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EXPERIMENTAL ARTICLES

Isolation and Characterization of a New Low-Molecular Antibacterial Peptide of the Lantibiotics Family

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Abstract—The physicochemical and biological properties of the low-molecular antibacterial peptide isolated from the cultivation medium of *Staphylococcus warneri* IEGM KL-1 were studied. The peptide was obtained in a homogenous state by the methods of ultrafiltration, ion exchange, and reversed phase chromatography. The peptide contained a substantial quantity of cationic and hydrophobic amino acid residues and an uncommon amino acid lanthionine. The molecular mass of the peptide was 2999 Da. A bactericidal effect of the isolated peptide on the cells of *S. epidermidis* 33 was exhibited in a wide pH range, being completely preserved upon heat treatment. In accordance with the characteristics, origin, and species affiliation of the producer, the peptide was named warnerin. The available data allow us to consider warnerin as a new representative of the family of lantibiotics, promising antibiotic agents of microbial origin.

Key words: low-molecular cationic peptides, antibacterial effect, lantibiotics, *Staphylococcus*.

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Bacteria are producers of a vast number of various biologically active compounds. Particular attention of researchers is drawn to small cationic peptides synthesized by both gram-positive and gram-negative microorganisms [1, 2]. To date, numerous such peptides have been characterized; their pronounced antibacterial effect is based on disturbing the membrane structural organization or hitting intracellular targets with lethal consequences [3].

Among antibacterial peptides, low-molecular bacteriocins secreted by some of gram-positive bacteria are objects of intense study [4]. They are peptides with the molecular mass of about 1.5–5 kDa and distinctive atypical structural properties: the presence of dehydrated serine and threonine residues in peptide chains and rare thioester amino acids: lanthionine and 3-methyllanthionine, which induce the formation of strong intramolecular ring structures during posttranslational modification. These uncommon peptides sometimes also contain D-alanine, lanthionine sulfoxide, *allo*-isoleucine, and other rare compounds [4]. The total positive charge allows the peptide molecules to bind to anionic groupings of bacterial cell envelopes. At the same time, the excess of anionic zones on the inner surface of cytoplasmic membranes facilitates further advance of the peptides: sorption on the outer side of the membranes and incorporation into the lipid matrix of membranes with the formation of unregulated channels and pores, which finally results in the death of attacked cells [5]. The available data show the ability of peptides to facilitate penetration into bacterial cells of different biologically active agents—in particular, beta-lactams, vancomycin, and other antibiotics—significantly increasing their efficiency against pathogenic bacteria, including antibiotic-resistant strains [6, 7].

The growing interest in lanthionine-containing peptides as prospective antibacterial compounds with a wide range of practical applications is explained by the half-century-old use of one peptide of this group, nisin, as a food preservative, which did not result in emergence and distribution of resistant bacteria [8].

Previously, when searching for producers of novel low-molecular cationic antibacterial compounds, we isolated a strain of gram-positive cocci strain secreting a low-molecular antibacterial factor into the cultivation medium [9]. The goal of this work was isolation, purification, and characterization of the physicochemical and biological properties of this factor. In accordance with the data presented in this report, it may be considered as a new peptide of the family of lantibiotics.

MATERIALS AND METHODS

The strain *Staphylococcus warneri* IEGM KL-1 was used as a producer of the antibacterial factor (ABF)

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[10]. The strain *Staphylococcus epidermidis* 33 obtained from the Tarasevich State Research Institute of Standardization and Control for Medical Biological Preparations (Moscow) was used as an indicator.

The strain *S. warneri* IEGM KL-1 was grown in a special enriched liquid medium [11] in 250-ml flasks with 50 ml of the medium on a thermostatically controlled Certomat shaker (Sartorius, Germany) under stirring (150 rpm) at 37°C. Culture growth was assessed by the change of optical density (OD_{600}) measured in a PD-405 spectrophotometer (Apel, Japan). Bacteria were cultured until the maximum level of antibacterial activity in the growth medium. For ABF isolation, cells were removed by centrifugation (20 min, 4°C, 6000 *g*) on a Sigma 3K30 centrifuge (Sartorius, Germany); the supernatant was harvested and passed through a 0.4-μm Synpor membrane filter (Chemapol, Czech Republic). The filtrates were applied to an EC glass column (25 × 500 mm, BioRad, United States) with Toyopearl-SP 650M resin (Tosoh, Japan) preequilibrated with 10 mM Na–phosphate buffer, pH 7.2. For removal of filtrate components not bound to the sorbent, the column was washed with the same buffer in a volume tenfold higher than the volume of applied supernatant. ABF was eluted from the column with the linear NaCl gradient (0–0.5 M) in the same buffer at a flow rate of 2 ml/min. All eluate fractions were tested for antibacterial activity. The latter was revealed by the diffusion method, with aliquots (5 μl) of each collected fraction applied to a dense LB nutrient medium containing 0.8% agarose (Sigma, United States) and the cells of *S. epidermidis* 33 indicator culture (10^6 CFU/ml). The eluate fractions possessing antibacterial activity were combined and desalinated on PD-10-Sephadex™ G-25M columns (Amersham Biosciences, Sweden). The presence (absence) of NaCl in the samples after desalination was determined by a Vital Diagnostics kit (Russia) according to the manufacturer's prescription. Desalinated fractions were again tested for antibacterial activity by the method of terminal dilutions in immunological trays. A series of twofold sequential dilutions of tested samples in a liquid LB medium without KCl were prepared; then 10 μl of the cell suspension of *S. epidermidis* 33 ($1.5–2 \times 10^6$ CFU/ml) was added into each well containing 100 μl of LB medium with ABF. The trays were incubated for 16–18 h at 37°C. For higher sensitivity of antibacterial activity measurement, 10 μl of 1% 2,3,5-triphenyltetrazolium solution (BDH, United Kingdom) was added to the holes after the incubation, and the plates were additionally incubated in a thermostat at 37°C for 30 min for the staining of precipitated bacterial cells with formazan. The inverse value of the maximum dilution at which the growth of tested bacteria was completely inhibited, was taken as a conventional peptide activity unit (AU).

