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Lysogenic Conversion of *Salmonella typhimurium* Bacteriophages A3 and A4 Consists of O-Acetylation of Rhamnose of the Repeating Unit of the O-Antigenic Polysaccharide Chain

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Lysogenization of *Salmonella typhimurium* with either of the bacteriophages A3 and A4 results in O-acetylation of the L-rhamnose residues of the O-polysaccharide chain of the lipopolysaccharide of the bacterial cell envelope. The *O*-acetyl group is found on both O-2 and O-3 of the L-rhamnosyl residues. This lysogenic conversion prevents the adsorption of the A3 and A4 phages and also greatly reduces the rate of adsorption of phage P22 to the *O*-polysaccharide chain as measured by binding studies with whole bacteria. Isolated lipopolysacchaide from A3- and A4-lysogenized bacteria was also inefficient in inactivating these phages: the concentration required for 50% inactivation was 10,000-fold higher than that for lipopolysaccharide from *S. typhimurium* not lysogenized by any A phage. Binding of phages A3 and A4 is accompanied by hydrolysis of the α-1,3 linkage between rhamnose and galactose in the tetrasaccharide repeating unit of the *O*-polysaccharide. Phage hydrolysis generates saccharides of various lengths, the majority being dodecasaccharides, i.e., equivalent to three repeating units. It is surmised that O-acetylation of the rhamnosyl residue interferes with phage A3, A4, and P22 infection by preventing binding to and hydrolysis of the *O*-polysaccharide chain, the initial step in the phage infection cycle. The new *O*-acetyl-rhamnose entities did not elicit specific antibodies in rabbits in accordance with earlier experiences. The O-acetylation of O-2 and O-3 of rhamnose is a new, hitherto unknown, modification of the *O*-polysaccharide chain of *S. typhimurium*.

In the 1950s Boyd (2) isolated and characterized heat-stable bacteriophages, termed A phages, from several hundred strains of *Salmonella typhimurium*. These phages were assigned to 12 types which were finally classified into three groups: 10 types, including that originally termed A5, in group A1 to A2; and 1 type in each of groups A3 and A4 (4). The phages of group A1 to A2 (which includes phages P22 and A1b) are closely related serologically; phages A3 and A4 are less closely related, to each other and to the A1 to A2 phages. At about the same time certain temperate phages of *S. typhimurium* were found to effect lysogenic conversion (8, 9); when such phages lysogenized *S. typhimurium* strains lacking somatic (lipopolysaccharide [LPS]) antigen factor O1 they caused the appearance of this factor. One of us (15) reported that all the phages of group A1 to A2 effected this conversion but that phages A3 and A4 did not. Furthermore, a strain made lysogenic for phage A3 had no new antigenic specificity, or had it lost any such specificity (N. Zinder, personal communication). However, lysogeny with phage A3 or A4 was noted to affect ability to bind phage P22 (of group A1 to A2); the rate of phage adsorption by such lysogens was undetectable (5) or greatly reduced by comparison with the rate of adsorption to nonlysogenic bacteria (15).

All the A phages of *S. typhimurium* are O specific, that is, they do not attack strains which lack the *O*-polysaccharide side chain (of appropriate antigenic character) of LPS. Phage P22, and presumably all the A phages, adsorb to the *O*-polysaccharide of the LPS of susceptible strains. The chemical basis of O factor 1, and thus of the change caused by lysogeny with an A1 to A2 phage, is substitution by α-linked glucose of carbon 6 of the galactose of the oligosaccharide repeat unit of the O chain (14). Similarly, the chemical basis of factor 12_2 (determined by chromosomal genes and subject to form variation, that is, spontaneous alteration between expression and nonexpression) is substitution of O-4 of the same galactose unit by α-linked glucose. The substitution at O-6 (i.e., O-1 character) interferes with adsorption of phage P22, whereas the substitution at O-4 (i.e., $O12_2$ character) is compatible with unaltered adsorbing capacity. Similarly alteration of the galactose-to-mannose linkage between adjacent repeat units of the O chain from α-1,2 to α-1,6 (with the appearance of antigen factor O27) as a result of lysogenic conversion by phage P27 reduces the rates of adsorption of phage P27 and makes the *O*-polysaccharide nonsusceptible to the endorhamnosidase activity of phage P22 (more precisely of a component of the tail apparatus of this phage [18]).

