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LYSIS-FROM-WITHOUT OF *STAPHYLOCOCCUS AUREUS* STRAINS BY COMBINATIONS OF SPECIFIC PHAGES AND PHAGE-INDUCED LYTIC ENZYMES

DORIS J. RALSTON AND MARY McIVOR[1]

Department of Bacteriology, University of California, Berkeley, California

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ABSTRACT

RALSTON, DORIS J. (University of California, Berkeley) AND MARY McIVOR. Lysis-from-without of *Staphylococcus aureus* strains by combinations of specific phages and phage-induced lytic enzymes. J. Bacteriol. 88:676-681. 1964—Several typing phages, adsorbed in sufficient concentrations to their homologous propagating strains, altered the cell surface so as to render the cells sensitive to rapid and synergistic lysis by extracellular additions of wall lysins. Lysis was effected both by lysins induced by the individual phages and by phage K_1 virolysin. Phage K_1 also rendered cells sensitive to the lysins of the typing phages. With the exception of lysins from PS 53, 70, and 77, none of the lysins nor purified phages tested separately caused significant lysis of living cells. Lysis-from-without in *Staphylococcus aureus* appears to be a stepwise process: sensitization by phage followed by digestion of the wall by lysin.

In previous studies with the polyvalent staphylococcal phage K_1, it was shown that purified phage combined with extracellular additions of the phage-induced lysin, virolysin, or of autolysin from uninfected cells of strain K_1, causes a rapid lysis of living cells (Ralston et al., 1955, 1957*a*). Phage K_1 alone, even in amounts sufficient to saturate the cellular receptor material, causes no lysis; nor do strong concentrations of the enzymes. The change produced on the cell surface by phage which renders the wall susceptible to digestion by the lysins is termed sensitization. Sensitization is specific in that it does not increase the degree of cellular susceptibility to egg-white lysozyme or to several lysozyme-like agents capable of acting on heat-killed cells of *Staphylococcus aureus* or of *Micrococcus lysodeikticus* (Ralston, 1963). The rate of lysis of phage-sensitized cells is dependent upon both the phage concentration per cell and on the amount of extracellular lysin. Phage-sensitized cells produce no phage nor intracellular virolysin (Ralston et al., 1957*a*, 1961).

Several other staphylococcal phages cause the appearance of lytic enzymes, antigenically distinct from the phage K_1-host K_1-induced virolysin and from the normal cell autolysin (Ralston and McIvor, 1964). All of the lysins digest the mucopeptide portion of walls of strains of *S. aureus*.

This paper reports the ability of various staphylococcal-typing phages to sensitize living cells of *S. aureus*. It also presents data showing that phages and the induced lysins can be interchanged with the phage K_1 and the virolysin K_1 to produce lysis-from-without of living cells.

MATERIALS AND METHODS

Eleven phages representing members of the major phage-typing groups were studied, including those of A, B, F, and L serology (Blair and Williams, 1961). These were compared with the polyvalent phage K_1, of D serotype. The phages, lytic groups, and serotypes are listed in Tables 1 to 5. The preparation of phage lysates by shake culture in titers sufficiently high for concentration and partial purification was described previously (Ralston and McIvor, 1964). We experienced repeated difficulty in obtaining active preparations from phage 187 by the shake method, and therefore prepared this phage by the semisolid agar method at 30 C, suggested by Blair and Williams (1961).

Some of the phage lysates were concentrated sixfold by dialysis against dry polyethylene glycol (Carbowax 4000, Union Carbide Chemicals Co., New York, N.Y.). The lysates were then subjected to alternate cycles of centrifugation at 5,000 × *g* to sediment debris and at 20,000 × *g* to sediment phage. The phages in the

[1] Present address: Department of Biochemistry, University of California Medical School, San Francisco.

pellets were resuspended in Trypticase Soy broth (BBL) supplemented with 400 μg/ml of Ca^{++} (TSCa), and were centrifuged again to remove traces of lysins. The final titers ranged from 10^{11} to 10^{12} per ml, as assayed by plaque count on their respective homologous hosts. Phages and bacterial strains were obtained through the courtesy of John E. Blair (N.Y. Hospital for Joint Diseases, New York, N.Y.).

