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REVIEW PAPER

Peptide Autoinducers in Bacteria

A. O. Shpakov[1]

Laboratory of Molecular Endocrinology, Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, prosp. Toreza, 44, St. Petersburg 194223, Russia

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Abstract—The review classifies and analyzes the literature data on bacterial peptide autoinducers (AIs), responsible for intra- and interspecies communication (quorum sensing) between bacterial populations. The most important families of peptide AI are discussed, including a large group of bacteriocins, subdivided into lantibiotics (class I), unmodified heat-stable bacteriocins (II), large bacteriocins with $M_r > 30$ kDa (III), and "circular" bacteriocins (IV), as well as CSP peptides (Competence-Stimulating Peptides), peptides with thiolactone and lactone cycles, and short tryptophan-containing peptides with pheromone activity. The sensor systems are discussed, which recognize peptide AIs and regulate the activity of bacterial intracellular effector systems. For different families of peptide AIs, the typical features of structural organization are determined, which are responsible for their biological activity

Key words: autoinducer, bacteriocin, bacterium, histidine kinase, lantibiotic, intercellular communication, peptide, CPS peptide, quorum sensing.

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INTRODUCTION

In the populations of gram-positive bacteria, peptide autoinducers (AIs) play the key role in inter- and intraspecies communication (quorum sensing) [1–8]. Since they exhibit pronounced antimicrobial activity, they may serve as a basis for the development of highly efficient and highly selective preparations for treatment of bacterial infections. Most of peptide AIs bind selectively to receptor proteins on the cell surface; gene expression and other important vital processes are regulated via the signal stages coupled to these proteins. Some peptide AIs penetrate through the membrane inside the target cell; in the cytosol, they inhibit the synthesis of nucleic acids and proteins and control the enzymatic activity and cell wall synthesis [9].

Unlike gram-positive bacteria, in communities of gram-negative bacteria nonpeptide molecules usually act as autoinducers (N-acyl homoserine lactones, derivatives of 2-methyl-2,3,4,5-tetrahydroxy tetrahydrofuran, indole and quinoline derivatives) [10, 11], although some exceptions are known. For example, some *Escherichia coli* strains produce colicins, polypeptides with a molecular mass over 20 kDa, which inhibit growth of related microbial strains [12]. Relatively small peptide AIs, such as colicin V and microcins (below 10 kDa) were also found in enterobacteria; the size of these molecules is comparable to that of the peptide AIs from gram-positive bacteria (usually not exceeding 8 kDa) [13].

[1] Corresponding author; e-mail: alex_shpakov@list.ru

Peptide AIs of gram-negative bacteria include bacteriocins (subdivided into four classes according to their structure, functional properties, mechanisms of secretion and synthesis) [3, 7, 14, 15], CSP peptides (Competence-Stimulating Peptides) [16–18], peptides with thiolactone and lactone cycles [19, 20], and relatively short tryptophan-containing peptides with pheromone activity [21, 22]. In some cases, the functional differentiation between pheromones and bacteriocins is impossible, because many of them exhibit both the pheromone activity and the antimicrobial activity characteristic of bacteriocins [23].

Lantibiotics, the first class of bacteriocins, are small peptides which undergo posttranslational modification and contain unusual amino acid residues (AAR) (table). Heat-stable peptides containing no unusual AAR belong to the second (most numerous) class of bacteriocins. They are subdivided into three subclasses: IIa, pediocin-like bacteriocins with high bactericidal activity against *Listeria* (antilisteria pediocin-like bacteriocins); IIb, two-peptide bacteriocins; and IIc, bacteriocins without the leading N-terminal sequence. The third class comprises bacteriocins of significant size and is subdivided into subclasses of bacteriolysins (IIIa) and nonlytic proteins with $M_r > 30$ kDa (IIIb). Bacteriocins which undergo posttranslational modifications and form macrocyclic structures with the N- and C- termini connected by an amide bond form the fourth class (they are sometimes classified as subclass IId). The "circular" bacteriocins of class IV should be differentiated from the peptides which contain internal cycles (usu-

Classification of bacteriocins and their major representatives

Class	Type or subclass	Representatives
I: Lantibiotics	Type A (linear molecules with molecular mass below 4 kDa)	Nisin, subtilin, ericins A and S
	Type B (globular molecules with molecular mass from 1.8 to 2.1 kDa)	Mersacidin, mutacin I, astagardine, michiganin A
	"Atypical" lantibiotics	Sublancin, subtilisin A
II: Heat-stable unmodified bacteriocins with M_r < 10 kDa	Subclass IIa (antilisteria pediocin-like bacteriocins)	Pediocin, leucocin A, sakacin G, enterocin A, carnobacteriocin B2, mesentericin Y105, plantaricin 423
	Subclass IIb (two-peptide bacteriocins)	Plantaricins E/F and J/K, lactococcins F and G, mutacin IV, thermophilin 13
	Subclass IIc	Lactococcin 972
III: Polypeptide bacteriocins with M_r > 30 kD	Bacteriolysins	Lysostaphin, millericin B, zoocin A
	Nonlytic proteins	Helveticin J
IV: Cyclic bacteriocins (or subclass IId)		enterocin AS-48, circularin, uberolysin, gassericin A, acidocin B, reutericin 6, butyrivibriocin

ally formed by disulfide or structurally similar bonds), incorporating only a part of the polypeptide chain).

