STUDIES ON THE INTERACTIONS BETWEEN COMPONENTS OF STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCUS BACTERIOPHAGE*

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Although bacteriophage typing of staphylococci has been widely utilized in epidemiologic studies, there has been conflicting evidence regarding the locus and nature of the bacteriophage receptor. This problem was restudied using fractions of *Staphylococcus aureus* strain NYH-6 obtained by differential centrifugation of mechanically disrupted organisms.

Materials and Methods

Organisms and Culture Medium.—The prototype of *S. aureus* NYH-6 and the method of cultivation were those described in a previous communication (1).

Bacteriophage Strains.—The *S. aureus* bacteriophage strains and propagating organisms were kindly supplied by Dr. John E. Blair. The phage strains were propagated by the soft agar technique described by Blair (2).

RESULTS

Bacteriophage Inactivation by Fractions of S. aureus NYH-6.—Acetone-dried cells of *S. aureus* NYH-6 were disrupted in a Mickle disintegrator by the method previously described (1). However, the addition of tri-*n*-butyl phosphate was omitted. Components of the organism were then isolated by differential centrifugation in the following manner.

After removal of the glass beads by filtration, the homogenate was diluted to achieve an initial concentration of 150 mg/ml. The crude cell walls were isolated by centrifugation at 12,800 *G* for 20 minutes at 4°C. The turbid supernatant fluid was then clarified by centrifugation at 30,000 *G* for 2 hours.

Aliquots of all fractions were restored to original volume in distilled water. The ability of these fractions to inactivate bacteriophage was tested in the following manner.

0.3 ml of serial dilutions of the test substances in distilled water was added to 0.9 ml of trypticase soy broth. 1.5 to 4.5 $\times$ 10^4 viable bacteriophage units in 0.3 ml of broth were added, and the tubes placed at 30° for 30 minutes.

A portion of the entire reaction mixture was then diluted 1:10, and 0.2 ml of undiluted and diluted samples were added to 0.1 ml of a concentrated suspension of the propagating strain of *S. aureus*. After 10 minutes at room temperature, 2.5 ml of 0.9 per cent trypticase soy agar

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was added and after vigorous mixing the entire contents of the tubes were poured into a basal agar layer. Plaques were enumerated after overnight incubation at 30°C.

In each case, the fractions of disrupted NYH-6 cells were tested against two *S. aureus* bacteriophage strains. One of these, phage 81, lysed viable NYH-6 at the routine typing dilution. The other, phage 83, did not lyse the NYH-6 strain even at concentrations 1000 times the routine typing dilution.

Table I presents a composite of the results obtained in a number of similar experiments. The bacteriophage-inactivating properties of the homogenate were primarily located in the crude cell wall fraction. Activity was also found in the 30,000 *G* pellet. However, this fraction microscopically contained some intact cell walls and cell wall fragments in addition to membranes and the intracellular particulates. There was very little activity in the 30,000 *G* supernatant. Parallel results were obtained with both the lytic and non-lytic bacteriophage strains.

TABLE I

The Inactivation of S. aureus Bacteriophage Strains 81 and 83 by Components of S. aureus NYH-6

Fraction of *S. aureus* NYH-6	Highest dilution of fraction inactivating 95 per cent of added bacteriophage*	
	Phage 81	Phage 83
Total Mickle homogenate	1:1000	1:10,000
Crude cell walls (13,000 *G* pellet)	1:1000	1:10,000
Crude supernatant (13,000 *G* supernatant)	1:100	1:1000
30,000 *G* supernatant	<1:10	1:100
30,000 *G* pellet	1:100	1:1000

* Bacteriophage concentration 1 to 3 × 10^4/ml. Incubation at 30°C × 30 minutes.

The crude cell walls were then incubated successively with trypsin, ribonuclease, and pepsin in the appropriate buffers. The purified cell walls retained 60 to 80 per cent of their bacteriophage-absorbing properties. The loss of activity was associated with release at all stages of soluble phage-inactivating material which was effective only at low dilutions. This release was not a consequence of enzymatic activity since similar incubation in buffer without enzyme produced the same results.

Bacteriophage Inactivation by Purified NYH-6 Cell Walls.—The time course of inactivation of bacteriophage 81 by various concentrations of purified NYH-6 cell walls was next studied. As indicated in Fig. 1, as little as 1 μg/ml of the cell walls resulted in inactivation of 75 per cent of the initial phage population of 1.5 × 10^4/ml in 120 minutes.

The cell walls of *S. aureus* strain NYH-6 consist of two components: the mucopeptide and a polymer of ribitol phosphate and glucosamine (ribitol teichoic acid). The mucopeptide was prepared by extraction of the walls with hot

5 per cent TCA and the ribitol teichoic acid was isolated from cold TCA extracts (1). These cell wall components were then tested for phage-inactivating properties. In contrast to the marked inactivation of phage 81 by intact cell walls, neither the particulate mucopeptide (100 μg/ml) nor the soluble ribitol teichoic acid (500 μg/ml) caused a significant reduction in bacteriophage titer (Fig. 1). Attempts to prepare soluble receptor substance from staphylococcal cell walls in quantities suitable for detailed analysis have thus far been unsuccessful.

