Review

Staphopains in *Staphylococcus aureus* bacteremia: Virulence activities related to the onset of septic shock, coagulation disorders, and infectious endocarditis

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**A B S T R A C T**

**Background:** *Staphylococcus aureus* is an important oral bacterium that enters the bloodstream following dental procedures, causing bacteremia. Infectious endocarditis occurs due to the invasion of blood-borne pathogens into the endocardium. *S. aureus* is the most frequently isolated bacterium in Gram-positive sepsis, and shock and coagulation disorders are common and potentially fatal consequences of sepsis. Staphopains are the most abundant proteases among extracellular proteolytic enzymes produced by staphylococci, and their virulence activities related to the pathophysiology of *S. aureus* bacteremia have been elucidated.

**Highlight:** Staphopain A (ScpA)—not staphopain B (SspB)—releases bradykinin, and the two staphopains synergistically release a novel kinin, Leu-Met-Lys-bradykinin, directly from human plasma kininogens. These kinins cause vascular leakage. ScpA, similar to the two kinins, lowers blood pressure in guinea pigs in a bradykinin B2-receptor-dependent manner when administered intra-arterially, and produces septic shock symptoms in them. Kinin generation from human plasma by ScpA is enhanced in the presence of SspB, strongly suggesting a shock induction by the bacterial proteases. Staphopains, SspB being threefold more potent than ScpA, truncate fibrinogen by preferentially cleaving this coagulation factor at the C-terminal region of the Aα chain, which results in loss of fibrinogen clottability, thereby causing bleeding tendency. Staphopains showed a degradation activity for type I collagen similar to that observed in ScpA-mediated skin destruction, indicating that they participate in *S. aureus*-induced endocardium destruction.

**Conclusion:** Staphopains are potent virulence factors and are potentially involved in the onset of septic shock, coagulation disorders, and infectious endocarditis that occurs in *S. aureus* bacteremia.

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1. Introduction

Staphylococcus aureus is an important oral bacterium [1] that is isolated in approximately 20% of specimens obtained from the oral cavity and facial area [2]. S. aureus was isolated from 12% of supragingival plaque samples in healthy dentate adults [3]. Approximately 28% of tongue swabs obtained from children, who had no diseases other than dental disease, were S. aureus-positive [4]. The isolation rate from oral cavity increases to more than 30% in periodontal disease patients [5]. Some oral infections are caused, at least in part, by S. aureus, such as angular chelitis [6], parotitis [7], and staphylococcal mucositis [8]. Bacteria in the oral cavity enter the bloodstream in high numbers after dental procedures, causing bacteremia [8,9]. The bacteremia rates were 60% (range: 18–85) following tooth extraction, 88% (range: 60–90) after periodontal surgery, and 40% (range: 7–50) subsequent to brushing teeth or irrigation [10]. Periodontal probing can cause bacteremia in patients with periodontitis [11]. The risk of bacteremia increases in patients with periodontal disease due to the proximity of the organisms to the bloodstream via ulcers within gingival pockets or the gingival crevice epithelium [12]. S. aureus bacteremia is associated with various general diseases, including infective endocarditis and sepsis [10,14,15]. S. aureus is the most frequently isolated pathogen in Gram-positive sepsis, which is as common as Gram-negative sepsis [16–18]. Shock and coagulation disorders are the common and potentially fatal consequences of sepsis.

S. aureus infection-mediated diseases are caused by the virulence factors produced by this pathogen. In addition to enterotoxins and haemolysins, S. aureus secretes several extracellular proteases, including the serine protease V8 protease, the metallo-protease aureolysin, and the cysteine proteases staphopain A (ScpA) and staphopain B (SspB). These enzymes are considered as putative virulence factors [19,20]. Staphopains are the most abundant proteases among extracellular proteolytic enzymes of staphylococci [19]. This review focuses on the pathophysiology of S. aureus bacteremia and staphopain virulence activities associated with septic shock, coagulation disorders, and infectious endocarditis.

2. Properties of staphopains

ScpA is encoded by scpA, and the mature form has a molecular mass of 20 kDa and is composed of 174 amino acid residues [21]. SspB is encoded by sspB, and the mature form encompasses 174 or 168 amino acid residues sharing 46% identity with ScpA [21]. In spite of this similarity, the pl of SspB is acidic (pl 4.64). Both ScpA and SspB are processed from large proenzymes containing 363 and 357 residues, respectively [19]. The tertiary structure of ScpA resembles the overall fold of papain with two interacting domains. As in the papain-like proteases, the CYS124 and HIS125 (His21 in SspB) residues form the catalytic dyad of ScpA, with Gln12 being involved in forming the “oxyanion hole,” and Asn141 interacting with the imidazolium ring of the catalytic His. SspB has a very similar fold and a topology identical to that of the ScpA active-site cleft.