LANTIBIOTICS (CLASS I BACTERIOCINS)

The presence of unusual dehydrated amino acids and cyclic structures, lanthionine and β-methyllanthionine [24, 25]. Lanthionine consists of alanine residues bound via a sulfur atom; β-methyllanthionine consists of N-acetylbutyrine and alanine residues, also bound via a sulfur atom. Formation of lanthionine and β-methyllanthionine occurs in the course of posttranslational modification of precursor peptides, which includes dehydration of serine and threonine residues, respectively, and subsequent interaction with the nearby SH-group of cysteine resulting in formation of a sulfur bridge. Lantibiotics contain also a number of rare amino acids, didehydroalanine, didehydrobutyrine, and S-aminovinyl cysteine. All lantibiotics may be subdivided into two major groups.

The first group (type A) comprises relatively long polypeptides with flexible chains (21–38 AAR), which form potential-dependent pores in the plasma membrane determining their toxic effect against gram-positive bacteria. Nisin of *Lactococcus lactis* [23] and subtilin and ericins A and S of *Bacillus subtilis* [26] are the typical representatives of class A lantibiotics.

Nisin is a cationic hydrophobic peptide containing 34 AAR, including one lanthionine ring and four β-methyllanthionine rings, as well as dehydroalanine and dehydrobutyrine residues. Two variants of nisin (A and Z) were revealed, differing in position 27. Nisin synthesis depends on the stage of *L. lactis* growth; it is initially almost absent, increases significantly during the exponential growth phase, peaks at the late-exponential stage (when the biomass is maximal), and stops completely at the transition to the stationary phase. Nisin biosynthesis is controlled via a two-component signal system, including a NisK sensor histidine kinase and a coupled NisR response regulator, which regulates expression of the genes encoding the nisin precursor. The *de novo* synthesized nisin is modified by membrane-associated proteins NisB and NisC; this modification results in the formation of the derivatives of natural amino acids, which are typical of lantibiotics. Completely modified precursor peptide is then translocated through the membrane by the NisT ABC transporter. At the final stage of translocation, the leader sequence is cleaved from the precursor peptide by subtilisin-like protease NisP, resulting in formation of the biologically active nisin molecule. In the extracellular space, nisin acts as both an antimicrobial agent and an AI regulating the activity of the NisK–NisR system responsible for its synthesis (autoregulation mechanism) [27]. The cell producing nisin is protected from its toxic effect by the complex of the NisI lipoprotein, the ABC exporter consisting of two molecules of the NisF cytosol protein, and the NisE and NisG integral proteins.

B. subtilis subtilin (32 AAR) contains five cycles and is structurally close to nisin and ericin S, another lantibiotic from *B. subtilis* [28]. Similar to nisin, subtilin is synthesized as a precursor peptide, which is then modified by the SpaB and SpaC proteins, transported to the extracellular space by the SpaT protein, and undergoes proteolysis by the AprE extracellular serine protease in the course of translocation. Cleaving of the leading sequence by this protease results in the active form of subtilin. Extracellular subtilin activates a two-component system consisting of the SpaK sensory histidine kinase and the SpaR response regulator. SpaR binds to the promoter DNA site (*spa* box) and controls expres-

sion of the genes encoding the proteins responsible for subtilin synthesis; it also provides protection of the cell from the toxic effect of subtilin. Inactivation of *B. subtilis* subtilin is carried out by the complex consisting of the SpaI lipoprotein and the SpaFEG ABC transporter [29].

Two more type A lantibiotics were revealed in *B. subtilis*, namely ericin S which is similar to subtilin in function and differs only in four AAR, and ericin A, which differs from ericin S in both the organization of lanthionine rings and in its primary structure. Ericins S and A are synthesized by one EriBC synthetase and are secreted by *B. subtilis* cells in equivalent amounts [30].

