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Identification of ORF636 in Phage ϕSLT Carrying Panton-Valentine Leukocidin Genes, Acting as an Adhesion Protein for a Poly(Glycerophosphate) Chain of Lipoteichoic Acid on the Cell Surface of *Staphylococcus aureus*▽

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The temperate phage ϕSLT of *Staphylococcus aureus* carries genes for Panton-Valentine leukocidin. Here, we identify ORF636, a constituent of the phage tail tip structure, as a recognition/adhesion protein for a poly(glycerophosphate) chain of lipoteichoic acid on the cell surface of *S. aureus*. ORF636 bound specifically to *S. aureus*; it did not bind to any other staphylococcal species or to several gram-positive bacteria.

Staphylococcus aureus, a ubiquitous and harmful human pathogen, produces three types of bicomponent pore-forming cytotoxins, namely, γ-hemolysin (LukF and Hlg2), leukocidin (LukF and LukS), and Panton-Valentine leukocidin (PVL) (LukF-Pv and LukS-Pv) (16). Of these, PVL has been investigated as a virulence-related factor of some *S. aureus* infectious diseases (7, 11, 23, 24, 31, 37). PVL shows high cytolytic specificity against human polymorphonuclear leukocytes and macrophages, and it is closely associated with most cutaneous necrotic lesions, such as furuncles or primary abscesses, and severe necrotic skin infection (24, 31), as well as with severe necrotic hemorrhagic pneumonia (11, 23). LukF-Pv and LukS-Pv are expressed by the PVL locus (*pvl*), which is distinct from the γ-hemolysin locus (*hlg*) (16, 32). In previous research, we found that *pvl* genes are located in the genome of the lysogenic bacteriophage ϕPVL (17, 18). We also found another PVL-carrying temperate elongated-head *Siphoviridae* phage, ϕSLT, which has the ability to convert *S. aureus* to the PVL-producing strain from a clinical isolate (29). These findings indicated that at least two types of staphylococcal temperate phages are involved in the horizontal transfer of *pvl* genes among *S. aureus* strains (16, 29). Recently, the emergence of a single clonal community-acquired methicillin-resistant *S. aureus* (CA-MRSA), which produces PVL, was reported (7). Most CA-MRSA strains isolated in the United States and Australia carry the staphylococcal cassette chromosome *mec* (SCC*mec*) IV, and they were divided into five clonal complexes by multilocus sequence typing (30). The analysis of the CA-MRSA clones confirmed the presence of PVL genes and SCC*mec* IV in CA-MRSA and suggested that various CA-MRSA strains have arisen from the diverse genetic backgrounds associated with each geographic origin, rather than from the worldwide spread of a single clone (30, 37). Although there is great debate as to whether PVL is an important virulence factor, numerous studies support the hypothesis that PVL plays an important role in the pathogenesis of CA-MRSA necrotizing pneumonia (3, 6). In regard to the acquisition of PVL gene clusters and the proliferation of PVL-carrying CA-MRSA, the horizontal transfer of PVL via PVL-carrying phages, as well as that of SCC*mec*, has become the focus of intense research interest. To understand the horizontal transfer of PVL, the analysis of the infection ability of a PVL-carrying phage is important. If the phage has a wide host range, the PVL-carrying phage might threaten to become a source of emerging PVL-positive bacteria. Phage infection starts from an interaction between a phage virion and its host cell surface receptor. Nevertheless, little is known about phage receptors on the surface of *S. aureus*, and the mechanism of host cell-specific binding of staphylococcal phages has been poorly characterized. In addition, there is no information about staphylococcal phage proteins involved in host cell recognition and/or binding. Here, we identify ORF636, with a mass of 66 kDa, as a structural protein of the ϕSLT tail and determine that it acts as a protein for recognition/adhesion of a poly(glycerophosphate) moiety of lipoteichoic acid (LTA) on the cell surface of the host *S. aureus* in the first stage of infection by ϕSLT.

