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CELL-WALL LYSINS OF *STAPHYLOCOCCUS AUREUS* STRAINS INDUCED BY SPECIFIC TYPING PHAGES[1]

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ABSTRACT

RALSTON, DORIS J. (University of California, Berkeley), AND MARY McIVOR. Cell-wall lysins of *Staphylococcus aureus* strains induced by specific typing phages. J. Bacteriol. 88:667-675. 1964.—At least 12 different phages induced the formation of soluble lysins (separable from the phages by ultracentrifugation). The lysins caused rapid clearing of heat-killed cells of all strains of *Staphylococcus aureus* tested, irrespective of the capacity of the phage to form plaques on living cells of the strain. The uninfected cells of the 12 strains contained a second lysin, an autolysin, released upon cellular autolysis. The autolysin preparations differed from the phage-induced lysins, in that they exhibited relatively high activity for lysing *Micrococcus lysodeikticus* and low activity for strain *S. aureus* K_{IN}, and were each specifically inhibited by antiserum prepared against purified autolysin from strain K_1. A third kind of lysin, virolysin, induced by polyvalent phage K, was differentiated from the lysins of the specific phages on the basis of its antigenic specificity and lack of action on *M. lysodeikticus*. All three kinds of lysins digested the mucopeptide portion of staphylococcal cell walls. No evidence was found that any of these lysins possessed specific host ranges which could be correlated with the lytic host range of the inducing phage.

In previous studies, infection of *Staphylococcus aureus* strain K_1 by phage K_1 was shown to induce the formation of a lysin, virolysin, released at lysis and separable from the phage by ultracentrifugation (Ralston et al., 1955). The enzyme lysed heat-killed, but not multiplying, cells of all strains of *S. aureus*. It was differentiated from a second enzyme, autolysin, present in uninfected cells of strain K_1 on the basis of the following. (i) Virolysin fails to lyse living, heated, or acetone-extracted cells of *Micrococcus lysodeikticus*, whereas the normal cell autolysin does. (ii) Virolysin shows a pH optimum at pH 7.5; autolysin is more active at pH 6.5. (iii) Virolysin and autolysin show different antigenic specificities with neutralizing rabbit antisera prepared with the partially purified enzymes (Ralston et al., 1957*b*).

The present paper reports the production of phage-induced lysins and autolysin-like enzymes by specific phages and propagating strains of the staphylococcal phage-typing series. These lysins are compared with the phage K_1 virolysin. The study was carried out with 12 phages and hosts, representing members of four broad lytic groups and phages of A, B, F, and L serology (Blair and Williams, 1961). In addition, it includes an analysis of the lysins formed by these phages as a result of infection of a common host, *S. aureus* K_1, and of the lysins induced by phage K_1 in various strains of *S. aureus*.

Of particular interest has been the problem of determining whether the properties and specificity of lysins induced by a phage must be related to: (i) the general group lytic specificity of the phage, (ii) the narrow host range of the phage, (iii) the antigenic serotype of the phage, (iv) the kind of cell autolysin of the uninfected host, or (v) the temperate or virulent nature of the phage. Phage K_1 is a virulent phage of wide-host range; the typing phages are considered to be temperate (Rippon, 1956), and are known to be of restricted host ranges.

It is also anticipated that these studies will provide information on the production of additional enzymes for analysis of the structure of the staphylococcal wall.

MATERIALS AND METHODS

Phages and S. aureus host strains. Phages and host strains of the phage-typing series were obtained through the courtesy of John E. Blair

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(N.Y. Hospital for Joint Diseases, New York, N.Y.). The staphylococcal cultures were maintained on Trypticase Soy agar (BBL). The phages were produced in Trypticase Soy broth, supplemented with 400 μg/ml of calcium ion, designated TSCa, and were stored at 4 C. The phages and homologous host strains are listed in Table 1. *S. aureus* K_{1N} and K_{1Hi} represent variants of the strain K_1, originally isolated by A. P. Krueger in 1929 (*personal communication*); they were maintained in this laboratory on Tryptose Phosphate agar (Difco). They differ mainly with respect to their relative susceptibility to host-restricted phage K_{14} and to certain of the typing phages (Ralston and Baer, J. Gen. Microbiol. *in press*). Phage K_1 has a wide-host range, but fails to form plaques on strains PS 70 and 77. (PS designates propagating strain.)

