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Surface Proteins of *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a commensal bacterium that causes infections such as sepsis, endocarditis, and pneumonia. *S. aureus* can express a variety of virulence factors, including surface proteins. Surface proteins are characterized by presence of a Sec-dependent signal sequence at the amino terminal, and the sorting signal domain. Surface proteins are covalently attached to peptidoglycan and they are commonly known as cell wall-anchored (CWA) proteins. CWA proteins have many functions and participate in the pathogenesis of *S. aureus*. Furthermore, these proteins have been proposed as therapeutic targets for the generation of vaccines. In this chapter, different topics related to CWA proteins of *S. aureus* are addressed. The molecular structure of CWA proteins and their role as virulence factors of *S. aureus* are described. Furthermore, the involvement of CWA proteins in the processes of adhesion, invasion of host cells and tissues, evasion of the immune response, and the formation of biofilm is discussed. In addition, the role of CWA proteins in skin infection and the proposal to use them as potential vaccine antigens are described. The information contained in this chapter will help the readers to understand the biology of CWA proteins and to recognize the importance of surface molecules of *S. aureus*.

Keywords: *Staphylococcus aureus*, CWA proteins, surface proteins, vaccines, skin

1. Introduction

Staphylococcus aureus has been widely studied due to their ability to cause infections such as sepsis, endocarditis, and pneumonia. Therefore, it is relevant to find new therapeutic targets against this bacterium; since the treatments with common antibiotics are seldom effective due to the acquisition for multidrug resistance, such as methicillin-resistant *S. aureus* (MRSA)

strains [1, 2]. *S. aureus* expresses a variety of virulence factors and some of them are found in the bacterial surface (surface proteins). The surface proteins of *S. aureus* are covalently attached to the peptidoglycan, and for that reason, they are named as cell wall-anchored (CWA) proteins. Within the genus *Staphylococcus*, *S. aureus* has an average of 24 different CWA proteins, whereas *Staphylococcus epidermidis* and *Staphylococcus lugdenensis* have less CWA proteins. Not all strains of *S. aureus* have the same 24 CWA proteins on their surfaces, there are differences according with their genotypes. With regard to the expression of CWA proteins, it is dependent on the bacterial growth phase and growth conditions; for example, some proteins are expressed only under limited iron concentration [3, 4], while others are expressed predominantly in the exponential phase [5], or in the stationary phase of bacterial growth [6]. The CWA proteins are classified into four families according to a structure-function analysis and to their different motifs. Currently, the CWA proteins have taken great interest due to their multiple functions during the pathogenesis of *S. aureus*.

2. Structure of CWA proteins

All CWA proteins contain a Sec-dependent signal sequence at the amino terminal and at the carboxyl terminal a sorting signal and a hydrophobic domain (wall-spanning W). The sorting signal domain contains the characteristic motif for breaking by the sortase LPXTG (Leu-Pro-X-Thr-Gly; wherein X represents any amino acid). The hydrophobic domain retains the protein in the bacterial membrane during secretion, so that the sortase can join and carry out its transpeptidase function. Between the amino and carboxyl terminal domains, there are different regions or functional domains. Based on its molecular structure and arrangement, the CWA proteins of *S. aureus* have been classified into four families: the MSCRAMM family, the NEAT motif family, the three-helical bundle family, and the G5-E repeat family (**Table 1**).

2.1. The MSCRAMM family

The main feature of this family of proteins is its structural similarity and its mechanism for binding the ligand. The general structure of these proteins is a domain A at the amino terminal and a region R. The A domain is divided into subdomains: N1, N2, and N3, which integrate the ligand-binding domain. The N2 and N3 subdomains form folding structures IgG-like [7] that are important to form the ligand-binding site. With respect to ligand-binding mechanism of these proteins, they bind to fibrinogen through the mechanism “dock, lock, and latch” (DLLs) by N2 and N3 subdomains. The DLL mechanism occurs when the ligand dock to the open apo form and conformation changes create a closed form, in which the ligands are locked into a place [8]. Clumping factor A (ClfA) and ClfB proteins of *S. aureus* and serine-aspartate repeat-containing protein G (SdrG) of *S. epidermidis* are representative proteins of this family.