Identification of ORF636, a novel tail structural protein of ϕSLT. Previously, we determined the entire genome sequence of ϕSLT and found six open reading frames (ORFs) for structural proteins, which include ORF387, a capsid protein; ORF412, a portal protein; ORF151, -636, and -488, minor tail proteins; and ORF212, a major tail protein of the phage (18). Among the three minor tail proteins, ORF636 had two unique regions: (i) the region comprising 267 amino acid residues in a central region (Thr151 to Ile416), with 53% identity and 78% sequence homology to the region (Thr18 to Ile284) of TagC from *Bacillus subtilis* strain 168 that is involved in the assembly

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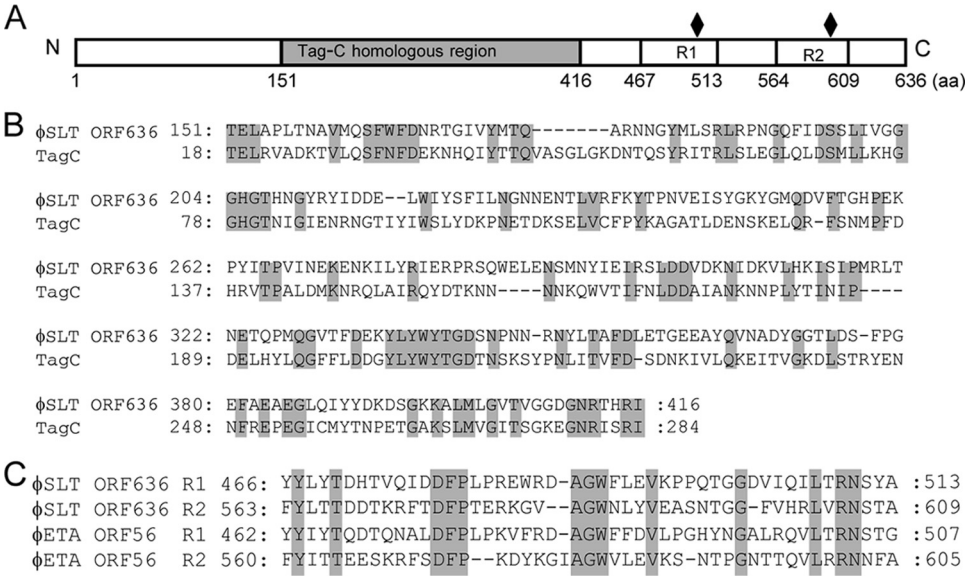

FIG. 1. Organization of ORF636. Protein and nucleotide sequences were compared with the sequence databases using the BLAST programs implemented at the EMBL/GenBank/DDBJ nucleotide sequence databases and SWISSPROT/NBRF-PIR protein sequence databases. ORF identification and multiple-sequence alignment were performed using the GENETYX program (Software Development Co., Tokyo, Japan) and the CLUSTALW program on the DDBJ site. (A) Schematic representation of ORF636. The shaded box represents the region having homology to the poly(glycerophosphate) teichoic acid biosynthesis protein TagC of *B. subtilis*. The two boxes in the C-terminal domain represent GW repeats consisting of 47-amino-acid (aa) (R1) and 46-amino-acid (R2) sequences with GW sequences (diamonds). The numbers indicate the positions of amino acids in ORF636. (B) Alignment of the predicted amino acid sequence of the product in the central region of ORF636 with that of the *B. subtilis* TagC product. Identical residues are shaded. (C) Comparison of the deduced amino acid sequences of two GW repeat sequences (R1 and R2) of ORF636 from ϕSLT and ORF56 from ϕETA. The numbers indicate the positions of amino acids in ϕSLT ORF636 and ϕETA ORF56.