Lantibiotics of type B are peptides with a rigid globular structure, uncharged or negatively charged. Mersacidin from *B. subtilis* [31], astagardine from *Actinoplanes* sp. [32], mutacin I from *Streptococcus mutans* [33], and michiganin A from *Clavibacter michiganensis* [34, 35] belong to this group. Mersacidin (20 AAR), the best known of these compounds, contains four internal cycles; one of them includes an ethylenamide bridge. Mersacidin is produced by stationary-phase bacteria; ten proteins participate in its biosynthesis. Among these, the proteins encoded by *mrsM* and *mrsD* genes are responsible for the posttranslational modification of mersacidin; the MrsT protein carries out its transport and proteolysis; and the complex of three immune proteins (MrsF, MrsE, and MrsG) protect the cell from the lantibiotic. The MrsD protein is a flavin-containing cysteine decarboxylase; it catalyses oxidative decarboxylation of the C-terminal cysteine residue in the mersacidin precursor. In the functionally active state it forms an oligomer containing 12 molecules of the MrsD protein. This lantibiotic affects expression of the genes encoding the proteins required for mersacidin synthesis via a two-component system including the MrsK2 histidine kinase and the MrsR2 response regulator; mersacidin synthesis is regulated by the MrsR1 protein which does not exhibit kinase activity [31]. Michiganin A (21 AAR) found in a phytopathogenic gram-positive bacterium *C. michiganensis* contains three sulfur bridges [34]. Similar to other B type lantibiotics, it affects lipid II incorporation in peptidoglycans and thus regulates the formation of bacterial cell wall. Since the biological effects of type B lantibiotics (including their action as AIs) are realized in the nanomolar concentration range, their efficiency is relatively high.

Unusual forms of lantibiotics have been revealed, which do not belong to types A and B. These are sublancin 168 and subtilisin A produced by *B. subtilis* [26] Sublancin (37 AAR) contains a β- methyllanthionine bridge and a disulfide bond unusual for lantibiotics; its formation is believed to depend on the thiol disulfide oxidoreductase encoded by the *bdbB* gene [36, 37]. Ssubtilisinubtilisin A is a macrocycle formed by binding of the N-terminal Gly^1 residue and the C-terminal Trp^{35} residue with an amide bond [38]. This structural organization is characteristic of class IV bacteriocins with a circular, macrocyclic structure. Subtilisin has also three internal cycles formed by highly unusual bonds between the sulfur atom of the cysteine residue and the α carbon atom of other AAR [39].

HEAT-STABLE, UNMODIFIED BACTERIOCINS (CLASS II BACTERIOCINS)

Subclass IIa Bacteriocins

Subclass IIa bacteriocins are small (M_r < 10 kDa), polycationic unmodified peptides resistant to heating. Their number of AAR varies from 37 (leucocin A and mesentericin Y105) to 48 (carnobacteriocin B2 and enterocin SE-K4). Over 25 representatives of this subclass are presently characterized; according to analysis of their primary structure, they fall into three or four groups. Their N-terminal domain contains β-folded structures stabilized by disulfide bonds between the cysteine residues in the $YGNGV(X)C(X)_4C(X)V(X)_4A$ sequence, which is the consensus sequence for all bacteriocins IIa (X is any AAR) [3, 40, 41]. The hydrophobic C-terminal domain contains one or two amphipathic α helices; in some bacteriocins (sakacin G, plantaricin 423, pediocin PA-a/AcH, divercin V41, and enterocin A), an additional disulfide bond is present, which plays an important role in the stabilization of their three-dimensional structure [42]. Formation of the β-folded structures in the N-terminal domain and of the helical structures in the C-terminal domain of bacteriocins IIa requires association with the hydrophobic membrane surface.

Bacteriocins IIa are synthesized as precursors with an N-terminal sequence which is removed in the course of translocation to the extracellular space. This sequence has two functions. While in the cytoplasm, it maintains the bacteriocin in its inactive state and thus protects the cell from its damaging action; it also acts as a signal for the ABC transporter responsible for bacteriocin transport through the cell membrane. The precursors of leucocin A, mesentericin Y105, and carnobacteriocin B2 are therefore, significantly less active than their analogues without the N-terminal sequence. Removal of this sequence or mutations (for example, substitution of other AAR for glycine in the highly conservative GG motif) results in impaired interaction between bacteriocins IIa and the N-terminal proteolytic domain of the ABC transporter; their secretion is therefore blocked [43]. As with other peptide AI, the rate of bacteriocin IIa synthesis is determined by the density of the bacterial population; it also depends on the temperature, ionic strength, and pH of the medium. Production of bacteriocins IIa usually peaks at the temperatures close to 20°C and is suppressed completely at 35–37°C [44].

Protection of the cell from bacteriocins IIa is achieved by immune proteins associated with the cytoplasmic side of the cell membrane and highly specific to the bacteriocins they inactivate. The immune pro-

teins interacting with structurally similar bacteriocins IIa may exhibit very low homology in their primary structures. For example, the immune proteins inactivating sakacin P and pediocin PA-1/AcH (which belong to the same group of bacteriocins IIa) exhibit 28% homology in their amino acid sequences [45]. According to presently available data, the immune protein (which is usually not an integral protein and is anchored in the cytoplasmic side of the cell membrane by a C-terminal helix) interacts with the bacteriocin IIa molecule associated with the external side of the membrane also by means of a hydrophobic C-terminal helix. Specific interaction between the C-terminal helices of an immune protein and bacteriocin IIa is believed to involve either the membrane lipids or certain integral proteins able to form complexes with bacteriocins [3]. Transmembrane permeases are the most probable candidates for the role of such intermediates; they are not only able to bind with bacteriocins IIa, but are in some cases responsible for bacterial sensitivity to these compounds [46].