The salt-free eluates with antibacterial activity were combined, frozen in liquid nitrogen, and lyophilized in Alpha-2 (Christ, Germany).

The nature of ABF in the samples was established by their treatment with different hydrolases. DNase preparation (Sigma, United States) for suppression of concomitant protease activities was preincubated at 37°C for 2 h in the buffer with 1.25 mM phenylmethanesulfonylfluoride (Sigma, United States) and added to the sample (36 U/ml). RNase preparation (Reanal, Hungary) was preheated at 100°C for 15 min to suppress the concomitant DNase activity and then, upon slow cooling to room temperature for renaturing, was added to the samples (20 U/ml). Proteolytic treatment was carried out with proteinase K (Sigma, United States), 3 U/ml; pepsin (Calbiochem, United States), 500 U/ml; trypsin (Serva, Germany), 35 U/ml; and carboxypeptidase A (Reanal, Hungary), 5 U/ml. The substrates were ABF preparations with the known antibacterial activities, with equal volumes of buffer solutions added for the pH optimum necessary for enzymatic reaction: 0.1 M CH_3COONa/0.1 M CH_3COOH, 0.05 M $MgCl_2$, pH 5.0, for DNase; 0.1 M Tris/0.1 M HCl, 0.15 M NaCl, pH 7.2, for RNase; 0.05 M Tris/0.05 M HCl, pH 7.5, for proteinase K and carboxypeptidase A; 0.01 M Tris/0.01 M HCl, pH 7.4, for trypsin; and 0.05 M HCl for pepsin. Two control samples were taken for each experimental sample: the first one contained a substrate with the buffer and 0.14 M NaCl solution instead of the enzyme, and the second one contained the enzyme, the buffer, and water instead of the substrate. The samples were incubated for 2 h at 37°C; then the aliquots of incubation media were taken to determine the antibacterial activity.

The degree of purification of the antibacterial peptide preparation isolated by ion exchange chromatography was assayed by vertical electrophoresis in 12.5% polyacrylamide gel in Tris–glycine buffer with 0.1% SDS, pH 8.3, according to Laemmli [12], using the Color Markers for SDS-PAGE (Sigma, United States).

Peptide samples were purified to a homogeneous state by reversed phase chromatography in a 140 V gradient system with a 785 A optical detector (Applied Biosystems, United States). The peptide was isolated in a BrownleeRP-Silica C18 column, 30 × 2.1 mm, 300 Å, with the carrier particles of 5 μm (PerkinElmer, United States). Vessel A: 0.1% trifluoroacetic acid (PerkinElmer, United Kingdom); vessel B: 70% acetonitrile (Sigma, United States) in 0.085% trifluoroacetic acid. The fractions collected manually were dried on a Speed-Vac cooling concentrator (Savant, United States), dissolved in water, and tested for antibacterial activity.

Mass spectrometric analysis of the samples obtained during purification and isolation of the peptide was carried out in a Voyager-DE STR Biospectrometry Workstation (PerSeptive Biosystems, United States).

Amino acid composition was determined in peptide samples (0.5–0.7 mg) hydrolyzed with 6 M HCl

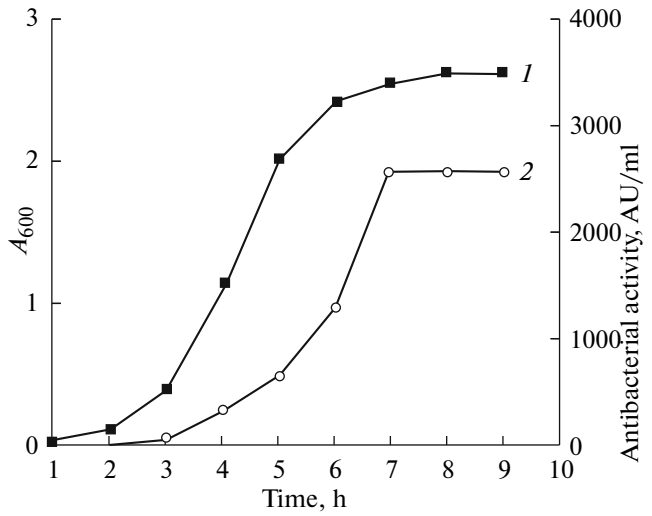

Fig. 1. Dynamics of *S. warneri* IEGM KL-1 growth and ABF production: optical density of bacterial culture (*1*); ABF content in the culture liquid (*2*).

in sealed ampoules in the nitrogen atmosphere, 60°C, and then analyzed in an Amino Acid Analyser 420A (Applied Biosystems, United States) in the automated mode including hydrolysate treatment with phenylisothiocyanate followed by separation of phenylthiocarbamoyl derivatives of amino acids in reversed phase on C18 columns. The whole procedure was calibrated using 29 pmol (500 ng) of the standard: horse myoglobin (hydrolysis stage) and a Standart H amino acid kit (Pierce, United States) containing 250 pmol of each amino acid with norleucine as the internal standard (derivation stage).

The effect of high temperatures on the biological characteristics of isolated peptide was studied upon incubation of its solutions in a boiling water bath for 5, 10, 30, and 60 min and autoclaving at 0.5 and 1.0 atm for 30 and 15 min, respectively, followed by assessment of antibacterial activities of the preparations by titration in microplates.