Production of phage-induced lysins. Production of the phage K_1-induced lysin from host K_{1N} has been described (Ralston et al., 1955, 1957*b*). Lysins induced by specific typing phages 3C, 55, 79, 42B, 42E, 53, 70, 77, 42D, and 73 were produced in TSCa broth by shake culture at 37 C. Bacteria at 5×10^7 cocci per ml were infected with phage at initial phage-cell ratios (P/B) optimal for each system, generally ratios of 1:100 to 1:1,000. A total volume of 50 ml of infected cells was placed in a 250-ml flask and was incubated until lysis, or for a maximum of 6 hr. The lysates were stored at 4 C or were centrifuged immediately at 20,000 $\times$ *g* for 2 hr at 4 C. The sedimented pellets containing phage were resuspended to one-tenth volume in TSCa broth and were centrifuged once more to remove residual soluble lysin. The lysins were found in the supernatant layers. Similar procedures were used to produce lysins and phages on host *S. aureus* K_{1Hi} and to produce phage K_1 lysates of the PS strains of *S. aureus* (Tables 1, 3, 4, and 5). Phages 80 and 187 produced low yields of both phage and lysin by shake culture, and were made by incubation on semisolid agar according to the phage production procedure recommended by Blair and Williams (1961). A lysin produced by phage 3C on host PS 3C is designated 3C(3C), etc.

Production of cell autolysin. Uninfected cells of each strain were added to TSCa broth in initial concentrations of 5×10^7 cocci per ml. The flasks were incubated on a reciprocal shaker at 37 C for 6 hr, and then were placed at 4 C to allow the cultures to autolyze. Lysis was followed by observing decreases in turbidity at 660 mμ in a Klett photoelectric colorimeter. When 50% clearing of the culture had occurred, the preparations were centrifuged at 20,000 $\times$ *g* at 4 C, the pellet material was discarded, and the supernatant layers were used as the source of autolysin. No activity was found in the pellets. The autolysins were stored at -15 C.

Assay of lysin activity. Enzyme activity was measured by a turbidimetric procedure at 660 mμ. The enzyme activity of any given preparation for standard cell suspensions was reported in terms of a relative velocity constant K min^{-1} ml^{-1}, calculated according to the method described previously for virolysin and autolysin (Ralston et al., 1957*a*). Unless otherwise stated, the lysins were tested at 1:2.5 final dilutions in TSCa broth. The symbol K_{K1N} $min^{-1}ml^{-1}$ designates the velocity constant K with respect to assay on test cells of strain K_{1N}.

Standard cell preparations for lysin assays. Heat-killed cells of strain K_{1N} were prepared from 4-hr cultures on Tryptose Phosphate (TP) agar (Difco). The cells were harvested in 0.85% solution (saline), washed once in saline, and heated immediately at 80 C for 1 hr. Heat-killed cells of the specific propagating strains (PS) of *S. aureus* were prepared similarly from growth on Trypticase Soy (TS) agar. Heat-killed, acetone-extracted cells of a laboratory strain of *M. lysodeikticus* were prepared from 24-hr cultures on TS agar. The cells were centrifuged, resuspended in saline, and then heated at 80 C for 1 hr, after which they were mixed with 80% acetone for 20 min at room temperature, centrifuged, resuspended in 100% acetone, dried in vacuo, and stored at room temperature.

Preparation of cell walls. Walls were prepared from 24-hr growth of strain K_{1N} on TP agar by disintegration in a Waring Blendor in the presence of glass beads (Super Brite Paving Beads, no. 130-5005; Minnesota Mining and Manufacturing Co., St. Paul, Minn.). Approximately 100 ml of cells at 10^{10} per ml were mixed with 100 g of beads and blended at top speed for 20 min. Unbroken cocci were separated from walls by differential centrifugation. The walls were washed in distilled water, heated at 100 C for 20 min to inactivate residual autolysin, and exposed to the enzymes ribonuclease, deoxyribonuclease, and trypsin (Worthington Biochemical Corp.,

Freehold, N.J.; 2 μg/ml at 37 C, in appropriate buffer) until materials adsorbing light at 260 and 280 mμ had been reduced to a minimum. They were then washed free from enzyme and were resuspended in distilled water to approximately 5 mg/ml (dry weight).