Taken together, these findings suggested that lysogeny for phage A3 or A4 reduced or abolished the ability to adsorb phage P22 by causing a change in the composition of the *O*-polysaccharide chain, even though no change in O-antigenic character was detectable. We therefore reexamined the effect of A3 and A4 lysogeny on O-chain constitution. Isolated LPSs were subjected to structural studies by nuclear magnetic resonance (NMR) spectroscopy and methylation analysis. Our data show that A3 and A4 phages cause lysogenic conversion of the *O*-polysaccharide chain by causing O-acetylation of O-2 or O-3 or both of the L-rhamnose of the repeating unit. This O-acetylation significantly reduces the rate of adsorption of phage P22 and abolishes adsorption of phages A3 and A4 and makes the

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TABLE 1. *S. typhimurium* strains used

Strain	Genotype	Origin or reference
SL696	LT2 *metA22 trpB2 H1*-b *H2*-e,n,x *flaA66 rps L120* cured of Fels2	17
SL1292	Q1 wild type	4
SL1749	Q1 (A3)	Wellcome Culture Collection as CN5146
SL1750	Q1 (A4)	Wellcome Culture Collection as CN5147
SL5146	Q1 *srl-2*::Tn*10 recA1*	From SL1292 by transduction[a]
SL5147	Q1(A3) *srl-2*::Tn*10 recA1*	From SL1749 by transduction[a]
SL5148	Q1(A4) *srl-2*::Tn*10 recA1*	From GL1750 by transduction

[a] *recA1* cotransduced with *srl-2*::Tn*10* by phage P22 HT105/1 *int* from strain TT521, with selection for tetracycline resistance.

O-polysaccharide resistant to the endorhamnosidase activity of all three of these phages. Immunization of rabbits with A3-lysogenized bacteria or with LPS from such bacteria did not elicit detectable antibody reactive only with LPS containing *O*-acetyl-rhamnose (*O*Ac-rhamnose).

MATERIALS AND METHODS

Bacterial strains and phages. Two strains were used as parents of lysogenic derivatives: SL696, an auxotrophic derivative of strain LT2, cured of prophage Fels2 (17) and with somatic antigens O4, -5, and -12; and strain Q1 with somatic antigens O4 and -12, the apparently nonlysogenic *S. typhimurium* strain used by Boyd and Bidwell (4) as the phage indicator and parent of lysogenic derivatives. Q1 lysogenic for phage A3 was obtained from the Wellcome Culture Collection as CN5146, and Q1 lysogenic for A4 was from the same source as CN5147. Strains Q1, Q1(A3), and Q1(A4) were made *recA1* by cotransduction with *srl-12*::Tn*10* by phage P22 HT105/1 *int*, with selection for tetracycline resistance determined by transposon Tn*10*. Purified tetracycline-resistant transductants, confirmed free of the transducing phage and of *recA* character, were assigned strain numbers (Table 1); however, below for clarity we refer to them as Q1 *recA*, Q1(A3) *recA*, and Q1(A4) *recA*.

Bacteriophages. Phages A3 and A4 were obtained from the supernatants of broth cultures of Q1(A3) and Q1(A4), respectively. Phage P22 *c2*, a clear-plaque mutant of P22, was from the collection at the National Bacteriological Laboratory, Stockholm, Sweden.

Preparation of phage stocks. All phages were prepared in submerged culture: A3 and A4 on *S. typhimurium* Q1 and P22 *c2* on *S. typhimurium* SL696. The procedure for growing and purifying the phages was essentially as described earlier (18).

Preparation of LPS. Each strain was grown in submerged culture in tryptone-yeast-glucose medium with aeration to the late-logarithmic growth phase, harvested, and washed, and the LPS was extracted from formaldehyde-killed bacteria by hot phenol-water (11).

Phage adsorption and inactivation measurements. In the phage adsorption experiments, phage (3×10^6 PFU/ml) and washed and cyanide-poisoned bacteria (2×10^8 CFU/ml) in a 0.05 M phosphate buffer (pH 7.4) containing 0.005 M KCN were mixed and incubated at 37°C in a water bath. Samples were withdrawn at regular intervals, immediately chilled to 0°C, and assayed for unbound phage by plaque counts by the soft-agar-layer method with strain Q1 as the indicator. The phage adsorption rate constant was then calculated as described earlier (12). The method measures irreversible phage adsorption. For estimation of phage irreversibly inactivated by LPS, various concentrations of LPS in the phosphate buffer and phage (2.5×10^3 PFU/ml) were mixed and incubated at 37°C for 60 min. The titers of viable phage were determined by the soft-agar-layer method, and the concentration required for 50% inactivation of the phage was calculated as described earlier (12).