The lysins were contained in the high-speed supernatant materials. These solutions were repeatedly centrifuged until the titer of phages was reduced to $<10^7$ per ml. They were stored frozen at -15 C. The lysins are described in terms of the phage and host of production; for example, lysin 42B (42B) designates a lysin obtained by incubating phage 42B with strain PS 42B.

Bacterial strains. Strains used as propagating hosts for the typing phages were maintained on Trypticase Soy agar. Strains K_{1Hi} and K_{1N} represent two variants of *S. aureus* K_1 that differ with respect to their relative ability to produce plaques with certain typing phages and with host-restricted forms of phage K_{14} (Ralston and Baer, *in press*). They appear to be alike with respect to production of virolysin when infected with phage K_1 and with respect to their sensitivity to this enzyme when in the heat-killed state. They were maintained on Tryptose Phosphate Agar (Difco).

Lysis tests. Tests of lysis were performed by methods described previously for the phage K_1-virolysin K_1 system (Ralston et al., 1957). Unless otherwise stated, TSCa broth was used throughout. Living cells harvested after 4 hr at 37 C on agar were collected in 0.85% (w/v) NaCl, centrifuged, and resuspended to 3×10^8 to 4×10^8 cocci per ml; purified phage was added to produce a final phage-to-cell ratio (P/B) of 20:1. The test lytic agent was then added, and the samples were placed at 37 C. The number of bacteria lysed in a given time was estimated from standard curves prepared from direct counts made of strain K_{1N}. It was assumed that these curves were closely related to those of other strains, although there has not been any comparative study of morphological similarities.

RESULTS

In the phage K_1-*S. aureus* K_1 system, increased numbers of adsorbed phage per cell produce increased proportions of sensitized cells. With rapidly dividing cells, harvested after 3 to 4 hr at 37 C, as few as three to five particles have proved sufficient to cause sensitization (Ralston et al., 1957*a*). In the present studies, cells were uniformly exposed to a multiplicity of 20 particles per cell (P/B = 20). In the lysis tests, phage (P), lysin (E), and phage plus lysin (PE) were mixed with cells. The per cent lysis within a given time, generally 20 min at 37 C, was calculated for each sample from standard curves relating optical density at 660 mμ to the number of bacteria per ml. Lysis-from-without of any given phage-host combination was observed as an immediate lysis. In the case of PE combinations, the lysis was considered to be synergistic when the lysis observed for PE was greater than the sum of P (observed) + E (observed).

The curves of lysis resemble those reported for the phage K-virolysin system (Ralston et al., 1957*a*), which was described as a stepwise lysis-from-without (sensitization by phage followed by digestion by virolysin). For this reason, the results of the present paper are described in similar terms. Data are presented for tests of specific typing phages and their phage-induced lysins (Table 1), for tests of cellular autolysins (Table 2), for tests of specific typing phages and the $K_1(K_{1N})$ virolysin (Table 3), and for tests of phage K_1 and the typing phage lysins (Table 4).

Specific typing phages and homologous phage-induced lysins. Washed preparations of the specific phages caused little or no lysis at P/B = 20:1 (Table 1). The lysins obtained from the typing phages caused no lysis, even at 1:2.5 dilution. In fact, the lysin-treated cells usually multiplied in the test medium at rates comparable to untreated control cells. When the specific typing phages were combined with their homologous phage-induced lysins, rapid lysis was observed with the following systems (Table 1): 3C(3C), 55(55), 42B(42B), 53(53), 70(70), 42D(42D), and 187(187). No lysis occurred with the phage 77-lysin 77 system. The rate and extent of lysis varied from system to system. Since the phage input per cell was constant for the various systems, the variations might be related to differences in the test cells and in the relative concentration (activity) of the various lysins.