Slide test for estimation of lysin activity. This procedure was essentially a "screening" test for assaying many enzymes or testing one enzyme on a series of substrates. Cells to be used as indicator substrates were suspended in 0.10 M K_2H-KH_2 phosphate buffer (pH 7.0) to a turbidity equivalent to 6×10^9 cocci per ml. The buffer was prepared in saline and contained 0.0002 M $MgCl_2$, $MnCl_2$, and $CaCl_2$. The cell-buffer mixture was diluted 1:2 with melted 0.75% agar in distilled water, and 1-ml amounts were placed on clean microscope slides arranged on a tray as described for assay of phage by plaque count (Ralston and Baer, 1960). Enzymes to be tested were placed on the surface of the solidified agar with a Pasteur pipette. The slides were incubated in moist chambers at 37 C for various times, and were observed for appearance of visible lytic zones (clearing).

Preparation of rabbit antisera. Rabbit antisera specific for the K_1 phage-induced virolysin and the autolysin from uninfected cells of *S. aureus* K_{1N} were prepared by methods previously described (Ralston et al., 1957*a*, *b*). The antisera contained a trace of material (probably serum lysozyme) lytic for *S. aureus* cells and for *M. lysodeikticus*. It was partially removed by heat at 56 C for 1 hr, followed by exposure to Super-Cel (Johns-Manville Corp., Buffalo, N.Y.).

Antiserum neutralization tests. Tests of enzyme inhibition by specific antisera were performed by mixing lysin and antiserum and incubating for a given time (0.5 to 2 hr) at 20 C. Virolysin antiserum was used at $\frac{1}{40}$ final concentration and autolysin at $\frac{1}{25}$ final concentration. Residual lysin activity was then determined by the usual lysis test procedure. All data were corrected for any lysis which occurred as a result of lytic material present in the serum-control tubes. The degree of lysin inhibition was found to be a function of the amount of antibody, the time of incubation, and the concentration of lysin. In testing an unknown lysin, the lysin exposed to antiserum was first adjusted to contain a lytic activity equal to that used for the known lysin (against which the specific antiserum had been prepared). A lysin was considered to be related to virolysin K_1 when its activity was depressed in the order of 50% by the antiserum. Tests for relatedness of a lysin to the phage K_1-induced virolysin were performed with standard *S. aureus* K_{1N} heat-killed cells as a substrate. Tests for relatedness of an unknown lysin to autolysin of uninfected K_{1N} cells were made with standard acetone-dried *M. lysodeikticus* cells.

RESULTS

Preparation and tests of lysins. In addition to the agents designated "autolysin" from autolysates of uninfected cells, lysates were prepared by infecting (i) a series of propagating strains (PS) of *S. aureus* with their respective typing phages, (ii) a series of the same strains (PS) with the polyvalent phage K_1, and (iii) strain *S. aureus* K_{1Hi} with individual specific typing phages. The lysins, prepared as described under Materials and Methods, were heat-labile (56 C for 15 min) and were inactivated by exposure to trypsin (2 μg/ml for 2 hr at 20 C). They lost activity within a few weeks at 4 C, but could be preserved for considerable periods at −15 C and withstood several cycles of freezing and thawing.

The following criteria were used to characterize each preparation: ability to lyse a standard preparation of heat-killed logarithmic-phase cells of *S. aureus* K_{1N}, ability to lyse heat-killed and acetone-extracted cells of *M. lysodeikticus*, susceptibility to inhibition by specific antiserum prepared against partially purified phage K_1-induced virolysin from strain K_{1N}, and inhibition with specific antiserum prepared against partially purified autolysin from uninfected strain K_{1N}. The enzyme activity was expressed as a relative velocity constant, K $min^{-1}ml^{-1}$, calculated as previously described (Ralston et al., 1957*b*).

