ISOLATION AND PROPERTIES OF A GROUP ANTIGEN OF STAPHYLOCOCCUS ALBUS*

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Recently a polymer of ribitol phosphate and acetyl glucosamine has been shown to be a group antigen of *Staphylococcus aureus* (1, 2). The antigen, isolated from *S. aureus* cell walls, belongs to the group of polyol phosphate compounds termed teichoic acids (3).

This report describes the isolation and properties of a group antigen of *Staphylococcus albus* (*Staphylococcus epidermidis*) which is also a teichoic acid. The antigen, prepared from acid extracts of *S. albus* cell walls, is composed of glucose and glycerophosphate.

Material and Methods

Staphylococcus albus Strains.—The prototype strain, *S. albus* Prengel, as well as strains Greaves and Mendita were originally isolated from human beings; *S. albus* Air was an isolate from room air (4). The organisms were maintained on penassay agar (Difco) and passed at frequent intervals.

Preparation of Cell Walls.—Organisms were grown in the casamino acids (Difco)-yeast extract medium previously described (2). After 18 hours' growth at 37°C the organisms were harvested by centrifugation, washed with distilled water, and acetone-dried. Cell walls were prepared in a manner identical with that used for the preparation of *S. aureus* cell walls (2). However, treatment with pepsin was omitted.

Chemical Analyses.—Nitrogen and phosphorus were determined by the methods of Koch and McMeekin (5) and Fiske and Subbarow (6) respectively. Hexosamine was assayed by the Elson-Morgan reaction (7), and glucose by a glucose-oxidase method (glucostat, Worthington) after heating samples in 2 N HCl at 100°C in sealed ampules for 4 hours. Amino acids were determined by the procedure of Mandelstam and Rogers (8).

Descending paper chromatography was carried out for 24 to 48 hours at room temperature on Whatman No. 1 paper. Hydrolysates for qualitative paper chromatographic analyses were prepared by heating samples in 2 N H_2SO_4 at 100°C for varying time periods. The hydrolysates were brought to pH 5.5 with $Ba(OH)_2$ and the resulting precipitate of $BaSO_4$ removed by centrifugation. The supernatant fluids were concentrated to dryness *in vacuo* at 25°C, and the dried material was dissolved in a small amount of water. Chromatographic analyses of the $BaSO_4$ precipitates dissolved in dilute mineral acid revealed that unrecognized compounds were not removed during the neutralization procedure. Standard substances were treated in the same way as the unknown materials.

N-Butanol–acetic acid–water (4:1:5) was the solvent system employed for separation of

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sugars, and isopropanol–formic acid–water (7:1:2) was used for the separation of sugar phosphates. The use of other solvent systems did not reveal the presence of compounds undetected in these systems. Silver nitrate was used for the detection of sugars (9). Phosphorus compounds were identified by spraying with the perchloric acid–ammonium molybdate reagent (10) and then exposing the paper to ultraviolet light for several minutes.

Serologic Techniques.—Rabbit antisera were prepared by repeated intravenous injections of suspensions of heat- or phenol-killed organisms. Qualitative precipitin tests were performed in capillary tubes and occasionally by the ring test. Gel diffusion studies were performed on standard microscope slides coated with 2 per cent agar in saline.

Quantitative precipitin tests were performed in the following manner. 0.2 or 0.25 ml of rabbit antiserum was mixed with various quantities of antigen dissolved in saline in a total volume of 2.0 ml. The tubes were kept at 37°C for 30 minutes and then at 4°C for 18 hours. The precipitates were centrifuged down, washed three times in chilled saline, and then dissolved in 0.1 N NaOH. Protein was determined on aliquots by the method of Lowry *et al.* (11).

Hapten inhibition studies were carried out by preincubating antiserum with hapten for 45 minutes at 37°C. Antigen at equivalence was added and the quantitative precipitin test performed in the usual manner.

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RESULTS

Preparation and Properties of the S. albus Antigen.—Although antigen could be extracted from cell walls of *S. albus* by cold 10 per cent trichloroacetic acid (TCA), brief extraction at 100°C with dilute HCl proved to be a more efficient process. The material isolated was fully as reactive as that prepared from cold TCA extracts.

