The Distribution of Teichoic Acids in Staphylococci

By A. L. DAVISON AND J. BADDILEY

Department of Chemistry, King's College, Newcastle upon Tyne

(Received 4 February 1963)

SUMMARY

Wall and intracellular teichoic acids were prepared from several strains of staphylococci. Intracellular glycerol teichoic acids were found in all the cases examined but their detailed chemical composition was not determined. The presence and structure of wall teichoic acids is characteristic of the different species; thus, strains of *Staphylococcus aureus* all possess a teichoic acid containing glucosamine in their walls; this was shown previously to be indistinguishable from the group-specific antigen. Similarly, *S. saprophyticus* strains contain in their walls a glycerol teichoic acid with glucosyl substituents probably identical to the group-specific precipitinogen, polysaccharide B. The species *S. lactis* is heterogeneous: three groups are distinguishable, one without wall teichoic acid and the others with a teichoic acid containing glucosamine or galactosamine.

INTRODUCTION

The teichoic acids are widely distributed in Gram-positive bacteria (Armstrong *et al.* 1958, 1959; Baddiley, 1961). An investigation of these glycerol phosphate or ribitol phosphate polymers containing alanine ester and sugar residues in *Lactobacillus* species indicated that those in the cell walls are of taxonomic significance (Baddiley & Davison, 1961). Moreover, the presence and structure of teichoic acids have been correlated with the serological behaviour of lactobacilli, and it has been shown that these compounds are indistinguishable from the group antigens in many cases (Baddiley & Davison, 1961; and unpublished work with Dr M. Elizabeth Sharpe). Similarly, in group D streptococci the group antigen is serologically identical with the intracellular teichoic acid, and differences in the serological behaviour of group D antigen preparations from several strains of organism have been attributed to small differences in their chemical structure (Wicken, Elliott & Baddiley, 1963).

In staphylococci the wall teichoic acids have also been associated with serological reactions. That in the walls of *Staphylococcus aureus* strain H is a ribitol phosphate polymer to which are attached D-alanine ester and N-acetylglucosaminyl residues, the latter being in predominantly β linkage (Baddiley, Buchanan, RajBhandary & Sanderson, 1962*a*; Baddiley, Buchanan, Martin & RajBhandary, 1962*b*). This teichoic acid is serologically identical with the group-specific polysaccharide A of this organism (Haukenes, Ellwood, Baddiley & Oeding, 1961; Haukenes, 1962). *S. aureus* strain Copenhagen possesses a chemically similar wall teichoic acid which contains a higher proportion of α glycosidic linkages, and serological differences between these compounds may be related to the chemical differences (Natherson & Strominger, 1962). Although serological specificity is associated with the glycerol

teichoic acid from the walls of *S. lactis* (*S. albus*) NCTC 7944 (Haukenes *et al.* 1961), in which the sugar is *N*-acetylgalactosamine (Ellwood, Kelemen & Baddiley, 1963), no teichoic acid has been found in certain other staphylococci, e.g. *S. afermentans* (*Micrococcus lysodeikticus*) and *S. roseus* (Salton & Pavlik, 1960; Armstrong, unpublished work). The present work was undertaken in an attempt to correlate the taxonomy of staphylococci with the presence and composition of teichoic acids in their walls, and as a preliminary study connected with their serological classification.

METHODS

The sources of bacteria, other than those from the National Collection, are given in Table 1. Freshly isolated strains were classified according to the method of Shaw, Stitt & Cowan (1951), and strains of *Staphylococcus aureus* were classified by the bacteriophage-type procedure in the Department of Bacteriology, Royal Victoria Infirmary, Newcastle upon Tyne.

Bacteria were grown in batches (15 l.) with forced aeration at 37° for 16 hr. in a liquid medium of the following composition: nutrient broth no. 2 (Oxoid), 25 g.; glucose, 10 g.; yeast extract (Oxoid), 5 g.; dipotassium hydrogen phosphate, 3 g.; olive oil, 0.3 ml.; water (demineralized), 1000 ml. Cocci were harvested in a Sharples refrigerated centrifuge and washed with cold 0.85 % (w/v) sodium chloride solution. Strains 15379, 15499 and 17261, which had been isolated from patients with staphylococcal infections, were grown in screw-capped bottles on solid medium of the same composition + agar for 16 hr. at 37°, killed with formol-saline and the cocci collected in a closed refrigerated centrifuge.

