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REVIEW PAPER

# QS-Type Bacterial Signal Molecules of Nonpeptide Origin

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**Abstract**—This review classifies and analyzes the literature data on bacterial autoinducers (AI), the signal molecules produced and secreted by bacterial cells and responsible for intercellular communication (quorum sensing, QS). The most important families of nonpeptide AI are discussed, including *N*-acyl homoserine lactones, derivatives of 2-methyl-2,3,4,5-tetrahydroxy tetrahydrofuran, indole and quinoline derivatives, and adrenaline-related compounds. The data is provided on the intracellular and membrane receptors specifically binding to AI, as well as on the effector systems that are activated by AI and mediate their regulatory effects. The possible role of some vertebrate hormones (adrenergic agonists, serotonin, etc.) as AI and their effect on bacterial activity are discussed. The data are presented suggesting a high efficiency of AI-based antibacterial preparations, which selectively disrupt the bacterial information network and thus suppress bacterial infections.

*Key words*: autoinducer, bacterium, histidine kinase, *N*-acyl homoserine lactone, 2-methyl-2,3,4,5-tetrahydroxy tetrahydrofuran, quorum sensing, signal system, quinoline.

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## INTRODUCTION

Bacterial intercellular communication (quorum sensing, QS) relies heavily on autoinducers (AI), endogenous chemical compounds which are synthesized and secreted into the environment. Since the number of cells in a growing bacterial population increases, AI concentrations in the medium increase as well. By assessing the extracellular AI concentration, bacteria determine the density of their own population (intraspecific communication) and sometimes the density of the populations of other bacterial species (interspecies communication). This mechanism underlies the choice for the optimal survival strategy both within a bacterial population and within a community including other populations of other microbial strains and species. Some AI are bound to the membrane receptors located on the cell surface, while others may penetrate the membrane and bind to the intracellular receptors [1–6]. After binding with the receptors, AI activate a broad spectrum of effector systems affecting the regulation of expression of a number of genes, including those encoding the signal proteins responsible for AI-generated signal transmission. Altered gene expression provides control of such important processes as conjugation, transformation, spore formation, synthesis and secretion of antibiotics and virulent factors, and formation of cell aggregates or surface biofilms. Thus, bacterial cells react adequately to the presence of AI and structurally similar compounds in the environment; the latter may be produced, apart from microorganisms, by vertebrates and invertebrates.

Apart from AI, physical factors (e.g. light or temperature) and simple chemical signals (exogenous ions and molecules) may act as regulators for bacterial effector systems. Such external signals include both inorganic ions (bicarbonate anion, potassium and calcium cations, etc.) and organic compounds (amino acids, monosaccharides, nucleotides, etc.) Molecular mechanisms of their action on effector systems usually differ from those of AI. Bacteria were recently shown to receive the signals initiated by vertebrate hormones and hormonelike factors, including biogenic amines, adrenergic receptor ligands, serotonin, and melatonin. Adrenaline and related catecholamines form a specific group of AI responsible for bacterial intercellular communication and interaction with animal cells [7, 8].

External signals are received by receptors which may be located on the membrane or in the cytoplasm. In the latter case, signal molecules must penetrate the cytoplasmic membrane to bind with the ligand-binding site of the intracellular receptor. In higher eukaryotes, the classical signal system usually includes three components: (i) a receptor in the plasma membrane, responsible for specific signal recognition; (ii) a coupling component, usually a heterotrimeric G protein; and (iii) an effector protein, either an enzyme generating secondary intermediates or an ion channel. In membrane-associated bacterial signal systems, the receptor, the coupler, and the effector are usually combined within one molecule. Multifunctional receptor molecules are therefore formed, with several modules with a

