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Review article

Mechanism of anchoring proteins on the cell envelope

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Abstract: *Surface proteins, essential structural components of bacterial cell wall, are synthesized as precursors equipped with specific functional domains. The N-terminal signal module enables translocation across the plasma membrane via Sec or Tat pathways, while sorting motif, located in the C-terminus, is responsible for protein attachment to the cell wall peptidoglycan. Only exception are lipoproteins which lipoylated cysteinyl residue connected with bacterial membrane is in N-terminal part of protein. Most of surface proteins, as surface (S-) layer proteins, internalins or autolysins, are linked to the different structures of cell wall through non covalent forces. From the other hand, molecules with LPXTG motif, which attachment involves sortase activity, are linked to the peptidoglycan by covalent bond. Due to structural, chemical and physicochemical properties, surface proteins are attractive components of diverse industrial or medical systems. Knowledge about mechanism of anchoring proteins to the cell envelope will open new possibility of their applications.*

Keywords: *surface proteins (S-layer proteins), LPXTG motif, sortase.*

Introduction

Cell walls, structural frames of prokaryotic organisms, are also mediate their interaction with the environment. Protection from chemical, thermal or mechanical agents, selective transport, providing supply of nutrients and inhibition of destructive macromolecules [1], moreover adhesion on diverse surfaces, including technical surfaces, artificial membrane interfaces [2] or host tissues involves external proteinaceous subunits bond with cell envelope [3]. Regular array of protein, termed S-layer, appreciated by Houwink in 1953 is widespread in domains of Archaea and Bacteria [4]. Particular units of lattice interact with each other as well as with cell wall components through non covalent forces, contrary, proteins with peculiar motif LPXTG which are typical among Gram-positive bacteria, are anchored via amide bond linking the Thr-residue to the peptidoglycan [5]. Motif, located on the C terminus, is recognized by enzyme sortase, called specific transpeptidase, associated with cell membrane.

Different system function in some *Streptococcus pneumoniae* proteins like LytA, which are attached to both teichoic (TA) and lipoteichoic acids (LTA)

through means of 20-amino acid repeats, whereas proteins of *Listeria monocytogenes* associate directly with LTA by stretch of 80 amino acid tandem repeats beginning with the dipeptide GW localized in the C terminus.

In case of membrane proteins relevant is presence of hydrophobic domain composed of about 20 amino acids followed by positively charged segment acting as stop-transfer signal, while lipoproteins detachment of signal peptide determines lipoylated cysteinyl residue which is subsequently connected with bacterial membrane [6,7].

Properties of surface layer proteins

Proteinaceous lattice, presently known as S-layer, was described first time by Houwink in 1953 as 'macromolecular monolayer' in the cell wall of *Spirillum* sp., surprisingly, term S-layer was introduced in 1987 during Second International Workshop on S-layers. Since then, S-layer have been detected in different species belonging to both earlier mentioned domains, analogous structures have been identified also on the surface of algae cell wall [4,6].

Main components of this crystalline arrays are single protein or glycoprotein species [8,9] with molecular weights of 40 kD to 200 kD specific to each microorganism, hence, they exhibit pores identical in size (2-8 nm) and morphology [2,9]. Layer of few species, such as *Bacillus anthracis*, *Clostridium difficile* or *Bacillus brevis* consist of two types of subunits, EA1 (extracellular antigen 1) typical to *B. anthracis* establish the main lattice, while Sap (surface layer protein) only support this construction [7]. There are also known microorganisms creating superimposed lattices composed with different subunit species [10].

In most cases S-layer is 5 to 25 nm thick and is composed of subunits aligned in lattices with oblique, square or hexagonal symmetry [9,10], its inner face is corrugated and generally net negatively charged, on the contrary to outer face, which is smooth and neutral at physiological pH [2]. Subunits are linked to each other and to the cell wall through non covalent forces, therefore can be isolated by addition of chelating agents (ethylenediaminetetraacetic acid, (EDTA)), chaotropic denaturants (guanidine hydrochloride and urea), or by changing pH values [4,8]. Elimination of denaturing factor provide their recrystallization into regular arrays in suspension [11] or technical surfaces like glass, quartz, Si, Pt and Au what is common feature of proteins isolated from *Bacillaceae*, moreover, it can be also achieved on artificial membrane interfaces like vesicles [2].