The action of bacteriocins IIa is based on their ability to form ion-selective pores in the cell membrane; through these pores, potassium cations, amino acids, and other low-molecular compounds escape from the cell. Exhaustion of intracellular ATP, decrease of the membrane potential, and impaired functioning of the proton pumps also occur; these events result in the death of a bacterial cell. The C-terminal part of bacteriocins (15 AAR) was shown to be responsible for pore formation; it forms a helix which is comparable in size with the classical transmembrane domain of integral proteins [44]. Synthetic peptides corresponding to this part compete with full-sized bacteriocin molecules and inhibit its bactericidal activity. Antimicrobial activity of bacteriocins IIa is determined to a considerable degree by the highly conservative YGNG(V/L)$^{3-7}$ fragment located in their N-terminal domain. For example, substitution of tyrosine for phenylalanine in carnobacteriocin B2 results in a drastic decrease in its antimicrobial activity [47]. Substitution of asparagine for lysine in the relevant motif of pediocin PA-1/AcH has a similar effect [48]. Pediocin PA-1/AcH and enterocin A, which have an additional SH-bond in the C-terminal domain, are known to be more active than sakacin P and curvacin A, which have only one disulfide bond. Introduction of the pediocin C-terminal fragment with a disulfide bond into the sakacin molecule increases its bactericidal activity, broadens the spectrum of its antibacterial action and results in resistance of the modified sakacin to high temperature [44].

Bacteria of the genus *Listeria* are the most sensitive to bacteriocins IIa; these compounds are therefore termed antilisterial bacteriocins. Bacteria of the genera *Enterococcus*, *Lactobacillus*, and *Clostridium* are also targeted.

Subclass IIb Bacteriocins

Two-peptide bacteriocins of subclass IIb are binary complexes consisting of two different, albeit complementary, peptide molecules in a 1 : 1 ratio [49]. Outside this complex, the peptide molecules are inactive or have very low activity. Formation of the complex results in a two-peptide bacteriocin with very high biological activity. For example, the binary complex of lactococcin G exhibits antimicrobial activity at pico- and nanomolar concentrations, while the individual complementary peptides are inactive even at the concentrations over 50 μM [50]. A similar pattern was observed for the *Lactobacillus plantarum* two-peptide bacteriocins, plantaricin E/F, comprising the E and F peptides, and plantaricin J/K, comprising the J and K peptides [51]. For the functioning of a two-peptide bacteriocin, which acts as both an AI and an antimicrobial agent, the complementary peptides should be physically associated together [52]. The genes encoding the complementary peptides of two-peptide bacteriocins are located sequentially within the same operon [53–57]; for each bacteriocin, only one immune protein exists, which binds to the binary complex but does not interact with the individual complementary peptides [49]. The complementary peptides are therefore active only within a complex.

Two-peptide bacteriocins are believed to originate from synergically acting bacteriocins, single peptides produced either by one species or by related bacterial species; in the course of evolution, the activity of the individual peptides outside the complex decreased. For example, one of the peptides (LafA) of the two-peptide bacteriocin lactacin F from *Lactobacillus johnsonii* VPI11088 is homologous to one of the peptides (PlsA) of the two-peptide bacteriocin plantaricin S from *L. plantarum* LCP010. Homology was found between the peptide A of mutacin IV and the peptide A of thermophilin 13, as well as between the peptide B of mutacin IV and peptide LafA of lactacin F. The assortment of single peptides, precursors for the complementary peptides of two-peptide bacteriocins, was shown to be limited. Thus, the diversity of the latter results mainly from the number of combinations of single peptides.

Molecular mechanisms of action of two-peptide bacteriocins on a cell are similar to those of bacteriocins IIa; they also increase the permeability of the membrane to ions and small molecules [49, 52, 58, 59]. For example, lactococcin G makes the target cell membrane permeable to monovalent ions (Na^+, K^+, Li^+, etc.) and choline, though not to protons, anions (including phosphate), and bivalent cations [50]. Plantaricins E/F and J/K cause a flow of protons and monovalent cations through the membrane but do not affect its permeability for anions and bivalent cations [60].

The ability of two-peptide bacteriocins to form ion-selective pores is determined by their ability to form amphipathic helices with the hydrophobic, positively charged side; therefore, they may bind to the negatively charged membrane surface, penetrate it, and form a

transmembrane channel. For example, in peptide α of lactococcin G, an α-helix 3–22 exists; in the complementary peptide β, a shorter α-helix exists including the 6–16 sequence. The α-helices of considerable length were revealed in lactacin F, plantaricins S and NC8. The ratio of the α-helical conformation in two-peptide polycationic bacteriocins increases in the presence of liposomes; this results from the "freezing" of their ordered structure due to the interaction with the negatively charged liposome surface and anchoring of the hydrophobic C-terminal site of bacteriocins [49].