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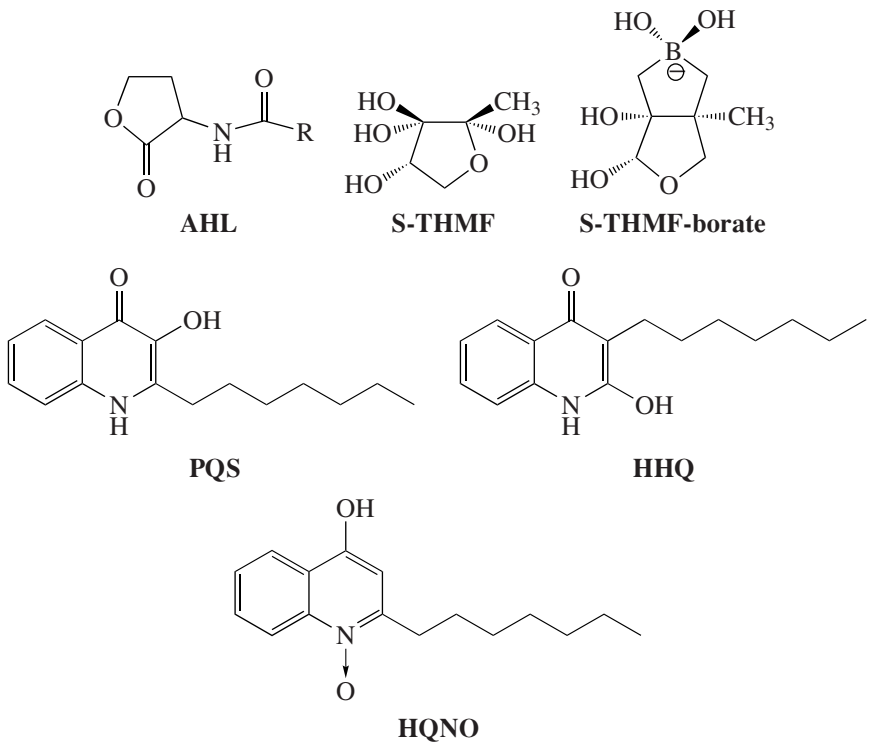


Molecular structure of *N*-acylated homoserine lactone (AHL), (2S,4S)-2-methyl-2,3,4,5-tetrahydroxytetrahydrofurane (S-THMF) and its borate ether (S-THMF borate), 2-heptyl-3,3-dihydroxyquinoline (PQS), 2-heptyl-2-hydroxyquinoline (HHQ), and its *N* oxide (HQNO).

sensor, effector, and regulatory functions. Most bacterial signal systems are two-component and contain a receptor molecule and a response regulator; the latter is functionally coupled to effector domains located in the cytoplasmic part of the receptor molecule [9–11]. However, one-component systems also exist (with the receptor molecule including a response regulator), as well as more complex, multicomponent systems resembling eukaryotic signal systems. This similarity may be the result of the origin of eukaryotic hormone signal systems from ancient prokaryotic signal systems due to endosymbiosis of bacterial cells with already developed signal cascades or their major components [12–15]. The most widespread effector blocks of bacterial signal systems include histidine kinases, methyl acceptor proteins, and enzymes generating cyclic nucleotides; the latter belong to two major families, adenylate cyclases (AC) and diguanylate cyclases. While both prokaryotic and eukaryotic organisms possess various forms of AC catalyzing cAMP synthesis, diguanylate cyclases catalyzing the synthesis of c-diGMP were found only in bacteria (see [16] for details).

The goal of the present review is classification and analysis of the literature data on QS-type signal molecules of nonpeptide origin responsible for intercellular communication in bacterial communities, as well as on the specific intracellular and membrane receptors involved in the regulation of bacterial growth and metabolism. As was mentioned above, AI and related molecules form the largest, relatively well-studied group of bacterial regulatory molecules. Depending on their structure and functions, AI are classified within several families; the most widespread and important are lactones of *N*-acylated homoserine (AI-1, AHL), derivatives of 2-methyl-2,3,4,5-tetrahydroxy tetrahydrofuran (Ai-2, THMF), adrenaline-related compounds (AI-3), and indole and quinoline derivatives.

## *N*-ACYL HOMOSERINE LACTONES (AI-1, AHL)

Gram-negative bacteria possessing the genes encoding LuxI synthase, the enzyme synthesizing AHL from S-adenosyl methionine, use AHL as AI (figure) [4–6, 17–19]. The AHL produced by different bacterial species share the same lactone cycle and have different acyl radicals (from 4 to 12, in some cases 14–18 carbon atoms). The C-3 atom can be unmodified or contain an oxo or hydroxyl group. For example, *Chromobacterium violaceum*, *Agrobacterium tumefaciens*, and *Pseudomonas aeruginosa* produce an AHL with medium-sized acyl radicals and the C-3 atom modified by the oxo group ($C_8$-3-oxo-AHL and $C_{12}$-3-oxo-AHL, respectively) [20–23]. *Pseudomonas fluorescens* produces six different AHL, including three with the hydroxyl group at C-3 position, $C_6$-3-hydoxy-AHL (the main product), $C_8$-3-hydoxy-AHL, and $C_{10}$-3-hydoxy-AHL [24]. *Paracoccus denitrificans*, *Rhodobacter capsulatus*, *Rhizobium leguminosarum*, and *Sinorhizobium meliloti*

produce AHL with acyl radicals over 12 carbon atoms long, which are hydrophobic molecules [25, 26].