Analysis of amino acid protein structures has revealed general features in their composition and surprisingly low identities among proteins of different species or strains [9].

Typically protein contain 40–60 mol% hydrophobic residues and 25 mol% charged amino acids [12], is characterized by abundance of glutamic and aspartic acid residues, while principal basic amino acid is lysine, contrary to arginine, methionine and histidine which content is usually low as well as degree of sulfur-containing amino acids [2,4,7]. According to this analysis, the isoelectric points

(pI) of most proteinaceous subunits are weakly acidic [7,10], however some are basic e.g., those of *Methanothermus sociabilis*, *Methanothermus fervidus*, *Methanosarcina mazei*, *Methanobacterium thermoautotrophicum*, and *Lactobacilli* with pI between pH of 8.0 to 10.0 [7,12]. Measure of secondary structure indicate that 40% of the amino acids are organized as β-sheets 10-20% as α-helices [7,10].

Bacterial cells need to 5×10^5 proteinaceous units for covering their envelope, which require productive gene expression system, proteins synthesis, their translocation and incorporation into the existing lattice [7,11-14]. Hence, genes encoding S-layer proteins requires efficient promoter, like this identified in *Lactobacillus acidophilus* [7]. Moreover some genes are preceding by multiple promoters which lead to high level and stability of messenger ribonucleic acid (mRNA), also provide opportunity to regulate expression depending on physiological conditions [10,14]. Stabilization of S-layer mRNAs accomplish 5' untranslated regions (5'UTR) composed of 300 bp. Significant are also efficient transcription factors represented by AbcA protein of *Aeromonas salmonicida*, bifunctional proteins involved in LPS synthesis and transactivation of gene encoding vapA protein [14].

According to current data, S-layer proteins undergo several well-documented post-translational modifications such as, proteolytic cleavage of N- and C-terminal fragments, phosphorylation, sulphurylation, glycosylation, and lipid transfer [7,12,13]. The most numerous and diverse group are glycosylated S-layer proteins characterized in Gram-positive bacteria and Archeae, where glycosylation varies between 2% and 10% (m/m). In most cases glycan chains contain 20 to 50 identical units of pentoses, hexoses, heptoses, 6-deoxyhexoses and amino sugars [7,10]. Whereas in bacteria dominate O-glycosidic bond, in archeae O-coexist with N-glycosidic bonds in the same strain [12]. Degree of remaining modifications is rather low, phosphrylation is typical for only one protein, AhsA of *Aeromonas hydrophila*, although currently report suggest that it is also common attribute of other motile aeromonads [10]. Similar situation takes place for lipid modification of S-layer subunits which has been detected only in the halophilic archaea *Haloferax volcanii* and *Halobacterium halobium*. Significance of this processes result from atypical electrophoretic properties of modified proteins and confusion about molecular mass of the monomer during comparing results from SDS–PAGE and gene sequences e.g., purified S-layer glycoprotein from *M. fervidus* demonstrates two size conformations on SDS gels [12].

Secretion of the S-layer proteins and their attachment to the cell envelope

Basic structure initiates translocation of S-layer proteins through the plasma membrane is signal peptide (SP) consist of 20 to 30 amino acids, which is cleaved after protein secretion. This structure seems to be characteristic for mentioned group of molecules, so far only proteins of *Campylobacter foetus*, *Caulobacter crescentus* and *Serratia marcescens* have been deprived of SP.

Localization of SP in the N-terminal part of protein chain indicates that Sec II secretion pathway is used, whereas its presence on C-terminal prove existence of a type I secretion signal [7,10], in Gram-negative called uncleaved C-terminal secretion signal [10].