and export of poly(glycerophosphate) teichoic acid (26), and (ii) the region consisting of 47- and 46-amino-acid repeating basic units with a glycine-tryptophan dipeptide (GW) sequence in the C-terminal region of the protein, which resembles the C-terminal region of the invasion protein InlB anchored to LTA on the surface of *Listeria monocytogenes* (15) (Fig. 1). Although no significant overall homology of amino acid sequences was observed among the characterized GW repeat sequences, various surface proteins with different biological functions possess GW repeats in gram-positive bacteria (12, 35). These findings led us to assume the possibility that ORF636 of ϕSLT is a recognition/adhesion protein for LTA on the *S. aureus* cell surface that acts in the first step of infection. To confirm this possibility, we first visualized ORF636 on the phage virion by using immunogold labeling, followed by electron microscopy. For the preparation of anti-ORF636 antibodies, we constructed a plasmid, pETH636, for the expression of His-tagged ORF636 (H636). The DNA fragment including full-length *orf636* was amplified from ϕSLT DNA by PCR using primer I (5′-GCCATATGGAAAATTTG TATTTAATAAAGGATTTGGGAGC-3′) and primer II (5′-AATGGATCCTTAACTTATAATTCTCCCTTCGTGTAAA GTC-3′), which contain the start and stop codons of *orf636*, respectively. These primers also contain NdeI and BamHI sites, respectively (underlined). The amplified fragment was digested with NdeI and BamHI and was inserted into the NdeI-BamHI site of the expression vector pET15-b (Novagen) to construct a plasmid, pETH636, containing the sequence corresponding to H636. *Escherichia coli* BL21 harboring pETH636 was grown in LB medium containing ampicillin (100 μg/ml) at 37°C with shaking to an optical density at 660 nm of 0.6. IPTG (isopropyl-β-D-thiogalactopyranoside) was added at a final concentration of 0.4 mM, followed by incubation with shaking for an additional 2 h at 30°C. Expressed H636 was accumulated as an inclusion body in the cells. Precipitated H636 was solubilized with 20 mM potassium phosphate containing 0.5 M NaCl (pH 7.0) (PBS) and 8 M urea and was dialyzed in steps to gradually reduce the urea concentration to obtain the refolded H636 preparation (Fig. 2A). An anti-H636 antiserum was used in the immunogold labeling of ORF636. The antiserum was raised in female BALB/c mice injected with a purified H636 preparation (60 ng) emulsified with Freund's complete adjuvant (Wako Pure Chemical Industry, Osaka, Japan), followed by four boosts (at 2-week intervals) with the same amount of H636 mixed with Freund's incomplete adjuvant (Wako Pure Chemical Industry, Osaka, Japan). Three days after the final boost, blood was collected to prepare the antiserum, and its titer was assayed by Western blotting. The 300 ng of purified H636 was clearly detected by the anti-H636 antiserum at a dilution of 1:10,000. For colloidal gold immunolabeling, the ϕSLT virions (3×10^7 PFU/10 μl), which were prepared and purified according to methods described previously (29) and dialyzed against an SMC buffer (29), were incubated for 2 h at room temperature with primary anti-H636 antibodies, which were diluted 1:100 in PBS in a 0.6-ml sample tube. Fifteen microliters of the reaction mixture was mixed with 15 μl of goat anti-mouse immunoglobulin G–5-nm gold conjugate solution (BBI, Cardiff, United Kingdom), diluted 1:40 (vol/vol) with PBS, and then applied to carbon-coated polyvinyl formal (Formvar) films on 400-mesh copper grid. After 1 h

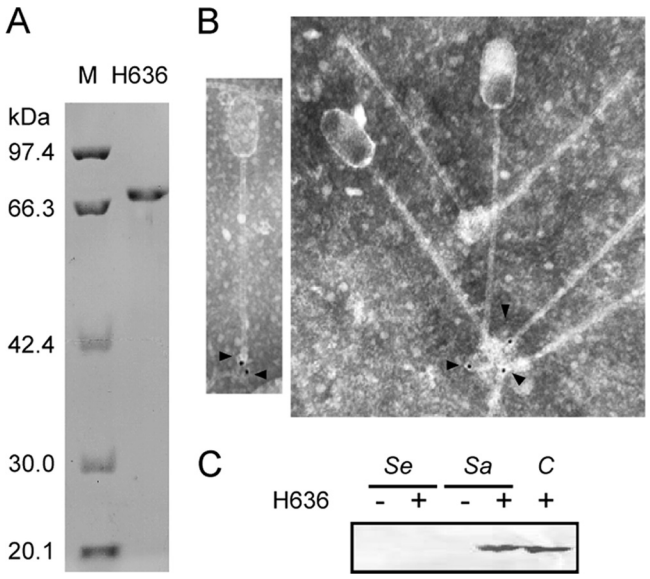

FIG. 2. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile of the purified H636 preparation from *E. coli.* ORF636 was purified as an N-terminal His_6-tagged fusion protein. The gel was stained with Coomassie brilliant blue R-250. The molecular mass standards (lane M) were phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), and bovine carbonic anhydrase (31 kDa). The second lane contained the H636 preparation. (B) Transmission electron micrographs of the immunogold-labeled ϕSLT minor tail protein ORF636. ϕSLT particles were incubated with anti-H636 antiserum and labeled with anti-mouse immunoglobulin G-conjugated gold particles. (C) Binding activity of the H636 preparation to staphylococcal cells. Protein bands on the cells were visualized with anti-H636 antiserum, and the amount of protein 636 was determined by quantitative comparisons with the signal generated by an amount of the purified protein 636 preparations. *Se*, *S. epidermidis* JCM2414T cells; *Sa*, *S. aureus* RN4220 cells; *C*, control H636.

of incubation at room temperature, extra gold conjugate solution was washed, and negative staining was performed as described previously, except for the use of 2% phosphotungstic acid (17, 29). Gold particles were observed only around the tip of the tail (Fig. 2B), indicating that anti-H636 recognizes the distal part of phage tail tip constituents. Moreover, anti-H636 appeared to promote the aggregation of phage tails at the tips of the tails (Fig. 2B, right). These results indicate that ORF636 is a constituent of ϕSLT tail tip structural proteins joining at the base plates of phage tails, possibly as tail fiber proteins.