Coccal walls were prepared by mechanical disruption with glass beads (Dawson, 1949; Salton & Horne, 1951); cocci were suspended in cold water (18–20 g. wet wt. in 100 ml. water) and shaken with no. 11 Ballotini beads in an International centrifuge with shaker head (Shockmann, Kolb & Toennies, 1957) at 1250 rev./min. for 45 min. at –8°. Beads were removed by filtering with a no. 1 sintered glass funnel, and the filtrate was centrifuged at 25,000g for 30 min. The cloudy supernatant fluid was separated from sedimented walls and a few unbroken cocci, and then centrifuged at 100,000g in a Spinco model L ultracentrifuge for 1 hr. The sedimented gel contained ribosomal material and intracellular teichoic acid; it was preserved by freeze-drying. The wall fraction was washed by centrifugation six times with 5 vol. of cold M-phosphate buffer (pH 7.0) and six times with cold water; material was recovered each time at 25,000g for 30 min., the wall fraction being carefully separated from any lower layer of unbroken cocci. Walls recovered by freeze-drying were homogeneous and of clean appearance under the electron microscope.

Teichoic acid was extracted from walls (0.5 g.) with 10 % (w/v) trichloroacetic acid (30 ml.) for 4 days at 2°. After centrifugation, the material was precipitated from the solution with 5 vol. cold ethanol, collected after 24 hr. at 2°, redissolved in 10 % trichloroacetic acid solution and precipitated as before. The precipitate was washed with acetone, ethanol and ether, then dried *in vacuo* (yield 10–15 % of the dry wt. of the walls.) A similar isolation procedure was adopted for the teichoic acid in the ribosomal gel (cf. Baddiley & Davison, 1961).

The chemical composition of teichoic acids was determined by hydrolysis with 2N-hydrochloric acid or 2N-sodium hydroxide solution for 3 hr. at 100° and exami-

nation of hydrolysates by several paper-chromatographic procedures described in detail by Armstrong *et al.* (1958, 1959), Baddiley *et al.* (1962*a*) and Ellwood *et al.* (1963); sugars and amino sugars were separated using a mixture of pyridine + ethyl acetate + aq. acetic acid (Fischer & Nebel, 1956). Glucosylglycerol was characterized by hydrolysis with a β -glucosidase (see Armstrong *et al.* 1959), glucosaminyl-ribitol by dephosphorylation with intestinal phosphomonoesterase and chromatographic comparison of the product with authentic glucosaminylribitol (Baddiley *et al.* 1962, *a, b*), and the saccharinic acid by its similarity on paper chromatograms with that obtained by heating 3-*O*-methylglucose (provided by D. A. Applegarth) in alkali under comparable conditions. Alanine ester residues were detected by reaction with aqueous ammonia and chromatography of the resulting alanine and its amide.

RESULTS

The composition of teichoic acids from walls and cell contents of different staphylococci is given in Table 1. Intracellular teichoic acids were examined with respect to glycerol and its phosphates but not sugars. The hospital strains of *Staphylococcus aureus* were not examined for the presence of intracellular compounds and alkali hydrolysis of their wall teichoic acids was not attempted because of shortage of material.

Table 1. *Intracellular and wall teichoic acids in staphylococci*

		Teichoic acid		Composition of wall teichoic acid	
		Intra-cellular	Wall	Sugar	Hydrolysis product
<i>Staphylococcus aureus</i>					
H	Dr J. T. Park, 52/52A/79/80, group 1	G	R	Glucosamine	Glucosaminylribitol
I 5379	Hospital isolate, 3B/55/71, group 2	—	R	Glucosamine	—
I 5499	Hospital isolate, 75/77, group 3	—	R	Glucosamine	—
A 1	Animal strain, 42D, group 4	G	R	Glucosamine	Glucosaminylribitol
<i>Staphylococcus saprophyticus</i>					
NCTC 7292	—	G	R	Glucosamine	Glucosaminylribitol
NCTC 7291	—	G	G	Glucose	Glucosylglycerol
C 1	Professor M. R. J. Salton (received as <i>S. citreus</i>)	G	G	Glucose	Glucosylglycerol
I 2	Fresh isolate from animal gland	G	G	Glucose	Glucosylglycerol
I 7261	Fresh isolate from hospital (pathogenic)	G	G	Glucose	—
NCTC 7617	—	G	G*	Galactos-amine	Galactosaminyl-glycerol
I 3	Fresh isolate from animal gland	G	G†	Glucosamine	Saccharinic acid
I 4	Fresh isolate from milk	G	G	Galactos-amine	Galactosaminyl-glycerol
NCTC 7564	—	G	—	—	—
NCTC 7567	—	G	—	—	—

* No alanine detected; † no glycerol on acid or alkali hydrolysis; G = glycerol teichoic acid; R = ribitol teichoic acid.