In some cases translocation is indepedent on the Sec apparatus and dependent on proton motive force and ATP, like in *Corynebacterium glutamicum*, or specific terminal branches e.g., in *A. hydrophila* and *A. salmonicida* [10].

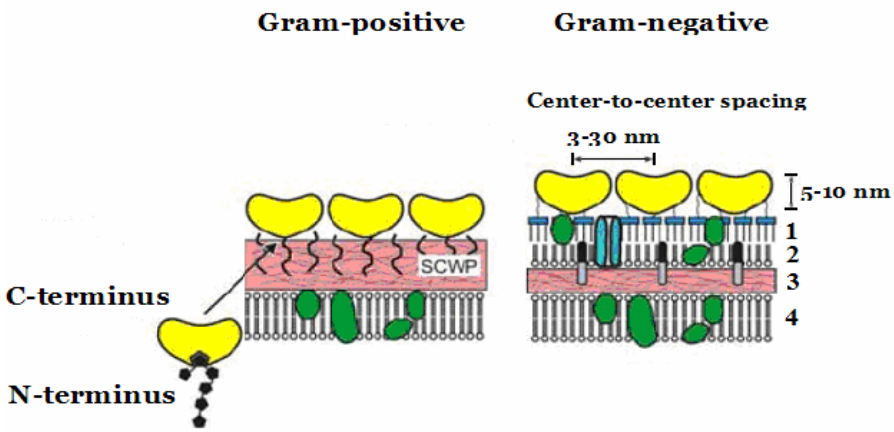

Figure 1. Attachment of S-layer proteins to the cell envelope of Gram-positive and Gram-negative bacteria. SCWP-secondary cell wall polymer; 1-lipopolysaccharide; 2-lipoprotein; 3-peptidoglycan; 4-lipid membrane

S-layer proteins are attached to the cell envelope components by non covalent forces, weaker than interactions between individual subunits and dependent on construction of the cell wall (Figure 1).

Cell envelope in Gam-positive bacteria is composed of peptidoglycan (PG) and secondary cell wall polymers (SCWPs): teichoic acids, teichuronic acids, lipoteichoic acids, or lipoglycans [7,10], which name is consequence of their role in cell wall function [15]. Their chains are linked to the peptydoglycan backbone via phosphodiester bond or attached to lipid anchor moiety [7].

In most cases S-layer proteins are anchored to the cell wall of Gram-positive bacteria through S-layer homology (SLH) domain located on the N or C-terminal part of subunits [12,16]. SLH motif originally identified by Lupas et al. [10] binds with SCWP, like in *Bacillaceae*, or with PG in the case of other bacteria [16]. So far, in most proteins three repeats of SLH domains are resent, each of them contains 50 to 70 amino acids with 10 to 15 conserved residues [12,16]. Sequence following specific motif is essential for the self-assembly process, as in genera *Bacillus* and *Geobacillus*, surprising is the fact that truncation about 200 amino acids at the C- terminus does not induce negative consequences [12]. SLH motifs are identified also in cell-associated exoenzymes, other than S-layer

proteins of Gram-positive bacteria and in outer membrane proteins of Gram-negative bacteria [10].

Proteins of lactobacilli, *C. glutamicum* and wild-type of *G. stearothermophilus* strains which does not possess SLH domains, are linked to the cell envelope in way drawing a comparision to aforementioned bond [10].

In some Gram-negative bacteria like *C. foetus*, *C. crescentus*, fragment responsible for attachment is located in the N-terminal region of S-layer, while in *A. hydrophila* in C-terminal part [10] and linking structure is lipopolysaccharide (LPS) consisting of a lipid A-moiety, core section and an O-polysaccharide region [6,10].

Functions and applications of S-layer proteins

S-layers, function as molecular sieves, provide adherence both bacterial cells (lactobacilli and *Bacillus cereus*) and exo-enzymes in *G. stearothermophilus* spp., also protect microorganisms such as *A. salmonicida*, *C. foetus*, *Aquaspirillum serpens*, and *C. crescentus* from attack by *Bdellovibrio bacteriovorus*, known to be popular bacterial parasite. S-layer can strengthen virulence of pathogens, for example only *B. cereus* cells with S-layer proteins adhere to matrix proteins and are resistant to polymorphonuclear leukocytes in the absence of opsonins [7,10,12].