Phage hydrolysis of LPS. The procedure used for phage hydrolysis of LPS was essentially as described earlier (18), but instead of alkali-treated LPS, *S. typhimurium* native LPS (20 mg) and phage (10^{11} PFU of P22 *c2*, A3, or A4 per ml) were mixed in 20 ml of 0.005 M ammonium carbonate buffer (pH 7.0), put in dialysis tubing, and incubated at 37°C for 18 h. The mixture was dialyzed twice against 250 ml of the same buffer. The dialysates were pooled and concentrated by evaporation on an RE-III Rotavapor (Büchi Laboratoriums-Technic AG, Flavil, Switzerland). The concentrated saccharide solution was purified and separated by gel chromatography on a Bio-Gel P2 column (1.6 by 100 cm) with water as the eluant.

Chemical analyses. Sugar and methylation analysis and separation and analysis of partially methylated alditol acetates were done as described earlier (18). ^1H and ^{13}C NMR spectra were recorded on a JEOL GX270 FT NMR Spectrometer (JEOL Ltd., Tokyo, Japan) at 70°C with solutions in ^2H_2O.

The terminal reducing sugar in oligosaccharides obtained after phage hydrolysis was determined as described previously (13). In brief, oligosacchrides were reduced with sodium borohydride, hydrolyzed, treated with hydroxylamine, and subsequently treated with a mixture of acetic anhydride and pyridine. The product was finally analyzed by gas-liquid chromatography-mass spectrometry.

The *O*-acetyl substitution site on the polysaccharide was determined as described previously (7). *S. typhimurium* Q1(A3) *recA* (100 mg) was condensed with methyl vinyl ether by using an acidic catalyst which creates acetol groups on free hydroxyl groups. Treatment of the product with a strong base followed by methylation with methyl iodide replaces the *O*-acetyl groups with *O*-methyl groups. After acidic hydrolysis, the monosaccharides were reduced and acetylated, and subsequently the sugars were separated by gas-liquid chromatography. Sugars bearing *O*-methyl groups were identified, and the location of the substitution was determined by gas liquid chromatography-mass spectrometry.

Immunization of rabbits. New Zealand White rabbits (2.0 to 2.5 kg and of both sexes) were immunized either with five injections 3 to 5 days apart of 2.0×10^{10} heat-inactivated *S. typhimurium* Q1(A3) *recA* bacteria or with rabbit erythrocytes coated with phenol-water-extracted Q1(A3) *recA* LPS, five injections given with weekly intervals. The rabbits were bled before, during, and 10 days after the last immunization. Sera were kept frozen at −20°C until assayed. Adsorption of antisera was done with heat-inactivated *S. typhimurium* Q1 *recA* as described previously (10).

Enzyme immunoassay. The enzyme immunoassay procedure was essentially as described previously (6). LPSs from *S. typhimurium* Q1 *recA* and Q1(A3) *recA* were used as coating antigens (1 μg/ml). Sheep anti-rabbit immunoglobulin conjugated to alkaline phosphatase (dilution, 1:1,000) was used to detect bound rabbit antibodies.

RESULTS

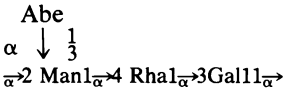
We at first used *S. typhimurium* SL696 and Q1 and their A3-lysogenic or A4-lysogenic derivatives in experiments on the rate of adsorption of phage P22 *c*2. Such experiments were complicated by the high concentration of phage A3 or A4 present in broth cultures of strains lysogenic for these phages and also by a high frequency of segregation of nonlysogenic variants. The frequency of spontaneous induction of various prophages is greatly reduced or abolished in *recA* strains. We therefore made *recA1* derivatives of strains Q1, Q1(A3), and Q1(A4) by transduction with phage P22 HT105/1 *int*, with selection for the tetracycline resistance determined by an adjacent transposon insertion, *srl-12*::Tn*10*. The *recA* derivatives were shown by their phage sensitivity patterns to be unaltered with respect to lysogeny or nonlysogeny. The rate of spontaneous production of phage in the two *recA* strains was extremely low; furthermore, no nonlysogenic segregants were encountered in experiments involving the *recA* versions of the A3- and A4-lysogenic strains.