ORF636 is essential for selective adhesion of ϕSLT to *S. aureus* cells. Generally, a bacteriophage adsorbs to its host cell at the tail tip. To ascertain that ORF636 is involved in recognition of the cell surface of *S. aureus*, we examined the binding activity of ORF636 to *S. aureus* cells compared to binding to *Staphylococcus epidermidis* cells. For assay of H636 protein binding to the cells, staphylococcal cells grown to exponential phase in 1 ml of heart infusion broth were collected and suspended in 1 ml of fresh heart infusion broth that contained 3.8 mg of H636 preparation and then incubated for 30 min at room temperature. The cells were harvested and washed five times with PBS and applied to 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by Western immunoblotting with anti-H636 antiserum. H636 was detected on *S. aureus* RN4220 cells, but not on *S. epidermidis* JCM2414T cells (Fig. 2C). ORF636 did not bind to *Streptococcus pneumoniae*, *Enterococcus faecalis*, or *B. subtilis* cells (data not shown), suggesting the cell specificity of ϕSLT binding through ORF636. To investigate whether the host specificity of ϕSLT depends on cell recognition for the first step of phage infection, the adhesion activity of ϕSLT was tested against 150 methicillin-susceptible *S. aureus* strains originating from food poisoning in our laboratory collection and 13 types of *Staphylococcus* isolate strains from humans or animals purchased from the Japan Collection of Microorganisms. The cells were grown aerobically at 37°C in heart infusion broth (Difco) until the cell density reached 8×10^8/ml. Then, ϕSLT was mixed with host cells at an multiplicity of infection of 0.01 and incubated for 20 min at 37°C. After centrifugation, free phage remaining in the supernatant were counted. As a result, ϕSLT was found to adhere to all of the assayed *S. aureus* cells. In contrast, ϕSLT never adhered to other staphylococci, e.g., *Staphylococcus intermedius* (JCM2422T; ATCC 29663), *S. epidermidis* (JCM2414T; ATCC14990), *Staphylococcus warneri* (JCM2415T; ATCC27836), *Staphylococcus haemolyticus* (JCM2416T; ATCC29970), *Staphylococcus cohnii* (JCM2417T; ATCC29974), *Staphylococcus homius* (JCM2419T; ATCC27844), *Staphylococcus capitis* (JCM2420T; ATCC27840), *Staphylococcus hyicus* (JCM2423T; ATCC11249), *Staphylococcus simulans* (JCM2424T; ATCC27848), *Staphylococcus sciuri* (JCM2425T; ATCC29062), *Staphylococcus saprophyticus* (JCM2427T; ATCC15305), *Staphylococcus felis* (JCM 7496T), *Staphylococcus schleiferi* (JCM2425; ATCC49545), or *B. subtilis* strain Marburg. This resulted in no formation of plaques on the lawns of the staphylococci or *B. subtilis*.

To assess the necessity of ORF636 for ϕSLT infection, the neutralization of ϕSLT plaque formation by the anti-H636 antibody was investigated. When the purified ϕSLT particles (1.5×10^3 PFU/100 μl) were incubated with anti-H636 antiserum at 37°C for 30 min before infection, plaques forming on the lawn of the indicator strain RN4220 were neutralized, depending on the serum dosage (Fig. 3A), whereas a decrease in plaque numbers was not observed with normal mouse serum (data not shown). Moreover, more than 80% of ϕSLT particles (1.2×10^3 PFU) were inactivated by the addition of a 1:3,300-diluted anti-H636 antiserum and were completely restored by excess amounts of H636 (5 μg) (Fig. 3A). This indicates that the neutralization was competitively inhibited by additional amounts of H636 preparation at each serum concentration. These results clearly show that masking of ORF636 in the phage tail tip inhibits phage infection. Competition of ϕSLT binding to the host cell by H636 protein was also performed. The purified H636 protein (9 to 180 μg) was preincubated with *S. aureus* RN4220 cells (4×10^6 cells) for 10 min at 37°C. Then, ϕSLT particles (3×10^3 PFU) were mixed and incubated for 5 min at 37°C. After centrifugation, free phage remaining in the supernatant were counted. The adsorption of ϕSLT to the cell was prevented in a dose-dependent manner by the addition of H636 protein (Fig. 3B). When 90 μg (7.2×10^{14} molecules) of H636 was added to the mixture, more than 95% of ϕSLT did not bind to the host cell. Thus, we concluded that ORF636 in the ϕSLT tail structural protein plays a crucial role in the recognition of the cell surface of *S. aureus*.