The R region of the Sdr- and Clf proteins is composed by repeated Ser-As, known as the SD region. However in the fibronectin-binding protein A (FnBPA) or (FnBPB), the R region contains repeated fibronectin-binding, which have the function of mediating ligand binding. The SdrC, SdrD, SdrE, and bone sialo-binding protein (BBP) proteins, which are MSCRAMM of *S. aureus*, have two or more repeated subdomains named as B_{SDR} ranging from 110 to 113 amino

Protein family	Structural motifs and domains*	Proteins	Function during infection
(1) MSCRAMM Clf-Sdr	N-terminal A region (comprises subdomains N1, N2, N3); B _{SDR} repeats (in SdrC, SdrD and SdrE); R region (known as SD region, contains serine-aspartate repeats)	ClfA ^a	Adhesion to fibrinogen; degradation of C3b. Immune evasion
		ClfB	Fibrinogen, keratin and loricrin binding. Nasal colonization by adhesion to desquamated epithelial cells
		SdrC	β-Neurexin binding. Adhesion to desquamated nasal epithelial cells
		SdrD	Adhesion to desquamated nasal epithelial cells
FnBp	A region (subdomains N1, N2, N3); R region (contains fibronectin-binding repeats)	FnBpA	Fibrinogen, fibronectin and elastin binding. Adhesion to extracellular matrix; cell host invasion.
		FnBpB	Fibronectin-binding. Adhesion to extracellular matrix; cell host invasion
Cna	A region (subdomains N1, N2, N3); B _{CNA} repeats	Cna	Collagen binding. Adhesion to extracellular matrix
(2) NEAT	Near iron transporter motif; C-terminal hydrophilic stretch (in IsdA)	IsdA	Heme, fibrinogen, fibronectin, cytokeratin and loricrin binding. Heme capture and iron uptake; nasal colonization
		IsdB ^a	Heme, hemoglobin and 3β integrins binding. Heme capture and iron uptake; invasion of non-phagocytic cells
		IsdH	Heme, hemoglobin binding. Heme capture and iron acquisition; immune evasion by C3b degradation
(3) Three helical bundle	Tandemly linked triple-helical bundle domains (known as EABCD); repeat-containing Xr region; nonrepetitive Xc region	Protein A	IgG, IgM and TNRF1 binding. Evasion of immunity; increased inflammation during pneumonia and skin infection
(4) G5-E	A region; alternating repeats of G5 and E domains	SasG	Adhesion to desquamated epithelial cells; formation of biofilm

*All CWA proteins share an N-terminal signal sequence and a wall-spanning region and sorting signal at the C-terminal region.
^aAntigens as potential vaccines.

Table 1. Structure-function of CWA proteins.

acids and that are located between the region A and the SD region. The repeated B_{SDR} are folded separately and form a rigid bar and it is Ca²⁺ dependent for structural integrity [9, 10].

An atypical MSCRAMM protein is collagen adhesin (Cna). This protein binds to collagen, also has a domain A in its N-terminal and it is divided into three subdomains N1, N2, and N3. The Cna differs from the other members MSCRAMMs because its ligand-binding domain (IgG-folded) is composed of the N1 and N2 subdomains, and not composed of the N2 and N3

subdomains as other MSCRAMMs typical. Furthermore, the space between domain A and the cell wall-spanning W domain consists of a variable number of repeated B_{CNA} domains, which are different from B_{SDR} subdomains. Another difference is that the Cna has a different ligand-binding mechanism named as collagen hug.

2.2. NEAT motif family

The main feature of this family is the presence of near iron transporter (NEAT) motifs, which recognize and bind to heme or hemoglobin. Proteins iron-regulated surface (Isd) A, B, and H contain NEAT motif (one NEAT motif for IsdA, two NEAT motif for IsdB, and three NEAT motif for IsdH) and these proteins are involved in the capture of heme from the hemoglobin. Isd is important for the survival of the bacterium into the host, where the iron is limited. Besides, Isd is involved actively in the metabolism of heme. Heme binds to Isd, and then heme binds to a membrane transporter protein, which transfers heme into the cytoplasm. In the cytoplasm, the iron is released from heme by hemoxygenases [4, 11]. The structure of the NEAT domain has been elucidated and the molecular mechanism of ligand-binding was determined [12]. Other Isd proteins can bind different ligands to the heme group, as the case IsdA that binds to fibrinogen, fibronectin, cytokeratin 10, and loricrin; and IsdB that binds to 3β integrins.