Current knowledge about S-layer structure, genetics, chemistry and physicochemical properties suggests that this specific crystaline lattice has many applications in molecular nanotechnology, biotechnology, nanobiotechnology and biomimetics [10,13]. Broad spectrum of them is connected with ability of S-layer proteins to assemble into regular array both in suspension and on diverse surfaces (e.g., silicon wafers, metals, and polymers) and interfaces like lipid films and liposomes area after elimination of factor used for their isolation [7]. They have been used as matrices for immobilization of macromolecules such as antibodies, allergens oligosaccharides haptens, biochemically and biomedically functional proteins [10,13]. Moreover, S-layers can be applied as matrices for the development of dipstick-style immunoassays, templates for the formation of regularly arranged metallic nanoparticles [13] or as stabilizing structures for solid-supported lipid membranes [10]. Lattice structure, connected with it repeatability of physicochemical properties and presence of pores identical in size and morphology, enable exploatation of S-layer proteins from *Bacillaceae* as ultrafiltration membranes. Since pore areas of S-layer possess functional groups such as amino or hydroxyl groups, using repetitive modifications cause optimisation of filtration parameters and nonspecific adsorption properties [7]. Another direct application is use of S-layer reassembly products as a combined carrier-adjuvant system against infection, in the immunotherapy of cancer or in antiallergic immunotherapy. In addition, cells or their purified products can be used as attenuated vaccines [7].

Specially important are research on S-layer proteins of *G. stearothermophilus* and *B. cereus*, and their fusion with desired functional molecules. Forms of

G. stearothermophilus proteins SbsA and SbsB which are truncated and subsequently enriched in insertion of up to 500 amino acids without negative effects on their properties, subunits still have possibility to recrystallize into regular arrays [13]. Significant are also conception tested on *Lactobacillus* S-layer protein genes which assume using of promoters and/or signal sequence for both intra- and extracellular heterologous protein. Studies have revealed that promoters of *Lactobacillus brevis* ATCC 8287 and *L. acidophilus* ATCC 4356 function very well in intracellular protein as well as secretion signal of the slpA gene of *L. brevis* ATCC 8287. Bacteria from *Lactobacillus* genus are also topic for studies on adhesive properties of proteins, which has fundamental meaning for prevent the adhesion of pathogens [13].

Analysis of covalent attachment sortase-dependent surface proteins with LPXTG motif

Gram-positive bacteria possess several types of mechanism for anchoring proteins to the cell envelope, which are dependent on specific structural motifs in the sequence of the molecule [17,18]. Although, among them exists only one type of surface protein covalently linked to the cell wall, its prototype is protein A of *Staphylococcus aureus* [19]. Covalent character of this bond is connected with presence of motif LPXTG in C-terminal part of more than 60 proteins from various Gram-positive bacteria such as lactococci, enterococci and *Listeria* [18].

Attachment of aforementioned proteins is connected with activity of sortase, known as specific transpeptidase, which is found in almost all Gram-positive bacteria with high frequency [20]. Bioinformatic analysis performed by Konto-Ghiorghi et al. (2009) suggests existence of 4 classes of sortases called A, B, C and D. While class A sortase is responsible for binding of proteins with LPXTG motif, others are involved in such function as iron acquisition, pilus assembly and developmental processes including sporulation [21].

Sortase was described for the first time by Schneewind et al. during their studies on *S. aureus*, which revealed that sortase, called cystine protease, is responsible for covalent binding of protein A [19]. Enzyme represents group of functional proteins associated with membrane, what was corroborated by results of immunoblot analysis, moreover assumes type II membrane topology, where N terminus is located in the cytoplasm while C-terminal part located across plasma membrane [22].