3.4 gm of *S. albus* Prengel cell walls were suspended in 180 ml of distilled water and the pH brought to 2.0 with 1 N HCl. The suspension was heated at 100°C for 20 minutes with vigorous stirring, cooled, and the pH adjusted to approximately 6.0 by the addition of 1 N NaOH in the presence of phenol red. The residual cell walls were removed by centrifugation, and extracted twice more.

Two volumes of acetone were added to each of the extracts and the precipitates which formed after standing for 24 hours at 4°C were harvested by centrifugation, washed twice with cold acetone, and dried *in vacuo* at room temperature.

Although serologically reactive material of similar chemical composition was obtained following each extraction, the bulk (84 per cent) was isolated from the initial pH 2.0 extract (Table I). Therefore, antigen recovered from one extraction procedure was utilized for further studies.

The antigen was readily soluble in water and saline yielding clear, colorless solutions without appreciable viscosity. It did not pass through standard cellophane tubing.

Chemical Composition of the Antigen.—As indicated in Table II, hexosamine and five amino acids accounted for approximately 8 per cent of the antigen

suggesting slight contamination with cell wall mucopeptide. The major portion of the compound was composed of glycerophosphate and glucose; the ratio *P*:glucose was 1.4:1.

All of the glucose was released from the antigen after 2 hours of hydrolysis in 2 N HCl. Oligoglucosides were not detected by paper chromatographic analyses of samples hydrolyzed for 10, 30, and 60 minutes in 2 N H_2SO_4.

A strongly staining phosphate ester with the mobility of glycerophosphate in several solvent systems was observed in chromatographic studies of samples heated in 2 N H_2SO_4 for 4 hours. This material was isolated after separation on paper. Treatment with acid phosphatase (wheat germ phosphatase, Worthington) resulted in the formation of inorganic phosphorus and a compound which moved with glycerol in several solvent systems.

TABLE I

Composition and Serologic Reactivity of Material Isolated from Cell Walls of S. Albus Prengel

Extraction*	Antigen recovered		Per cent composition				Precipitate with Prengel antiserum		
	Weight	Per cent of total	Phosphorus	Nitrogen	Moisture	Ash	100 μg/ml	10 μg/ml	1 μg/ml
	mg								
1	590	84.2	7.1	1.0	7.0	28.5	++++	+++	+
2	74	10.6	7.6	0.9	7.0	30.7	++++	+++	+
3	37	5.2	7.1	1.1	6.6	33.1	++++	++	±

* Starting material = 3.4 gm of *S. albus* Prengel cell walls. Extractions at pH 2.0 at 100°C × 20 minutes.

A second, slower moving, weakly staining phosphate ester was also present. Treatment of this component with acid phosphatase resulted in the release of inorganic phosphorus, glycerol, and a small amount of glycerophosphate. The effects of phosphatase and its mobility suggested that this phosphate ester was glycerodiphosphate (12).

Under the hydrolytic conditions employed, only small amounts of free phosphorus and glycerol were liberated.

Free glucose was not released from the intact antigen by the action of either alpha or beta glucosidases, nor could enzyme-susceptible units be prepared by heating the antigen in alkali. Less than 0.5 per cent of the phosphorus was released from the antigen by the action of acid phosphatase.

Serologic Reactivity of the Antigen.—The antigen isolated from *S. albus* Prengel reacted at low dilutions with both homologous and heterologous *S. albus* antisera. Representative quantitative precipitin curves are depicted in Fig. 1. The antigen did not react with *S. aureus* antiserum nor with ten normal rabbit serum. Six adult human sera were also unreactive.

Because of the presence of glycerophosphate in both antigens, it was of interest to examine the possibility of a cross-reaction between the *S. albus* antigen and streptococcal polyglycerophosphate.

As shown in Table III, streptococcal antiserum which reacted with 0.001 mg

TABLE II

Chemical Constituents of the Cell Wall Antigen of S. albus Prengel

	per cent		*per cent*
Glucose	28.9	Non-phosphorus ash	21.4
Glycerophosphate*	38.8	Moisture	7.0
Hexosamine	3.1	Amino acids	4.7

* Estimated on the basis of 7.1 per cent phosphorus found.