2.3. Three-helical bundle family

The main feature of this family is the presence of several single separately-folded three-helical bundles. Protein A of *S. aureus* is the common prototype of this family. Protein A has five homologous modules in its amino terminal, known as EABCD (each module has a folding three-helical bundles) which can bind to different ligands; then there is a Xr region composed by repeated octapeptides, which are highly variable number, and finally in the constant Xc region [13, 14]. Protein A is a multifunctional CWA protein ubiquitous in *S. aureus* and frequently it is used to subtype strains, based on the variation of the DNA sequence-encoding Xr.

Other proteins of *S. aureus* containing three-helix bundles are: the binder protein IgG (Sbi) with four three-helix bundles which is not covalently linked to the cell wall [15, 16], and the proteins that have a single three-helix bundle, the staphylococcal complement inhibitor (SCIN) and the extracellular fibrinogen binding protein (Efb) [17], which are involved in the immune evasion.

2.4. The G5-E repeat family

The basic structure of this family is G5-E repeat domain. Each domain G5 has five conserved glycine residues, which adopt a folding of β -triple helix- β -like structure. Currently, it is unknown whether this domain is involved in the ligand-binding function. The region E is known as the spacer region and is composed of a sequence of 50 amino acid residues [18, 19]. The domain G5 and the region E form the structure of this family. The G5-E unit is repeated in a tandem arrangement. In addition, proteins of this family have a domain A in the amino terminal. The surface protein G (SasG) of *S. aureus* and the accumulation-associated protein (Aap) of *S. epidermidis* are closely homologous and are members of this family; both proteins

are involved in biofilm formation. The G5-E repeated of Aap and SasG are exposed on the surface of the bacterium. For both proteins become functional that must be processed; in the case of Aap, the domain A of the amino terminal is removed by proteolytic processing and in the case of SasG occurs by limited breaking within G5-E domains [20].

2.5. Other CWA protein families

There are other CWA proteins with different functional domains such as the legume lectin and the nucleotidase. These two groups of CWA proteins are classified outside the four families mentioned above, because they are not exclusive of *S. aureus*. CWA proteins with a legume lectin domain are represented by the serine-rich adhesion of platelet SraP [21]. SraP is composed of a BR region and a short serine-rich region (SSR1). The BR region is formed of three different structural domains: the legume lectin-like, the β -grasp fold (β -GF) and the cadherin-like (CHLD). It has been observed that the function of the BR region is to recognize Neu5Ac-containing glycoproteins of mammalian cells; such as the salivary glycoprotein gp340 [22]. In addition, the SraP is involved in bacterial adhesion and the invasion of mammalian cells. On the other hand, the nucleotidase domain has been identified in CWA protein SasH of *S. aureus*. The nucleotidase motif is enzymatically active and contributes to evade the host immune response [23, 24]. It has been shown that when *S. aureus* is phagocytosed, the SasH (also named as synthase adenosine, AdsA) dephosphorylates intracellular ATP to adenosine, where the adenosine is immunoregulatory because the adenosine inhibits the oxidative burst and promotes the survival of *S. aureus* within neutrophil [25, 26].

3. Posttranslational modifications of CWA proteins

The MSCRAMMs proteins achieve proteolytic posttranslational modification in the domain A. Proteases that remove subdomain N1 of MSCRAMMs are located on the bacterial cell surface. Proteolytic processing is conducted by a staphylococcal protease, called aureolysin, which cleaves between the subdomains N1 and N2 of ClfB and ClfA. For FnBPA, there is not a staphylococcal protease, the responsible of this processing is the thrombin of the host. Removal of N1 of ClfB can decrease the length of the protein and cause lack of binding fibrinogen [5]. It is thought that the elimination of N1 subdomain reduces the ability of *S. aureus* to adhere to fibrinogen, loricrin, and cytokeratin 10. The biological importance of the elimination of the subdomain N1 of ClfA and FnBPA is unclear, since experiments suggest no reducing biofilm formation by FnBPA; or no decrease in adhesion to fibrinogen by ClfA and FnBPA processed proteolytically [27, 28]. However, it has been reported that under certain conditions, FnBPs are degraded by the *S. aureus* V8 protease, reducing the ability of the bacterium to adhere to fibronectin [29].

