acetylglucosamines as compared to the ratio, 1 to 7, found in the polymer (I). A large percentage of the α-N-acetylglucosaminyl-ribitols might, therefore, be located on the non-phosphorylated end of the polymer. Alkaline degradation of V led to recovery of 80% of the N-acetylamino sugar as glucosaminyl-ribitol, the remainder being recovered as phosphorylated products. This result, however, does not show unambiguously that α-acetyl-glucosaminyl-ribitol occurs predominantly at the end of the polymer chain, since non-phosphorylated products might be obtained from internal positions in V, particularly if the N-acetylglucosamine residues remaining in the polymer (V) had a directing effect on the course of alkaline hydrolysis.

[3] Cell walls of S. aureus (Copenhagen) were also agglutinated by antiserum to Group A streptococcal carbohydrate (kindly provided by Dr. M. McCarty (7)). This heterologous agglutination was inhibited by I, II and III and by β-phenyl-acetylglucosaminide, but not by V or by α-phenyl-acetylglucosaminide. The heterologous agglutination is presumably due to the β-acetylglucosaminide residues, as is the homologous reaction of Group A carbohydrate with its own antiserum (7).

[4] The possibility that teichoic acids might be antigenic in Lactobocilli has, for example, been suggested (8).

Table II

Agglutination of Cell Walls by Rabbit Antiserum

Haptenes indicated below were added to 0.04 ml. rabbit antiserum (1:40 dilution in normal saline phosphate buffer, pH 7.4) and incubated for 1 hr. at 37°. Then, 0.03 mg. purified cell wall in 0.02 ml. was added to each tube. Agglutination was followed at room temperature. Tubes were graded as follows: 0 = homogeneous milky suspension; 1+ = granular milky suspension; 2+ = marked granularity; 3+ = cell walls clumped and partially settled; 4+ = completely clumped and settled, supernatant clear.

Additions	Final Conc., M	Degree of agglutination 5 min.	1 hr.	2 hrs.	24 hrs.
None	-	4+	4+	4+	4+
Acetylglucosamine	0.01	0	0	1+	3+
α-phenyl-acetylglucosaminide	0.0025	0	0	1+	3+
β-phenyl-acetylglucosaminide	0.0025	4+	4+	4+	4+
Teichoic acid (I)	0.0013	0	0	1+	3+
Alanine-free teichoic acid (II)	0.0008	0	0	1+	3+
II after treatment with β-acetylglucosaminidase (V)	0.0003	0	0	0	0
Acetylglucosaminyl-ribitol phosphates (III)	0.003	0	0	2+	4+
Acetylglucosaminyl-ribitol (IV)	0.003	0	0	2+	4+
D-ribitol-5-phosphate	0.01	4+	4+	4+	4+
Normal rabbit serum substituted for antiserum	-	0	0	0	0

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