DISCUSSION

The taxonomic and serological significance of the intracellular teichoic acids in staphylococci is not known. Like all others found in underlying regions of bacterial cells, they are glycerol phosphate polymeric derivatives, but in only one case has detailed structural work been carried out; that from *Staphylococcus aureus* strain H is a 1,3-polymer of glycerol phosphate with D-alanine ester residues at position 2 on most glycerol units and a small number of gentiobiosyl (6-O- β -D-glucopyranosyl-D-glucosyl) and N-acetylglucosaminyl residues situated at position 2 (RajBhandary & Baddiley, 1963). All the coagulase-positive and coagulase-negative staphylococci contained intracellular teichoic acid, again illustrating the widespread occurrence of these compounds in Gram-positive bacteria (Baddiley & Davison, 1961; Baddiley, 1961). All accompanied the ribosomal RNA on centrifugation at 100,000g, a property which has been utilized in the isolation of these substances from many different bacteria (Baddiley & Davison, 1961; Critchley *et al.* 1962; Wicken & Baddiley, 1963).

The composition of teichoic acids in the walls of staphylococci may be used in the classification of these bacteria, a problem which often presents difficulty (see Hill, 1959). Shaw *et al.* (1951) assigned all coagulase-positive members to the species *Staphylococcus aureus*, but separated fermentative coagulase-negative members (*S. albus*) into two species *S. saprophyticus* and *S. lactis*, according to their behaviour in the Voges-Proskauer test. The composition of wall teichoic acids in staphylococci (see Table 1) supports this general classification into three species, but indicates that the species *S. lactis* is heterogeneous. Yellow staphylococci (micrococci) of Gibson's groups 3A and 3B (Abd-el-Malek & Gibson, 1948) which contain no teichoic acid in their walls could be distinguished from those strains of *S. lactis* with a wall teichoic acid. Moreover, two distinct types of *S. lactis* containing wall teichoic acids were observed: strains isolated from milk (14, NCTC 7617) had a glycerol teichoic acid containing galactosamine, whereas a strain from an infected animal gland, which probably corresponded to *Gaffkya tetragena*, had a glycerol teichoic acid containing glucosamine. This last teichoic acid probably differs structurally from the others, since it yields a compound resembling a saccharinic acid on alkali hydrolysis and no glycerol is produced under these conditions.

Strains of *Staphylococcus saprophyticus* were clearly distinguishable from those of *S. aureus* and *S. lactis* since they possess a glycerol wall teichoic acid containing glucose. The chemical similarity between this teichoic acid and an unidentified phosphoric ester containing glucose (polysaccharide B) isolated by Wieghard & Julianelle (1935) from a strain of *S. albus* suggests that the group-specific precipitinogen, polysaccharide B, is the wall teichoic acid; serological studies are in progress to examine this point. In this connexion, Morse (1962) has differentiated the wall teichoic acids of *S. aureus* and *S. albus* by haemagglutination, and has shown that the wall teichoic acid from a strain of *S. albus* is a glycerol phosphate polymer containing glucose.

The type strain of *Staphylococcus saprophyticus* (NCTC 7292) proposed by Shaw *et al.* (1951) possesses a ribitol teichoic acid containing glucosamine, apparently identical or very similar to that from the walls of species of *S. aureus* representing the four main bacteriophage groups. We conclude that strain 7292 is atypical,

whereas the freshly isolated pathogenic strain of *S. saprophyticus* (17261) contained the characteristic glycerol wall teichoic acid with glucosyl substituents as found in other members of the species. Thus, knowledge, of the composition of wall teichoic acids in staphylococci is valuable for the accurate classification of this important group of organisms.

We thank the staff of the Department of Bacteriology, The Royal Victoria Infirmary, Newcastle upon Tyne, for cultures and bacteriophage typing, and Dr J. T. Park and Professor M. R. J. Salton for cultures. The work was supported by grants from the Department of Scientific and Industrial Research, the Medical Research Council and the Nuffield Foundation.