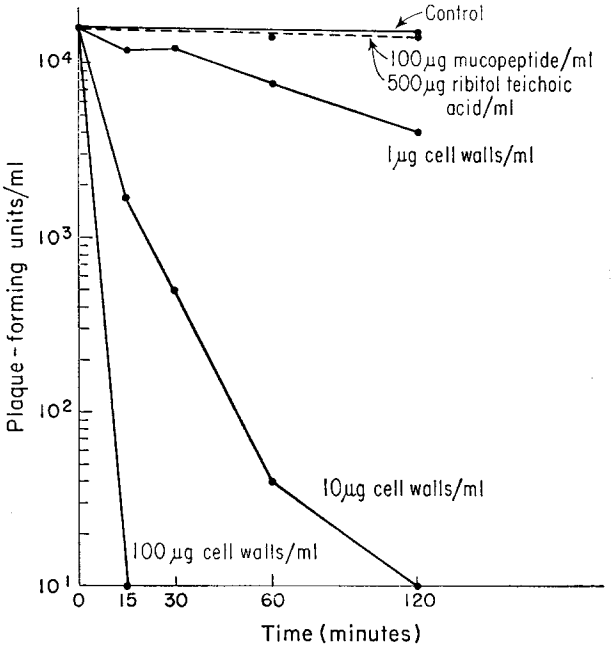

FIG. 1. The inactivation of *S. aureus* bacteriophage (81) by purified cell walls of *S. aureus* strain NYH-6.

The Inactivation of Various S. aureus Bacteriophages by Cell Walls of Strain NYH-6.—Table II presents in summary form studies on the capacity of purified NYH-6 walls to inactivate several strains of *S. aureus* bacteriophage. Some of these bacteriophage strains were capable of lysing the parent organism (80 and 81) and others were capable of lysing NYH-6 at 100 to 1000 times the routine typing dilution (42E, 52A, 83). In each instance a marked reduction in the number of viable plaque-forming units occurred during 30 minutes of incubation.

Other experiments utilizing cell walls of two other *S. aureus* strains, indicated that the cell walls of a strain of one phage type could inactivate all *S. aureus*

bacteriophage strains tested. These results were irrespective of the ability of the test phage to lyse the parent organism.

In contrast cell walls of *S. albus*, Groups A and C streptococci, and *E. coli* K-12 did not absorb or inactivate staphylococcal phage 81 or phage 83. These cell walls were tested at concentrations tenfold higher than that of NYH-6 cell walls required to produce 99 per cent bacteriophage inactivation in 30 minutes.

TABLE II

The Inactivation of Various Strains of S. aureus Bacteriophage by NYH-6 Cell Walls

S. aureus bacteriophage strains	Lytic activity for *S. aureus* strain NYH-6	Reduction of viable Bacteriophage	
		30 minutes	120 minutes
		per cent	*per cent*
83	0	>99	>99
81	+	99	>99
42E	0	95	>99
80	+	40	86
52A	0	58	82

Initial bacteriophage titer = 8×10^3 to 1.5×10^4. Cell wall concentration = 10 μg/ml. Incubation was at 30°C.

DISCUSSION

Hotchin *et al.* reported that after disruption of intact staphylococci, bacteriophage receptor activity was restricted to the particulate cell wall fraction and was not solubilized (3). In contrast, Freeman reported the isolation of partially purified bacteriophage receptor released by mechanical disruption of staphylococci in a ball mill apparatus (4). Freeman believed that the active material was a polysaccharide whereas Rountree found the phage receptor activity of staphylococcal lysates in a nucleoprotein fraction (5).

The results presented in this communication indicate that staphylococcal bacteriophage strains are inactivated by the cell walls. However, small amounts of material with receptor activity are solubilized during the preparative procedures.

The cell walls of *S. aureus* NYH-6 are free of both nucleic acids and aromatic amino acids. Therefore, it is unlikely that the phage receptor is a nucleoprotein as suggested by Rountree.

It is not possible to demonstrate bacteriophage receptor activity for either the mucopeptide or the teichoic acid prepared by TCA extraction of staphylococcal cell walls. Perhaps the acid extraction required for the isolation of teichoic acid in some manner alters the structure and renders it inactive. Alternatively,

the configuration of the teichoic acid as it is linked to the mucopeptide may serve an important role in permitting the attachment of bacteriophage.

The cell walls of one strain of *Staphylococcus aureus* are capable of absorbing all strains of *S. aureus* bacteriophage tested. These observations conform with the known ability of heat-killed *Staphylococcus aureus* strains to absorb a variety of staphylococcus bacteriophage strains (5, 6). Differences in bacteriophage typing would therefore seem to be dependent upon one of two mechanisms; *1.* differences in the ability of bacteriophage to penetrate the cell wall of certain organisms or, *2.* differences between strains of staphylococci in their ability to replicate the various bacteriophage strains.

SUMMARY

The cell walls of *Staphylococcus aureus* are capable of inactivating *S. aureus* bacteriophage. Furthermore, the cell walls isolated from *S. aureus* of a given phage type inactivate a variety of different staphylococcal bacteriophages. Under the conditions employed neither the isolated mucopeptide nor teichoic acid components of the cell walls act as bacteriophage receptor.

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