AI-1 penetrate within bacterial cells and specifically interact with LuxR proteins which act as intracellular receptors. The ligand-activated LuxR proteins bind to AHL-regulated promoters and initiate transcription of QS-controlled genes [27]. The specificity of AHL interaction with receptors is determined by the size of their acyl radical and the presence of a functional group in the C-3 position; these are the molecular determinants responsible for AI-1 binding to specific LuxR proteins and for their activation. Some AI-1 are able to interact with surface receptors; this was demonstrated for the AHL of *Vibrio harveyi* and *V. cholerae*, binding with the LuxN receptor protein functionally related to LuxR [28].

Although communication between bacteria of the same species is the major function of AHL, they may also participate in interspecies communication. For example, gram-negative bacteria *V. harveyi* use AHL to control the formation of compact colonies by gram-positive bacteria [29]; AHL produced by *P. aeruginosa* regulate gene expression in *Burkholderia cepacia* [30].

Regulation of the interactions between bacterial species, as well as between bacteria and eukaryotic cells can occur by AHL modification or by synthesis of their analogues with altered characteristics. For example, many *Bacillus* species release an enzyme, AiiA, which cleaves the AHL lactone ring and makes it inactive; development of other bacteria utilizing AHL as AI is therefore suppressed [31]. AI-1 synthesized by *P. aeruginosa* suppress formation of hyphae by *Candida albicans* [32]. It is still unclear whether this phenomenon results from suppression of fungal growth by bacteria or is an adaptive response of fungi to the presence of antagonistic bacteria. The surface of *Delisea pulchra* algae is covered by a mixture of halogenated furanones structurally similar to bacterial AHL [33]. Upon contact with algal cells, bacteria absorb halogenated furanones which efficiently bind bacterial LuxR proteins and disrupt their structure. The expression of LuxR-controlled genes is therefore impaired, resulting in suppressed bacterial growth and reduced virulence. It was shown ten years ago that the *P. aeruginosa* lactone of *N*-(3-oxododecanoyl)-L-homoserine has a pronounced immunomodulator activity; mammalian cells may specifically recognize this bacterial signal [34]. Human epithelial cells were later found capable of inactivation of this AI, thus preventing infection with *P. aeruginosa*. Suppression of intracellular communication by blocking the AHL-generated signals may therefore be considered a new approach in treating bacterial infections, QS-oriented therapy. AHL molecules can also act as chemoattractants. For example, marine algae are able to receive these molecules and thus move in the direction of bacteria [35].

In *V. cholerae*, (S)-3-hydroxytridecane-4-one (CAI-1) was found; it has some structural and functional similarities to AI-1 [36]. CAI-1 synthesis requires the enzyme CqsA synthase, homologous to pyridoxal phosphate aminotransferase. The length of the molecule directly affects the CAI-1 biological activity; shortening the natural analogue ($C_{13}$) to the molecules with 11 and 12 carbon atoms results in a eight-fold and 60-fold decrease in activity, respectively. CAI-1 binds to the CqsS receptor's protein, thus causing inactivation of LuxO protein which regulates the functional activity of the HapR transcription factor and therefore controls expression of the genes responsible for *V. cholerae* virulence and capacity for biofilm formation. The TCP (toxin co-regulated pilus) virulence factor is one of the targets for CAI-1; at enhanced concentrations of this AI, TCP expression decreases or is completely blocked, resulting in a loss of virulence in *V. cholerae* [36].

## 2-METHYL-2,3,4,5-TETRAHYDROXY TETRAHYDROFURAN AND ITS DERIVATIVES (AI-2, THMF)

AI-2 are widespread among bacteria; they control a number of processes, including production of toxins and pathogenic factors and formation of surface films; they also determine intra- and interspecies relations between bacteria underlying the "social" behavior of bacterial cells and populations [3, 19, 37–45]. AI-2 were recently shown to play an important role in bacterial survival under oxidative stress conditions. In some bacteria (*Porphyromonas gingivalis* and *Streptococcus mutans*), blocking of the biosynthetic pathways responsible for AI-2 synthesis results in enhanced resistance to hydrogen peroxide [40, 41]; in other species (*Photorhabdus luminescens*), AI-2 exhibit a pronounced antioxidant effect [47]. In *Bacillus subtilis*, activation of AI-2 biosynthetic pathways was observed under oxidative stress [48].

