Bacteriophage receptors on *Listeria monocytogenes* cells are the *N*-acetylglucosamine and rhamnose substituents of teichoic acids or the peptidoglycan itself

Günther Wendlinger, Martin J. Loessner and Siegfried Scherer

Author for correspondence: Siegfried Scherer. Tel: +49 8161 71 3516. Fax: +49 8161 71 4492.
e-mail: 100424.2340@compuserve.com

Institut für Mikrobiologie, Forschungszentrum für Milch und Lebensmittel Weihenstephan, Technische Universität München, Vöttinger Str. 45, 85350 Freising, Germany

Different approaches were used to examine the function of teichoic acids (TA) as phage receptors among selected *Listeria* strains, and to identify and characterize specific receptor structures of host cells belonging to different serovars. This included successive removal of cell wall constituents, preparation and purification of TA, and GLC analysis of TA components. Adsorption of *Listeria monocytogenes* bacteriophages could be inhibited by polyvalent antisera, specific lectins and addition of purified TA. The results confirmed the necessity of TA in general and of rhamnose and glucosamine in particular for adsorption of *Listeria* phage A118, which is a temperate Siphovirus (morphotype B1), attacking predominantly serovars 1/2. Host binding of siphoviral phage A500 (predominantly lysing serovars 4b), was also dependent on cell wall TA. A phage-resistant *L. monocytogenes* strain was shown to lack glucosamine in its TA. These results support the view that TA substituents may play an important role not only in antigenicity of *Listeria* cells, but also in specificity of host recognition by two temperate *Listeria* phages. In contrast, the broad-host-range virulent phage A511 (Myovirus, morphotype A1) uses the listerial peptidoglycan as primary receptor. This corresponds well with the observation that A511 is capable of lysing the majority of *L. monocytogenes* strains.

Keywords: *Listeria monocytogenes*, bacteriophages, phage receptors, teichoic acids

INTRODUCTION

Attachment of bacteriophages to the surface of bacterial host cells requires host-specific recognition and depends on certain cell surface structures. In Gram-positive bacteria (streptococci, lactobacilli or lactococci), phage adsorption almost always involves cell wall carbohydrates (Douglas & Wolin, 1971; Ishibashi *et al.*, 1982; Keogh & Pettingill, 1983; Sijtsma *et al.*, 1988; Valyasevi *et al.*, 1994; Yokokura, 1971). In particular, cell wall teichoic acids (TA) have been found to be important for phage adsorption in *Staphylococcus* spp. and *Bacillus* spp. (Archibald, 1976; Chatterjee, 1969; Coyette & Ghuysen, 1968; Givan *et al.*, 1982; Glaser *et al.*, 1966; Schleifer & Steber, 1974; Young, 1967). Exceptions are the reports of

membrane proteins as phage receptors in *Lactococcus* (Monteville *et al.*, 1994; Oram, 1971; Valyasevi *et al.*, 1991).

The primary interaction of *Listeria* phages with their host cells and the nature of the phage receptor sites have not been studied before. *Listeria* phages can be subdivided into several host range groups of distinct specificity, which correlate with the serovar of the host cells. This makes it possible to discriminate listerial strains in different phagovars by means of phage typing (Loessner, 1991). Correlation between TA structures and serogroups of *Listeria* strains was described by Fiedler *et al.* (1984), clearly pointing to the key role of cell wall TA for the determination of specific serovars. Several authors have reported the antigenicity of *Listeria* cells to be dependent on a variable combination of four monosaccharide substituents, which are covalently bound to the ribitol

units of the TA: L-rhamnose, D-glucose, D-galactose and N-acetylglucosamine (Fiedler *et al.*, 1984; Fiedler & Ruhland, 1987; Kamisango *et al.*, 1983; Knox & Wicken, 1973; Ullmann & Cameron, 1969). These carbohydrate substituents are therefore likely to be involved in determining O-antigenic specificities. It was interesting to determine whether listerial TA are also responsible for specific phage attachment. In this paper, we demonstrate the role of cell wall components in adsorption of three selected phages for *Listeria monocytogenes*, namely A511 (broad host range, genus-specific), A118 (serovar 1/2 strains), and A500 (serovar 4b strains).