According to current data, sortase A*S. aureus* consists of eight β-barel sectors, one or two helices and several loops, where strands 7 and 8 form a hydrophobic area with active site (Figure 2.). Characteristic feature of mentioned structure is presence of conserved Cys184 in β-7, His120 located within a helical region connecting β-2, β-3 and Asn98 at the C-terminal part of β-4. Surprisingly, they are positioned in a configuration analogous to that of the Cys25-His159-Asn175 triad typical to cysteine proteases in the papain family. Data suggest that Arg197

anchored in β-8 activate Cys184, exactly Arg197 enables thiol ionization during catalysis [22].

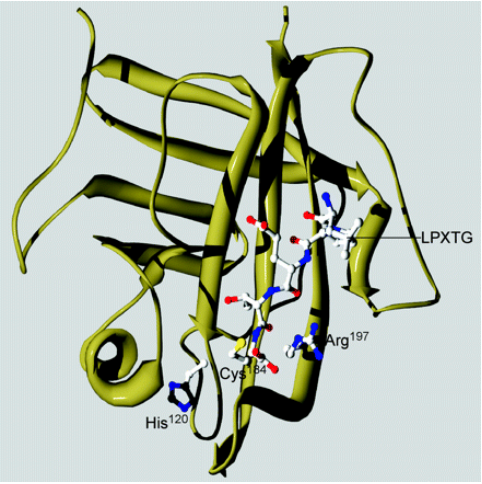

Figure 2. Structure of sortase A [22]. H120, C184, R197-residues conserved among sortases located in a configuration analogous to the triad characteristic of cysteine proteases; structure includes eight β-barel sectors, one or two helices and several loops, strands 7 and 8 create catalytic cleft with active residues

Substrate for sortase A from *S. aureus* is protein A used as model of anchoring proteins to the cell envelope [23]. Protein A, as the rest of surface proteins possess an N- terminal signal peptide (SP), member of the YSIRK-G/S family, which is required for the translocation through the Sec pathway and a C-terminal cell wall sorting signal [3,17,23], consisting the specific LPXTG motif, hydrophobic domain and positively charged tail [18,20,22]. Deletion experiments suggested that both the charged tail and specific motif are indispensables for complete cell wall anchoring [18], surprisingly, sorting signal from one species fulfil its role in another microorganism [22].

Synthesized in cytoplasm protein A precursor is cleaved between 36 and 37 residues of SP, what enables its translocation across the plasma membrane [3,19,24]. In consequence the N terminus of molecule is on the bacterial surface, while C-terminal part of protein is buried in the cell wall what provide protection from extracellular protease [22,25]. Specific LPXTG is now recognized by sortase, membrane-anchored transpeptidase with activated by Arg197 thiol residues of Cys184, which cleaves the peptide linkage between the threonine (T) and the glycine (G) generating acyl intermediate [3,20]. Afterwards, amino group of Gly 5 from the peptidoglycan biosynthesis precursor performs nucleophilic attack on product of reaction between enzyme and threonine [24], in consequence active site of sortase is regenerated and C-terminal threonine of the surface proteins is linked to the bacterial peptidoglycan (Figure 3) [19,25].