Specific typing phages and autolysins from uninfected hosts. None of the autolysins produced lysis of living cells of their strain of origin (Table 2). When combined with samples of purified typing

TABLE 1. *Lysis-from-without of strains of Staphylococcus aureus by the combined action of specific typing phages and phage-induced lysins*

Phage group	Phage serotype	Phage and lysin source*	Test strain	Per cent lysis of logarithmic phage PS† cells after 20 min at 37 C		
				Phage	Lysin	Mixture of phage + lysin
I	B	79(79)	79	0	0	23
II	A	3C(3C)	3C	0	0	32
	B	55(55)	55	0	0	50
III	A	42B(42B)	42B	4	0	38
	A	70(70)	70	10	0	21
	A	73(73)	73	0	0	27
	B	53(53)	53	0	0	14
	F	77(77)	77	0	0	0
IV	D	42D(42D)	42D	9	0	22
Miscellaneous	L	187(187)	187	26	0	39

* Phage number (host strain number).

† PS designates specific propagating strain.

TABLE 2. *Lysis-from-without of strains of Staphylococcus aureus by the combined action of specific typing phages and autolysins from the uninfected host cells of the various strains*

Phage group	Phage serotype	Phage source*	Autolysin source (PS)	Test strain (PS)	Per cent lysis of logarithmic phase PS cells after 20 min at 37 C		
					Phage	Lysin	Mixture of phage + lysin
I	B	79(79)	79	79	0	0	10
II	A	3C(3C)	3C	3C	0	0	38
	B	55(55)	55	55	0	0	27
III	A	42B(42B)	42B	42B	4	0	26
	A	70(70)	70	70	5	0	0
	A	73(73)	73	73	0	0	12
	B	53(53)	53	53	0	0	20
	F	77(77)	77	77	0	0	0
IV	B	42D(42D)	42D	42D	4	0	16
Miscellaneous	L	187(187)	187	187	26	0	42

* Phage number (PS). PS designates specific propagating strain.

phages, most of the autolysins produced lysis, indicating that the phages had sensitized the cell surfaces to lysis by these soluble agents. The autolysins from strain 70 and 77 failed to produce any clearing; however, neither lysin was very active, as measured by its action on heat-killed cells. From these tests it was not possible to tell whether the phages had actually sensitized the cells. That phage 70 and 77 did, in fact, possess a sensitizing mechanism was shown by the lysis studies of host K_1 (Table 3).

Specific typing phages and the phage $K_1(K_{1N})$ *virolysin.* Most of the specific phages tested were capable of sensitizing cells of *S. aureus* K_{1Hi} to lysis by the phage-induced virolysin $K_1(K_{1N})$. As in the previous tests, the phages and lysins used alone produced little or no lysis of the cells, whereas the combination of phage and lysin produced significant and synergistic lysis. Phage 187 adsorbed poorly to the test cells of *S. aureus* K_{1Hi}, and could not sensitize the strain. Phage 55 produced sensitized cells but formed no plaques on strain K_{1Hi}, showing that sensitization by the specific typing

TABLE 3. *Lysis-from-without of strain K_{1Hi} by the combined action of specific typing phages and phage K_1 virolysin**

Phage group	Phage serotype	Phage (PS)	Per cent lysis of logarithmic phase K_{1Hi} cells after 20 min at 37 C		
			Phage	Lysin	Mixture of phage + lysin
I	7B	79(79)	14	12†	75
II	3A	3C(3C)	22	21	100
	B	55(55)	4	8	38
III	A	42B(42B)	5	12	26
	A	70(70)	25	8	48
	A	73(73)	NT‡	NT	NT
	B	53(53)	4	0	33
	F	77(77)	16	8	89
IV	B	42D(42D)	25	0	100
Miscellaneous	L	187(187)	0	12	7
Wide-host	D	$K_1(K_{1N})$	5	0	90

* The lysin source (phage PS) was $K_1(K_{1N})$ for all serotypes.

† Differences in tests with lysin only are due to the fact that tests were performed on separate days with different batches of test cells.

‡ Not tested.

phages was a property independent of the ability of the phage to replicate and form plaques on a particular host.

In general, each typing phage did not cause as extensive sensitization of its homologous host as it did of strain K_{1Hi}, despite the fact that the two kinds of test cells were of the same age on harvest and were exposed to identical amounts of phage per cell. These differences possibly were related to variations in the ages at which the different strains of *S. aureus* become most highly susceptible to sensitization, to variations in the rates of adsorption of the phages, or to intrinsic differences in the cell surfaces.