METHODS

***Listeria* strains and bacteriophages.** All listerial strains used were taken from the Weihenstephan *Listeria* Collection (WSLC): *L. monocytogenes* WSLC 1003 (serovar 1/2b) and WSLC 1042 (serovar 4b) as bacteriophage host strains and, in addition, *L. ivanovii* WSLC 3009 (serovar 5) as a negative control. *L. monocytogenes* WSLC 1442 (serovar 1/2a) was also included in this investigation because of its insensitivity to lysis by any of the phages used in phage typing in this laboratory (unpublished data).

Bacteriophages A118 (*L. monocytogenes* 1003), A500 (*L. monocytogenes* 1042) and A511 (*L. monocytogenes* 1003) were selected from a set of typing phages employed in this laboratory (Loessner & Busse, 1990; Loessner, 1991). Propagating strains are given in parentheses. Propagation of phages was done according to Loessner & Busse (1990). For adsorption assays, host strains were grown overnight in tryptose broth (Merck) at 30 °C. Cells were washed with SM buffer (Sambrook *et al.*, 1989), resuspended in equal volumes, and titrated. One millilitre volumes of suspensions were used for adsorption assays.

Preparation of listerial cell walls. Broth cultures (late exponential phase) were inactivated by steaming (100 °C, 10 min), followed by concentration and washing by ultrafiltration (Sartocon module, cellulose acetate, pore size 0·2 µm, Sartorius). Finally, cells were harvested by centrifugation (10000 *g*, 15 min), resuspended in SM buffer, and mechanically disrupted by double passage through a French pressure cell (SLM Aminco) at 40000 p.s.i. (276 MPa). Cell walls were prepared by methods similar to those described by Fiedler *et al.* (1984) and Valyasevi *et al.* (1990). Crude cell walls were sedimented by differential centrifugation (15000 *g*, 30 min), resuspended in SM buffer, and treated with DNase/RNase (3 h) and proteinase K (3 h) (each at 0·1 mg per g wet cell walls; all enzymes from Sigma). Digested cell walls were then extracted with hot SDS solution (4%, w/v, 100 °C, 30 min), washed with water, and lyophilized. The resulting preparations were cell walls free of protein, as determined by the lack of detectable material on silver-stained SDS gels (data not shown).

Extraction of TA. TA were extracted from cell walls by treatment with 25 mM glycine/HCl buffer, pH 2·5, at 100 °C for 10 min (Fujii *et al.*, 1985). Cell walls were then sedimented at 30000 *g* for 30 min. The pellet was resuspended and the procedure repeated. Finally, cell walls were washed in purified water, lyophilized and used as cell walls with reduced TA content. Prior to each treatment, TA content was quantified photometrically as total phosphate according to Ames (1966), indicating the grade of extraction. The TA-containing supernatants of the extractions were combined, dialysed against purified water (16 h, 4 °C, three changes of water), and lyophilized (crude TA fraction).

To obtain cell walls free of TA, lyophilized walls after mild TA extraction were resuspended in water (15 mg in 10 ml) at 80 °C. Then, 2·5 ml 2% (v/v) N,N'-dimethylhydrazine in water (neutralized to pH 7·0 with dilute formic acid) was added (Hancock & Poxton, 1988). The suspension was stirred vigorously at 80 °C for 2 h. TA contents were again quantified as total phosphate before and after extraction. Cell walls were sedimented and washed after double extraction. The lyophilized preparations were used as TA-free cell walls.

Purification of TA. Crude TA fractions were purified according to Fiedler *et al.* (1984), with slight modifications. Anion-exchange column chromatography was carried out on DEAE-Sephacel (Pharmacia) at 4 °C, equilibrated with 10 mM Tris/HCl buffer pH 7·5. Purified TA were eluted with a linear gradient from 0 to 1 M NaCl. The phosphorus-containing fractions (Ames, 1966) were pooled, dialysed against distilled water (16 h, 4 °C), and lyophilized.