Phage binding to whole bacteria or isolated LPS. Phages P22, A3, and A4 adsorbed efficiently to the nonlysogenic strain *S. typhimurium* Q1 *recA* (Fig. 1A and B; Table 2). The adsorption was as efficient as to the parent strain Q1 (data not shown). Stable lysogenization with either phage A3 [strain Q1(A3) *recA*] or phage A4 [strain Q1(A4) *recA*] greatly reduced the rate of adsorption of phage P22 (Fig. 1A) and abolished adsorption of phages A3 and A4 (Fig. 1B). The adsorption rate constant for P22 was reduced by more than 80%, and that for A3 and A4 was reduced by more than 95% (Table 2).

The efficiency of plating (EOP) of phages P22, A3, and A4 was tested on *S. typhimurium* Q1 *recA*, Q1(A3) *recA*, and Q1(A4) *recA* (Table 2). The EOP of P22 was only slightly reduced on the A3- and A4-lysogenic strains. On the other hand, no plating of A3 or A4 was seen on either of the two lysogenic strains (EOP, $<10^{-9}$). However, this may result from lysogenic immunity.

That the lysogenic conversion caused by phages A3 and A4 and affecting adsorption of the phages was localized to the LPS was subsequently tested in phage-inactivation experiments (Table 2). Different concentrations of LPS were mixed with the phages and incubated, and the percentage of noninactivated phage was assayed. LPS from strain Q1 *recA* caused 50% inactivation of all three phages at concentrations of 0.1 to 0.8 μg/ml. The LPS from strain Q1(A3) *recA* was inactive in comparison; at least 10,000-fold-higher concentrations were required for 50% inactivation. Since none of the phages lyse, or adsorb to, rough mutants of *S. typhimurium*, the results suggested that any phage conversion should affect the *O*-polysaccharide chain of the LPS.

Chemical analyses of LPS. The LPSs from strains Q1 *recA* and Q1(A3) *recA* were subjected to sugar and methylation analyses. Sugar analyses on peralditol acetates revealed the presence of the expected four sugars of the repeating unit, i.e., abequose, mannose, rhamnose, and galactose in both LPSs. After weak acid hydrolysis, the isolated O-antigenic polysaccharides from strains Q1 *recA* and Q1(A3) *recA* were studied by methylation analysis (data not shown). The methyl ethers detected were the same in both preparations studied, and the structure was as expected in an *S. typhimurium* *O*-polysaccharide, i.e.,



where Abe is abequose, Man is mannose, Rha is rhamnose, and Gal is galactose. The analyses failed to detect any significant difference in the average length of each O chain (data not shown).

The LPSs were subsequently studied by NMR spectroscopy. In the ^1H NMR spectrum recorded on the Q1(A3) *recA* LPS, two signals at about 2.15 ppm were found (Fig. 2A). In this region signals from *O*-acetyl protons are detected. The two signals were not found in the spectrum on the Q1 *recA* LPS (Fig. 2B). In the ^{13}C NMR spectrum of the Q1(A3) *recA*, LPS signals from two acetyl groups were detected, demonstrating the presence of two different *O*-acetyl groups. These two signals were not found in the LPS from the nonlysogenic Q1 *recA* strain (data not shown).

Isolated *S. typhimurium* Q1(A3) *recA* polysaccharide,

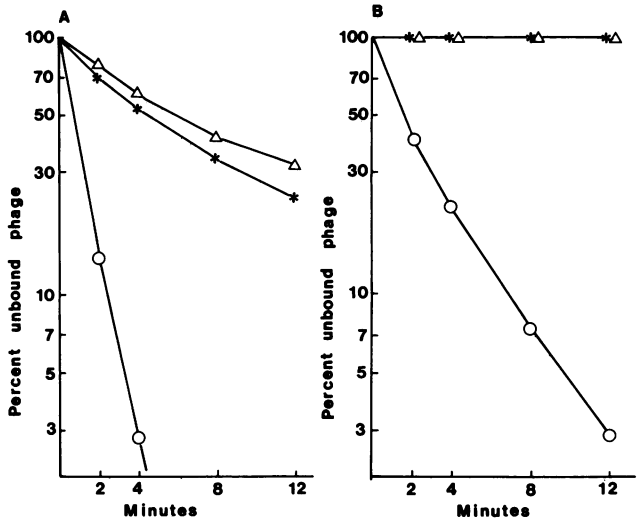


FIG. 1. Adsorption of phage to cyanide-poisoned bacteria. (A) Phage P22 *c*2; (B) phage A3. Symbols: ○, *S. typhimurium* Q1 *recA*; *, *S. typhimurium* Q1(A3) *recA*; △, *S. typhimurium* Q1(A4) *recA*.