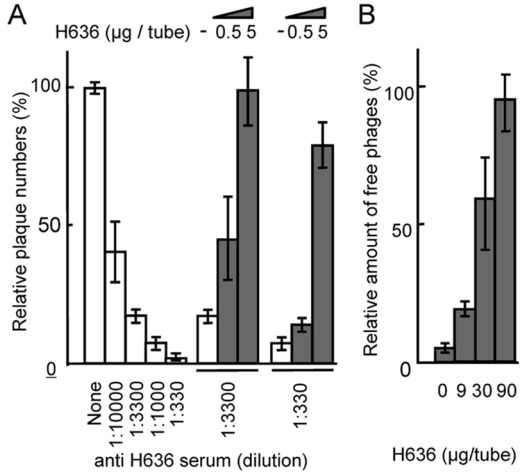

FIG. 3. (A) Inhibition of phage infection by anti-H636 antiserum. ϕSLT particles (1.5×10^3 PFU) were preincubated with various concentrations of anti-H636 antiserum for 30 min at 37°C. Then, the plaques of ϕSLT on the lawn of *S. aureus* RN4220, which was treated with anti-H636 antiserum with (shaded bars) or without (open bars) H636 as a competitor, were counted, and the relative plaque numbers were calculated. (B) Competition of infection by ORF636. Purified H636 protein (9 to 180 μg) was preincubated with RN4220 cells (4×10^6 cells) for 10 min at 37°C. Then, ϕSLT particles (3×10^3 PFU) were mixed and incubated for 5 min at 37°C. After centrifugation, free phage that remained in the supernatant were counted by plaque formation on the lawn of *S. aureus* RN4220. The data are shown as the average values from at least three independent measurements. The error bars represent standard deviations.

ORF636 interacts with staphylococcal LTA. From the features of the amino acid sequence of ORF636, we assumed that LTA and/or cell wall teichoic acid (WTA) on the surface of the host cell wall was the candidate molecule that interacted with ORF636 in the phage particles at the first stage of phage infection. To assess the possibility, inhibition of phage infection by staphylococcal LTA and WTA was examined. Staphylococcal WTA was prepared from strain RN4220 by the method of Jenni and Berger-Bachi (14). WTA was extracted from the dried cell wall preparation (443 mg) with 17.7 ml of 10% trichloroacetic acid at 60°C with shaking for 12 h. An insoluble peptidoglycan fraction was removed by centrifugation (15,000 × *g*; 30 min), and the supernatant containing WTA (13 ml) was mixed with 5 volumes of ethanol and kept at 4°C for 72 h. After centrifugation (16,000 × *g*; 30 min; 4°C), the WTA was reextracted from the precipitate with 10% trichloroacetic acid and collected by ethanol precipitation. The WTA preparation was washed three times with ethanol (20 ml) and twice with diethyl ether (20 ml) and then vacuum dried. A deacylated LTA derivative was prepared by hydrolyzing the *S. aureus* LTA preparation (Sigma) with 0.2 M NaOH in 1.5 ml of 50% aqueous methanol at 37°C for 16 h, according to the method described by Koch and Fischer (21). Fatty acids that were released from the LTA preparation were extracted with petroleum ether. The deacylated LTA preparation thus obtained was confirmed to be fatty acid free. For neutralization of ϕSLT infection by staphylococcal LTA, the purified ϕSLT particles (1.5×10^3 PFU/tube) were treated in 100 μl of reaction mixture with staphylococcal LTA, WTA, or *Bacillus* LTA diluted with PBS buffer (pH 7.2). After a 30-min incubation at

