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SHORT COMMUNICATIONS

Activation of Autolytic Activity of *Staphylococcus epidermidis* 33 by a Low-Molecular Weight Cationic Peptide Warnerin

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The mechanisms of antimicrobial activity of low-molecular weight cationic peptides produced by some bacteria [1] are poorly studied at present. Available data suggest that the intracellular apparatus of biopolymer synthesis and various membrane structures are targets of these compounds. Peptides attacking cellular membranes form complexes with lipid II, generating short-living pores and unregulated ion channels. As a result of the membrane damage induced by antibacterial peptides, the electric component of the membrane potential and intracellular ATP levels decrease rapidly, which triggers autolytic hydrolysis of peptidoglycan and osmotic cell death in unprotected medium [1].

Peptidoglycan is a major component of a gram-positive bacterial cell wall and a dynamic structure with components being synthesized and cleaved in coordination in processes of cell growth and division. The main catalysts of these processes are various glycan and peptide hydrolases, which are represented in staphylococci by *N*-acetylmuramyl-L-alanine amidases, *N*-acetyl glucose aminidases, *N*-acetyl muramidases, endopeptidases, and transglucosidases. For some of them, specific physiological functions and genetic determination are known. Presumably, glycan hydrolases play an important role in the processes of cell wall growth, cell division and segregation, muropeptide recycling, induced cell lysis, and production of pathogenic factors [2]. Bacterial cell integrity during processes of growth and division is ensured by strict control of activity of autolytic hydrolases at the level of transcription by means of global negative and positive regulatory factors and two-component signal transduction systems [3, 4]. Upon completion of translation events, autolysin activity is modulated by stress factors, salts, and protease activity [4]. Immobilization of autolysins on anionic molecules of teichoic and lipoteichoic acids plays a significant in suppression of autolytic processes [5]. Dissociation of such complexes results in the induction of lysis of the biopolymers of bacterial cell wall.

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The aim of the work was to perform an enzymographic study of the enzymes of the autolytic complex exhibiting sharply increased activity in *Staphylococcus epidermidis* 33 subjected to the bacteriolytic effect of a cationic peptide warnerin.

Bacteria used in the experiments were grown on LB medium to the mid-exponential growth phase ($OD_{600} = 0.8$). Cells were sedimented from 30 ml of the culture by centrifugation (Sigma, 3K30, 10000 rpm, 10 min) and washed with 0.01 M Tris–HCl, pH 7.2. The pellet was resuspended in the same buffer to yield $OD_{600} = 1.0$. Autolysis was activated in washed cells suspensions by addition of equal volumes of 0.2% Triton X100 and 100 μM warnerin solutions. The samples were incubated for 4–5 h on a Sertomat shaker (Sartorius, Germany) at 150 rpm, 37°C. After the incubation, sample aliquots (1 ml) were centrifuged as described and the supernatants were subjected to PAGE under renaturation conditions [6]. Polyacrylamide gel contained the cells of *S. epidermidis* 33 killed by boiling and washed with distilled water (1.6 mg dry cells/ml gel) as substrates for autolysins excreted into the environment by staphylococci cells in the process of bacteriolysis. Each sample was analyzed in two variants: (1), after introduction of phenylmethanesulfonyl fluoride (Sigma, United States) to the final concentration of 100 μM to stop proteolytic cleavage of autolysins, and (2), without the addition of protease inhibitor. After electrophoresis separation, the gel was washed with distilled water for 30 min, placed into the renaturing buffer containing 50 mM MES–NaOH, pH 6.0, and 0.1% Triton X-100; incubated for 16 h at 37°C; washed with water; stained with 0.1% methylene blue in 0.01% KOH for 1 h; and washed again with water to let transparent zones of cell lysis develop on the blue background (nonlysed cells stained with methylene blue).