Autolysins. We had previously shown that the autolysin from host K_1 was antigenically related to the autolysin of strain 145 (now designated strain PS 51). This relationship suggested that all of the autolysins of the staphylococci might be alike (Ralston et al., 1957*b*). In contrast, the lysin virolysin induced by phage K_1 (also by its mutant phage K_{14}) was unaffected by antiserum to autolysin. In the present studies, autolysins of several additional strains of *S. aureus* appeared to be antigenically related to the K_1 autolysin. Table 1 records the number of days required for the cells to undergo 50% autolysis, together with

TABLE 1. *Autolytic enzymes released from Staphylococcus aureus strains at 4 C*

Propagating strain	Cells per ml at 6 hr and at 37 C × 10^9	Days to reach 50% autolysis at 4 C	Relative enzyme activity (K $min^{-1}ml^{-1}$ at 37 C)		Ratio K_{1N}/*M. lysodeikticus*	Inactivation by antiserum	
			Heat-killed *S. aureus* K_{1N}	Acetone-extracted *Micrococcus lysodeikticus*		Virolysin phage K_{1N}	Autolysin K_{1N}
79	2.54	3	0.010	0.060	0.17	−	+
80	3.14	>10	0.010	0.075	0.12	−	NT*
3C	2.37	2	<0.005	0.038	<0.14	−	+
55	2.39	>10	0.015	0.040	0.33	−	+
42B	1.64	>10	0.025	0.055	0.50	−	+
42E	2.23	≫10	0.010	0.023	0.50	NT	NT
70	2.89	9	0.038	0.070	0.50	−	+
73	3.14	1	<0.005	<0.005	0	NT	NT
53	2.58	9	<0.005	0.025	<0.20	−	NT
77	2.35	≫10	<0.005	0.028	<0.17	−	+
42D	2.52	5	0.045	0.080	0.50	−	+
187	3.09	5	0.017	0.060	0.33	−	+

* Not tested.

the relative lysin activity for the two test cells. Different strains autolyzed at widely varying rates, but these rates were not related to the phage group of the strain or to the ultimate yield of lysin. Strain 73 usually lysed very rapidly, but released very low amounts of lysin.

Each preparation was tested for its ability to lyse the two test cells (substrates). The rate of lysis of *M. lysodeikticus* generally exceeded that of strain K_{1N}—a relationship already noted for the K_1 autolysin (Ralston et al., 1957*b*). However, individual lysins showed differences in the extent of the ratio K_{1N}/*M. lysodeikticus*. Until the various autolysins are purified, it is not possible to state whether these variations represent differences in a family of closely related materials or differences in the activity of a single enzyme (or enzyme complex) due to interfering materials in the culture lysates.

Phage-induced lysins: (i) *Specific typing phage-homologous host (PS) systems.* When produced on their respective propagating strains, the typing phages caused the appearance of active lysins (Table 2). We found that the 42E(42E) lysin usually contained low activity regardless of the phage titer. For example, the preparations listed in Table 2 contained 10^{10} phage particles per ml, but the relative enzyme activity, K_{K1N} $min^{-1}ml^{-1}$, was 0.001. We also obtained low yields of active lysin from phages 80 and 187. In most instances, several lysates were made under conditions of varying initial phage-cell ratios (P/B) before we obtained lysates with substantial lytic activity. Several lysins showed high K_{1N}/*M. lysodeikticus* activity ratios, the values exceeding 1: 79(79), 80(80), 55(55), 70(70), 53(53), 77(77). Some systems showed the reverse relationship (values < 1): 42B(42B) and 73(73). Still others seemed to have almost equal activity for both substrates (Table 2).

The phage-induced lysins were not affected by antiserum to the phage K_1 virolysin; neither were they extensively inhibited by antiserum to strain K_1 autolysin. However, autolysin antiserum caused a partial inhibition in some cases, suggesting that the phage lysates contained small but significant quantities of cell autolysin mixed with larger amounts of other induced lysins. These observations indicate that the typing phages caused the formation of agents distinct from the phage K_1 virolysin and the strain K_1 autolysin, making it probable that separate genes are responsible for inducing each kind of lysin.