For the study of pH resistance, the antibacterial peptide was dissolved in different buffer solutions: 0.02 M KCl/0.02 M HCl, pH 2.0; 0.01 M CH_3COONa/0.01 M CH_3COOH, pH 4.0; 0.01 M KH_2PO_4/0.01 M NaOH, pH, 6.0; 0.01Tris/0.01 M HCl, pH 8.0; and 0.01 M glycine/0.01 M NaOH, pH 9.0–10.0. Peptide solutions were incubated for 2 h and then neutralized with 0.1 M HCl or 0.1 M NaOH to pH 7.0–7.2; antibacterial activity was determined with the corresponding neutralized buffer solutions used as a control.

The dynamics of peptide binding to microbial cells was studied on obtaining of the labeled peptide variant from the supernatants of producer cultures grown in the presence of [U-^{14}C]protein hydrolysate (Amersham LIFE SCIENCE, United Kingdom). Solution of the labeled peptide preparation in 0.01 M K-phosphate buffer, pH 7.2 (100 μl), was quickly mixed with an equal volume of "cold" peptide with the same biological activity and added to 800 μl of the cell suspension of *S. epidermidis* 33 (10^9 CFU/ml) in 0.25 M sucrose. The final antibacterial activity of the peptide in the samples was 1024 AU/ml. After mixing the peptides, an aliquot (50 μl) was immediately taken from the sample and placed into a vial with 5 ml of Ecolite scintillation fluid (ICN, United States) for total radioactivity detection. The cells were incubated with the peptide for 100 s. The aliquots (100 μl) were taken every 20 s, the cells were precipitated (10 s, 4°C, 12000 *g*) on a 5414R centrifuge (Eppendorf, Germany), and the level of radioactivity was measured in the supernatant (50 μl) by a Wallac 1414 liquid scintillation counter (Guardian, Finland).

The dynamics of bactericidal effect of the peptide was studied in the cells of *S. epidermidis* 33 indicator culture grown in the liquid LB medium to the middle of the logarithmic growth phase. The cells were centrifuged as described above, washed twice with saline solution, and suspended in deionized water to the concentration of 10^9 CFU/ml. Prepared cell suspension was distributed by 1.5 ml into polypropylene test tubes and centrifuged; the supernatant was removed, and the precipitate was resuspended in 1.5 ml of peptide solution with an activity of 1024 AU/ml. Peptide solutions did not contain salt in the first variant of experiments and contained 0.14 M NaCl in the second variant. In the control samples, water or 0.14 M NaCl were added to cell precipitates instead of the peptide solutions. Samples were taken in certain time intervals to determine the quantity of viable bacteria by the micromethod of point inoculations of respective tenfold dilutions on agarized LB medium in petri dishes [13].

RESULTS

The dynamics of antibacterial activity was studied during cultivation of *S. warneri* IEGM KL-1 under aerobic conditions. It demonstrated that ABF was secreted into growth medium during the exponential phase of bacterial culture development. On its transition to the stationary phase after 6–7 h of growth, no further increase of antibacterial activity in the medium was observed (Fig. 1).

The peptide was purified from the culture liquid ultrafiltrates on the chromatographic column with a strong cation exchanger Toyopearl-SP 650M by elution with the increasing NaCl gradient (0–0.5 M). Antibacterial activity was eluted from the column compactly, in a narrow salt gradient of 0.35–0.5 M NaCl (Fig. 2a). The high efficiency of this cation-exchange resin used for chromatography indicates that the molecules of the compound under study have a total positive charge in the neutral medium.

The fractions containing antibacterial activity were combined and desalinated by gel filtration, the results