GLC of neutral- and amino-sugar alditol acetates. The methods of Fox *et al.* (1983) were used. Two-step hydrolysis of purified TA with 50% HF (0 °C, 16 h, Riedel-de-Haen) and 2 M HCl (100 °C, 3 h) was followed by sodium borohydride reduction of the monosaccharides. Subsequent peracetylation of the resulting sugar alcohols with acetic anhydride was carried out as described by Fiedler *et al.* (1981). As references, ribitol, rhamnose, glucose, galactose, glucosamine and inositol (as an internal standard) were derivatized accordingly. The resulting alditol acetate derivatives were separated on a fused silica megabore capillary column (Durabond DB-225, length 30 m, inside diameter 0·53 mm, film thickness 1 µm; J&W Scientific Fisons), connected to a Perkin Elmer 8420 gas chromatograph equipped with a flame ionization detector. Isothermal column temperature was 220 °C, carrier gas N_2 and split ratio 1:100. Detector and injector temperatures were 250 °C. Determination of retention times and integration of peak areas enabled qualitative and quantitative determination of TA components.

Phage binding to viable host cells and cell walls. The ability of viable host cells to adsorb bacteriophages was tested under standardized conditions as follows. To 1 ml host cells in SM buffer (approximately 10^8 cells ml^{-1}), 100 µl of a phage suspension (approximately 10^8 p.f.u. ml^{-1}) was added, resulting in a multiplicity of infection of 0·1. Phage binding capacities of the different cell wall preparations were assayed using a procedure similar to that of Valyasevi *et al.* (1990). Cell wall preparations were homogeneously suspended and used at concentrations that had been found in preliminary experiments (data not shown) to bind more than 90% of the phages (0·05%, w/v, in 1 ml SM buffer). After 5–20 min incubation at room temperature, assays were centrifuged (16000 *g*, 2 min) in order to sediment host cells or cell walls together with phages already adsorbed and to stop further phage adsorption. Residual unbound viruses in the supernatants were then enumerated as described by Loessner & Busse (1990), using the corresponding host strain. Phage adsorption rates and phage binding activity of cell walls were calculated as percentage reduction of p.f.u. in relation to phages added.

Adsorption kinetics and competitive phage desorption with antisera. Infective phage particles were enumerated at defined time points during the adsorption process and plotted on a time axis. Competitive exclusion of phages already bound to their receptors on either intact host cells or cell walls was examined by subsequent addition of polyvalent antiserum 1/2/4, which is a mixture of antisera against *Listeria* serovars 1/2 and 4 (Difco). At defined time points after addition of phages, antiserum (10 µl of a 1:10 dilution) was added to 200 µl aliquots of corresponding adsorption assays, and resulting phage titres in the supernatants

were determined. Controls were carried out without addition of antiserum.

Inhibition of phage attachment by antisera, TA, lectins or monosaccharides. Antisera against *Listeria* serovars 1/2 and 4 (Difco) were tested for their effectiveness in suppressing phage adsorption. Viable cells were preincubated with antisera (diluted 1:10 and 1:100, 15 min, 25 °C).

To test for inhibition of adsorption by TA, phages were preincubated with purified TA (5%, w/v) for 15 min, before addition of host cells.

Using the method of Valyasevi *et al.* (1990), host cells in 1 ml SM buffer were pretreated by addition of 100 µl of a lectin solution (0·4%, w/v, in SM buffer, room temperature, 5 min) from either *Canavalia ensiformis* (Concanavalin A, ConA), *Helix pomatia* (HPA) or *Ricinus communis* (RCA 120) (all from Boehringer Mannheim; for binding specificities see Results). Phages were then added and the mixture incubated for 15 min. Lectin activity (slight cell agglomeration) was monitored by light microscopy.

The abilities of monosaccharides present in listerial TA to prevent phage adsorption were tested by a method similar to that of Yokokura (1977). L-Rhamnose, D-glucose, D-galactose, D-glucosamine and *N*-acetyl-D-glucosamine (all from Sigma) were added to obtain final concentrations of 40 mM or 400 mM.

Unadsorbed phages in the supernatants and adsorption rates were determined as described above. All results of inhibition experiments were calculated in comparison to negative controls without inhibitor and expressed according to the formula:

Percentage inhibition =

$$1 - \frac{\text{(initial titre)} - \text{(titre after assay with inhibitor)}}{\text{(initial titre)} - \text{(titre after assay without inhibitor)}} \times 100$$

Electron microscopy. After addition of polyvalent antiserum and sedimentation of host cells, samples of the supernatants were ultracentrifuged (120000 *g*, 2 h), in order to sediment liberated phage A511 particles and to distinguish between irreversible phage adsorption (no viruses detectable) and nonspecific inactivation of contractile phage A511, which would also have resulted in low phage titres. Negative staining of sediments with uranyl acetate and electron microscopy was carried out according to Zink & Loessner (1992).