Whenever the induced enzymes acted on cells of strain K_{1N}, they also acted on cells of the homologous strain (PS), i.e., the strain on which the lysin was produced. The relative activity of individual lysins for strains K_{1N} and PS varied, however (Table 3, columns a and b), many of the individual lysins showing much more rapid lysis of their homologous strain than of strain K_{1N}. This suggested either that the lysins were

TABLE 2. *Lysin production by specific phages on homologous propagating hosts*

Lytic group of phage	Phage (host)	Serotype of phage	Phage yield $\times 10^9$	Enzyme yield (K $min^{-1}ml^{-1}$ at 37 C)		Ratio K_{1N}/*M. lysodeikticus*	Inactivation by antiserum	
				Heat-killed *Staphylococcus aureus* K_1	Acetone-extracted *Micrococcus lysodeikticus*		Virolysin phage K_{1N}	Autolysin K_{1N}
I	79(79)	B	3.8	0.139	0.035	4	—	—
	80(80)	B	22.0	0.040	0.002	20	NT*	NT
II	3C(3C)	A	1.6	0.013	0.005	3	NT	(?)
	55(55)	B	16.0	0.122	<0.001	>100	—	NT
III	42B(42B)	A	39.0	0.020	0.050	0.4	—	(?)
	42E(42E)	A	10.0	<0.001	<0.001	NT	—	NT
	70(70)	A	120.0	0.190	0.025	8	—	—
	73(73)	A	45.0	0.153	0.380	0.4	—	—
	53(53)	B	19.0	0.075	0.013	6	—	—
	77(77)	F	100.0	0.115	0.013	10	—	—
IV	42D(42D)	B	3.0	0.058	0.040	1	—	±
Miscellaneous	187(187)	L	1.8	0.022	0.013	2	—	—

* Not tested.

TABLE 3. *Relative sensitivity of heat-killed cells of Staphylococcus aureus K_{1N} and specific propagating strains (PS) to phage-induced lysins*

Source of lysin phage (host)	Lytic group of phage	Serotype of phage	Individual lysin		Mixture of lysins		Adjusted; activity of individual lysin PS‡
			K_{1N} (a)	PS* (b)	PS (c)	Ratio K_{1N}/PS for lysin mixture (d)	(e)
79(79)	I	B	0.12†	0.04	0.06	2.5	0.10
80(80)	I	B	0.07	0.05	0.12	1.2	0.06
3C(3C)	II	A	0.07	0.17	0.29	0.5	0.09
55(55)	II	B	0.25	0.39	0.12	1.2	0.47
42B(42B)	III	A	0.05	0.07	0.21	0.7	0.05
42E(42E)	III	A	0.03	0.12	0.27	0.5	0.06
70(70)	III	A	0.29	1.06	0.23	0.6	0.63
73(73)	III	A	0.03	0.09	0.22	0.7	0.06
53(53)	III	B	0.05	0.21	0.30	0.5	0.10
77(77)	III	F	0.07	0.16	0.23	0.6	0.10
42D(42D)	IV	B	0.04	0.09	0.12	1.2	0.11
187(187)	Miscellaneous	L	0.02	0.07	0.17	0.9	0.06
$K_1(K_{1N})$	Nontyping	D	0.27	—	0.15	—	—

* PS designates the specific propagating strain for a given phage.

† Calculated from: $K_{PS} \cdot K_{K_{1N}} \div K_{PS}$.

‡ Indicates relative lytic activity, K min^{-1} ml^{-1} at 37 C. Lysins were diluted 1:5 in 0.02 M phosphate buffer (pH 7.0) containing 0.002 M $MgCl_2$. Heat-killed cells were added to 5.0 ml of enzyme to turbidity equivalent to 1.2×10^9 cocci per ml.

