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Streptolysin S Activation by Lipoteichoic Acid

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Lipoteichoic acid from streptococci and *Staphylococcus aureus* both activated membrane-bound precursor streptolysin S and induced the formation of extracellular streptolysin S. Lipoteichoic acid could replace other substances, such as yeast ribonucleic acid core, that act as carriers for hemolysin but which are not components of the streptococcus or the host. Lipoteichoic acid may play a role as the physiological carrier of streptolysin S to host tissues.

Among the extracellular substances produced by group A streptococci, streptolysin S (SLS) is unique in that it is expressed only when a carrier, such as ribonucleic acid (RNA) core (pancreatic ribonuclease-resistant fraction of yeast RNA) or a nonionic detergent, is added to stabilize the hemolytic moiety produced by the growing culture or resting cell suspension (11). This hemolytic moiety can be transferred from one carrier to another but any procedure which separates it from the carrier or destroys the carrier irreversibly inactivates the hemolysin. The extracellular SLS is formed from SLS precursor which is tightly bound to the streptococcal membrane (6) and can be activated in vitro only in the presence of carrier by sonification or blending on a Vortex mixer with glass beads (8).

The role of SLS in streptococcal infections is uncertain but may involve membrane toxicity (11). Production of SLS during infection would require an appropriate carrier or a surface-associated ligand. Although both RNA core and nonionic detergents are excellent carriers of SLS and other substances such as streptococcal RNA and serum albumin are far less efficient (11), neither RNA core nor nonionic detergents are native components of streptococci or mammalian cells, nor are they formed under in vitro or in vivo conditions.

In the present study, we tested lipoteichoic acid (LTA) as an SLS carrier because of its potential as a carrier of streptococcal proteins (14, 19) and its affinity for binding to mammalian cell membranes (9, 17). LTA is present in membranes of most gram-positive bacteria and has been shown to bind spontaneously to erythrocytes and other mammalian cells via fatty acids-ester linked to the polyglycerol phosphate backbone of the LTA molecule (2, 16, 19). Also, several studies have demonstrated that LTA is exposed on the surface on gram-positive bacteria (3, 15, 19) and is involved in the adherence of group A streptococci to epithelial cells (2, 3, 15, 16).

We used *Streptococcus pyogenes* strains CB112252 and C203S throughout this study to test for the activation of membrane-bound SLS precursor and the formation of extracellular SLS. Cultivation of cells, preparation of RNA core, production of extracellular SLS, and hemolytic assays have been described previously (7). Membranes containing SLS precursor were isolated from protoplasts prepared in hypertonic buffer with a phage-associated lysin and were activated by blending on a Vortex mixer with glass beads in the presence of an added carrier (8).

LTA was extracted from whole cells of *Staphylococcus aureus* ATCC 6538P and different groups of streptococci by chloroform-methanol-hot aqueous phenol treatment (10), purified by gel chromatography on Sepharose 6b (10), and assayed serologically by passive hemagglutination inhibition (12) with antisera prepared against purified LTA coupled to methylated bovine serum albumin (4). LTA lacking the fatty acid portion of the molecule was isolated either from the culture fluids or by deacylation of the LTA with NH_4OH (18). Deacylated LTA could be differentiated from the acylated form by its elution profile on Sepharose 6b (10) and inability to sensitize sheep erythrocytes (14). Ribitol teichoic acid was extracted from purified cell walls of *S. aureus* with 10% trichloroacetic acid (1, 13).

When staphylococcal LTA was added to membranes containing SLS precursor and the mixture was activated, hemolysin was produced (Fig. 1). SLS could be activated with as little as 0.05 mg of LTA, and with 2.0 mg, a 60-fold increase in titer occurred which was equal to or better than that obtained with the same amount of RNA core (1,800 hemolytic units/ml). One