TABLE 2. EOP, adsorption rate constants, and phage-inhibiting activity for phages P22 *c*2, A3, and A4

S. typhimurium strain	P22 *c*2			A3			A4		
	EOP	ARC[a]	PhI_{50}[b] (μg/ml)	EOP	ARC	PhI_{50} (μg/ml)	EOP	ARC	PhI_{50} (μg/ml)
Q1 *recA*	1.0	888	0.1	1.0	402	0.40	1.0	351	0.80
Q1(A3) *recA*	0.40	145	6.0×10^3	$<10^{-9}$	<5	$>10^4$	$<10^{-9}$	<5	$>10^4$
Q1(A4) *recA*	0.35	105	ND[c]	$<10^{-9}$	<5	$>10^4$	$<10^{-9}$	<5	$>10^4$

[a] ARC, Adsorption rate constant ($k \times 10^{-11}$ ml min^{-1}).
[b] PhI_{50}, Concentration of LPS required to inactivate 50% of the phages under experimental conditions.
[c] ND, Not determined.

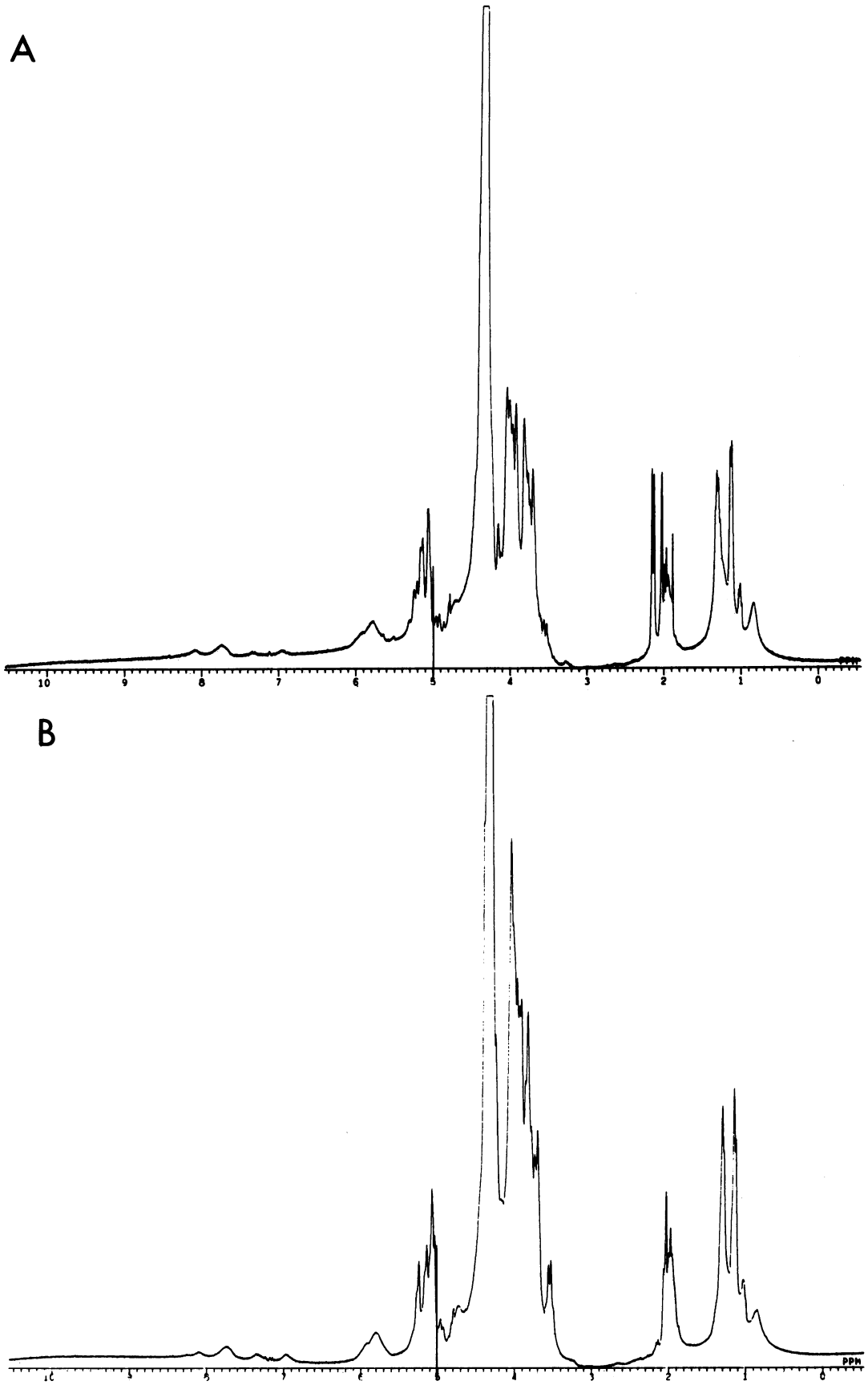


FIG. 2. Proton NMR spectra of Q1(A3) *recA* LPS (A) and Q1 *recA* LPS (B). Notice the additional signals for the *O*-acetyl group at 2.15 ppm in spectrum A.