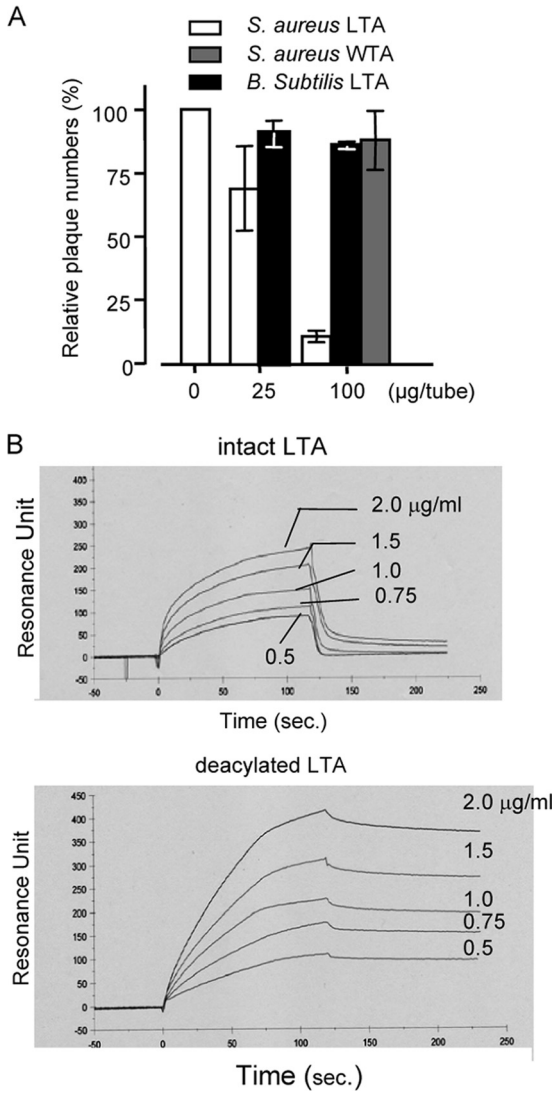

FIG. 4. (A) Inactivation of phage ϕSLT with LTA preparations. ϕSLT particles (1.5×10^2 PFU) were preincubated with an LTA preparation from either *S. aureus* or *B. subtilis* or with an *S. aureus* WTA preparation at room temperature for 30 min in 100 μl of bicarbonate buffer (pH 9.6). The reaction mixture was added to strain RN4220, which was grown in 1 ml of heart infusion broth medium to exponential phase, followed by incubation at 37°C on the lawn of RN4220, and the plaques were counted. The level of inhibition was expressed as a percentage of ϕSLT infection. The data represent the means and standard errors from at least three experiments. (B) BIAcore sensorgrams. The purified H636 protein (1.4 mg) was captured across the activated surface of the CM5 sensor chip. The amounts of *S. aureus* LTA and deacylated *S. aureus* LTA, used as an analyte, are indicated.

room temperature, the reaction mixture and 100 μl of the indicator strain were added to 6 ml of soft agar and immediately plated. The number of plaques formed on the lawn of strain RN4220 was determined. When the ϕSLT particles were incubated with an LTA preparation from *S. aureus*, a dose-dependent decrease in the number of plaques on the lawn of RN4220 was observed (Fig. 4A). In contrast, the staphylococcal WTA preparation did not cause a decrease in plaque num-

bers (Fig. 4A). These results indicate that ORF636 selectively interacts with LTA, but not with WTA, on the cell surface of *S. aureus*. In the case of *S. aureus*, LTA consists of a 1,3-linked poly(glycerophosphate) chain, while WTA has a poly(ribidol phosphate) backbone. ORF636 may recognize the difference between LTA and WTA through their backbone structures. The LTA of *B. subtilis* has a poly(glycerophosphate) backbone in its core structure that bears a close resemblance to that of *S. aureus* LTA (9). However, the LTA preparation from *B. subtilis* (Sigma) did not prevent infection by ϕSLT of the indicator cells (Fig. 4A).