Subclass IIc Bacteriocins

This small subclass includes lactococcin 972 from *L. lactis* and enterocin from *Enterococcus faecium* L50, which are characterized by the absence of a leader sequence [61, 62]. The most studied polycationic peptide lactococcin from *L. lactis* (66 AAR) inhibits peptidoglycan synthesis in the course of cell wall formation and has almost no effect on the permeability of the membrane [62, 63]. Similar to type B lantibiotics, the molecular mechanisms of its inhibitory effect on cell wall synthesis include binding of this bacteriocin to the precursor of lipid II.

BACTERIOCINS AND NON-LYTIC PROTEINS WITH M_r > 30 KDA (CLASS III BACTERIOCINS)

Some species of bacteria produce large bacteriocins (M_r > 30 kDa), bacteriolysins with peptidoglycan hydrolase activity, which decompose the cell wall peptidoglycans. Most of bacterial peptidoglycan hydrolases are used against competing microorganisms, especially under nutrient limitation. Lysostaphin from *Staphylococcus simulans*, millericin B from *Streptococcus milleri* NMSCC 061, and zoocin A from *Streptococcus zooepidemicus* belong to bacteriolysins [64]. Helveticin J from *Lactobacillus helveticus*, which has no lytic activity, is a member of another group of large bacteriocins [65]. Bacteriocins III are believed to regulate the cellular signal systems, as well as control their own synthesis and some other processes, although no direct proof is presently available.

BACTERIOCINS WITH CIRCULAR STRUCTURE (CLASS IV BACTERIOCINS)

Bacteriocins of class IV are macrocycles formed by binding of the N- and C-terminal sites; they resemble a circular structure [7]. The presently identified and characterized members of this group are enterocin AS-48 from *Enterococcus faecalis* S-48, it homologue circularin A from *Clostridium beijerinckii* ATCC 25752, uberolysin from *Streptococcus uberis* 42, gassericin A from *Lactobacillus gasseri* LA39 and reutericin 6 from *L. reuteri* LA6 (these two peptides are close in primary structure), acidocin B from *L. acidophilus* M46, and butyrivibriocin from *Butyrivibrium fibrisolvens* AR10.

Enterocin AS-48 is a polypeptide (70 AAR), rich in positively charged AAR; the N-terminal amine group of Met^1 and the C-terminal carboxylic group of His^{70} form a peptide bond, thus closing the whole polypeptide chain into a macrocycle. Five sequential α helices, 9–21 (α_1), 25–34 (α_2), 37–45 (α_3), 51–62 (α_4), and 64–70 (α_5), are the major elements of the secondary structure. The $KAYLKKEIKKKGKR^{52–65}$ fragment, consisting of the α_4 helix and a part of α_5 helix, is responsible for its antibacterial activity; it binds to the negatively charged membrane surface and orients the hydrophobic α_1-, α_2-, and α_3 helices normally to the membrane area. This orientation is required for formation of an ion-selective pore by the enterocin [66]. The secondary structure of highly hydrophobic gassericin A and reutericin 6 is also mostly formed by α helices; they, however, do not contain polycationic motifs. This fact indicates different molecular mechanisms of pore formation by these bacteriocins and enterocin AS-48 [67]. Enterocin, gassericin, and reutericin were found to be highly resistant to heating; they retained activity at 100°C, probably due to their macrocyclic, circular structure.

Unlike lantibiotics and bacteriocins IIa, molecular mechanisms of processing and secretion of class IV bacteriocins, as well as the mechanisms of their regulatory effect on bacterial cells are poorly studied [7]. According to some data, translocation of "circular" bacteriocins through the membrane into the extracellular space is carried out by a complex of integral proteins containing ATP-binding sites, which have similar structural and functional organization to the ABC transporters transferring other classes of bacteriocins. For example, translocation of enterocin AS-48 is believed to be carried out by a complex containing the As48B protein with 12 transmembrane domains (TM) grouped in two hydrophobic domains (6 TM in each), the As-48C protein with 6 TM, and the ATP-binding As-48D protein [68]. Inactivation of the genes encoding the components of this complex results in impaired enterocin production. Translocation of butyrivibriocin is also believed to be carried out via an ABC-like transporter including the ATP-binding BviB protein and two hydrophobic proteins, BviC and BviD with 6 and 2 TM, respectively [69]. Although immune proteins and their complexes which protect the cells from bacteriocins IV were revealed, the mechanisms of their action are not known.