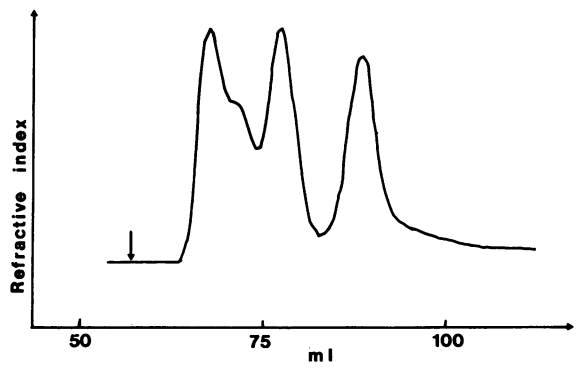


FIG. 3. Gel filtration of *S. typhimurium* Q1 *recA* oligosaccharides released after phage A3 hydrolysis on Bio-Gel P2. The column (1.6 by 100 cm) was eluted with water and trichlorobutanol (0.05%) at 7.0 ml/h. Arrow indicates void volume.

obtained by weak acid hydrolysis which cleaves off the lipid A part, still showed the signal at 2.15 ppm, which confirmed that the *O*-acetyl groups are located in the *O*-polysaccharide chain. Saponification of the polysaccharide resulted in the disappearance of the *O*-acetyl signals, and the NMR spectrum of the Q1(A3) *recA* polysaccharide became superimposable on the Q1 *recA* polysaccharide spectrum. ^{1}H and ^{13}C NMR spectra recorded on Q1(A3) *recA* and Q1(A4) *recA* LPSs were identical. The analyses show that lysogeny with either phage A3 or phage A4 causes *O*-acetylation of the polysaccharide chain.

The locations of the *O*-acetyl groups in the repeating units of the Q1(A3) *recA* polysaccharide chain were then determined. The analyses showed the presence of 2-*O*-methyl and 3-*O*-methyl rhamnose residues not present in the LPS from the nonlysogenic strain. The ratios between 2-*O*-methyl, 3-*O*-methyl, and nonmethylated rhamnose residues were 10:12:7, respectively. This demonstrates that the *O*-acetyl groups in the polysaccharide chain of LPS from *S. typhimurium* lysogenized by phage A3 are linked to the rhamnose residues and that at least 75% of the rhamnose residues are O-acetylated. The presence of both 2-O- and 3-O-methyl derivatives may seem surprising. However, the *O*-acetyl group is known to migrate to vicinal hydroxyl groups even at moderate pH values, and such conditions were met in our *O*-acetyl determinations. The observation is also in agreement with the split *O*-acetyl signals seen in both ^{1}H and ^{13}C NMR spectroscopy of the Q1(A3) *recA* LPS.

Endoglycosidase activity of phages A3 and A4. The presence and specificity of phage A3 and A4 glycosidases was investigated by incubation of the phages with Q1 *recA* LPS in dialysis bags. Saccharides released into the dialysis fluids were collected after gel chromatography on Bio-Gel P2 (Fig. 3). The peak fractions were pooled, lyophilized, and analyzed for reducing sugar residues. Both phages released oligosaccharides which were separated into three major peaks. In all of them rhamnitol pentaacetate was the only alditol acetate derivative detected. This demonstrates that both phages A3 and A4 hydrolyze the α-1,3 linkage between the rhamnose and galactose residues within the repeating unit. The first peak after the void volume contained four and five repeating units, the next three, and the last two repeating units.

We tried to hydrolyze LPSs from strains Q1(A3) *recA* and Q1(A4) *recA* by phage treatment. Phage P22 *c2*, A3, or A4 and LPS were mixed and incubated as described above. None of the phages caused the release of detectable amounts of sugars from either of the two LPSs (20 mg in the incubation mixture). This implied that <1% of the *O*-polysaccharide chains had been hydrolyzed. LPS which had been pretreated with alkali to remove *O*-acetyl groups from L-rhamnose was susceptible. Each of the three phages released >90% of the *O*-saccharide from alkali-treated Q1(A3) *recA* LPS. This demonstrates that the presence of *O*-acetyl groups on O-2 and O-3 of L-rhamnose prevents hydrolysis of the *S. typhimurium* *O*-polysaccharide chain.