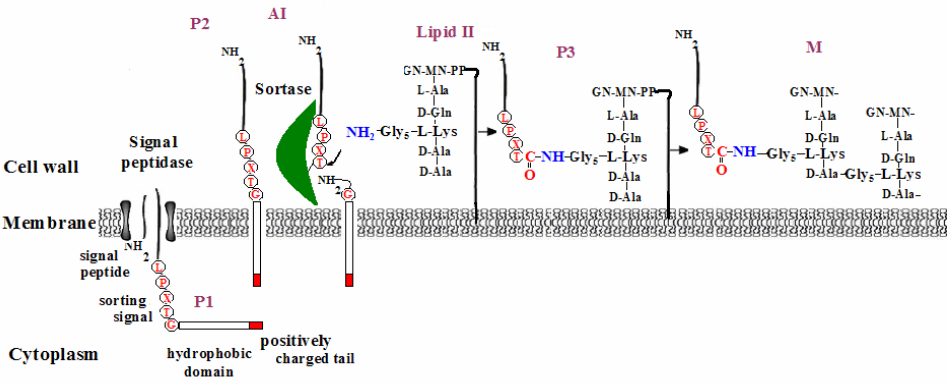

Figure 3. Model for cell wall sorting pathway of surface proteins with LPXTG motif in Gram-positive bacteria. P1-surface proteins precursor with N-terminal signal sequence and C-terminal sorting signal; P2-product of enzymatic reaction catalyzed by signal peptidase; AI-acyl intermediate generated by sortase; Lipid II-the peptidoglycan biosynthesis precursor; P3-substrate for penicillin binding protein sequently incorporated into the cell wall surface; M-mature anchored surface protein.

Other types of anchoring proteins on the cell wall of Gram-positive bacteria

Totally different system function in some *S. pneumoniae* proteins which are attached noncovalently to phosphorylcholine of both teicholoic and lipoteicholic acids [26]. The presence of this group was indicated recently on the surface of other respiratory tract patogens like *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus constellatus*, *Neisseria meningitidis* or *Pseudomonas aeruginosa* [27].

Choline-binding proteins are connected with the cell wall by highly conserved choline-binding module (CBM) consisting of 2 to 10 tandem repeats of a 20 amino acids identified in such exported proteins as toxins A and B of *C. difficile* [27-30]. While binding domain is localized in the C terminus of molecule followed by a flexible linker peptide built from a proline-rich segment, N-terminal part confer distinct function of protein [28,31]. So far, in pneumococci have been identified six surface proteins binding already mentioned phosphorycholine moiety through their CBM domain [27], however the best characterised is LytA, major autolysin of pneumococcus required for daughter cell separation and lysis in stationary phase and in the presence of penicillin [27,28,32]. The C-terminal domain of this molecule is equipped with seven repeats of CBM and consequently adopts a characteristic β-solenoid structure, with choline groups binding between β-hairpin ‘steps’ of the staircase-like structure [30].

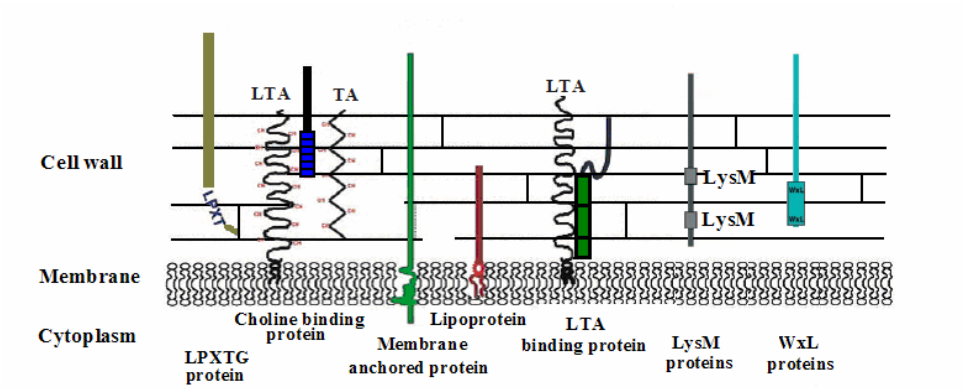

Figure 4. Types of anchoring surface proteins to the cell envelope in Gram-positive bacteria. LTA-lipoteichoic acids; TA-teichoic acids

Similarly, choline binding protein A identified in the genome of the TIGR4 strain has eight repeats of this motif [30]. Free choline is specific inhibitor of the activity of CBPs, moreover, addition of an excess of this compound to culture media prevents daughter cell separation.

According to current data both choline and its analogues act as inhibitors of the linkage to the cell envelope what give possibility to use them as potential drugs against *S. pneumoniae* [32].