comprised of mixtures of enzymes with different activities for the substrate of the test cells or that the substrate of a given PS strain was different from that of strain K_1. To compare the substrates under constant enzyme conditions, individual strains were exposed to constant amounts of a mixture of the lysins. Some strains were more resistant to the mixture than were others (Table 3, column c): PS 79, 80, 55, 42D, and 187. Some strains were more susceptible to the lysin

mixture than to the standard K_{1N} substrate: PS 3C, 42B, 42E, 70, 53, 77, and 73. The ratio K_{K1N}/K_{PS} indicates the magnitude of the difference. This ratio was used to calculate the rate at which a given single lysin would act on a given strain if that strain were equal in sensitivity to strain K_1: enzyme activity, K_{PS}, individual lysin $\times K_{K1N} \div K_{PS}$, lysin mixture. Most of the individual lysins worked equally well on the two kinds of cells, but some, such as 187(187) and 42D(42D), were quite different (Table 3, columns a and e). It is not yet possible to conclude that all the lysins are identical in activity; however, it seems reasonable to assume that they are much alike and that strain K_{1N} may be used as a common and sensitive test substrate.

(ii) *Phage K_1-PS systems.* Phage K_1 induced the appearance of a virolysin-like enzyme in various strains. The enzyme was considered to be a virolysin when it was inhibited by antiserum to K_1 virolysin, and showed little or no activity for *M. lysodeikticus* cells (Table 4). (Two strains, PS 70 and 77, were resistant to phage K_1 and could not be studied.) This information made it possible to conclude that, when a given typing phage failed to induce the appearance of virolysin, the failure was not due to an inherent inability of the strain to produce virolysin.

(iii) *Specific typing phage-host S. aureus K_{1Hi} systems.* Many phages of the typing series were shown to be capable of forming plaques on *S. aureus* K_1 (Ralston and Baer, *in press*). Two variants of this strain have been isolated: K_{1N}, which is relatively more resistant to plaque formation by phages of B serotype and also by phages lytic for group II strains of the *S. aureus* typing series; and strain K_{1Hi}, which is more susceptible to these phages (Ralston and Baer, *in press*). Strain K_{1Hl} was used to prepare lysates of the typing phages. Five of ten lysates, each containing at least 10^9 particles per ml, as assayed on strain K_{1Hi}, contained significant amounts of lysins: 70, 73, 79, 42B, and 42D (Table 5). In general, lysates of K_{1Hi} contained much less activity than did lysates of the respective typing strains (PS). Most of the typing phage K_{1Hl} lysates showed relatively low activity for *M. lysodeikticus*, but 42D(K_{1Hi}) and 79(K_{1Hi}) were active for this strain, possibly because of contaminating amounts of cell autolysin. The lysin 42B(K_{1Hi}) was partially inhibited by antiserum to virolysin, whereas all the others were not affected. Inhibition of 42B(K_{1Hi}) lysin occurred only in tests with K_{1N} cells, perhaps indicating a nonspecific effect. In general, it was difficult to evaluate these antibody tests, because the lysins were of such low activity.

Host range, site of action, and biochemical nature of the substrate for the phage-induced lysins and cellular autolysins. (i) *Heterologous genera.* Earlier tests established that the phage-induced virolysin and the K_1 autolysin exhibited host-range differences: virolysin would lyse only suitably altered strains of *S. aureus*, whereas autolysin would

TABLE 4. *Lysin induction by polyvalent phage K_1 on Staphylococcus aureus hosts*

Phage (host)	Phage yield × 10^9	Enzyme yield (K $min^{-1}ml^{-1}$ at 37 C)		Ratio K_{1N}/*M. lysodeikticus*	Inactivation by antibody to phage K_1 virolysin
		Heat-killed *S. aureus* K_{1N}	Acetone-extracted *Micrococcus lysodeikticus*		
K_1(79)	9.3	0.095	<0.001	>95	+
K_1(80)	7.0	0.135	<0.001	>135	+
K_1(3C)	0.6	0.015	<0.001	>15	+
K_1(55)	1.5	0.033	0.008	4	+
K_1(42B)	5.8	0.095	<0.001	>95	+
K_1(42E)	1.7	0.162	<0.001	>160	+
K_1(70)	No infection	NT*	NT	NT	NT
K_1(73)	1.0	0.030	<0.001	>30	+
K_1(53)	0.03	0.058	<0.001	>58	+
K_1(77)	No infection	NT	NT	NT	NT
K_1(42D)	0.2	0.118	0.036	3	+
K_1(187)	6.7	0.288	<0.001	>280	+

* Not tested.