AI-2 biosynthesis is turned on by glucose and other nutrients activating the methyl cycle. During the first stage of the cycle, methyl transferases remove the methyl radical from S-adenosyl methionine, the main donor of methyl radicals and the direct precursor to AI-2. S-adenosyl homoserine obtained by demethylation is hydrolyzed by Pfs nucleotidase to S-ribosyl homocysteine, the substrate for the hydrolytic enzyme LuxS. Cleavage of the thioester bond by LuxS results in formation of 4,5-dihydroxy-2,3-pentanedione; its cyclization leads to the two major AI-2 forms [49–51]. The first form is (2S,4S)-2-methyl-2,3,4,5-tetrahydroxy tetrahydrofuran (S-THMF) or, more often, its borate ether (S-THMF borate) (Figure); the second form is (2R,4S)-2-methyl-2,3,4,5-tetrahydroxy tetrahydrofuran (R-THMF) or its phosphate ether (R-THMF phosphate). Bacteria of a given species usually synthesize a specific form of AI-2. For example, *V. harveyi* produces S-THMF borate, while *Salmonella enterica*, R-THMF. AI-2 are then secreted into the extracellular space where they may be intercepted by other bacteria producing these AI by means of an ABC transporter [52]. In spite of the differences in AI-2 structures, most

bacteria can receive the signals generated both by their inherent THMF and by their homologues synthesized by other bacteria [49, 50]. The total AI-2 content in a bacterial cell is determined by de novo synthesis and capture from the intercellular space; these processes are mutually independent [42]. The factor determining the THMF balance in the cell is the glucose content in the medium; glucose both induces the biosynthetic pathways leading to THMF production and regulates their transport from the extracellular space [52].

Wide occurrence of signaling by AI-2 is suggested by the fact that the genes encoding the proteins required for THMF synthesis, primarily LuxS hydrolase (AI-2 synthase) have been revealed in 80% of genomes of gram-positive and gram-negative bacteria. Even the absence of these genes does not rule out AI-2 synthesis, since alternative biological pathways were recently discovered, which do not require Pfs nucleotidase and LuxS hydrolase. For example, AI-2 synthesis was observed in bacterial mutants with functionally inactive *pfs* and *luxS* genes; the rate and intensity of this process depended on glucose content [42]. Molecular mechanisms of alternative AI-2 synthesis may be similar to those revealed in eukaryotes, namely in tomato fruits, where 4,5-dihydroxy-2,3-pentanedion was formed from D-ribuloso-5-phosphate, rather than from S-ribosyl homocysteine. Detection of AI-2 in eukaryotes suggests that they, as well as bacteria, can synthesize AI-2 and use them to affect bacterial vital processes. There are reasons to believe that eukaryotic cells are able to control bacterial signal pathways involving THMF; this ability may be important for the preservation of normal gastrointestinal microflora [5].

Two signal systems are presently known, which have a regulatory effect on bacterial cells via AI-2. The first was described for *V. harveyi* and *V. cholerae* [2, 3, 36, 49]. It includes the membrane LuxP receptor protein, specifically binding to S-THMF borate; this results in phosphorylation, involving LuxQ histidine kinase coupled to the LuxP receptor, LuxU phosphate transferring kinase, and LuxO response regulator. In *S. enterica* and *P. luminescens*, another system operates. It includes an ABC transporter (Lsr) transferring AI-2 inside the cell, where its molecule is modified by two enzymes (encoded by *lsrFG* and *lsrK*); the modified AI-2 then activates effector proteins, the regulators for bacterial gene expression [47, 50, 53]. In the case of *S. enterica*, the nonesterified R-THMF acts as AI-2; addition of the etherified analogue (5 mM borate) results in a decreased response to AI-2. Bioluminescence of *P. luminescens* is decreased in the presence of 1 mM borate; this process is regulated by AI-2. These data indicate that etherified AI-2 acts via receptor proteins similar to LuxP, while nonesterified forms act via ABC transporters. However, other mechanisms of the AI-2 effect on bacterial cells may exist as well.

In some bacteria the signal systems regulated by AI-1 and AI-2 (as well as by closely related AI) and differing in the initial, proximal stages were shown to converge subsequently into a common signal stage [3, 36, 44]. For example, in *V. cholerae* and *V. cholerae*, at least three different AI's act as activators for the LuxU phosphate-transporting protein, namely AI-1 (HAI-1), CAI-1, and AI-2 (S-THMF borate). HAI-1 binds to the LuxN receptor protein; CAI-1 binds to the CqsS receptor protein; and S-THMF borate, binds to the complex of the LuxP receptor protein and the LuxQ histidine kinase. Together with LuxU, the common blocks of the distal stages of signal transfer include the LuxO response regulator and LuxO-dependent expression of small RNA genes; these, in turn, determine expression of the HapR/LuxR translation regulators and may act as AI [44].