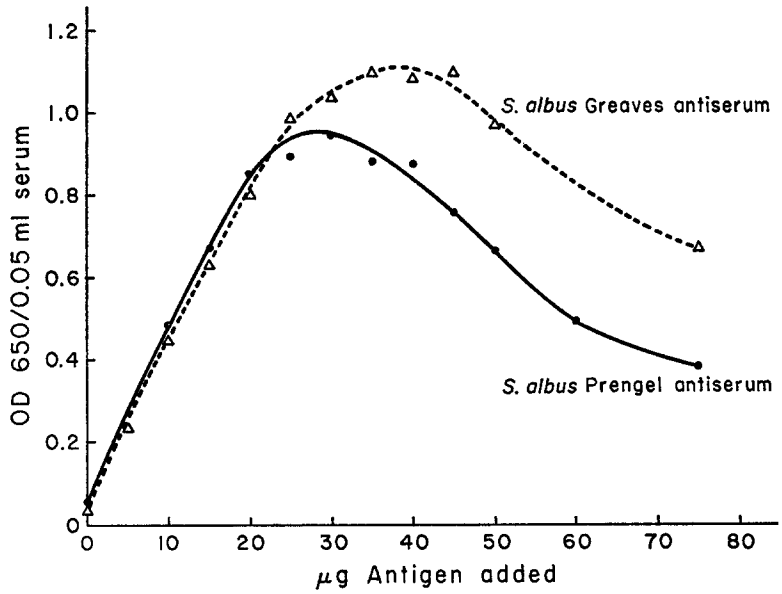

FIG. 1.—Quantitative precipitin reactions between *S. albus* Prengel and *S. albus* Greaves antisera and the cell wall antigen of *S. albus* Prengel.

of streptococcal polyglycerophosphate reacted only minimally with 1000 × that amount of the *S. albus* antigen. This result indicated the absence of significant numbers of glycerophosphate determinants in the *albus* antigen obtained from Prengel cell walls. Antiserum prepared against intact *S. albus* Prengel organisms reacted weakly with streptococcal polyglycerophosphate. This observation conformed with the knowledge that immunologically reactive polyglycerophosphate is present in *S. albus*, but not in the cell wall (13).

The *S. albus* antigen did not show significant cross-reactions with the Group D streptococcal antigen which also contains glucose and glycerophosphate (14).

Extraction of Antigen from Differing S. albus Strains.—Because of variability

TABLE III

Cross-Reactions between S. albus Prengel Antigen and Polyglycerophosphate

Antiserum	Antigen	Antigen concentration, *mg/ml saline*			
		1	0.1	0.01	0.001
Group A streptococcus	Polyglycerophosphate	++++	++++	+++	+
" " "	*S. albus* Prengel	Trace	0	0	0
S. albus Prengel	Polyglycerophosphate	Trace	+	+	Trace
" "	*S. albus* Prengel	+++	+++	++	+

Ring tests recorded at 2 hours.

TABLE IV

Studies on the Inhibition of the Reaction between S. albus Prengel Antigen and Antiserum

Inhibitor	Per cent inhibition	
	μ moles added	
	10	25
D-Glucose	17.4	25.2
D-Mannose	4.6	7.4
D-Galactose	5.3	5.3
Glycerol	3.0	3.7
α-Methyl-D-glucopyranoside	27.8	33.0
β-Methyl-D-glucopyranoside	1.2	9.7
α-Methyl-D-mannopyranoside	6.1	8.0
Maltose	27.1	30.4
Cellobiose	3.4	6.0
α-Glycerophosphate-Na_2	12.4	25.0
β-Glycerophosphate-Na_2	14.0	22.4
β-Phosphoglyceric acid-Na_2	—	17.6
Glucose-6-phosphate-Na_2	12.3	20.8
Fructose-6-phosphate-Na_2	18.7	19.7
Mannose-6-phosphate-Na_2	9.9	22.3
Glucose-1-phosphate-Na_2	25.3	38.5
Polyglycerophosphate	14.6*	—

* 5 mg of synthetic Ca polyglycerophosphate with average of 6 chain units added.

in the production of antiserum directed against the *S. albus* Prengel compound, further evidence for the group nature of the antigen was obtained by examining material isolated from cell walls of other *S. albus* strains.

Cell walls of *S. albus* strains Mendita, Greaves, and Air were extracted at

pH 2 and 100°C for 20 minutes. After removal of the residual walls by centrifugation, 2 volumes of acetone were added to the supernatant fluids and the resulting precipitates were washed with acetone and dried *in vacuo*.