TABLE 5. *Lysin production by specific phages on common host Staphylococcus aureus* $K_{1H\,i}$

Group of phage	Phage (host)	Serotype of phage	Phage yield × 10^9	Enzyme yield (K $min^{-1}ml^{-1}$ at 37 C)		Ratio K_{1N}/*M. lysodeikticus*	Inactivation by antibody	
				Heat-killed *S. aureus* K_1	Acetone-extracted *Micrococcus lysodeikticus*		Virolysin phage K_{1N}	Autolysin K_{1N}
I	79($K_{1H\,i}$)	B	4.0	0.035	0.010	4	—	—
	80($K_{1H\,i}$)	B	10.0	0.017	<0.001	>17	NT*	NT
II	3C($K_{1H\,i}$)	A	6.0	0.017	<0.001	>17	NT	NT
	55($K_{1H\,i}$)	B	No infection	NT	NT	NT	NT	NT
III	42B($K_{1H\,i}$)	A	0.2	0.030	<0.001	>30	(?)†	—
	42E($K_{1H\,i}$)	A	5.5	0.003	<0.001	>3	NT	NT
	70($K_{1H\,i}$)	A	1.0	0.065	<0.001	>65	—	—
	73($K_{1H\,i}$)	A	2.0	0.025	<0.001	>25	—	—
	53($K_{1H\,i}$)	B	0.08	0.008	<0.001	>8	NT	NT
	77($K_{1H\,i}$)	F	Poor	0.001	<0.001	—	NT	NT
IV	42D($K_{1H\,i}$)	B	4.0	<0.022	0.002	11	—	—
Miscellaneous	187($K_{1H\,i}$)	L	No infection	NT	NT	NT	NT	NT

* Not tested.

† Lysin 42B was partially inactivated by virolysin antiserum when tested on K_1 host, but not when the lysin was tested with the 42B host.

lyse *M. lysodeikticus* as well. The enzymes lysed a wide range of heat-killed strains of *S. aureus* (Ralston et al., 1955). In the present studies, lysis tests were performed by a spot technique on agar layers spread on microscope slides in a manner similar to that employed for plaque counts (Ralston and Baer, 1960; see Materials and Methods). Lysins were spotted undiluted on heat-killed cell substrates of the following: *Streptococcus lactis*, *Vibrio metchnikovi*, *Pseudomonas fluorescens*, *Clostridium sporogenes*, *Bacillus subtilis*, *Neisseria catarrhalis*, *Corynebacterium xerosis*, *Sarcina lutea*, *Lactobacillus casei*, and *Saccharomyces cerevisiae*. None of the lysins caused clearing of these bacterial or yeast preparations, although control lysins spotted on heat-killed cells of strain K_{1N} showed extensive lysis.

(ii) *S. aureus strains.* In general, the lysins were without lytic effect on dividing cells of all *S. aureus* strains. Three lysins, however, 53(53), 70(70), and 77(77), showed ability to lyse living cells of strain K_{1N} in TS broth. After heating for 15 min at 65 C, this activity was destroyed, but two of the lysins (53 and 77) still inhibited cell growth. The lysins apparently did not act on living cells of their respective homologous strains.

A preliminary study by spot-test technique was made of the host range of each lysin for heat-killed cells of different *S. aureus* strains. This study was performed to determine whether there were any specificities in lytic range which might be correlated with either the plaque range or serotype of the inducing phage. Lysins resulting from infections of the specific phages and their homologous hosts were cross-spotted on heat-killed preparations of 12 strains of *S. aureus*. Significant clearing was produced by all lysins; there were only minor differences in the relative degree and rate of lysis. When tested by this procedure, strains PS 80 and 55 were more resistant to the lysins than were the other strains (see also Table 3).

(iii) *Cell-wall preparations.* All the phage lysins were spotted on turbid layers of purified cell walls of strain K_{1N}. The walls had been prepared by disintegration with glass beads as described under Materials and Methods. All the lysins produced clearing of the walls, showing that the site of action is on the staphylococcal wall.