RESULTS

Cell wall preparation and analysis of TA

Successive removal of cell wall components and purification of TA was done in order to perform several experiments: (i) GLC analysis of TA components; (ii) phage adsorption and competitive desorption assays with cell walls of different preparation steps (crude, free of protein, reduced TA content, free of TA); and (iii) inhibition of phage adsorption to the viable host cell by addition of purified TA.

Using glycine/HCl buffer for mild extraction of TA yielded a high proportion of undegraded polymers. This TA fraction was purified and subsequently used for component analysis as well as for inhibition experiments. However, approximately 50% of TA remained in the cell walls even after repeated extraction, as measured by determination of residual phosphate in relation to the initial content. For preparation of cell walls free of TA, a
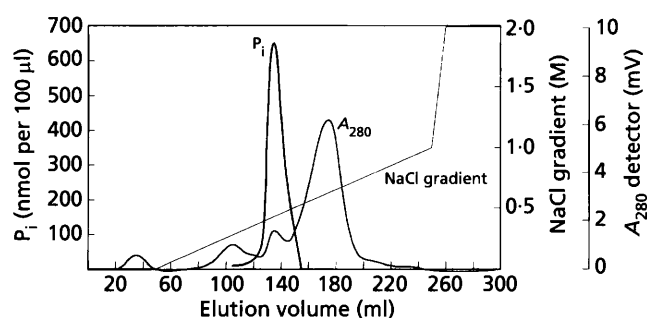

Fig. 1. Purification of glycine/HCl-extracted TA of *L. monocytogenes* WSLC 1003 by DEAE-cellulose column chromatography. For detection of TA, total phosphate was determined in each of the 10 ml fractions collected.

more caustic procedure with dimethylhydrazine was applied; this led to much higher extraction rates in cell walls of listerial strains after twofold extraction: WSLC 1003 (92%), WSLC 1042 (96%), WSLC 1442 (91%). The decrease in cell wall-associated phosphorus, given in parentheses, indicates an almost quantitative removal of TA. Extracted polymers were not used for subsequent experiments. Electron microscopic examinations confirmed that the remaining cell walls were not destroyed or visibly hydrolysed by this procedure (not shown).

A typical anion-exchange chromatogram of glycine/HCl-extracted TA from *L. monocytogenes* WSLC 1003 is presented in Fig. 1. In all cases, the phosphate-containing acidic polysaccharide (i.e. purified TA) eluted as a single peak in the fractions containing approximately 400 mM NaCl.

Results of GLC separations and quantifications of per-acetylated sugars and hexosamines found in hydrolysed TA are detailed in Table 1. Since ribitol or ribitol 1-phosphate may be converted into 1,4-anhydroribitol during acid hydrolysis (Kamisango *et al.*, 1983), peak areas of ribitol and anhydroribitol were summed before calculation. Surprisingly, strain WSLC 1442 completely lacked glucosamine in its TA.

Phage adsorption to *Listeria* cells and cell walls

Phage adsorption to different cell wall preparations after successive removal of certain components was investigated (Table 2). Crude cell walls and preparations free of protein retained their ability to adsorb phages; in fact cell walls free of protein showed improved phage binding. A clear loss of adsorption capacity for phages A118 and A500 was seen using cell walls with diminished TA contents. Removal of TA from cell walls of strain WSLC 1003 almost completely prevented attachment of phages A118 and A500, even though the final concentrations of cell walls were higher and the incubation times were prolonged. In contrast, the ability of cell walls to adsorb phage A511 was not affected by partial or total removal of TA. Adsorption assays of phages A118, A500 and A511 with viable cells of WSLC 1442 revealed that these phages were unable to bind to this strain.

Table 1. Composition of cell wall TA from strains of *L. monocytogenes*

Quantitative determination of substituents as alditol and hexosaminitol acetates is given in nmol per mg TA. Figures in parentheses indicate the molar ratios to ribitol. tr., trace.