Another posttranslational modification is the glycosylation of proteins Clf-Sdr. It has been shown that the glycosyltransferases SdgB and SdgA of *S. aureus* are responsible to modify the SD region of the Clf-Sdr family. This modification involves adding N-acetylglucosamine residues in the region SD protecting it from the degradation by neutrophil serine protease, cathepsin G [30]. Thus, the glycosylation of repeated SD is crucial for functional maintenance of MSCRAMMs on the surface of *S. aureus*.

4. CWA proteins as virulence factors

The generation of mutants is a useful tool to know the function of a gene; however, the study of CWA proteins has been complicated because the generation of defective mutants of CWA protein had generated, in some cases, unexpected results due to functional redundancy. For example, *S. aureus* expresses some CWA proteins that bind to fibrinogen, and most of the strains can produce two proteins with the same function; in consequence, a mutant protein could be replaced by a protein with the correct function. Another difficulty that occurs in the study of CWA proteins is to obtain mutants in the isolates of clinical relevance, since the studies have been conducted with laboratory strains; such as *S. aureus* Newman and derivatives of NCTC8325 strains. Finally, there is the problem of species because some CWA proteins have a specific function in mice but in humans have different functions or behavior [31].

Despite the difficulties mentioned above, the role of CWA proteins in virulence has been studied. Human population (20%) is permanently colonized by *S. aureus* in the nasal cavity [32], where the bacterium is able to grow exponentially and to express high levels of mRNA encoding CWA proteins [33]. ClfB [34] and IsdA [35] contribute in the nasal colonization in rodents, and ClfB also in humans [36]. ClfB is capable of binding to keratin 10 of mouse and human [37]. Keratin 10 is the largest component of squamous cells. ClfB also binds to the loricrin protein [38]. Some other CWA proteins (such as SdrC, SdrD, SasG, and SasX) can promote adhesion to squamous cells but the ligand or ligands involved are not known [39–42].

4.1. CWA proteins in the invasion of epithelial and endothelial cells

Recently, *S. aureus* was recognized as an intracellular pathogen and its ability to survive inside neutrophils. *S. aureus* can be taken directly by nonphagocytic cells and host cells; subsequently, it can cause damage to the above mentioned cells by the production of cytotoxins. In addition, intracellular bacterium is protected against the attack by the host because *S. aureus* acquires a state of semidormancy known as small colony variants, which yields intrinsic resistance to antibiotic therapy [43].

In the case of FnBPA and FnBPB proteins, the binding of these proteins to fibronectin facilitates *S. aureus* internalization [44–46]. Fibronectin is composed of three different types of structural modules, called 1, 2, and 3, of which the modules type 1 contain two β sheets involved in interactions with the binding domains of FnBPs [47]. In addition, an arginine-glycine-aspartate sequence of one of the modules type 3 of fibronectin is recognized by integrins. Particularly, the interactions of fibronectin with FnBPs and integrin $\alpha 5\beta 1$ initiate the activation of a signaling cascade that triggers a cytoskeletal rearrangement in the host cell, which causes endocytosis of *S. aureus* [48].

4.2. Immune system and inflammation

The CWA proteins are involved in immune evasion. Protein A binds to the Fc region of IgG, this binding leads to an incorrect orientation of IgG antibody, preventing the recognition of the bacterium by neutrophils and the activation of the classical complement pathway [49].

Furthermore, it has been demonstrated that in pulmonary epithelial cells, protein A is capable of interacting with tumor necrosis factor receptor 1 (TNFR1), triggering the production of interleukin-8 (IL-8) and the neutrophil recruitment, promoting inflammation and tissue damage [50]. Also it has been reported the involvement of protein A in the production of interferon β (IFN β) and IL-6 in a mouse pneumonia model [51].