To obtain direct evidence of molecular interaction between ORF636 and the poly(glycerophosphate) chain of *S. aureus* LTA displayed on its cell surface, BIAcore surface plasmon resonance analysis was performed. The BIAcore analysis was done with purified H636 using the method described by Karlsson et al. (19). The purified H636 preparation (1.4 mg) was immobilized on a CM5 chip (BIAcore) by a standard amine-coupling procedure. The deacylated preparations of LTA from *S. aureus* (Sigma) were used as analytes. Injection and binding experiments were carried out with a 5-μl/min flow of 10 mM HEPES buffer (pH 7.4) containing 0.15 M NaCl. Regeneration after each round of binding was achieved using a glycine buffer. As shown in Fig. 4B, a significant increase in resonance in BIAcore sensorgrams was observed when an LTA preparation or a deacylated LTA preparation was added as an analyte to the immobilized H636. Intact LTA exhibited a higher binding rate than deacylated LTA, but the interaction intensity between LTA and ORF636 was about 30% less than that obtained in the deacylated LTA. In contrast, very rapid dissociation was observed in intact LTA, whereas deacylated LTA dissociated slowly from ORF636. These sensorgram patterns indicate that the LTA interaction with intact H636 is quickly reversible, while the interaction of the H636-polysaccharide chain of LTA is slower and seems to be essentially irreversible. The insufficient inhibition of plaque formation by ϕSLT in the presence of excess amounts of ORF636 or LTA (Fig. 2B) may be explained by this rapidly reversible interaction between H636 and LTA. In contrast, no interaction between H636 and *B. subtilis* LTA was observed (data not shown). As described above, ϕSLT could not adsorb to *B. subtilis* cells. LTAs of *S. aureus* and *B. subtilis* have poly(glycerophosphate) backbones with different side chain structures. Our results support the notion that ϕSLT selectively recognizes the whole polysaccharide chain structure of polysaccharide LTA on the host cell surface via H636.

Discussion. We have shown that many lactococcal phages adsorb initially to the cell wall and subsequently to a host cell membrane protein, PIP, which leads to the ejection of the phage genome into the host cell (27). The initial adsorption of the phage to the cell wall carbohydrates is a reversible process, and subsequent adsorption of the phage to PIP is irreversible. Although the protein receptor of ϕSLT is not yet clear, the adsorption of ϕSLT is presumed to consist of two stages, like that of lactococcal phages, that is, an initial reversible interaction between ORF636 and LTA, followed by an irreversible binding of the phage tail to the receptor on the cell surface.

So far the phage receptors on the surface of *S. aureus* have been explored by a combination of selective cell wall lytic enzymes and chemical degradation methods. Murayama et al. identified a complex of glycopeptide and ribitol teichoic acid as a receptor for phage 3C (28). Coyette et al. reported the importance of the 4-*O*-β-(*N*-acetyl-D-glucosaminyl) moiety in ribitol teichoic acid in the infection of phages 3C, 71, and 77 (5), and their data were supported by Shaw and Chatterjee (36). Chatterjee showed the importance of the GlcNAc moiety in teichoic acid for phage inactivation, using a bacteriophage-resistant mutant (4). However, no additional research regarding the phage receptor(s) for staphylococcal phages has been available, because the phage protein allowing the phage to attach to the staphylococcal surface receptor had not been characterized. The cell wall of *S. aureus* consists of (i) peptidoglycan; (ii) carbohydrate; (iii) surface proteins; (iv) WTA, which consists of a repeating poly(1,5-ribitol diphosphate) chain linked through a phosphodiester linkage to the peptidoglycan; and (v) LTA, which consists of repeats of a 1,3-linked poly(glycerophosphate) chain linked to the cytoplasmic membrane through a fatty acid (20, 21). LTAs are hydrophobically anchored to the membrane through their fatty acids, and the carbohydrate chain of LTAs is transfixed and associated with the peptidoglycan layer (8). It is known that the LTAs of *S. aureus* and *B. subtilis* have the same 1,3-linked poly(glycerophosphate) backbone, but the degree of glycosylation and D-alanine substitution of the polyglycerophosphate backbone in *S. aureus* is higher than that in *B. subtilis* (8–10). Our results favor the notion that this difference in LTA backbone modifications between *S. aureus* and *B. subtilis* may be involved in the selective recognition by ORF636. All staphylococci, including the species to which ϕSLT could not bind, contain poly(glycerophosphate) backbones modified with glucose, *N*-acetylglucosamine, and/or N-acetylgalactosamine in their WTAs (20). Thus, we suggest that ORF636 of ϕSLT has the function of distinguishing the degree of modification of poly(glycerophosphate) backbones with D-alanine and *N*-acetylglucosamine of *S. aureus* LTA from those of other staphylococcal LTAs/WTAs.