## ADRENALINE AND ITS DERIVATIVES (AI-3)

In bacterial mutants with the functionally inactive *luxS* gene, S-ribosyl homocysteine is accumulated and, consequently, the intracellular level of homocysteine decreases, which is the methionine precursor highly important for bacterial metabolism. Bacterial cells therefore use other pathways of methionine synthesis and accumulate oxaloacetate, the precursor of aspartate and some other amino acids. This modification of the biosynthetic pathways results in changes in amino acid metabolism and blocks AI-3 production. Since analysis of the AI-3 structure revealed that these compounds are aromatic and do not contain the furan ring typical for AI-2, their amino acid nature was hypothesized [54]. This conclusion is supported by the fact that AI-3 properties were revealed in typical eukaryotic hormones, catecholamines adrenaline and norepinephrine (phenylalanine derivatives capable of efficient action on bacterial receptors [39, 55, 56].

Adrenaline and norepinephrine compete with the AI-3 secreted by bacteria and thus act as AI-3-like agonists [39]. Both catecholamines are present in the gastrointestinal tract; norepinephrine is synthesized in adrenergic neurons of the abdominal nervous system, while adrenaline is delivered with blood flow. This fact agrees well with the presence of AI-3 related to adrenergic receptor ligands mostly in enterobacteria, both pathogenic (enteropathogenic strains of *Escherichia coli*, *Shigella* sp. and *Salmonella* sp.) and nonpathogenic, forming the normal intestinal microflora (nonpathogenic strains of *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*) [54, 56, 57]. Bacterial AI-3 and the catecholamines produced by host cells regulate the expression of bacterial genes responsible for virulence and capacity for colonization of the gastrointestinal tract. For example, they enhance the expression of the *LEE* gene family in the enteropathogenic strain *E. coli* O157:H7, increasing its virulence and pathogenicity. Adrenergic agonists were also shown to enhance bacterial motility, their chemotaxis, and capacity for biofilm formation. Indole, another *E. coli* extracellular signal, decreases bacterial motility and capacity for biofilm

formation and prevents attachment to epithelial cells; it is therefore an antagonist of AI-3 [57].

AI-3 specifically bind to the QseC and QseE receptor histidine kinases; exogenous AI-3 mainly activate QseC kinase [54, 55]. The QseC histidine kinase contains, apart from the catalytic (histidine kinase) domain, also the sensory domain (limited by two TM at N- and C-termini), the EAL domain (with c-diGMP phosphodiesterase activity), and the ATPase domain with phosphatase activity. It is functionally coupled to the QseB response regulator, which binds to the *flhDC* promoter and thus activates the genes responsible for bacterial motility. QseB also binds to its own promoter and carries out the autoregulation of the *qseB* gene transcription. Another histidine kinase, QseE, after binding to AI-3 activates the QseF response regulator, which controls the expression of the *LEE* gene family via two transcription factors, QseA and QseD [54]. The QseD transcription factor is responsible for cross-talk between the signal cascades including both QseC and QseE histidine kinases. The understanding of molecular mechanisms underlying AI-3 action is promising for the development of efficient preparations for treating bacterial gastrointestinal infections [58].

Detection of the genes related to the QseC histidine kinase, which is the receptor for AI-3 and their exogenous analogues (adrenaline and norepinephrine), in the genomes of a number of bacterial species, not always inhabiting the vertebrate gastrointestinal tract, confirms wide distribution of AI-3-regulated signal systems. Apart from the already mentioned *Shigella* sp. and *Salmonella* sp., these species comprise *Erwinia carotovora, Haemophilus influenzae, Pasteurella multocida, Actinobacillus pleuropneumoniae, C. violaceium, Rubrivivax gelatinosus, Thiobacillus denitrificans, Ralstonia eutropha, R. metallidurans*, and *Psychrobacter* sp. This system is therefore important both for intra- and interspecific bacterial communication. High homology levels between the primary structures of QseC sensor domains of various bacterial species indicates pronounced conservatism of the structural and functional organization of AI-3 signal systems. Among eukaryotic organisms, only the fungus *Aspergillus nidulans* was found to possess a homologue of QseC histidine kinase; its functions, however, remain unclear.