ClfA and Can are involved in evading the immune system by recruiting regulators of complement pathway [52]. Furthermore, ClfA is involved in bacterial survival by binding to fibrinogen, because in a sepsis model this interaction reduces the probability of *S. aureus* to be eliminated by neutrophils [53]. It has also demonstrated the importance of modifying ClfA by glycosyltransferases, which add N-acetylglucosamine to the SD region, thereby preventing the proteolysis by cathepsin-B from human neutrophils [30].

4.3. Biofilms

One of the major virulence factors of *S. aureus* is its ability to form biofilms on implanted medical devices, which favors resistance to antibiotics, survival, and dissemination [31]. In the formation of biofilms, a polysaccharide matrix is involved, particularly the molecule poly-N-acetylglucosamine (PNAG) or also called adhesin intercellular polysaccharide (PIA), whose production depends on the proteins encoded by the operon *icaADBC* (intracellular adhesion) [54, 55]. Furthermore, the CWA proteins of the cell-wall are also involved in biofilm formation, such as Bap, ClfB, FnBPs, SasC, SasG, and protein A [31]. It has been shown that Bap and SasC are involved in adhesion of *S. aureus* to polystyrene surfaces and the bacterial accumulation in biofilm formation [56, 57]. In the case of FnBPs, it has been proposed that N2 and N3 subdomains of the domain A are required to promote the bacterial accumulation in biofilm formation [27]. The mechanism of biofilm formation involving SasG consists of an array of loop structures, which are capable of interacting with other SasG located on the surface of another bacterium, thus allowing the accumulation of *S. aureus* [18].

5. Involvement of CWA proteins in skin infections

The study of the participation of CWA proteins in skin infections and abscess formation has been achieved mainly in animal models with CWA protein mutant strains of *S. aureus* (Table 2). *S. aureus* strains deficient in sortase proteins, which lost all CWA proteins, are unable to form abscess in mice [58, 59]. Mice inoculated with *S. aureus* strain Newman, deficient in ClfA, showed a lower bacterial load in skin abscesses compared with the wild-type strain [60]. The ClfA protects the bacterium from phagocytosis by neutrophils because ClfA recruits fibrinogen to the surface of the bacterium, thereby preventing the opsonization and recognition by receptors of phagocytic cells.

In a murine skin abscess model, infected with *S. aureus* LS-1 strain, mutant in FnBPA and FnBPB, the bacterial load decreased [60]. FnBPs is also able to adhere and invade the skin keratinocytes [61], thus contributing to the development of skin infection. SasX contributes to skin infection, which was demonstrated in a murine skin infection model in challenge with a SasX-deficient

Infection model	Mutant CWA in <i>S. aureus</i>	Result
Murine kidney abscess	Sortase	No abscess formation in the kidneys
Murine skin infection	ClfA	Decreased CFU in the skin abscess
Murine skin infection	FnBPA and FnBPB	Decreased CFU in the skin abscess
Murine skin infection	SasX	Smaller abscesses in the skin
Mice inoculated subcutaneously	Protein A	Decreased CFU in the skin abscesses
Rabbit skin infection	Wild-type	High transcription level of the <i>isdB</i> gene in abscesses

Table 2. CWA protein infection models.

strain of *S. aureus*, who produced smaller abscesses compared with those infected with the wild-type strain. In mice inoculated subcutaneously with the *S. aureus* Newman mutant strain, protein A-deficient, the bacterial load on the skin abscesses was significantly lower than abscesses infected the wild-type strain. The role of protein A during skin infection by *S. aureus* is probably by evading the immune response, since protein A binds to IgG and decrements B cells, prolonging the time of bacterial binding with the ligand of the skin. Protein A leads a proinflammatory response in the skin, because the protein A binds to TNFR1 of human keratinocytes and upregulates the expression of COX-2 and IL-8, driving shot downstream of the kinases, which results in the activation of NFκB and AP-1 [62]. IsdA has a function of resistance against the mechanisms of human innate immune defense and its presence on the surface of *S. aureus* causes that the bacterium to be more hydrophilic and is negatively charged [63, 64]. In a rabbit skin infection model, transcription levels of *isdB* were increased 24 h post infection [65], suggesting that it may have a role during infection of the skin. The Isd proteins bind to the ligands on skin cells, and it is probably Isd that is involved in the skin infection.