Strain (WSLC)	Serovar	Content in TA [nmol per mg TA/(molar ratio to ribitol)]						
		Ribitol*	Glycerol	Rhamnose	Galactose	Glucose	Glucosamine	Phosphate
1003	1/2	974/(1)	tr.	226/(0·2)	0	tr.	611/(0·6)	1045/(1·1)
1042	4b	964/(1)	tr.	tr.	213/(0·2)	328/(0·3)	1060/(1·1)	885/(0·9)
1442	1/2a	1625/(1)	tr.	249/(0·2)	0	tr.	0	1561/(1·0)

* Sum of ribitol and anhydroribitol.

Table 2. Phage adsorption to cell wall preparations of host strains

Cell wall concentrations (%, w/v) are given in parentheses. Phage adsorption data are shown as percentage adsorption rates after 5 min incubation (100 % corresponds to no phages detectable in the supernatant).

Phage	Host strain (WSLC)	Serovar	Phage adsorption to cell wall preparation of the respective host strain:			
			Crude cell walls (0·05%)	Cell walls free of protein (0·05%)	Cell wall TA content reduced* (0·05%)	Cell walls free of TA (0·1%)
A118	1003	1/2	90	97	58	5
A500	1042	4b	76	95	88	8
A511	1003	1/2	99	100	100	86

* After extraction with glycine/HCl buffer.

Table 3. Inhibition of phage adsorption to host strains using polyvalent antisera

Phage	Host strain (WSLC)	Serovar	Inhibition (%)* by antiserum against serovar:			
			1/2		4	
			Dilution:		Dilution:	
			1:100	1:10	1:100	1:10
A118	1003	1/2	41	ND	0	0
A500	1042	4b	0	0	20	100
A511	1003	1/2	0	0	0	0

*0, No inhibition detectable; ND, not determined.

Inhibition of phage adsorption and competitive exclusion of bound phages by antibodies

Binding of the Siphoviruses A118 and A500 could be inhibited with specific antisera corresponding to the serovars of the respective host strains (Table 3). Adsorption capacities of strains pretreated with antiserum for a different serovar were not reduced. In contrast, A511 adsorption was not affected by either antiserum.

For kinetic adsorption and desorption studies, samples were taken during the assay and phage counts plotted against time (Fig. 2). Regarding the Siphoviruses A118 and A500, adsorption to live host cells was completed after 20 min (Fig. 2a, full line). After that time, phage adsorption was irreversible, as competitive exclusion experiments performed by addition of polyvalent antisera revealed no detectable phages in the supernatants. Up to this point, phages already adsorbed could be excluded from intact host cells by subsequent addition of antiserum, resulting in an immediate increase of free phages in the supernatant (Fig. 2a, dotted lines). Within the first 20 min, the proportion of liberated, infective particles (after application of antiserum) gradually decreased as incubation continued, with an increase in the fraction of phages irreversibly bound to their receptors. Preparations of cell walls were unable to irreversibly adsorb A118 and A500 particles: phages could be liberated even after 30 min to a very high degree (Fig. 2b).