Uniquely, the sensory domains of histidine kinases as well as the architecture and structural and functional organization of these enzymes have nothing in common with the adrenergic receptors of higher eukaryotes, which penetrate the cytoplasmic membrane seven times and are functionally coupled to heterotrimeric G proteins. However, the affinity of adrenaline and norepinephrine binding by bacterial histidine kinases is comparable to those for adrenergic receptors of vertebrates. The similarity between ligand-binding sites of bacterial histidine kinases and vertebrate adrenergic receptors is confirmed by blocking the AI-3-mediated activation of QseC histidine kinase with phentolamine (an α-antagonist) [55].

Thus, the host organism and the bacteria inhabiting it are in permanent interaction via signal exchange generated by adrenergic agonists and their bacterial analogues (AI-3). Stress and other factors resulting in increased levels of catecholamines affect bacteria in the gastrointestinal tract and possibly in other organs and tissues. AI-3 produced by bacteria may also exhibit a hormone-like action on the effector systems of host cells and influence the activity of other bacterial communities, thus changing the microflora in intestine and other organs. The application of the ligands of adrenergic receptors for treating various pathologies may have a direct effect on bacterial virulence and may provoke bacterial infections as a response to such therapy. The possibility for such side effects of adrenergic ligands was not even considered until recently.

## INDOLE DERIVATIVES

In *E. coli* populations, indole is one of the major extracellular signals; it is produced via tryptophan hydrolysis by tryptophanase TnaA [59, 60]. Indole regulates the expression of a number of *E. coli* genes responsible for survival and pathogenicity and decreases the capacity for biofilm formation [59–62]. The regulatory effect of indole on gene expression is probably due to its binding with the SdiA sensor protein, a homologue of the LuxR protein, which is known to be an AHL (AI-1) receptor. Although AHL produced by other bacteria bind with SdiA and use it for the regulation of a number of important processes in *E. coli*, including resistance to acidification [63, 64], the gene encoding AHL synthase and required for synthesis of endogenous AI-1 is not present in *E. coli* genome [65]. Indole therefore possibly functionally replaces AI-1 and controls the effect of AI-1 produced by other bacteria on *E. coli.* Indole suppresses biofilm formation in *E. coli* and thus acts as an antagonist to its AI-2 and AI-3, as well as to adrenaline and norepinephrine, which are related to bacterial AI-3; these compounds stimulate biofilm formation [57, 66]. Indole therefore controls the effect of both endogenous (AI-2 and AI-3, intraspecific communication) and exogenous (AI-1 and catecholamines, interspecific communication) signal molecules on *E. coli*; at the molecular level it is responsible for fine regulation of biofilm formation and other processes determining survival and pathogenicity of these bacteria [57, 60].

Indole affects biofilm formation in *P. aeruginosa* as well; however, unlike *E. coli*, their formation is enhanced 1.4- and 2.2-fold at 0.5 and 1.0 mM, respectively [60]. Expression of the gene encoding toluene-*ortho*-monooxygenase, which transforms indole into insoluble isoindigo, results in a 5.6-fold decrease in the biomass of *P. aeruginosa* biofilms. In contrast to *E. coli, P. aeruginosa* is capable of AI-1 synthesis and lacks the enzymes required for indole synthesis. In this

case, therefore, indole is an exogenous signal produced by other organisms, including bacteria. The genes encoding tryptophanase with a high primary structure homology with *E. coli* tryptophanase (30% similarity) were found in the genomes of at least 27 bacterial species. Some bacteria possibly use indole as an interspecific communication factor in order to control formation of biofilms and layers by other bacteria. Bacterial indole-binding proteins may possibly interact with such indole-containing compounds of higher eukaryotes as indolyl-3-acetic acid, serotonin, and melatonin; this may have a direct effect on bacterial survival and pathogenicity [60]. The neurohormones, melatonin and serotonin were shown to suppress *Chlamydia*-induced infections [67]. *Pseudomonas putida* is known to use the plant hormone indolyl-3-acetic acid as an energy source and to incorporate it into its metabolic pathways [68].

## QUINOLINE DERIVATIVES

In gram-negative *P. aeruginosa*, a family of AI was revealed, which are chemically quinoline derivatives [69–71]. Together with AI-1, they regulate the expression of the genes responsible for virulence and biofilm formation in this species; they therefore play an important part in the development of infections caused by *P. aeruginosa.* The quinoline derivatives produced by *P. aeruginosa* exhibit antibiotic activity against many species of gram-positive bacteria [70].