CSP PEPTIDES

Linear peptides of the CSP family (Competence-Stimulating Peptides) form a special group of peptide AIs. Similar to bacteriocins, they are synthesized as precursors, which in the course of translocation through the membrane are cleaved at the site containing two sequential glycine residues by a protease located in the N-terminal part of the ABC transporter. The CSP peptides of *S. mutans* [16, 17] and the functionally related ComC peptide from *Streptococcus pneumoniae* [70], as

well as bacteriocin-inducing peptides PlnA from *L. plantarum* [53], IP-673 from *Lactobacillus sake* [71], CTC492 from *E. faecium* [18, 72], CbnB2 and CbnS from *Carnobacterium piscicola* [47], and CbnS from *C. maltaromaticum* [18, 73] are the typical representatives of this group of peptide AIs. Since synthetic CSP peptides are comparable in efficiency to the natural forms, they are believed not to form cycles and not to undergo modification by hydrophobic radicals.

All CSP peptides contain significant amounts of positively charged AAR; they form polycationic motifs in the C-terminal part of the molecule ($RKK^{15–17}$ in *S. pneumoniae* ComC and $KKLFKK^{18–23}$ in *L. plantarum* PlnA). The ratio of positively and negatively charged AAR in *S. pneumoniae* ComC (17 AAR), *L. plantarum* PlnA (26 AAR), *S. mutans* CSP-1 (21 AAR), and *C. maltaromaticum* CS (24 AAR) is 6/2, 6/0, 3/0, and 5/0, respectively. It should be noted that many bacteriocins are also polycations. For example, the amino acid sequence of the lantibiotic nisin from *L. lactis* (34 AAR) contains five residues of positively charged amino acids and no negatively charged ones. The polycationic character of peptide AIs increases the efficiency of their interaction with ligand-binding sites of receptor proteins resulting from association of their polycationic motifs with the negatively charged membrane surface. The C-terminal polycationic motifs and isolated positively charged AAR (Arg^3 in ComC from *S. pneumoniae*) were shown to determine the efficiency and selectivity of the interaction between CSP peptides and receptor proteins [74]. In this process, α helices formed by the central sites of CSP peptides (the 6–12 helix in *S. pneumoniae* ComC), rich in hydrophobic residues, and highly conservative, play an important role. Apart from polycationic motifs and helical regions, other molecular determinants are involved in specific interaction with receptors. Even the structurally similar CSP peptides of *S. mutans* and ComC of *S. pneumoniae*, which have highly conservative polycationic motifs and α helices, exhibit highly specific interaction with a certain type of receptors and practically no interaction with other types [17].

PEPTIDES WITH THIOLACTONE AND LACTONE CYCLES

The YSTCDFIM octapeptide from *Staphylococcus aureus* (AgrD) is the best studied among the peptides with thiolactone cycles. It contains a thiolactone bridge connecting the Cys^4 SH group and the C-terminal Met^8 COOH group [19]. The 11-component peptide GBAP (Gelatinase Biosynthesis-Activating Pheromone) from *E. faecalis* contains the lactone bridge connecting the Ser^3 hydroxyl group with the Met^{11} carboxylic group [20]. Peptides with lactone and thiolactone cycles contain practically no positively charged AAR, and the molecular mechanisms of their interaction with receptor proteins are therefore different from those of polycationic peptide AIs.

Unlike bacteriocins and CSP peptides, the transport of *de novo* synthesized precursor peptides (precursors of AgrD and GBAP) into the extracellular space is performed not by ABC transporters, but rather by specific transport protein channels, which are also responsible for modifying the structure of these peptides. Transport of the precursor of the *S. aureus* AgrD peptide is performed by the AgrB protein; the precursor of the *E. faecalis* GBAP peptide is transported by the FsrB protein. The FsrB protein is a molecule comprising the N-terminal domain responsible for peptide transport and the C-terminal domain containing the GBAP peptide; the latter is released in the process of translocation of this domain into the extracellular space [20].

SHORT PEPTIDES WITH PHEROMONE ACTIVITY

Short peptides with pheromone activity are characterized by the presence in the third or fourth position from the C-terminal end of a tryptophan residue with the side chain modified by a hydrophobic isoprenyl group and by the presence of a site (target for degradation by proteases) in the N-terminal part of the molecule. The primary structure of these peptides varies significantly even within a single bacterial species. For example, in *B. subtilis* strains 168, RO-C-2, RO-E-2, RS-B-1, RO-H-1, and RO-B-2, a group of pheromone peptides (ComX pheromones) was revealed, containing five to ten AAR: ADPITRQWGD, TREWDG, GIFWEQ, MMDWHY, MLDWKY, and YTNGNWVPS, respectively [21, 22]. The peptides ComX-168 and ComX-RO-C-2 are modified by the C_{15} farnesyl group, while ComX-RO-B-2 ComX-RO-E-2, by the C_{10} geranyl group; these features also determine high specificity of the interaction between pheromone peptides and the cellular sensor systems. In *B. subtilis*, isoprenylation of the pheromonal peptide AIs is performed by the ComQ protein, which contains the domain specifically binding to the isoprenyl group and acts as an isoprenyl transferase. This was demonstrated by both in vivo experiments and the structural homology between ComQ and the IdsA farnesyl-geranyl transferase form *Methanobacterium thermoautotrophicum* [75].