Phage K_{1N} and specific phage-induced lysins. All the lysins induced as a result of infections of the phage propagating hosts (PS) with their respective phages caused lysis of strain K_{1Hi} cells that had been sensitized by phage K_{1N} (Table 4). Three of the lysins lysed living K_{1Hi} cells in the absence of phage: lysins 53(53), 70(70), and 77(77). The lysin in lysates of phage 70 disappeared within a few days at 4 C. The lysins 53(53) and 77(77) were inactivated by heat at 56 C for 30 min; yet the heated preparations retained the ability to inhibit cell growth, a property different from the heat-inactivated virolysin of phage K_{1N}. We observed that living cells of K_{1N} occasionally become spontaneously sensitized to the $K_1(K_{1N})$ virolysin, lysing when exposed to it in the absence of phage (Ralston, 1963). Possibly, the spontaneously sensitized cells are also sensitive to the lysins of 70, 53, and 77. These three preparations did not lyse living cells of their strain of origin. We also noted that the autolysins prepared from these three strains did not lyse strain K_{1Hi} living cells (Table 5).

TABLE 4. *Lysis-from-without of strain K_{1Hi} by the combined action of phage K_1 and specific lysins induced by typing phages**

Lysin source (phage PS)	Per cent lysis of logarithmic phase K_{1Hi} cells after 20 min at 37 C		
	Phage	Lysin	Mixture of phage and lysin
79(79)	0	0	91
80(80)	0	0	13
3C(3C)	0	0	90
55(55)	0	31	100
42B(42B)	0	9	21
70(70)	0	60	100
73(73)	0	0	16
53(53)	0	48	80
77(77)	0	35	71
42D(42D)	0	10	30
187(187)	0	7	77
$K_1(K_{1N})$	0	0	90

* The phage source was $K_1(K_{1N})$.

TABLE 5. *Lysis of Staphylococcus aureus strains by material solubilized from PS 53, 70, and 77*

Agent	Per cent lysis in 20 min*			
	Living K_{1Hi}	Living homologous	Heat-killed K_{1N}	Acetone-treated *Micrococcus lysodeikticus*
Phage-induced				
53(53)	100	0	NT	NT
70(70)	100	9	37	NT
77(77)	100	0	15	NT
Autolysin				
PS 53	0	NT	22	16
PS 70	0	NT	6	22
PS 77	0	NT	3	9

* Lysins were tested at 1:2.5 dilution in Trypticase Soy Broth supplemented with 400 μg/ml of Ca^{++}.

These studies indicate that many staphylococcal phages can cause sensitization of the surface of staphylococci to lysin action. The change can be produced by both polyvalent phages, as the K phage, and by the more specific typing phages. Many of the phages, the phage-induced lysins, and cell autolysins can be interchanged and still act together to cause lysis, suggesting that common phage receptors and substrate materials are involved. There does not appear to be any specificity in phage sensitization by the various phages, which can be ascribed to their lytic range or antigenic grouping.

DISCUSSION

Many of the typing phages of this study exhibit specific host ranges (Blair and Williams, 1961), although most of them are known to cross-adsorb widely to many strains of *S. aureus* (Rountree, 1947; Morse, 1962). Presumably, they adsorb to a common receptor material.

The relationship of the phage-receptor areas to the chemical changes involved in sensitization is not yet defined. In the phage K_1-virolysin K_{1N} system, phage appears to lessen the ability of cells to reduce triphenyl tetrazolium chloride to its red formazan, suggesting that a certain level of dehydrogenase activity is required to maintain cellular resistance to virolysin; but the evidence for a causal relationship is not clear-cut (Ralston and Baer, *in press*, Ralston and Perry, 1963). Preliminary tests of specific phages and their hosts suggested that some of these phages also depress dehydrogenase activity, as measured by the same technique; but the phages seem to be less effective in this respect than the phage K (*unpublished data*).

All the lysins used in these studies have been shown to digest the mucopeptide material prepared from purified cell walls of strain K_{1N} (Ralston and McIvor, 1964). Since many of the specific phages sensitize cells to $K_1(K_{1N})$ virolysin, it is not surprising that these phages also sensitize cells to their induced lysins, especially when a common substrate material is implicated.

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