Immunization of rabbits with *S. typhimurium* Q1(A3) *recA* bacteria and LPS. Rabbits were immunized with either heat-inactivated *S. typhimurium* Q1(A3) *recA* bacteria or with rabbit erythrocytes coated with phenol-water-extracted LPS from this strain. The immune response was studied by the enzyme immunoassay with LPSs from Q1 *recA* and Q1(A3) *recA* as antigens. Three rabbits were immunized with each immunogen. The $\log_{10}$ endpoint titers were equally high against the two LPS antigens: 4.7 to 5.0 when immunized with inactivated bacteria, and 3.5 to 4.1 when immunized with LPS. Absorption with heat-inactivated Q1 *recA* bacteria reduced the titers 100-fold or more: 2.3 to 2.5 for sera elicited with bacteria, and 1.5 to 1.6 for sera elicited with LPS. No difference could be detected between Q1 *recA* and Q1(A3) *recA* LPSs as coating antigens. We thus failed to detect any antibody specificity directed against the 2-*O*- and 3-*O*Ac-rhamnosyl determinants present in the *O*-polysaccharide chain of the LPS of the A3-lysogenic *S. typhimurium* Q1(A3) *recA* strain. The Q1 and Q1 *recA* strains lack O antigen 5 specificity which is caused by O-acetylation of O-2 of abequose. The A3- and A4-lysogenized Q1 *recA* strains were also O5 negative in slide agglutination tests (data not shown).

DISCUSSION

Lysogenization of *S. typhimurium* with either phage A3 or phage A4 causes the appearance of *O*-acetyl groups on the rhamnosyl residues of the O repeating unit of the *O*-polysaccharide of the LPS. More than 75% of the rhamnose residues were O-acetylated. The hydroxyl groups of O-2 and O-3 of the L-rhamnosyl residues are vicinal, and hence *O*-acetyl migration between these two carbons can be expected to occur. It was therefore not surprising to find in the chemical analysis, with replacement of the *O*-acetyl groups with *O*-methyl groups, that there was an almost equal distribution between O-2 and O-3. A little more surprising was the observation that in native LPS also the *O*-acetyl groups seem to be evently distributed between O-2 and O-3 as judged from NMR spectroscopy data. We consider it unlikely that the *O*-acetyl groups are transferred to each position during LPS biosynthesis, since this probably would demand two transferases. This would imply that the *O*-acetyl group migrates easily, i.e., during bacterial growth or at LPS extraction. In general, *O*-acetyl migration takes place in a direction away from the anomeric carbon which would suggest that during biosynthesis the *O*-acetyl group is transferred to O-2 of the L-rhamnosyl residue.

The presence of an *O*-acetyl group on the rhamnosyl residue of the *S. typhimurium* repeating unit has not been detected earlier. Indeed, studies to understand the basis for the lysogenic conversion caused by A3 and A4 phages were unsuccessful (B. A. D. Stocker, unpublished data; N. Zinder, personal communication). One reason for this was that no new antigenic determinant was found, nor had any existing specificity disappeared. We were able to confirm

these earlier reports. Neither heat-inactivated Q1(A3) *recA* bacteria nor their LPSs (from the same preparation used in the chemical studies) elicited in rabbits an antibody response with detectable anti-*O*Ac-rhamnosyl specificity. The immunological basis for this remains unexplained.

Another reason for earlier failures to detect the *O*Ac-rhamnosyl may have been the unstable character of the lysogenic state. When using A3- and A4-lysogenic bacteria for batch preparation of LPS for chemical analysis we frequently observed a significant portion of nonlysogenic segregants in the cultures. This made the detection of the *O*-acetyl signal in the NMR spectra difficult. It was not until the stable integration of A3 and A4 phages was made possible by the introduction of the Tn*10*-linked *recA* mutation that cultures grown were found to have OAc-rhamnosyl residues in their *O*-polysaccharide chains (Fig. 2B). A retrospective analysis of several NMR spectra unambiguously showed that all LPS extracted from A3- and A4-lysogenic *S. typhimurium* strains had various amounts of *O*-acetyl groups on their rhamnose residues. Alkaline treatment of the LPS removed the alkali-labile *O*-acetyl groups.