6. CWA proteins as vaccines

Currently, there is a proposal to use recombinant CWA proteins as potential vaccine antigens. In animal models, the use of CWA proteins has induced immunological protection against *S. aureus* through the production of anti-*S. aureus* antibody [66–68]. However, it has been documented that IsdB vaccine produced immunity in animals, but not in patients with severe infections after cardiothoracic surgery (phase III test) [69, 70]. A strategy to increase vaccine efficacy is to develop multiple vaccines, a vaccine containing four antigens of CWA proteins resulted in greater protection in mouse [68] compared to a single antigen vaccine. Furthermore, the stimulation of humoral immunity is insufficient for protection in humans; subsequently adjuvants that trigger an immune response mediated by T helper 1 (Th1) and/or T helper 17 (Th17) cells, as well as recruit of neutrophils by IL-17 and IFNγ would be important.

6.1. CWA proteins such as T-cell antigen

Up to date, the mechanism of immune system activation by CWA proteins is unknown except for the protein A that binds to TNFR1 and induces the production of interleukin-8 (IL-8)

and the neutrophil recruitment [50]. The anti-*S. aureus* vaccines are capable for activating the effector T-cell subsets [71, 72]; however, the epitopes of *S. aureus* that recognize T-cells are unknown. T-cells not activated (virgin) increase their cellular proliferation and production of cytokines (phenotype of activated T-cells) when they are stimulated with extracellular proteins of *S. aureus*, but not when they are stimulated with intracellular proteins of *S. aureus* [73]. Stimulation of virgin T-cell with membrane proteins from *S. aureus* produces a high activation of T-cells, the same takes place when they are stimulated with ClfA [74]. ClfA triggers immunity antibody-mediated in a murine model of *S. aureus* arthritis [67]. On the other hand, ClfA nanoparticle applied to the nasal cavity of mice results in a significant protection against systemic infection by *S. aureus*, and an increase cellular immune response Th1 and Th17 [68]. Immune cellular response type Th17 has an important function in systemic protection against *S. aureus*, because knockout mice to IL-17 cytokine are not immunized with ClfA [75]. These studies suggest the potential role of ClfA as the major antigen to activate T-cells.

Currently, ClfA protein is used in multivalent vaccines. Thus, the vaccine designed by Pfizer, with the status of Phase II clinical trials, is made with ClfA antigens, capsular polysaccharide MNTC, and two proteins (CP5 and CP8) [76, 77]. This vaccine induces a high production of antibodies; however, there are no studies on its cellular immunity. NovaDigm I developed a vaccine with homologues of ClfA and Als3p [78]; in phase I clinical test, the vaccine showed an increase in the production of specific antibody titer and induced Th1 and Th17 cell response in humans [77]. ClfA is emerging as a potent stimulator of T-cells and it is a promising antigen vaccine development; however, there is little research on the potential of other CWA proteins to activate T-cells. Therefore, studies to determine which CWA proteins cause a high T-cell response should be performed to identify potential proteins for future vaccines.

7. Conclusions

Although it has recognized the role and ligands for some CWA proteins of *S. aureus*, there are other CWA proteins whose function in humans is unknown. The structural analysis of CWA proteins is a powerful tool to determine their role in *S. aureus*; however, the use of molecular techniques and animal models are essential for discovering new ligands and/or functions of the CWA proteins, which could be relevant in *S. aureus* pathogenicity.

On the other hand, the immune response of the CWA protein also requires more studies, since the mechanism by which CWA proteins interfere with the host innate immune response is unknown, in particular regulation of complement activation. In addition, determining CWA proteins causing a cellular immune response is crucial for the generation of new vaccines.

Most studies of CWA proteins have been conducted with laboratory strains. These studies should be extended in clinical isolates, where the variation of ligand binding of the CWA proteins is considerable. Additionally, the regulatory system of the expression of CWA proteins is still insufficient, because the expression of CWA proteins depends on the strain under study.

Surface proteins have a wide range of functions that are essential for colonization and survival of *S. aureus* in the host. Although the structural analysis of the CWA proteins has been crucial to define the mechanism of these processes and has provided the classification of the

CWA proteins, there are many questions to understand completely the functions of the CWA proteins in the pathogenesis of *S. aureus*.

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