The mucopeptide component of the wall was prepared by extraction of cells with hot trichloroacetic acid, followed by digestion with trypsin, according to the method of Hancock and Park (1958). Fractions prepared by this method were digested by the phage K_1 virolysin from strain K_1 (Ralston, Baer, and Elberg, 1961). All the lysins of the specific typing phage-PS

host systems also digested this mucopeptide material. It may be concluded that at least one component of each impure lysin is specific for biochemical linkages present in this polymer. All the lysins also digested residues of walls after extraction with hot formamide for 15 min at 145 C. This extraction removed approximately 50% of the phosphorus of the wall, leaving significant amounts of mucopeptide; but, in contrast to the hot trichloroacetic acid procedure, it did not destroy the capacity of the wall to adsorb phage K_1 (*unpublished data*). At present, no definitive studies have been completed of the chemical composition of the wall fragments released by the various lysins. A preliminary test of partially purified virolysin K_1 showed that significant amounts of materials reacting as hexosamines and reducing sugars were released, and that they differ in size and quantity from the fragments released from an equivalent percentage of walls by egg-white lysozyme (*unpublished data*).

On the basis of host-range and antigenic specificity, these studies allowed us to distinguish at least three kinds of lysins from strains of *S. aureus:* a lysin present in autolysates of all strains, highly active for *M. lysodeikticus*, usually less so for *S. aureus* cells, and related antigenically to the K_1 autolysin of uninfected cells; a lysin induced by phage K_1 (and its mutant phage K_{14}) in all strains of *S. aureus* which support multiplication of this phage, i.e., the phage K_1 virolysin, which has little or no activity for *M. lysodeikticus* cells but which lyses all strains of *S. aureus* when they have been suitably damaged by heat, etc.; and a group of phage-induced lysins appearing in lysates of most, if not all, of the specific typing phage infections of hosts in which the phages can reproduce. These lysins are not closely related antigenically to the K phage virolysin nor to the cellular autolysins of uninfected host cells, but they probably comprise a family of enzymes. For the most part, these lysins do not act on living cells of *S. aureus*, although preparations of 53(53), 70(70), and 77(77) have been lytic for strain K_{1N} (and not for their homologous cells), suggesting, perhaps, a fourth kind of lysin. In conclusion, the antigenic nature of the lysin appears to be genetically controlled by the inducing phage; the specificity of the lysin does not appear to be related to any particular phage group-lytic range or phage serotype.

DISCUSSION

It is of interest that all the phage lysates tested so far in these studies contained considerable quantities of lytic materials for *S. aureus* strains in a soluble form which may be separated from the different phages. In a subsequent paper, it is shown that the purified phages themselves do not cause lysis (Ralston and McIvor, 1964), but together with the soluble lysins produce a rapid lysis-from-without of living cells, like virolysin (Ralston et al., 1957*a*). It has been suggested that the lysin virolysin from phage K_1 probably functions at the end of the latent period of infection to digest the wall (presumably also the receptor material) and to release intracellularly formed phage (Ralston et al., 1955, 1961). Since the lysins studied in the present series also digest the mucopeptide of the wall, it may be surmised that phage infections of the staphylococci all involve a common mechanism for phage release.

The phages studied in these experiments are known to possess characteristic lytic ranges. It has been our interest to determine whether phages which were of narrow host ranges induced lysins of narrow lytic ranges. Under the assumption that a given lysin was essential for escape, this would have helped to explain the specificity of the phage. However, our tests show that the lysins are active for a wide range of *S. aureus*, regardless of the specificity of the inducing phage or the capacity of the phage to multiply on the host used as lysin substrate. The explanation for the specificity of the phage probably lies elsewhere.

Although materials in the various phage lysates all act on the mucopeptide of the staphylococcal wall, it is possible that individual lysins may cause this polymer to be broken into different fragments. It is also possible that the lysins are comprised of mixtures of enzymes, some of which will prove to be of different lytic specificity when they have been purified.

The lysin described by Richmond (1959) in cell cultures of *S. aureus* 524 SC may be related to the autolysins described in this paper, since it is active on *M. lysodeikticus*. In view of the antigenic differences in the various phage-induced lysins, it will be of interest to determine what

kind of lysins appears in cell supernatant materials after lysogenic induction.

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