Different results were obtained with phage A511. Particles bound to their receptors on both intact cells and cell walls
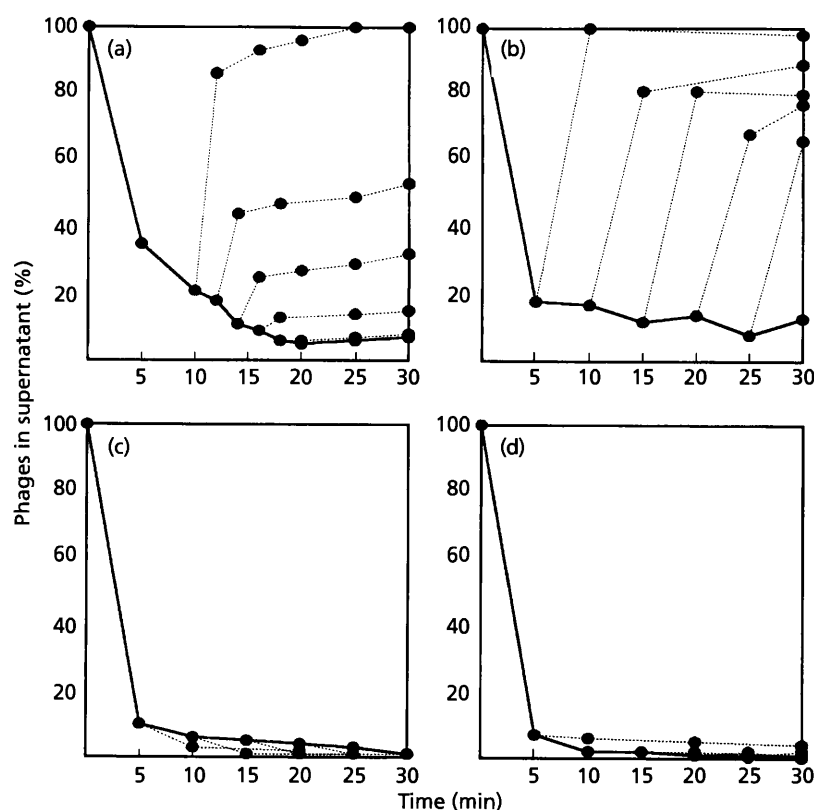
Fig. 2. Competitive exclusion (dotted lines) of adsorbed phages (full lines) from viable cells and from cell wall preparations of *L. monocytogenes* by addition of a mixture of polyvalent antisera 1/2 and 4 at different times. Results for phage A118 were comparable to those given for phage A500. (a) Phage A500/viable cells of host strain WSLC 1042; (b) phage A500/crude cell wall of host strain WSLC 1042; (c) phage A511/viable cells of host strain WSLC 1003; (d) phage A511/crude cell wall of host strain WSLC 1003.

very quickly, but could not be released by antiserum treatment (Fig. 2c, d). Quick and irreversible adsorption was also observed with both crude cell walls and cell walls with reduced TA content (results not shown, similar to Fig. 2d).

Electron microscopy showed that the supernatants after antiserum treatment and cell sedimentation were essentially free of unbound A511 particles. This indicates that true binding of the viruses was occurring, rather than inactivation of their infective capacity.

Inhibition of phage adsorption by TA, lectins and monosaccharides

With the exception of phage A511, adsorption rates of the phages tested were significantly reduced after pre-incubation with the corresponding TA (Table 4). TA prepared from strains not susceptible to A118 or A500 (e.g. *L. ivanovii* WSLC 3009) had no effect.

The inhibition of phage adsorption by lectins was found to correlate with the sugar substituents determined in the TA of host cell walls (Table 5). Adsorption of phage A118 was somewhat reduced after pretreatment of host cells with ConA, which has binding specificities to α-mannose > α-glucose > α-N-acetylglucosamine with decreasing affinities (Goldstein & Hayes, 1978; Lis & Sharon, 1986). Since mannose and glucose are not present in listerial TA, N-acetylglucosamine is the probable target of ConA and the critical determinant in the phage binding site. Pretreatment of host cells with HPA (α-N-acetylglucosamine

Table 4. Inhibition of phage adsorption after preincubation with purified TA

Phage	Host strain (WSLC)	Inhibition (%)* using TA isolated from strain:		
		1003	1042	3009
A118	1003	65†	0	0
A500	1042	0	76†	0
A511	1003	2†	3†	0†

* 0, No inhibition detectable.

† Phage host.

> α-galactosamine > α-galactose) almost quantitatively inhibited the adsorption of phages A118 and A511, and substantially decreased that of A500. RCA 120, which binds to β- and α-galactose substituents, decreased adsorption of A500 to host strain WSLC 1042, which contains galactose in its TA.

We also examined the influence of the TA monosaccharide substituents rhamnose, glucose, galactose and glucosamine on phage binding (Table 6). Inhibition of phage binding was dependent on the concentration of the monosaccharides. Complete blocking of phage A118 receptors was achieved with rhamnose and glucosamine. In contrast, N-acetylglucosamine slightly improved adsorption of phage A118 to its host. Glucosamine also

Table 5. Inhibition of phage adsorption using different lectins

Phage	Host strain (WSLC)	Monosaccharides found in TA*	Inhibition (%) by lectin†:		
			ConA	HPA	RCA 120
A118	1003	Rha, GlcN	21	92	3
A500	1042	Glc, Gal, GlcN	4	35	29
A511	1003	Rha, GlcN	0	93	2

* Gal, D-galactose; Glc, D-glucose, GlcN, D-glucosamine; Rha, L-rhamnose.
† See Results for specificities of lectins.