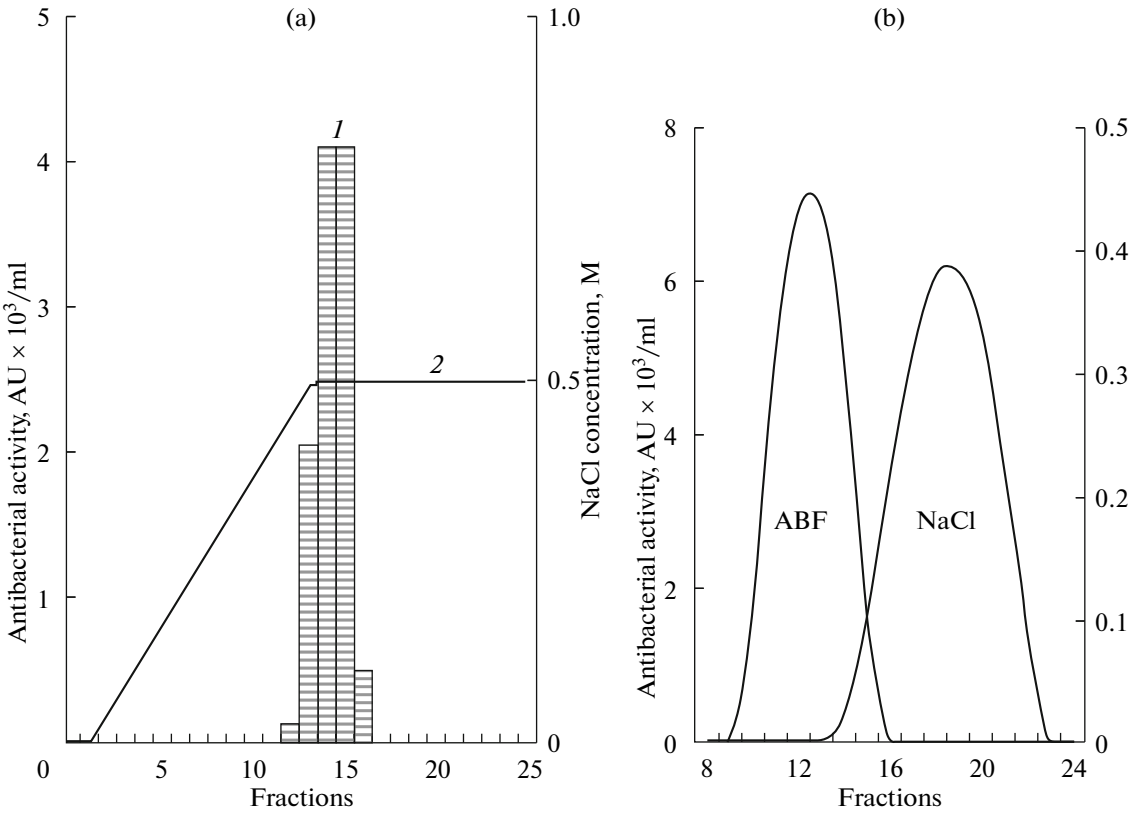

Fig. 2. Chromatography of the ultrafiltrate of the ABF-containing supernatant of *S. warneri* IEGM KL-1 culture on a Toyopearl-SP 650M column: (a) elution of ABF (*1*) by NaCl gradient (*2*); (b) desalination of integrated eluted ABF fractions by gel filtration on Sephadex G25M.

of which are presented in Fig. 2b. The eluates with no more than 10–15 mM NaCl were combined and lyophilized. The characteristics of the preparations at various stages of ABF isolation are given in Table 1. Lyophilization of desalinated ABF preparation (10 ml) yielded loose white powder. Its solution in 1 ml of deionized water contained 40–70 mM chlorides and its activity was 1024–2048 AU/ml. The data from Table 1 show that up to 65% of antibacterial activity present after the stage of ion-exchange chromatography was lost during desalination of the preparation.

The peptide nature of ABF synthesized by *S. warneri* IEGM KL-1 was confirmed by the study of its sensitivity to the action of different hydrolases. The results of these experiments showed that incubation of the desalinated preparation for two hours with nucleases (RNase and DNase) and carboxypeptidase A had no effect on its antibacterial activity. On the contrary, treatment of the preparation with proteolytic enzymes (trypsin, pepsin, and proteinase K) resulted in its complete inactivation.

Electrophoretic analysis of the desalinated peptide preparation showed the presence of a single fraction of low-molecular peptide compounds (Fig. 3a). However, the dye was bound in the gel in a wide zone between the bands of markers with the molecular masses of 1.06–3.49 kDa, which was evidence of insufficient purification of the peptide. This conclu-

Table 1. Protocol of ABF isolation from the culture liquid of *S. warneri* IEGM KL-1

Purification stages	Volume, ml	Total protein, (A_{214})	Total activity, AU	Specific activity, AU/A_{214}	Degree of purification	Yield, %
Supernatant	50	36400	128000	3.52	1.00	100.0
Filtration (0.4 μm)	49	35100	127200	3.62	1.03	99.4
Chromatography (Toyopearl-SP 650M)	10	4400	102300	23.25	6.60	79.9
Desalination (Sephadex G-25)	18	770	46000	59.74	16.97	35.9