The absence or virtual absence of free phage in broth cultures of the *recA* derivatives of strains Q1(A3) and Q1(A4) was as expected. It is well established that spontaneous induction of prophages such as λ, like their induction by DNA-damaging agents (UV, mitomycin C, etc.), depends on host *recA* function, presumably because spontaneous induction is a consequence of the inactivation of phage repressor protein by activated *recA* protein, present at sufficient concentrations in some cells, instead of in large amounts in all cells in cultures giving the SOS response to DNA damage. An unanticipated advantage of the introduction of the *recA* mutation into the lysogenic strains was that accumulation of nonlysogenic segregants was no longer observed. We propose a hypothetical explanation. Excision of prophage λ from the *Escherichia coli* chromosome is by a precise recombination event which produces a circular genome and an intact bacterial chromosome lacking the original insertion. If the liberated prophage for any reason fails to multiply and kill its host, some, at least, of the progeny of the cell concerned will not have prophage in the chromosome or as an extra chromosomal element, i.e., will be cured of lysogeny. Ordinarily the reinfection of such segregants by free phage would be expected to prevent their detection, but in the present case, of phages which alter host LPS to a form which does not adsorb the phage, nonlysogenic segregants will presumably be phage resistant during a period of phenotypic lag. A high frequency of spontaneous induction might then result in accumulation of cured segregants. By this hypothesis, making the lysogenic strain *recA* by preventing spontaneous induction would also prevent accumulation of nonlysogenic segregants.

Binding of the A3 and A4 phages to the *S. typhimurium* *O*-polysaccharide is accompanied by hydrolysis of the α-1,3 linkage between the rhamnose and the galactose residues of the repeating unit. Saccharides of various lengths were produced with the mean size of a dodecasaccharide which is equivalent to three repeating units. The catalytic activity of A3 and A4 is the same as that of phages P22, KB1, P27, 9NA, and all the O-antigen-specific phages of the collection of Lilleengen used for phage typing of *S. typhimurium* (16, 18). The rhamnose 1→3 galactose linkage is accessible in the O chain as evident from the conformational studies (1). This availability may make it a preferred site for phage-associated endoglycosidase activity. We indeed failed to detect a *Salmonella* serogroup A, B, and D phage specific for their *O*-polysaccharide chains with hydrolytic activity for any other linkage.

Lysogenization with A3 or A4 makes bacteria nonsusceptible to superinfection with the same phages (3). This nonsusceptibility perhaps results from lysogenic immunity, but would presumably be seen even in the absence of such immunity because of the observed inability of the phages to adsorb to the *O*-polysaccharide chain (Fig. 1A and B). Evidently, the *O*-acetyl group on O-2 and O-3 of rhamnose efficiently interferes with the binding of the phages. Also, in experiments on the phage-inactivating capacity of LPS extracted from A3 or A4 lysogenic bacteria or from the nonlysogenic parent strain, we could demonstrate that O-acetylation of O-2 and O-3 of rhamnose was incompatible with phage A3, A4, and P22 inactivation under experimental conditions (Table 2). Lysogenization with P22, which results in the addition of an α-linked glucose residue to O-6 of galactose, was also incompatible with the binding of P22, A3, and A4 phages (data not shown). Glucosylation of O-4 of galactose of the repeating unit, recognized as the $O12_2$ antigenic determinant, was, however, compatible with A3, A4, and P22 adsorption (data not shown). It is evident that the conformational changes imposed on the basal tetrasaccharide repeating unit may or may not prevent phage binding to and hydrolysis of the *O*-polysaccharide chain. Phages 9NA and KB1 bind to and hydrolyze *O*-polysaccharides glucosylated at O-6 but not at O-4 (18).

Phage P22 gave a rather high EOP on the phage A3- or A4-lysogenized strains (Table 2). Despite this observation adsorption to these bacteria was slow, and no release of saccharide was detected when P22 was incubated with LPS from strain Q1(A3) *recA* or Q1(A4) *recA*. The reason for this is probably that the O-acetylation interferes with the P22 endorhamnosidase activity so that its action produces large saccharides which are not readily dialyzed. Phage A3 and A4 lysogenization causes O-acetylation of L-rhamnose as shown above. The parent strain *S. typhimurium* Q1 and its *recA* derivative SL5146 both lack *O*-acetyl groups on O-2 of abequose, i.e., O antigen 5 (Fig. 2A). It is evident that A3 or A4 lysogenization did not cause O-acetylation of the abequosyl residues since no 2-*O*-methyl-abequose was found in the *O*-acetyl assay.

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