Table 6. Inhibitory effects of monosaccharides on phage adsorption to viable host cells

Phage	Host strain (WSLC)	Inhibition (%)* by monosaccharide (at 40 or 400 mM):									
		L-Rhamnose		D-Glucose		D-Galactose		D-Glucosamine		N-Acetyl-D-glucosamine	
		40	400	40	400	40	400	40	400	40	400
A118	1003	22	100	0	0	1	2	15	100	0	−6
A500	1042	0	0	0	4	4	5	8	84	0	0
A511	1003	0	0	3	5	0	1	0	0	0	0

* 0, No inhibition detectable.

inhibited A500 adsorption, while the other sugars had no effect. Interestingly, binding of A511 to its host was not affected at all.

DISCUSSION

Components of listerial TA

Generally, the TA components determined in this study were in accordance with earlier reports for *Listeria* species (Fiedler *et al.*, 1984; Fiedler & Ruhland, 1987; Fujii *et al.*, 1985; Kamisango *et al.*, 1983). These previous studies also found glucosamine to be a main component in TA of all listerial serovars. However, *L. monocytogenes* WSLC 1442 completely lacks glucosamine in its TA (Table 1). The possible implications of this phenotype are discussed further below.

Kamisango *et al.* (1983) deduced the probable structure of listerial TA from NMR spectrometry and found that glucosamine residues were fully N-acetylated in the native wall. This is in agreement with our results. Inhibition of phage adsorption occurred with the lectins ConA and HP, which bind to N-acetylglucosamine (Table 5). In contrast, pure N-acetylglucosamine was not able to hinder phage binding, whereas glucosamine did (A118 and A500, Table 6). The same phenomenon was described by Keogh & Pettingill (1983), who explained the failure of N-acetyl-glucosamine to inactivate phages as being due to unsuitable molecular conformation or charge under the experimental conditions.

The primary receptors for phages A118 and A500 are O-antigenic TA substituents

The effects of antisera on the kinetics of phage adsorption to host cells indicate that there is a lag period before viruses become firmly associated with host cells, as postulated by Watanabe & Takesue (1975) for *Lactobacillus casei*. Since bacteriophages can be liberated by antibodies from their hosts in a competitive fashion (Fig. 2a, b), the phage receptor structures involved can be regarded as strongly immunogenic. Cleary *et al.* (1977) considered the inhibition of phage adsorption by specific antisera to be misleading and debatable, as antibody bound to carbohydrate could sterically hinder adsorption by masking adjacent or underlying receptors. However, in our investigations, phage A511 bound to its host (WSLC 1003) in the presence of polyvalent antiserum, whereas A118, under the same conditions, did not (Table 3). Our results support the hypothesis that identical molecules determine both phage adsorption and specific O-antigens, a phenomenon which is known to occur among other bacterial species (for a review, see Lindberg, 1973).

Coyette & Ghuysen (1968) and Shaw & Chatterjee (1971) found that glucosamine substituents in TA serve as primary phage binding sites in *Staphylococcus aureus* walls, whereas Valyasevi *et al.* (1990) showed that phage binding to *Lactococcus lactis* subsp. *cremoris* involves the rhamnose of the extracellular wall polysaccharide. Keogh & Pettingill (1983) found rhamnose, N-acetylgalactosamine

and N-acetylglucosamine to be major immunogenic factors in two strains of *Streptococcus cremoris*, and these sugars and amino sugars also inhibited bacteriophage adsorption. Regarding *Listeria*, Ullmann & Cameron (1969) and Kamisango *et al.* (1983) reported that the major antigenic determinant in *L. monocytogenes* serovar 1/2 is rhamnose, but for serovar 4 the determinants are galactose and/or glucose. We showed that for phage A118 glucosaminyl and rhamnosyl components of ribitol TA are involved in primary and reversible adsorption; for phage A500 glucosaminyl residues are necessary (Table 6).