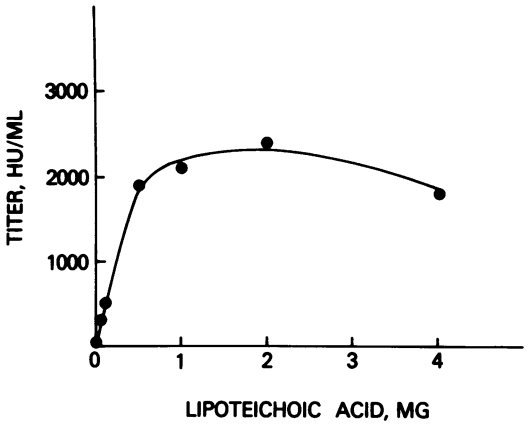

FIG. 1. *Activation of membrane-bound streptolysin S precursor by lipoteichoic acid. A mixture of strain CB112252 streptococcal membranes and staphylococcal LTA suspended in 2.0 ml of buffer (0.03 M potassium phosphate [pH 6.5], 0.85% NaCl) was added to 3.0 ml of glass beads (MS-XLX; Cataphote-Ferro Corp., Jackson, Miss.) in a tube (15 by 125 mm) and blended on a Vortex mixer for 15 min in the cold. After another 2.0 ml of buffer was added and the solution was mixed, the beads and membranes were removed by centrifugation at 20,000 × g for 15 min, and the supernatant fluid was assayed for SLS activity by using freshly washed sheep erythrocytes. The membrane concentration (usually 0.1 ml = 0.8 mg of protein) was adjusted so that addition of 2.0 mg of yeast RNA core yielded a titer of 1,800 hemolytic units (HU)/ml.*

difference noted between the two carriers was in the stability of the complexes. RNA core-SLS remained stable after overnight incubation in the cold while LTA-SLS lost approximately 25% of its activity. Such a loss has also been described for nonionic, detergent-activated precursor SLS (5) and is not unexpected since LTA in the micellar form behaves as a detergent (20).

LTA prepared from the same or a different strain of group A streptococci also activated precursor SLS as did the LTAs isolated from groups B, C, and G streptococci (Table 1). When deacylated LTA or ribitol teichoic acid was tested as a carrier, SLS precursor activation was minimal. Also, no inhibition of SLS activity occurred when increasing concentrations of deacylated LTA (0.5 to 2.0 mg) were added to an activation mixture containing 1.5 mg of LTA. These results suggest that the fatty acid portion of the LTA molecule is involved in the binding of the hemolytic moiety and closely parallels other studies showing that LTA with an intact glycolipid moiety is necessary for biological activity (20). Further proof that the hemolytic moiety is bound to LTA was shown by the inhibition of SLS activity when LTA was treated with anti-LTA immunoglobulin G either before or during the activation step. Based on the above studies, one might assume that an added carrier would not be required for the activation of SLS precursor in membranes since LTA is also present. However, this was not the case. We found that in the activation of membranes with glass beads in the absence of added carrier, acylated LTA remained with the particulate fraction after centrifugation while only deacylated LTA was released into the supernatant fluid. In contrast, all of the SLS activity was present in the supernatant fraction after the activation of membrane SLS precursor.

In testing for the production of extracellular SLS, we used resting cells prepared from a late log phase culture of strain C203S. Upon addition of 5 mg of streptococcal LTA to 5×10^{11} cells, a total of 360,000 hemolytic units were formed compared with 300,000 produced by a similar amount of RNA core. In the absence of carrier, no hemolysin could be detected even though LTA was present in the supernatant fluids. As shown with the membrane-activated preparation, LTA secreted by the resting cells was the deacylated or inactive form, as determined by its inability to sensitize sheep erythrocytes and its elution profile on Sepharose 6b.

The results of this study demonstrate for the first time that a native component of streptococcal membranes, LTA, may function as a physiological carrier of SLS. Interestingly, LTA resembles RNA core in having a negatively charged repeating polymer backbone, and it resembles nonionic detergents in being micellar. The amphipathic nature of LTA in possessing a polar glycerol teichoic acid moiety linked covalently to a glycolipid provides for both hydrophobic and hydrophilic interactions with other

TABLE 1. *Activation of membrane-bound SLS precursor from group A streptococcal strain CB112252 by streptococcal and staphylococcal LTAs*[a]

LTA source	Hemolytic units per ml
Group A *Streptococcus* CB112252	2,800
Group A *Streptococcus* C203S	2,400
Group B *Streptococcus*	550
Group C *Streptococcus*	1,200
Group G *Streptococcus*	1,600
S. aureus	1,900
S. aureus (deacylated)	80
S. aureus (ribitol teichoic acid)	160
Yeast RNA core	1,840
No addition	65

[a] Membranes were treated with 2 mg of LTA as described in the legend to Fig. 1.

molecular species. LTA could function as a carrier of SLS and deliver it to host sites distant from the site of production or, as a surface component, could mediate a bacterial-to-host cell interaction. For example, LTA may be the binding site between streptococcal surface SLS (called cell-bound hemolysin) and host cells (1). Reexamination of the pathological properties of SLS when bound to LTA rather than to the artificial yeast RNA core may provide a more realistic insight into SLS effects on host cells.

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