MOLECULAR MECHANISMS OF ACTION OF PEPTIDE AIS

At the first stage of signal transduction, most of peptide AIs (class I and II bacteriocins, CSP peptides, peptides with lactone and thiolactone cycles, and tryptophan-containing peptides with pheromone activity) bind to the membrane receptors, usually to receptor histidine kinases. This is their major difference from non-peptide AIs, which usually penetrate into the cell and interact with the intracellular sensor proteins. Binding of the peptide AIs with an extracellular sensory domain of a receptor histidine kinase causes the enzyme autophosphorylation at the histidine residue and starts the

transphosphorylation reaction. This reaction results in phosphate transfer from the histidine residue to the aspartate residue in the response regulator molecule; the latter is functionally coupled to histidine kinase. The activated response regulator controls the expression of bacterial genes, including those responsible for AI synthesis. The two-component signal system of streptococci regulated by CSP peptides is the best studied [17, 33]. It includes the ComD receptor histidine kinase and the ComE response regulator, which activates the expression of over 190 genes controlling the adaptation and transformation of bacterial cells, their virulence, and synthesis of peptide AIs.

The CSP peptides of *S. mutans* are responsible for formation of the film closely associated with the dental surface and thus preventing removal of bacterial cells from the oral cavity. They also stimulate formation of mutacins, which belong to different classes of bacteriocins and act as strong toxins causing death of other bacterial species; colonization of the oral cavity by *S. mutans* is therefore unhindered, especially under nutrient limitation [33, 76, 77]. For example, the lantibiotic mutacin I synthesized by *S. mutans* after treatment with CSP peptides, completely suppresses growth of pathogenic bacteria, including *E. faecalis, Staphylococcus epidermidis*, and eleven species of streptococci [33]. The CPS peptides lyse incompetent *S. mutans* cells incapable of transformation [78]. Their lysis releases DNA and other molecules which may be used as nutrients or genetic material by the competent cells capable of transformation. High survival of competent bacterial cells and the high rate of their adaptation to varying environmental conditions are therefore ensured. Investigation of this process is an important problem, because numerous toxins with cytolytic activity which are liberated in the course of lysis may damage the host cells [79]. On the other hand, introduction of CPS peptides was shown to enhance survival of *S. pneumoniae*-infected mice due to their pronounced antimicrobial effect against this bacterial species [80].

The AgrD octapeptide from *S. aureus* and the GBAP undecapeptide from *E. faecalis*, which belong to the group of peptide AIs with lactone and thiolactone cycles, are specifically bound to the AgrC and FsrC receptor histidine kinases, respectively. The AgrC histidine kinase activated by the AgrD peptide is autophosphorylated and transfers phosphate to the AgrA response regulator which activates transcription of the *agr* operon (responsible for the synthesis of the precursor of the AgrD peptide) and of the genes responsible for secretion of bacterial toxins and proteolytic enzymes [81]. Unlike bacteriocins, the peptide AIs containing lactone and thiolactone cycles exhibit low specificity of interaction with receptors. Several structurally similar cyclic peptides may bind to one receptor, while a single peptide may activate several receptors, albeit with different efficiency. This is the basis for hard competition even between structurally similar forms of peptide AIs with lactone and thiolactone cycles.

PEPTIDES OF THE CSF FAMILY

The peptides of the CSF family (Competence and Sporulation Factor) form an isolated group of peptide AIs [82–84]. In *B. subtilis,* three pentapeptides were revealed, ARNQT, ERGMT, and SRNVT, which are the products of cleaving of their polypeptide precursors, PhrA, PhrC, and PhrE (acting as phosphatase regulators. Seven such regulators are presently known in *B. subtilis*, although only three of them are known to contain pentapeptides with AI functions. They are all localized in the C-terminal segment of the Phr regulator and are formed in the course of two-stage processing. First, the signal sequence is cleaved from the polypeptide precursor (40–44 AAR). The resulting precursor containing 19 AAR is secreted into the extracellular space via a SecA-dependent system. It then undergoes proteolytic cleavage resulting in formation of a functionally active polypeptide, which is then returned to the cell via an oligopeptide transport system [83]. Thus, unlike most of the other peptide AIs, CSF peptides do not interact directly with receptor histidine kinases. These peptides, however, inactivate the phosphatases responsible for dephosphorylation of the response regulator in the two-component signal system, which is regulated by the ComX pheromone peptides and includes a receptor histidine kinase as the first component. Thus, CSF peptides are responsible for positive regulation of the ComX-competent signal cascade. The effect of the pentapeptides is highly specific. For example, the SRNVT peptide specifically inhibits the RapE phosphatase and does not affect the activity of RapA and RapB phosphatases, while the ERGMT peptide inhibits the RapC phosphatase and does not affect RapE phosphatase [83].