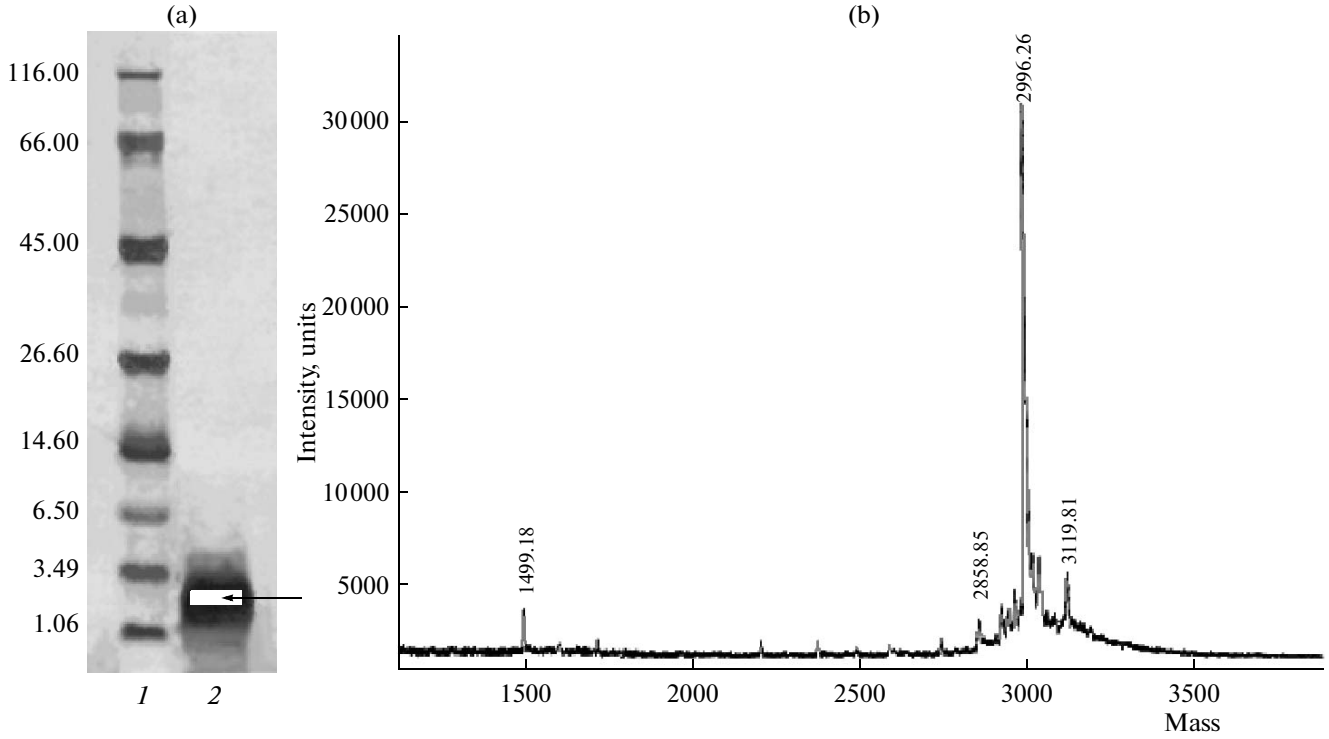

Fig. 3. Electrophoretic (a) and mass-spectrometric (b) characteristics of the preparation of desalinated peptide produced by *S. warneri* IEGM KL-1. (a): *1*, markers (kDa): bradykinin (1.06); chain B of bovine insulin (3.49); bovine aprotinin (6.50); bovine α-lactalbumin (14.20); rabbit muscle triose phosphate isomerase (26.60); chicken egg ovalbumin (66.0); and β-galactosidase, *E. coli* (116.00); *2*, peptide preparation; the arrow shows the gel zone used for mass spectrometry (b).

sion was confirmed by the results of mass-spectrometric analysis of the material eluted from the stained zone of the gel (Fig. 3b): the sample contained several peptide compounds with the prevalent one of 2999 Da.

The peptide was further purified by reversed phase high-pressure chromatography using various profiles of acetonitrile gradient. The pure peptide fraction was obtained after several steps of purification (Fig. 4).

Homogeneity of the peptide preparation obtained at the last stage of reversed phase chromatography (Fig. 4b) was confirmed by mass spectrometry (Fig. 5).

The amino acid analysis of hydrolysates of the homogenous peptide preparation showed the presence of various amino acids, with predominant lysine, glycine, threonine, serine, alanine, valine, leucine, and isoleucine (Table 2). Importantly, a rare amino acid, lanthionine, was found. Thus, the peptide was classified within the group of lantibiotics, low-molecular cationic peptides currently considered as an alternative to classical antibiotics [15].

The presence in the peptide structure of lanthionine residues forming tight thioester bonds explains its high resistance to external actions. Antibacterial activity of the peptide was preserved after the boiling water bath for 60 min, autoclaving (0.5 atm, 105°C, 30 min and 1.0 atm, 119.6°C, 15 min), and incubation at pH 2.0–8.0, but abruptly decreased under alkaline conditions (pH 9.0–10.0).

The dynamics of bactericidal effect of the peptide was studied in relation to the indicator strain *S. epidermidis* 33. Considerable antibacterial effect was observed already 1 min after introduction of the peptide into bacterial cell suspension (Fig. 6a). The quantity of viable cells decreased by 30–40% in 0.14 M NaCl solution and halved in water. The peptide's effect was most pronounced at the later terms: after 6-h incubation of bacteria with the peptide, the number of viable cells decreased by more than six orders of magnitude in a hypotonic medium (water) and by two orders of magnitude after incubation in saline solution (Fig. 6b).

Rapid manifestation of antibacterial activity of the peptide is apparently determined by the high rate of its binding to the cells of sensitive bacteria, as confirmed by the results of experiments with the ^{14}C-labeled peptide. The label is bound to the bacteria immediately after introduction of the peptide into the cell suspension. As is shown in Fig. 7, a substantial amount of introduced label is bound after 5 s of interaction between the peptide and the cells and the sorption saturation occurs after 1 min. The high rate of binding of positively charged peptide molecules with the cells probably results from ionic interactions with teichoic