Modules responsible for binding of the autolysin LytA and the toxin ToxB of *C. difficile* are related to GW domain of *Listeria* sp. The first characterized protein of this group is internalin B (InlB), which belongs to the internalin family including about nine members [33]. Module essential for infection of diverse mammalian cells creates 213 N-terminal amino acids, for which typical are tandem 22 amino acid Leucine Rich Repeats (LRR) [33,34,35]. The C-domain (231 amino acids) consists of three highly conserved modules of about 80 amino acids with the dipeptide Gly-Trp termed 'GW module' responsible for interaction with LTA on the bacterial cell envelope [36,37]. Moreover, the number of GW modules influence on strength of the attachment, thus autolysin Ami possessing eight modules binds more efficiently to the cell surface than InlB [36]. Recent research have revealed presence of Listeria-like GW modules in other Gram-positive proteins as: autolysin Alt (*S. aureus*), AltC (*Staphylococcus caprae*), AltE (*Staphylococcus epidermidis*) Aas (*Staphylococcus saprophyticus*) which have the same overall organization, it is equal with possessing two amidase domain connected by central GW module [36,37].

Interesting properties possess also P60 and P60-like proteins found in some Gram-positive bacteria like *Bacillus halodurans*, *Bacillus subtilis*, *Lactococcus lactis*, *S. aureus*, *Mycobacterium leprae* and *Mycobacterium tuberculosis*, which are predicted to be cell wall hydrolases. P60, a representative protein of surface protein group with molecular mass 60-kDa, is known as a molecule involved in

the invasion of mammalian cells by *L. monocytogenes*. Protein possess two LysM module, a bacterial Src homology 3 (SH3) domain and a C-terminal NLPC/P60 domain. The LysM domain, consisting of about 40 residues, what is typical for enzymes essential for degradation of the bacterial cell wall. It is supposed that LysM could have a general peptidoglycan-binding function. Bacterial SH3 domains, homologous to eukaryotic SH3 domain, are present in diverse bacterial species like *B. subtilis*, *Escherichia coli*, *Chlamydia trachomatis*, *Haemophilus influenzae*, *Helicobacter pylori*, *S. aureus*, *Streptococcus pyogenes* and *Synechocystis* PCC6803. Although function of this domain is unknown, it is predicted that *Staphylococcus simultans* lysostaphin is attached with cell wall by mentioned motif. The latter, NLPC/60 domain counting 100-110 amino acids, was first revealed in the *E. coli* putative lipoprotein precursor, NlpC, and in Listeria P60 it has also been found in many other prokaryotic surface proteins and lipoproteins. Function of NPLC/60 like SH3 is still unknown [37].

Another type of surface protein family, Csc, creates molecules with WxL domain which contains two conserved motifs with Trp-x-Leu sequence. WxL proteins was identified in many low-GC Gram-positive bacteria, recently presence of Csc gene clusters have been discovered in genome of *Lactobacillus plantarum* and *Enterococcus faecalis*. Csc encode CscA protein with a conserved DUF916 domain, which function is still unknown and C-terminal transmembrane anchor, CscB and CscC that display the WxL domain located on C terminus that is responsible for with interactions with peptidoglycan as in EF2686 protein of *E. faecalis* and CscD a small LPXTG protein [36].

Surface proteins containing a signal peptide can be bound in the membrane by hydrophobic domain located on the N or C terminus [36]

Group with a carboxyl-terminal module of 22 hydrophobic amino acids followed by positively charged tail used as stop-transfer signal, represents the protein ActA of *L. monocytogenes* that is responsible for the bacterial motility in the cytosol of eukaryotic cells [38]. ActA is a 639 amino acids protein exposed on the bacterial cell envelope in a polarized fashion [39] and can be removed with SDS under condition which do not cause lysis of the bacterial cells [40]. This protein has typical modular organization, while the C-terminal region links ActA to the cell surface, the N domain (234 amino acids) and four proline rich repeats are involved in actin polymeryzation and bacterial movement [40,41]. Each repeat consists proline surrounded by four acidic amino acids and is separated from next by one of three sequences termed the 'long repeats'. The first two of them are 24 amino acids in length, while the third is composed of 33 amino acids with no sequence similarity to the two long repeats [38].