Reversible and irreversible adsorption of phages A118 and A500

Crude cell walls failed to irreversibly bind phages A118 and A500 (Fig. 2b), but the potential for reversible adsorption was not affected (Fig. 2b, Table 2). Irreversible binding occurs only to living cells (Fig. 2a) and might be dependent on cell membrane components (Cleary *et al.*, 1977) or a wall protein (Keogh & Pettingill, 1983; Lindberg, 1973). However, receptors for reversible attachment of phages were not affected by heat treatment or removal of proteins (Table 2). Crude cell walls as well as preparations free of protein retained their ability to adsorb phages, indicating that the receptors for reversible binding are not proteinaceous.

In accordance with Valyasevi *et al.* (1990, 1991) and Cleary *et al.* (1977), our results indicate that adsorption of the Siphoviruses A118 and A500 occurs in a two-step reaction, requiring the TA units on the cell wall for initial, reversible attachment and a putative secondary component for the irreversible binding step.

An intact complex between TA and pepticoglycan is not necessary for binding to A118 and A500 receptors

We found that the adsorption of Siphoviruses A118 and A500 could be effectively prevented using a purified TA preparation (Table 4). Here, an intact complex of TA to peptidoglycan is obviously not necessary. In contrast, bacteriophage receptor sites in *Bacillus subtilis* (Glaser *et al.*, 1966; Young, 1967), *Staphylococcus aureus* and *Staphylococcus epidermidis* (e.g. Schleifer & Steber, 1974) were reported to involve both the glycosylated TA and peptidoglycan components of the cell walls. Although part of the receptor, purified TA itself generally had no effect (Lindberg, 1973). Thus, the configuration and chemical composition of the peptidoglycan–TA complex seems to be important for bacteriophage adsorption among the genera mentioned, but not in *Listeria*.

Irreversible adsorption of phage A511 to peptidoglycan

In contrast to the Siphoviruses A118 and A500, the broad host-range contractile Myovirus A511 apparently uses the peptidoglycan itself as a primary receptor, because neither deproteinization and lipid extraction nor removal of cell

wall TA led to a loss of its rapid and irreversible adsorption capability (Table 2, Fig. 2a, b), indicating that its receptor has been retained. This was also found for bacteriophage PL-1 of *Lactobacillus casei* (Watanabe & Takesue, 1975). It is notable that completely deproteinized cell walls showed a somewhat increased phage binding, which might be due to better accessibility of receptor molecules.

The fact that efficient and irreversible attachment of A511 to purified murein was independent of TA glycosyl substituents (Table 6) or the presence of TA itself (Table 2), and could not be inhibited with purified TA (Table 4), is in accordance with data of Cleary *et al.* (1977), who reported that highly purified peptidoglycan, devoid of phosphorus, retained its irreversible reactivity with phages for group A streptococci.

The blocking of A511 receptors after application of HPA lectin (Table 5) is explained by binding of the lectins to the N-acetylglucosamine components of the peptidoglycan. This hypothesis was supported by the observation that, after addition of HPA lectin, cell walls of strain WSLC 1003, free of TA, showed agglomeration to the same extent as crude cell walls.

Phage resistance may be caused by a lack of glucosamine in the TA

Listeria monocytogenes WSLC 1442 is resistant to lysis by more than 60 *Listeria* phages used in phage typing in this laboratory (unpublished observation). Moreover, the three phages tested here were even unable to bind to viable cells of this strain. Carbohydrate analysis of its TA revealed an unusual lack of glucosamine (Table 1), which we speculate determines the phage resistance of this strain. In accordance, Glaser *et al.* (1966) and Young (1967) reported that transformation from phage sensitivity to phage resistance in *Bacillus subtilis* is accompanied by deletion of glucose from the respective TA. It is, however, unclear why A511 did not bind to WSLC 1442 cells, since it was not inhibited by glucosamine (Table 6), which presumably is not part of the phage receptor. It might be assumed that the lack of TA-linked glucosamine is due to mutations and associated with other uncharacterized changes in the chemical composition of WSLC 1442 cell walls.

It would be interesting to determine in further investigations whether the serologically correlated phage adsorption specificities can be generalized within the serovar groups. Furthermore we want to examine whether phage resistance of other listerial strains is due to decreased adsorption capability caused by a lack of glucosamine or other sugar components in the cell wall TA.

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