The results of the study indicate that the given concentrations of both the neutral detergent (Triton X-100) and warnerin exhibited pronounced lytic activity (Fig. 1). The effect of the detergent, measured by a decrease in optical density, was considerably weaker

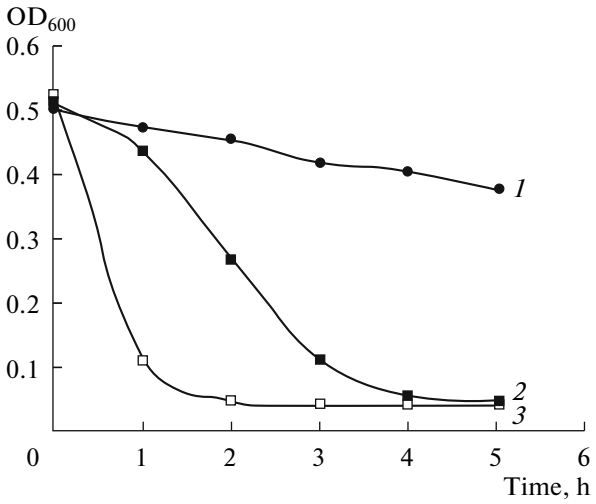

Fig. 1. Lytic effect of Triton X-100 and warnerin on *S. epidermidis* 33:, control (*1*), Triton X-100 (*2*), and warnerin (*3*).

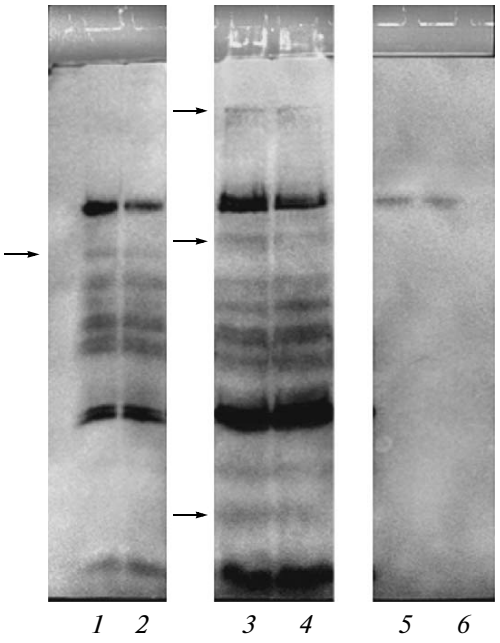

Fig. 2. Enzymographic study of autolytic enzymes released into the medium by bacteria treated with various lytic agents: Triton X-100 (*1, 2,*), warnerin (*3, 4,*), incubation in 0.01 M Tris–HCl buffer, pH 7.2 (*5, 6,*), and incubation in the presence of protease inhibitor PMSF (100 μM) (*2*, *4*, *6*). Arrows indicate peptidoglycan hydrolases specifically activated by each of the lytic agents.

than that of warnerin during the first hours of incubation; however, at late incubation stages, the cells were almost completely lysed in both experimental variants. As the peptide did not exhibit any enzymatic activity, its bacteriolytic effect is probably indirect and may be related to the rapid drop of the electric component of the membrane potential $\Delta\psi$ after warnerin introduction into the medium, as we have demonstrated previously [7], or to the activating effect of warnerin on autolysins.

Analysis of the results of electrophoretic separation of the proteins exhibiting peptidoglycan hydrolase activity present in the supernatant of the media containing lytic agents (Triton X-100 or warnerin) makes the situation somewhat clearer (Fig. 2). The greatest variety of autolysins was detected in the sample obtained upon warnerin treatment. Multiplicity of cell wall hydrolases is characteristic of Triton autolysates as well. Apart from the fractions that are identical in both variants in terms of their electrophoretic mobility, comparison of electrophoresis data reveals bands of peptidoglycan hydrolases specific for the lysis agent (marked with arrows). Thus, the pathways of staphylococci cell wall autolysin activation by Triton X-100 and warnerin share common features (detergent activity), yet are different. This may be ascribed to dissociation of autolysins from complexes with teichoic, teichuronic, and lipoteichoic acids induced by cationic warnerin molecules, releasing free autolysins now capable of exerting their specific enzymatic activity.

Thus, comparison of bacteriolytic effects of the nonionic detergent (Triton X-100) and the cationic amphiphilic peptide (warnerin) allows the conclusion to be drawn that one of the mechanisms of the antibacterial activity of the peptide is activation of autolysins of the attacked bacterial cells.

Pronounced solubilizing activity of warnerin is of special interest for thorough study of its detergent properties, since peptide detergents are promising agents for preparation of nanovesicular vectors as Trojan horses for transport of various biologically active cargoes in living organisms.

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