Surface proteins can also be anchored to the cell membrane by the N-tereminal hydrophobic domain, that can be the signal peptide (SP) itself if it remains uncleaved, this group include sortases SrtA, SrtB and proteins involved in protein binding or in the cell wall synthesis like penicilin binding proteins (PBPs) [36].

Opposite to above-mentioned molecules, lipoproteins in Gram-positive organisms are attached to the cytoplasmic membrane by lipoyed N terminus, whereas C-terminal part fulfils functions similar to periplasmic proteins in Gram-negative bacteria [42].

Lipidation is directed by presence a specific signal peptide [SP] sequence characterized as a lipobox [36], which is shorter than classical SP, has more hydrophobic amino acids in central region and is followed by a cysteine residue [37]. According to primary reports based on studies in *E. coli*, lipoprotein precursors (prolipoprotein) are synthesized with SP that initiate translocation across the cytoplasmic membrane via Sec pathway [43], it is also known that some molecules typical for Gram-positive bacteria are transported across the SecA2- dependent accessory Sec pathway. Interestingly, recently discovered Tat (twin arginine protein transport) system transports folded and oligomeric proteins, might be used in export lipoproteins in a fully folded state, what have been confirmed by analysis of the dimethylsulphoxide (Dms) reductase in *Shewanella oneidensis* [43].

The pathway for lipoprotein biosynthesis preparated by Wu and co-workers during research on *E. coli* takes into consideration two steps necessary for maturation of molecule [43]. Prolipoprotein is translocated across the cytoplasmic membrane via Sec or Tat pathway, subsequently, the prolipoprotein diacylglycerol transferase (Lgt) catalyzes the displacement of a diacylglycerol residue from phosphatidylglycerol from the membrane to the SH-group of the cysteine present in lipobox sequence, which then becomes the amino terminus of the lipoprotein. The synthesis precursor is cleaved by specific peptidase, the prolipoprotein signal peptidase or signal peptidase II (Lsp), what enable to release mature protein, which is then anchored to the membrane long-chain fatty acids, with the protein part exposed at the cell envelope [36,37].

Conclusions

Surface proteins create a diverse group of molecules connected with the cell wall surface which determining its interaction with the environment including selective transport providing the exchange of nutrients and waste products as well as the resistance to external destructive factors. Moreover, they are involved in such cellular processes as cell wall turnover, motility, proteins secretion and their binding or pathogenicity.

Molecules can be linked both to the components of bacterial cell wall (teichoic or lipoteichoic acids) and cell membrane through covalent or non covalent binding. Proteins attached by amide bond, molecules with specific motif LPXTG located on the C terminus, are recognized by membrane enzyme sortase which cleaves the peptide linkage between the threonine (T) and the glycine (G), while non covalent interactions are connected with presence in proteins structure repeated domains like GW or LysM module. Especially important are genetics, chemistry and physicochemical properties of S-layer proteins, proteinaceous lattice widespread in domains Archaea and Bacteria, which as well as unusual

strength of T-PG (peptidoglycan) linkage has diverse applications in nanotechnology, biotechnology, nanobiotechnology and many other scientific areas. Due to their ability to assemble into regular arrays in suspension, on diverse surfaces (e.g., silicon wafers, metals, and polymers) or liquid-surface interfaces S-layer proteins have been used as isoporus ultrafiltration membranes and matrices for immobilization of macromolecules such as antibodies, allergens oligosaccharides haptens, biochemically and biomedically functional proteins.

Among mentioned properties the most interesting is extremely productive gene expression system, proteins synthesis and connected with it high level of translocation and incorporation S-layer subunits into the existing lattice what allows for synthesis, translocation and incorporation approximately 500 S-layer proteins per second (bacteria with a generation time of 20 min). Surprisingly, this unusual system can be fused with desired functional properties of other molecules increasing its productivity, as indicate promising research on S-layer proteins of *G. stearothermophilus* and *B. cereus*.

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