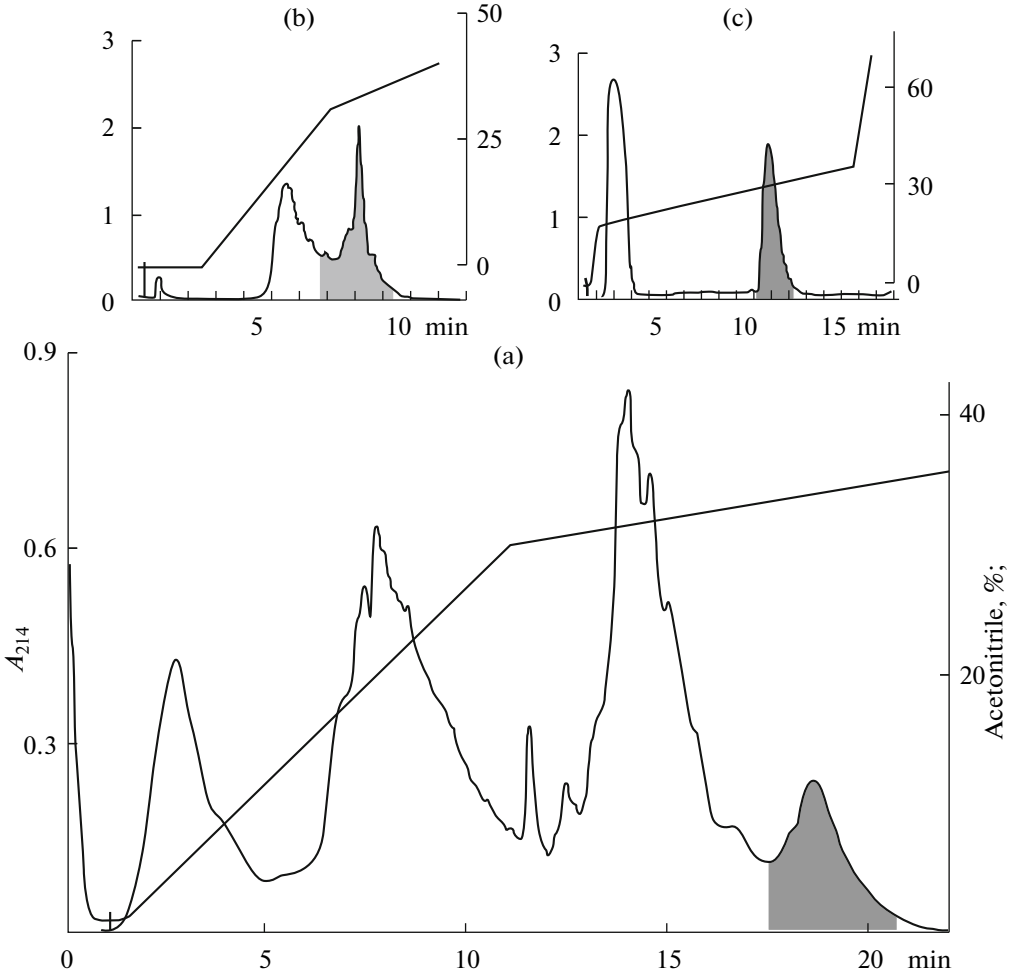

Fig. 4. Reversed phase chromatography of the desalinated antibacterial peptide preparation; a, b, and c: successive stages of purification of the peptide preparation using different types of acetonitrile gradient for obtaining the homogenous peptide preparation (c). Highlighted regions of chromatograms are eluate fractions containing antibacterial activity.

and lipoteichoic acids, the negatively charged components of bacterial cellular membrane.

DISCUSSION

Production of cationic antibiotic peptides is inherent to various gram-positive bacteria. It is largely typical of bacteria of the genus *Staphylococcus*, among which the most active producers of low-molecular bacteriocins are *S. epidermidis* strains secreting epidermin [16], Pep5 [17], epicidin [18], and epilancin [19], and *S. aureus* strains producing antibacterial peptides aureocins A70 and A53 [20], staphyllococcins C55 [21], Bac 1829 [22], Bac201 [23], and Bac R1 [24]. Previously, we have demonstrated [9] that one of the *S. warneri* strains is a producer of a low-molecular compound suppressing the growth of *S. epidermidis* 33. It was shown that only the supernatants of producer cultures possessed antibacterial activity, because it was not revealed in the sonicated cell extracts [7]. Based on these data, it may be supposed that the peptide synthesized in the cells acquires antibacterial activity during its transport across the bacterial membrane into the medium. Existence of an intermediate inactive form of the peptide is an essential condition for preventing the death of bacterial cells caused by their own metabolic products, which is typical of most systems of synthesis of low-molecular antibacterial peptides [14, 25].

The synthesis and secretion of the peptide by *S. warneri* IEGM KL-1 occur during the logarithmic growth phase, and both processes stop upon transition to the stationary phase. A similar dynamics of biosynthesis is typical of some other bacteriocins [22] and the antibacterial peptide factor of *Brevibacterium linens* [26].

The evident interrelation between peptide production and the developmental stage of the bacterial population seems to be determined by the functioning of an intrapopulation system of autoregulation of its synthesis, which is similar to regulation with the involvement of pheromones [27]. For the systems of synthesis of lanthionine-containing peptides nisin and mersaci-