REFERENCES

- ABD-EL-MALEK, Y. & GIBSON, T. (1948). Studies in the bacteriology of milk. II. The staphylococci and micrococci of milk. *J. Dairy Res.* **15**, 249.
- ARMSTRONG, J. J., BADDILEY, J., BUCHANAN, J. G., CARSS, B. & GREENBERG, G. R. (1958). Isolation and structure of ribitol phosphate derivatives (teichoic acids) from bacterial walls. *J. chem. Soc.* p. 4344.
- ARMSTRONG, J. J., BADDILEY, J., BUCHANAN, J. G., DAVISON, A. L., KELEMEN, M. V. & NEUHAUS, F. C. (1959). Composition of teichoic acids from a number of bacterial walls. *Nature, Lond.* **184**, 247.
- BADDILEY, J. (1961). *Immunological Approaches to Problems in Microbiology*, p. 91. Ed. by M. Heidelberger, O. J. Plesica and R. A. Day. New York: Rutgers University Press.
- BADDILEY, J. & DAVISON, A. L. (1961). The occurrence and location of teichoic acids in lactobacilli. *J. gen. Microbiol.* **24**, 295.
- BADDILEY, J., BUCHANAN, J. G., RAJBHANDARY, U. L. & SANDERSON, A. R. (1962*a*). Teichoic acid from the walls of *Staphylococcus aureus* H. *Biochem. J.* **82**, 439.
- BADDILEY, J., BUCHANAN, J. G., MARTIN, R. O. & RAJBHANDARY, U. L. (1962*b*). Teichoic acid from the walls of *Staphylococcus aureus* H. *Biochem. J.* **85**, 49.
- CRITCHLEY, P., ARCHIBALD, A. R. & BADDILEY, J. (1962). The intracellular teichoic acid from *Lactobacillus arabinosus* 17-5. *Biochem. J.* **85**, 420.
- DAWSON, I. M. (1949). In *The Nature of the Bacterial Surface. Symp. Soc. gen. Microbiol.* **1**, 119.
- ELLWOOD, D. C., KELEMEN, M. V. & BADDILEY, J. (1963). The glycerol teichoic acid from the walls of *Staphylococcus albus* NCTC 7944. *Biochem. J.* **86**, 213.
- FISCHER, F. G. & NEBEL, H. J. (1956). Detection and estimation of glucosamine and galactosamine on paper chromatograms. *Anal. Abstr.* **3**, 1101.
- HAUKENES, G., ELLWOOD, D. C., BADDILEY, J. & OEDING, P. (1961). Serological cross-reactivity between polysaccharide A and teichoic acid of *Staphylococcus aureus*. *Biochim. biophys. Acta*, **53**, 425.
- HAUKENES, G. (1962). Immunochemical studies on polysaccharide A of *Staphylococcus aureus*. *Acta path. microbiol. scand.* **55**, 463.
- HILL, L. R. (1959). The Adansonian classification of the staphylococci. *J. gen. Microbiol.* **20**, 277.
- MORSE, S. I. (1962). Studies on the chemistry and immunochemistry of cell walls of *Staphylococcus aureus*. *J. exp. Med.* **116**, 229.
- NATHERSON, S. G. & STROMINGER, J. L. (1962). Enzymatic synthesis and immunochemistry of N-acetylglucosaminylribitol linkages in teichoic acids of *Staphylococcus aureus* strains. *J. biol. Chem.* **237**, P.C. 3839.
- RAJBHANDARY, U. L. & BADDILEY, J. (1963). The intracellular teichoic acid from *Staphylococcus aureus* H. *Biochem. J.* **87**, 429.
- SALTON, M. R. J. & HORNE, R. W. (1951). Methods of preparation and some properties of cell walls. *Biochim. biophys. Acta*, **7**, 177.

- SALTON, M. R. J. & PAVLIK, J. G. (1960). Studies of the bacterial cell wall. VI. Wall composition and sensitivity to lysozyme. *Biochim. biophys. Acta*, **39**, 398.
- SHAW, C., STITT, J. M. & COWAN, S. T. (1951). Staphylococci and their classification. *J. gen. Microbiol.* **5**, 1010.
- SHOCKMAN, G. D., KOLB, J. J. & TOENNIES, G. (1957). A high speed shaker for the disruption of cells at low temperature. *Biochim. biophys. Acta*, **24**, 203.
- WICKEN, A. J. & BADDILEY, J. (1963). Structure of intracellular teichoic acids from group D streptococci. *Biochem. J.* **87**, 54.
- WICKEN, A. J., ELLIOTT, S. D. & BADDILEY, J. (1963). The identity of streptococcal group D antigen with teichoic acid. *J. gen. Microbiol.* **31**, 231.
- WIEGHARD, L. A. & JULIANELLE, C. W. (1935). The immunological specificity of staphylococci. *J. exp. Med.* **62**, 23.