*P. aeruginosa* produces approximately 55 derivatives of quinoline and the related quinolinone [70, 72]; while the initial stages of biosynthesis are common for these derivatives, the terminal ones are different for each compound. For example, inhibition of the *pqsH* gene, which encodes the protein responsible for the terminal stage of the synthesis of one of these compounds, inhibits the production of this compound alone and has no effect on the synthesis of related derivatives [73]. Moreover, at least some quinoline derivatives are responsible for regulation and control of specific processes and are unique since they can not be replaced by related compounds. For example, the mutants with the functionally inactive *pqsH* gene, in spite of the presence of all the quinoline derivatives except the one synthesized via the PqsH protein, are unable to form membrane vesicles. Addition of the missing derivative results in the restoration of vesicle formation and in the normalization of vesicular transport in the cell [73]. Three quinoline derivatives are most common in *P. aeruginosa*, namely 2-heptyl-3,4-dihydroxyquinoline (PQS), 2-heptyl-4-hydroxyquinoine (HHQ), and its *N*-oxide (HQNO) (figure) [70]. Quinoline derivatives (HHQ) and the *pqsA-E* operon responsible for their synthesis were recently revealed in *Burkholderia pseudomallei, B. thailandensis, B. cenocepacia*, and *B. putida*, as well as in the *P. aeruginosa* strains pathogenic to humans [74]. This is an indication that quinoline derivatives like AI are not restricted to one organism.

Since, unlike AI-1, quinoline derivatives are packed into membrane vesicles prior to their secretion into the medium; they play the key role in the regulation of vesicle formation and transport. For example, in *P. aeruginosa* about 86% of all the quinoline derivatives are contained within extracellular vesicles; the vesicles are used to deliver them to target cells [73, 75]. Thus quinoline derivatives control their own synthesis and secretion by controlling the vesicle formation. Packing of quinoline derivatives into membrane vesicles has several goals. First, these hydrophobic molecules, easily reacting with hydrophobic surfaces, are thus protected from dispersion in the medium; their delivery to bacterial cells is therefore facilitated. Second, fusion of the vesicles with the cell membrane results in high concentrations of quinoline derivatives on the membrane, which is required to obtain an adequate cell response. Third, quinoline derivatives are thus protected from hydrolytic decomposition by prokaryotic and eukaryotic cells. Disruption of the AI package, which leads to their degradation, may be promising for treating *P. aeruginosa*-caused diseases.

The fact that both natural (dynorphin) and synthetic (U-50488) antagonists of κ-opioid receptors mimic the action of quinoline derivatives and increase *P. aeruginosa* virulence is unique; these compounds act via the signal cascade activated by PQS, HHQ, and HQNO [76]. Since dynorphin and other endogenous antagonists of opioid receptors are released from the mucous membranes of the mammalian gastrointestinal tract under stress, their activating effect on the QS signal system competent to quinoline derivatives enhances *P. aeruginosa* virulence; this results in suppression of nonpathogenic enterobacteria and colonization of the gastrointestinal tract by *P. aeruginosa.* Direct connection was thus revealed between stress situations and the development of bacterial infections. As was mentioned above, other vertebrate hormones (catecholamines, serotonin, and melatonin) also act similar to AI on contact with bacterial cells; production of these hormones depends on stress factors to a substantial degree.

## OSMOLYTES AND LOW-MOLECULAR IONS

Osmolytes, small organic and inorganic molecules, most of which stimulate the activity of receptor histidine kinases, constitute a big group of bacterial extracellular regulators. Unlike AI, osmolytes do not exhibit a highly specific effect. Molecular mechanisms of their regulatory effect on histidine kinase activity are still unclear. Activity of the *E. coli* EnvZ receptor histidine kinase was shown to depend on the osmolyte concentration in the medium. However, removal of the EnvZ sensory domain (located in the periplasm) had practically no effect on the regulation of histidine kinase activity by osmolytes [77]. This is possibly due to the fact that osmolytes carry out the regulation of the functional interaction of EnvZ histidine kinase with other sensor proteins. They may probably change the physic-

ochemical characteristics of the lipid phase of the membrane, resulting in the structural changes of the histidine kinase transmembrane channel and for the related catalytic domain of this enzyme. The possible involvement of the transmembrane channel in the regulation of the EnvZ histidine kinase activity by osmolytes is indirectly confirmed by the fact that activation of the *Saccharomyces cerevisiae* Sln1p histidine kinase by osmolytes involves its transmembrane parts [78].