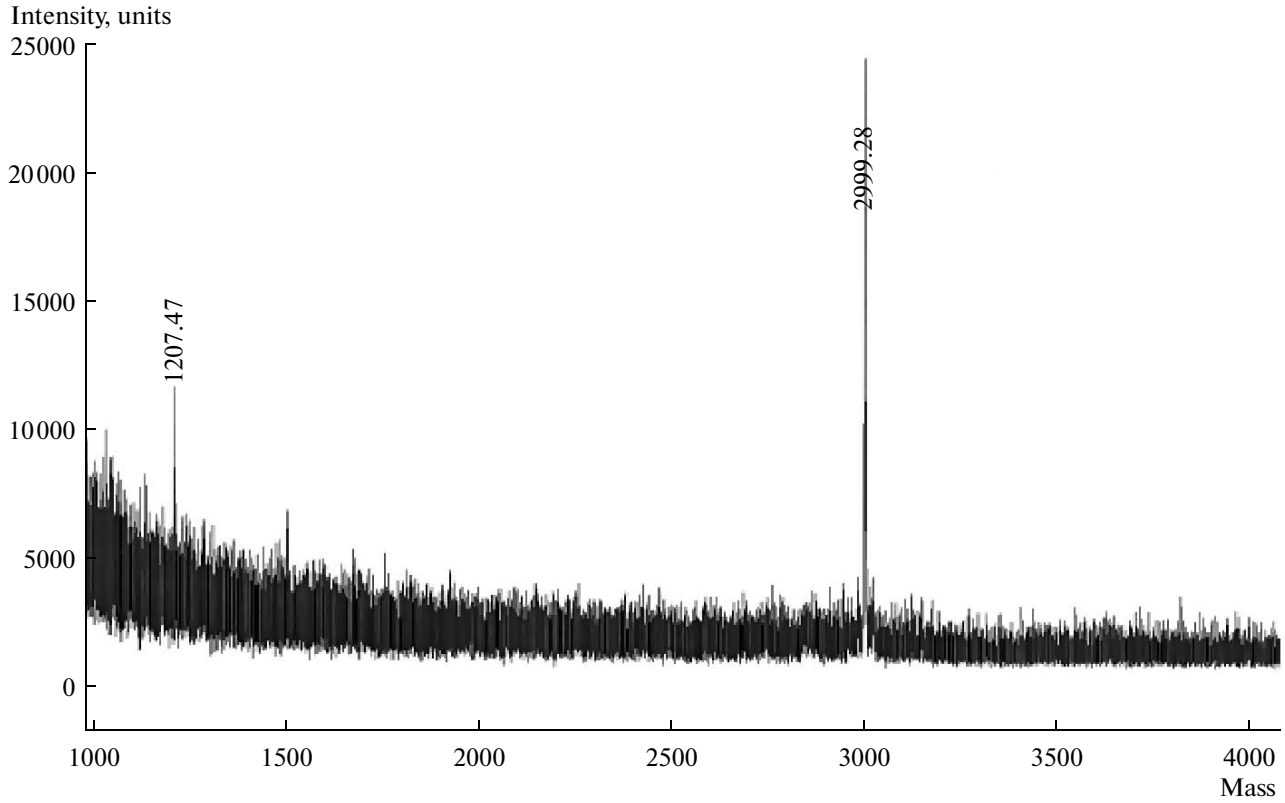

Fig. 5. Mass spectrometry of the homogenous antibacterial peptide preparation.

din, the autoregulatory role of peptides was shown in activation of the genes of their precursors and immunity factors with transcription initiation during exponential growth and with the maximum reached at the transition into the stationary phase. Extremely high efficiency of the regulatory activity of the peptides was demonstrated: only five nisin molecules are sufficient for the activation of its operon through the cascade of signal kinase and response regulator [28].

The antibacterial peptide was isolated from the supernatants of the cultivation medium by chromatography on Toyopearl-SP 650M cation-exchange resin. The high efficiency of peptide isolation with this sorbent containing sulfopropyl groups unambiguously indicates that peptide molecules both carry a total positive charge and possess pronounced hydrophobic properties. Elution of the peptide with antibacterial activity from the column as a compact peak in the narrow interval of NaCl gradient was an indirect evidence of relative homogeneity of the desorbed peptide fraction. Indeed, electrophoretic analysis of the peptide preparation desalinated on Sephadex G-25 showed the presence in gel of a zone of low-molecular peptide compounds with molecular masses in the range of 1.1–3.5 kDa, while mass-spectrometric analysis of the material eluted from this gel region showed the presence of several small peptides with molecular masses from 1.7 to 3.2 kDa. The antibacterial peptide was purified by multistage reversed phase chromatography on –C18 columns using various profiles of acetonitrile gradient. The peptide fraction obtained at the last stage of this procedure was homogenous and contained only one peptide with the molecular mass of 2999 Da. Based on the species affiliation of the producer, the isolated peptide was termed warnerin.

Amino acid analysis of hydrolysates of the homogenous warnerin preparation showed considerable quantity of hydrophobic amino acid residues, threonine and lysine, and an uncommon amino acid lanthionine. This compound (β-amino-β-carboxyethyl sulfide) was initially isolated during the treatment of wool by weak sodium carbonate solutions and named with the Latin name of the material used (*lana* in Latin) [29]. The presence of *lan*thionine in the structure of a number of peptide an*tibiotics* was a basis for isolation of a particular family of antibacterial peptides: *lantibiotics*. Unlike cystine, the structure of sulfur-containing lanthionine contains not a disulfide, but a thioester (*C-S-C*) bond, which is more resistant to different external actions [29]. Lantibiotic molecules obtain therefore substantial rigidity, which allows them to maintain their structure under high pressure, heating, low pH values, and the action of some proteases. The formation of thioester bond in lanthionine-containing peptides is a result of enzymatic posttranslational dehydration of the radicals of hydroxy

amino acids (serine and threonine) in the chain of a precursor peptide with formation of 2,3-dehydroalanine and 2,3-dehydrobutyirin residues, respectively. The subsequent nucleophilic attack of unsaturated bonds of these amino acids by sulfhydryl groups of cysteine with the involvement of a special cyclase results in the synthesis of lanthionine and 3-methyllanthionine, respectively, with the formation of cyclic intramolecular structures [4]. It should be noted that no residues of tryptophan and aromatic amino acids were found in warnerin composition (Table 2), which considerably complicates the quantitative assessment of this peptide both in different biological preparations and at all stages of its isolation, because conventional methods of protein assay, which are based on near-UV absorption, biuret test, and Folin reaction, cannot be applied.

The high contents of lipophilic amino acids (alanine, valine, glycine, leucine, and isoleucine), 40% total, attracts particular attention; it must contribute to the formation of a marked hydrophobic domain in the peptide molecule.

One more important molecular characteristic of the peptide is the absolute predominance of lysine, a positively charged amino acid accounting for about 23% of all amino acid residues. The tentative calculation shows that lysine residues under acidic and neutral conditions can give the molecule a pronounced positive charge (up to five or six units). Thus, the analysis of the amino acid composition of warnerin shows the evident hydrophobic and cationic properties of this compound, i.e. its amphiphilicity, which can provide high affinity of peptide molecules both to negatively charged surface structures of bacterial cells and to the lipid membrane components. This is essential for the interaction between the peptide and bacterial cell structures.

The whole scope of revealed physicochemical properties, peculiarities of amino acid composition and molecular mass characteristics make it possible to consider warnerin as a new representative of the family of lantibiotics, natural antimicrobial cationic peptides.