Qualitative analyses of acid hydrolysates of the substances isolated from the cell walls of these three strains of *S. albus* revealed the presence of both glycerophosphate and glucose in each. In gel diffusion studies, all formed a precipitate band when tested with Prengel antiserum. Furthermore these bands formed reactions of identity with each other and with the Prengel antigen.

Studies on the Antigenic Determinant of the S. albus Antigen.—Numerous monosaccharides, oligosaccharides, and phosphate esters were tested for the capacity to inhibit the reaction between the *S. albus* Prengel antigen and antiserum at equivalence.

As indicated in Table IV, D-glucose was a more potent inhibitor of the precipitin reaction than any of the other monosaccharides tested. Moreover, the alpha glucosides, maltose and α-methyl-D-glucopyranoside, were more inhibitory than the beta analogs, cellobiose, and β-methyl-D-glucopyranoside.

Although both alpha and beta glycerophosphate surpressed the precipitin reaction, synthetic polyglycerophosphate was not as effective as might be predicted were glycerophosphate an antigenic determinant (13). This suggested that the inhibition was a non-specific effect of the phosphate group (15). The supposition was borne out by the inhibitory effect of a variety of sugar phosphates (Table IV). The most effective compound of the group was glucose-1-phosphate, which is an alpha glucoside of phosphate.

The results presented in Table IV which suggested that alpha-linked glucose was the determinant of the antigen isolated from *S. albus* Prengel were supported by inhibition studies utilizing a heterologous *S. albus* antiserum.

DISCUSSION

In 1935, Wieghard and Julianelle (16) reported the isolation of phosphorus-containing group antigens from acid extracts of virulent and avirulent staphylococci. Although they were unable to completely characterize the antigens it seems likely that one was the same as the polymer of ribitol phosphate–acetylglucosamine recently isolated from *S. aureus* cell walls (1, 2) and the other the glycerophosphate-glucose *S. albus* antigen described in this report.

The immunogenicity of polyol phosphate compounds (teichoic acids) show a wide range of specificity. On the one hand McCarty demonstrated that polyglycerophosphate is an antigenic component of a variety of Gram-positive species (13). In contrast, other teichoic acids are apparently group- or species-specific as in the case of *Staphylococcus aureus* (1, 2) and Group D streptococci (14). Although the glycerophosphate-glucose compound described in this study is one group antigen of *S. albus*, it has been reported that an immunologically reactive glycerophosphate-galactosamine compound has been

isolated from the cell walls of other *S. albus* strains (1). Thus, the *S. albus* species may be divided into subgroups, depending upon the nature of the cell wall teichoic acid.

The absence of oligoglucosides and glucose phosphates in acid hydrolysates and the formation of glycerol diphosphate are most readily explained by an antigen structure with a backbone of glycerol doubly linked by phosphorus to which are appended monoglucoside units. Thus far attempts to prepare homogeneous subunits of the antigen have been unsuccessful. Moreover, it is not clear whether the molecule is branched.

The hapten inhibition studies indicate that the antigenic determinant is alpha-linked glucose. Although glycerophosphate inhibits precipitation, a polymer of glycerophosphate is no more effective than the monomer and polyglycerophosphate antiserum does not react with the antigen. Moreover other sugar phosphates are equally inhibitory, supporting the view that the effect of glycerophosphate is non-specific. The potent inhibitory activity of glucose-1-phosphate is most likely a result of its structure as an alpha-glucoside.

Despite the wide prevalence of *S. albus* as a component of normal human flora, it is of note that adult human sera do not react with the isolated *albus* antigen. This may be due to a variety of factors including insufficient antigenic mass, inaccessibility of the organism to antibody-forming sites, or relative lack of immunogenicity of the antigen.

SUMMARY

An antigen composed of glycerophosphate and glucose has been isolated from the cell walls of a strain of *Staphylococcus albus*. The antigen reacted with homologous and heterologous *S. albus* antisera. Similar material was isolated from the cell walls of three other *S. albus* strains. The antigen did not react with *S. aureus* antiserum nor with normal human or rabbit serum.

The antigen appeared to have a backbone structure of glycerophosphate with appended monoglucoside units. The antigenic determinant was shown to be glucose in alpha linkage.

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