Available data indicate that activity of some forms of such bacterial adenylate cyclases as *Spirulina platensis* CyaC, *Stigmatella aurantiaca* CyaB, as well as *Mycobacterium tuberculosis* Rv1319c and Rv3645c depends on bicarbonate anion concentration in the medium. Bicarbonate is believed to bind with the extracellular sensory sites of the enzymes and stimulate synthesis of the secondary intermediate, cAMP [79]. Hydrogen cations may also act as activators for bacterial AC; this was shown for *M. tuberculosis* AC Rv1264 [80]. Hydrogen cations change the charge of the side chains of lysine and aspartate residues forming the catalytic center of the enzyme; this results in stimulation of AC activity.

## PROSPECTS IN THE STUDY OF BACTERIAL SIGNAL MOLECULES

Development of a new generation of medical preparations, which are capable of highly selective inhibition of bacterial species and strains, is one of the promising fields in the investigation of bacterial signal molecules, primarily AI. Significant success has already been achieved in the creation of such preparations and development of approaches for treating bacterial infections which are based on the regulation and control of bacterial intercellular and interspecific communication at the molecular level.

A series of works dealt with the creation of synthetic AHL (AI-1) derivatives with antagonistic properties; these compounds can disrupt communication between gram-negative bacteria [33, 81–86]. The main directions in the creation of AI-1 antagonists are replacement or modification of the lactone ring, modification of the fatty acid radical, and its replacement by aryl radicals. Modification of the fatty acid radicals in AHL molecules results in disrupted intercellular communication in *Vigrio fischeri*, which relies on AI-1. Such modified AHL (2–10 μM) inhibit the transmission of the AI-1-generated signal by 50% or more [81]. AHL analogues with fatty acid radicals replaced by aryl radicals with various substitutes in the *meta-* and *para-* positions of the benzene ring (halogens, alkyl, phenol, hydroxy, amino, and nitro groups) [84, 85]. At 5 μM concentration they cause a 30–70% decrease in the intercellular communication of *V. fischeri, A. tumefaciens*, and *P. aeruginosa.* Small structural modifications result in significant changes in the properties of these analogues. For example, the analogue with a nitro group in the *para-* position of the benzene ring acts as an antagonist, while the analogue with a nitro group in the *meta-* position is a superagonist (its efficient concentration is ten times lower than for natural AI-1.

Bacterial AI-1 were also found capable of directly influencing the functional activity of mammalian cells. For example, the $C_{12}$-3-oxo-AHL from *P. aeruginosa* modulates the immune effect in mammalian cells infected with this organism. This AI promotes production of interleukine-8, cyclooxygenase 2, and prostaglandin E2 in human fibroblasts and intensifies apoptosis in mammalian macrophages and neutrophiles [87–89]. The $C_{12}$-3-oxo-AHL from *C. violaceum* stimulates production of anti-inflammatory cytokines in human and murine monocytes by activation of the NF-κB-dependent signal pathway [23].

The ideology of construction of AI-2 inhibitors is based on discovery of brominated furanones in the alga *D. pulchra*; these compounds prevent bacterial colonization of the alga by disrupting bacterial communication, which relies on both AI-1 and AI-2 [33, 90]. Numerous analogues of THMF (AI-2) and their natural brominated derivatives were subsequently developed; they proved highly efficient inhibitors of growth and biofilm formation by bacteria utilizing AI-2 for communication [91–93]. For example, (Z)-5-bromomethylene-2(5H)-furanone, the compound which can be easily and relatively cheaply synthesized, suppresses the biofilm formation and motility of staphylococci *S. epidermidis* and streptococci *Streptococcus anginosus, Str. intermedius*, and *Str. mutans*, the organisms causing dental caries, endocarditis, infections of the skin and gastrointestinal tract, as well as creating conditions for the development of cancer [93, 94]. Attachment of furanones to various carriers result in a material capable of preventing infections for a prolonged period; this was demonstrated using *S. epidermidis.* Biofilm formation by *S. epidermidis* on disks with furanones attached to polystyrene was inhibited by 89%. Furanones may control infections efficiently for long time periods, since, unlike most antibacterial preparations, they do not cause development of bacterial resistance. The latter is due to the fact that the natural and synthetic AI analogues target intercellular communication between bacteria, rather than bacterial cells as such [93]. The furanones of relatively simple structure, such as (Z)-5-bromomethylene-2(5H)-furanone, are not highly selective; they suppress development of various bacterial species which utilize AI-2 and sometimes AI-1 for intercellular and interspecies communication. They are therefore broad-spectrum preparations, while their analogues with more complex structure may exhibit high selectivity of action on specific bacterial species [93, 95].

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