Recently, LTA of *Lactobacillus delbrueckii* was identified as the receptor of phage LL-H (33). It is suggested that phage LL-H adsorbs to *L. delbrueckii* via its gp71 protein (34) and that the D-glucosyl and D-alanyl substitutions of the 1,3-linked poly(glycerophosphate) of *L. delbrueckii* LTA affect LL-H or related phages (33). In these reports, the authors also observed that deacylated lactococcal LTA showed a higher capacity for inactivation of phage LL-H infection. Although they did not mention the interaction between the polysaccharide chain of lactococcal LTA and gp71 of phage LL-H, the slow and irreversible interaction between them is assumed to be involved in the inactivation of LL-H infection by deacylated lactococcal LTA. Even though the primary structure of ORF636 shows no homology with gp71, we observed a slow and strong interaction between H636 and the polysaccharide chain of staphylococcal LTA. Most recently, Baptista et al. indicated that glucosylated poly(glycerophosphate) WTA is the major recognition target for *Bacillus* phage SPP1 reversible binding and precedes binding to YueB, a membrane protein that irreversibly binds to SPP1 (2). SPP1 binds to the surfaces of *yueB* mutants, but in a completely reversible way. They constructed the WTA synthesis mutants, and the adsorption kinetics of phage particles were studied to clarify the reversible binding factor(s) on bacterial cell walls. However, it is not clear which protein(s) on the

virion of SPP1 recognizes the structure of WTA. No homology between ORF636 and SPP1 proteins was observed, and ϕSLT could not bind to *B. subtilis* cells. These facts suggest that the systems for recognizing poly(glycerophosphate) cores and their side chain structures of host cell LTA differ among ϕSLT, LL-H, and SPP1. To clarify the differences among the recognition systems of host cell LTAs at the molecular level, further investigation, including structural analysis, is required.

In regard to the horizontal transfer of PVL genes in *S. aureus*, it is notable that ϕSLT adheres to all of the *S. aureus* isolates. It has been shown that ϕSLT or related PVL-carrying phages have the ability to infect almost all *S. aureus* cells and convert them to PVL-producing cells. We have identified two morphologically different PVL-carrying phages, ϕPVL and ϕSLT, which have a hexagonal and an elongated head morphology, respectively. We also suggested that at least two types of PVL-carrying phages are involved in the horizontal transfer of PVL genes (29). In fact, we found that PVL-carrying phages in Japanese CA-MRSAs were classified into two morphological groups (25). Horizontal transfer of PVL genes by these PVL-carrying phages should hold the key to the appearance of PVL-producing CA-MRSA around the world.

In this study, the phage protein involved in the first step of representative PVL-carrying ϕSLT infection was identified. Although it adsorbed to all PVL-negative *S. aureus* species tested, ϕSLT was able to form plaques with about 3% of these strains (29). To understand all of the mechanisms allowing successful infection by ϕSLT of the selective staphylococcal cells, the functions of the numerous other ϕSLT ORFs and host factors related to the steps of phage adhesion to the host cells need to be characterized.

Whole genomic DNA sequences of several types of *S. aureus Siphoviridae* phages have been determined (16). All of the sequenced PVL-carrying phages of the elongated-head type, such as ϕSLT, ϕSa-2958 (25), ϕSa2MW (1), and the related phage ϕ12 (13), have the same (99% identity) 636-amino-acid possible tail fiber proteins. From the homology search study, the presence of amino acid sequences corresponding to the C-terminal region of ORF636 containing GW repeats of 632- to 636-amino-acid proteins with 43 to 44% identity was found in the following staphylococcal phage tail proteins: ORF56 of ϕETA (38) and corresponding proteins of ϕ11 (13) and ϕMu50B (22), as well as other phages belonging to *pac*-type and icosahedral-head-type phage groups (Fig. 1C). These phages may recognize the *S. aureus* cell surface through these tail proteins. However, possible corresponding phage tail proteins of other PVL-carrying phages, ϕPVL and ϕ108PVL (25), and other staphylococcal phages, ϕ13 (13), or related *cos*-type icosahedral-head-type phages, showed no homology to ORF636. The deduced amino acid sequences of minor tail proteins of ϕPVL are almost identical to those of staphylococcal phage 77, which may recognize the 4-*O*-β-(*N*-acetyl-D-glucosaminyl) moiety of ribitol teichoic acid for infection, as mentioned above (27, 36).

Further studies of the host recognition/adhesion proteins among the phages of *S. aureus* are also needed to clarify the phage infection mechanism and the horizontal transfer of PVL genes.

Nucleotide sequence accession number. The sequence of ϕSLT has been deposited in the DDBJ database with accession no. AB045978.

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