ScpA has very broad substrate specificity, with no preference for a particular amino acid at any of the several positions on either side of the scissile peptide bond [22]. Despite the broad specificity, this protease shows a limited ability to cleave synthetic peptide substrates, which include ABZ(aminobenzoic acid)-Phe-Gly-Ala-Lys-ANB-NH2amide of 5-amino-2nitrobenzoic acid) [23], Z(benzylxycarbonyl)-Leu-Leu-Glu-MCA, and Z-Leu-Leu-Arg-MCA. Thiol compounds are necessary for the protease activity. In addition to heavy metals and oxidizing agents, ScpA is irreversibly inhibited by E-64 and human a2-macroglobulin [24]. SspB has far more restricted substrate specificity in comparison with ScpA, and it cleaves ABZ-Ile-Ala-Ala-Gly-ANB-NH2 [23] and Z-Phe-Arg-MCA. E-64 inhibits SspB, but no effective inhibitors for this protease in human plasma have been identified thus far.

3. Virulence activities of staphopains in bacteremia/sepsis

In bacteremia/sepsis, staphopains, released by S. aureus in the bloodstream, cleave plasma proteins, thereby exerting their virulence activities.

3.1. Induction of vascular leakage and hypovolemic hypotension

The pathogenesis of Gram-positive sepsis has not been fully elucidated. However, the plasma levels of the plasma kallikrein/kinin system components, factor XII, prekallikrein, and high molecular weight kininogen (HK), are low in sepsis patients, indicating activation and subsequent consumption of these components [25–28]. The activation of the plasma kallikrein/kinin system in an animal bacteremia model causes lethal hypotension [29,30]; hence, plasma kallikrein/kinin system activation appears to contribute to septic shock. In human plasma, S. aureus induces the release of bradykinin (BK) [31], the final product of the plasma kallikrein/kinin system activation, which causes vascular leakage (VL) [32] leading to hypotension. This bacterium has a high negative net surface charge because of the presence of cell wall teichoic acid and lipoteichoic acid [33], and can activate the plasma kallikrein/kinin system as efficiently as lipopolysaccharide and lipid A from Gram-negative bacteria do in vitro [34]. Thus, these cell wall molecules have the potential to activate the plasma kallikrein/kinin system in S. aureus bacteremia. However, heat-labile extracellular products of S. aureus were found to be far more potent as lethal factors than its cell wall components when evaluated in a mouse sepsis model [35]. Among the secreted proteases, V8 proteinase was shown to release kinin from HK; however, this activity was not affected by the presence of serine proteinase inhibitors [36], suggesting that contaminating proteolytic enzymes have been responsible for kinin generation. Therefore, heat-labile staphopains were examined for their role in kinin release.

Intradermal injection of ScpA into guinea pigs induced VL in a concentration-dependent manner starting at an enzyme concentration of 20 nM (2 pmole/100 μl) [37]. Inhibition of this activity by E-64 and treatment of the animals with the BK B2-receptor antagonist HOE 140 suggested that BK was generated by ScpA proteolytic activity. Indeed, this enzyme induced VL via release of BK directly from both human HK and low molecular weight kininogen (Fig. 1) but not through the activation of factor XII or
prekallikrein. Interestingly, although SspB enhanced VL activity induced by ScpA in a concentration-dependent manner, it showed no VL activity alone; this is attributed to the synergistic cleavage of kininogen. SspB cleaves HK at -Ile-Ser-j-Leu-Met-, and together with cleavage at the C-terminus of the BK sequence by ScpA, it releases Leu-Met-Lys-BK (Fig. 1), which exhibits VL activity almost equal to that of BK. Thus, coexistence of the two staphopains increases VL activity through the additional release of Leu-Met-Lys-BK. An important pathophysiological mechanism of septic shock is hypovolemic hypotension, caused by plasma leakage into the extravascular space. ScpA, similar to the two kinins, lowers blood pressure in a BK B2-receptor-dependent manner when administered as an intra-arterial injection to guinea pigs and produces septic shock symptoms in them. Kinin generation from human plasma by ScpA is enhanced in the presence of SspB, strongly suggesting that these proteases can cause shock in patients with *S. aureus* sepsis.

### 3.2. Induction of coagulation disorders

Disseminated intravascular coagulation (DIC) is a common and potentially fatal consequence of sepsis. In as many as 40% of septic patients, DIC leads to multiple organ failure [38], and is directly linked to a high mortality rate. Clotting induction and the subsequent bleeding tendency due to the consumption of coagulation factors are the prominent clinical features of DIC. In *S. aureus* sepsis, staphopains may participate in the onset of the clotting disorder through the activation or inactivation of plasma coagulation factors by a proteolytic cleavage. However, the ability of staphopains to affect plasma clotting has not been studied.