POLYPEPTIDE FACTORS

A polypeptide regulatory factor revealed in the phytopathogenic bacterium *Xanthomonas axonopodis* is homologous to the CMF factor (Conditioned Medium Factor) of the myxomycete *Dictyostelium discoideum* [85]. This endogenous factor is secreted into the extracellular space, and it binds these to the sensor domain of the *X. axonopodis* RpfC integral protein (which is a receptor histidine kinase) and stimulates the functional activity of the HD-GYP c-diGMP phosphodiesterase. Since the RpfG phosphodiesterase is responsible for the functional coupling between the RpfC histidine kinase and the receptor form of diguanylate cyclase, an unusual situation develops when one extracellular factor regulates the activity of three enzymes and thus controls various aspects of bacterial metabolism [85]. In the cells of *D. discoideum*, the CMF-1 and CMF-2 factors regulate the intracellular level of cyclic nucleotides (cAMP, cGMP), phosphoinositide metabolism, and intracellular Ca^{2+} concentration [86].

Our analysis by means of the BLAST software package revealed the polypeptide factors related to the

CMF of amoebae *D. discoideum*, *Hartmannella vermiformis* and *Acanthamoeba castellanii* not only in *X. axonopodis*, but also in other bacteria, *Xanthomonas campestris*, *Shewanella putrefaciens*, *Shewanella woodyi*, *Solibacter usitatus* and *Methylibium petroleiphilum* [87, 88]. The similarity in the primary structure of bacterial CMF-like factors exceeds 25%. High homology was revealed between the C-terminal half of the CMF factor molecules and the *Malawimonas jakobiformis* glucosyl hydrolase (gi|62766461). For example, the 382–542 part of *X. campestris* CMF and the 1–159 part of the glucosyl hydrolase are 47% similar. Since most of the glucosyl hydrolase molecule (a total of 164 AAR) is highly homologous to the CMF factors of bacteria and unicellular eukaryotes, the most conservative C-terminal half of the CMF factors may be considered a domain with glucosyl hydrolase activity; its function, however, is unclear.

CONCLUSION

Diversity of the forms of bacterial peptide AIs indicates that a highly organized and highly specialized chemical system of communication exists in bacteria, which includes information exchange both between bacterial cells of one species or even strain and between different bacterial species. In bacterial species well studied in this respect (*B. subtilis*, *L. lactis*, *S. pneumoniae*, and *E. faecalis*), the number of peptide AIs is comparable to the number of peptide regulators in higher eukaryotes. The number of these signals implies numerous signal systems responding to these signals. Receptor histidine kinases usually act as the sensor components of bacterial signal systems responsible for specific binding of peptide AIs; while the coupled response regulators, they control the expression of bacterial genes, including the genes responsible for the synthesis of peptide AIs. The main families of higher eukaryote receptors, i.e., serpentine type receptors penetrating the membrane seven times and receptors with tyrosine kinase activity, are believed to originate from various forms of bacterial receptor histidine kinases [87, 89]. This is because eukaryotic signal systems originated from prokaryotic chemical signal systems as a result of endosymbiosis of bacterial cells, which introduced some components of their signal systems or their complexes into eukaryotic cells [90, 91].

Most of bacterial peptide AIs are polycationic peptides with high helicity, and the ratio of the helix conformation increases sharply in the course of binding to receptor proteins and/or the negatively charged membrane surface. In higher eukaryotes, amphipathic polycationic peptides also act as regulators of the signal systems; they may act both at the receptor level and at the postreceptor stages of signal transduction, interacting with the down-stream signal proteins (for example, with heterotrimeric G proteins) [92]. The search for the origin of the functional activity of such polycationic peptides should deal with bacterial peptide AIs, the most ancient class of regulatory molecules. The functional and structural relations between bacterial and eukaryotic peptide regulators are confirmed by the fact that some vertebrate peptide hormones (somatostatin, melanocortin, natriuretic peptide) and immunomodulators (dynorphin) act as bacterial AIs [93]. They can regulate bacterial two-component signal systems, determine bacterial survival, growth, and virulence. These peptides from vertebrates are positively charged; some of them (somatostatin, natriuretic peptides) have a rigid cyclic structure; in these respects, they are similar to lantibiotics, cyclic bacteriocins, and other peptide AIs containing cycles of various chemical nature. In spite of the absence of direct evidence, it may be suggested that bacterial peptide AIs may also affect the functional activity of hormonal signal systems of higher eukaryotes in a way similar to that demonstrated for synthetic polycationic peptides [92]. This should be considered both in the development of the medical preparations based on peptide AIs and in the study of the pathogenetic basis of development of infections caused by gram-positive bacteria.

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