Previously, it was shown [9] that the culture liquids of the warnerin producer have a broad spectrum of antibacterial action against gram-positive bacteria of not only closely related species but also phylogenetically distant genera. The dynamics of peptide action on the cells of test strain *S. epidermidis* 33 revealed the development of bactericidal effect of warnerin from the very first minutes of its interaction with the cells (Fig. 6a). The quantity of viable cells decreases by half as early as 15 min after introduction of the peptide into bacterial suspension and by 2–2.5 orders of magnitude after 6 h of incubation (Fig. 6b). Application of ^{14}C-labeled warnerin demonstrated its practically instantaneous sorption on the attacked bacterial cells, with saturation reached during 1 min (Fig. 7). Such impetuous binding of the peptide to bacteria is apparently determined not only by the interaction between the cell wall anionic structures and positively charged warnerin molecules. The membrane potential, which facilitates quick directed movement of peptide molecules to the cytoplasmic membrane, and the change of physicochemical characteristics of the latter, resulting in substantial disturbance of its structural integrity, seem to play a substantial role in this process, as has been shown for some other lantibiotics [4]. Such course of developments during the attack of warnerin on bacteria is quite probable, because pretreatment of bacteria with valinomycin, which disperses the $\Delta\psi$ electric component of the membrane potential, significantly reduces the antibacterial effect of the peptide [30].

Table 2. Amino acid composition of the low-molecular peptide antibacterial factor produced by *S. warneri* IEGM KL-1

Amino acid	Number of residues
Aspartic acid/asparagine	0.8 ± 0.11
Serine	1.6 ± 0.13
Glycine	2.7 ± 0.15
Histidine	0.3 ± 0.05
Arginine	0.3 ± 0.28
Threonine	4.4 ± 0.46
Alanine	3.3 ± 0.11
Proline	0.3 ± 0.04
Tyrosine	0.2 ± 0.02
Valine	1.8 ± 0.15
Methionine	0.1 ± 0.03
Lanthionine	2.4 ± 0.04
Isoleucine	1.7 ± 0.14
Leucine	2.1 ± 0.01
Phenylalanine	0.2 ± 0.02
Lysine	6.8 ± 0.05
Total	29.0 ± 0.46

The findings substantiate the need for comprehensive research of low-molecular antibacterial peptides as an important biological phenomenon, which is not only of fundamental interest but also is of an essential value for development of the ways of application of these natural antibiotics in practice, considering their evident antibiotic activity.

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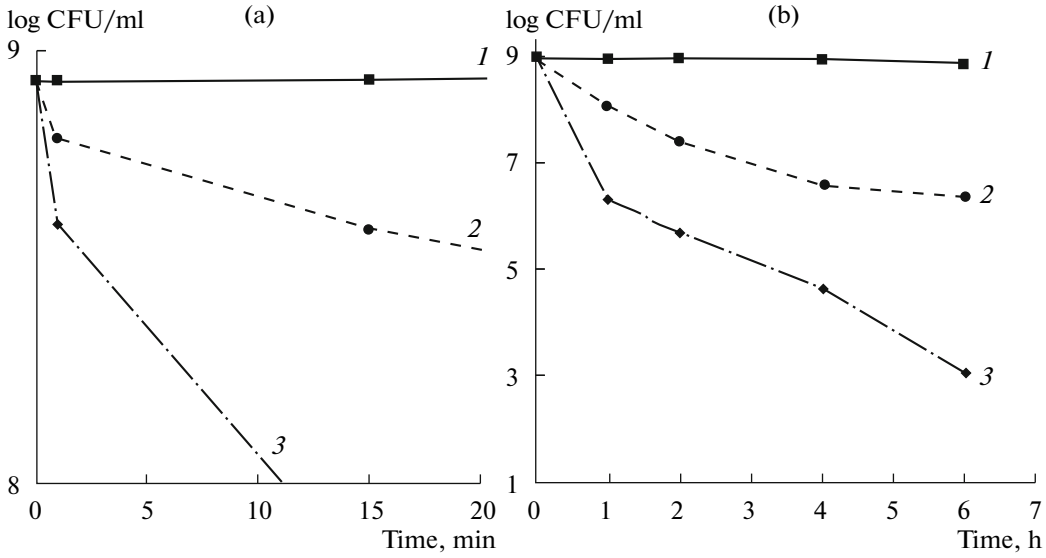

Fig. 6. The dynamics of death of *S. epidermidis* 33 cells under the action of the peptide: control, incubation in water (*1*), incubation in 0.14 M NaCl with the peptide concentration of 1024 AU/ml (*2*), and incubation in water with the peptide concentration of 1024 AU/ml (*3*); after 1 h (a); after 6 h (b).

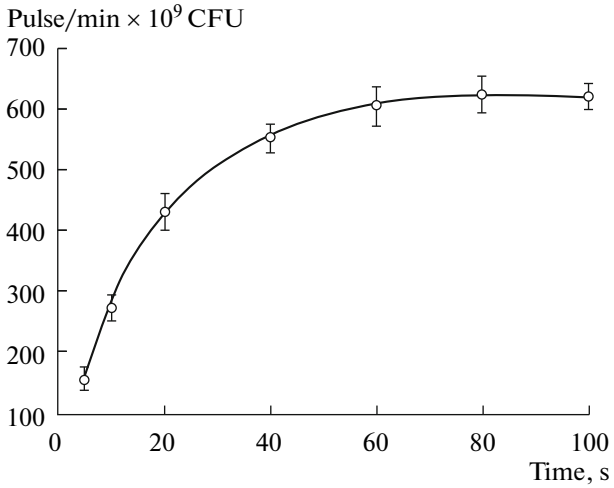

Fig. 7. Dynamics of ^{14}C-peptide binding by *S. epidermidis* 33.

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