Activated partial thromboplastin time (APTT) shows plasma clottability through the intrinsic coagulation pathway. Both staphopains prolonged APTT and SspB was found to be approximately threefold more potent than ScpA [39]. Plasma thrombin time demonstrates the clottability of fibrinogen and similar effects of the enzymes on this assay indicates that staphopains predominantly degrade fibrinogen to reduce its clottability. Staphopains, indeed, efficiently prolonged fibrinogen thrombin time in a concentration- and protease activity-dependent manner. SDS-PAGE analysis and N-terminal sequencing of fibrinogen fragments have revealed that staphopains preferentially cleave the αc-chain (Fig. 2). First, ScpA rapidly cuts out a 16-kDa fragment from the αc (C-terminal two/thirds region) of the αa-chain (66 kDa) [40], resulting in the formation of a 50-kDa fragment. Subsequent slow cleavage within this fragment reduces its molecular mass to 34 kDa. The αc is also targeted by SspB, which generates 41-, 37-, and 35-kDa fragments (Fig. 2). It is thought that SspB initially releases the 41-kDa fragment by proteolysis within the αc, at a rate slower than that of the ScpA-mediated cleavage to release the 16-kDa fragment. The 41-kDa fragment is further cut at the αc sequentially generating 37- and 35-kDa fragments. In contrast to the αa-chain, the Bβ- and γ-chain comparatively resist cleavage by staphopains. Plasmin degrades all three chains of fibrinogen, and cleaves the αa-chain at various sites, including peptide bonds at the C-terminal sites of Arg252, Arg424, Arg491, and Lys508 [41].

Proteolysis at these sites generates fragments with molecular masses different from those released by the staphopains-induced cleavage of the αa-chain. Therefore, it is clear that staphopains cleave the fibrinogen αa-chain at sites different from those at which plasmin cleaves. Thrombin cuts off fibrinopeptide A from the αa-chain, and fibrinopeptide B from the Bβ-chain [42], causing the polymerization of generated DesAA-fibrinogen into prototibrils. The prototibrils subsequently laterally associate with one another through the binding of the αc regions, (Fig. 3) [43], thus converting fibrinogen to a fibrin clot. Charged amino acid residues necessary for lateral association are located in the αc [44]. The very efficient cleavage of the αc by SspB truncates majority of the αc protein to disrupt the necessary interaction for lateral association of prototibrils (Fig. 3). ScpA also cleaves fibrinogen at the αc, more rapidly than SspB, generating the 50-kDa fragment first, but this cleavage seems unlikely to cause a reduction in clotting factor function. The next cleavage, by which a 34-kDa fragment is generated, is slow but renders fibrinogen uncleavable (Figs. 2 and 3). The difference in cleavage sites explains why SspB impairs fibrin clot formation more efficiently than ScpA. Thus, fibrinogen cleavage by staphopains in *S. aureus* sepsis results in the loss of plasma clottability, leading to a greater tendency to bleed, a fatal consequence of sepsis.

### 3.3. Collagen degradation

Invasion of *S. aureus* into the endocardium, leading to infectious endocarditis, requires the degradation of connective tissue extracellular
matrix proteins and is facilitated by proteases. In this context, staphylococcal proteases may contribute to this invasion, although little is known about the ability of S. aureus-derived enzymes to degrade matrix proteins. The extracellular matrix degradation essential for the pathogenicity of S. aureus is inferred from the ability of ScpA to induce widespread plasma leakage via BK release following its intradermal injection into guinea pigs [37]. This is in stark contrast to the localized plasma leakage induced by the injection of BK alone. The widespread leakage of plasma is thought to be due to degradation of extracellular matrix proteins, including elastin, which can be cleaved by ScpA [24]. The activity of SspB on matrix proteins has not been investigated.

Both staphopains degraded type I collagen in a concentration-dependent manner at concentrations as low as 10 nM and inactivation of these proteases inhibited their collagenolytic activity [39]. Although the two staphopains differ in their fibrinogenolytic activity, they exert the same collagenolytic activity. The ability to degrade collagen strongly suggests that staphopains can participate in endocardium destruction caused by S. aureus. Adherence of S. aureus to endothelial cells is the initial step in the development of endocarditis. In this process, fibrinogen acts as a bridging molecule between S. aureus and the endothelium [45], and the ability of this pathogen to bind to fibrinogen is recognized as an important factor for valve infection and invasion in experimental endocarditis [46]. Thus, the interaction of S. aureus with fibrinogen plays a crucial role in the pathogenicity of endocarditis caused by this bacterium. Moreover, a 135-kDa cell surface protein of S. aureus [47] binds specifically to collagen [48-51], constituting another important virulence trait in experimental endocarditis [52].

4. Conclusions

S. aureus in the oral cavity invades the bloodstream in high numbers following dental procedures and releases staphopains into the plasma. By cleaving kininogens and fibrinogen in plasma, staphopains lower blood pressure through kinin release and impair plasma clottability. Staphopains degrade type I collagen, which possibly contributes to endocardium destruction by S. aureus. These pathogenic activities of staphopains suggest that the S. aureus-produced proteases are potent virulence factors and are potentially involved in the induction of septic shock and coagulation disorders, and in the onset of infectious endocarditis in S. aureus bacteremia. In addition, such staphopain activities are associated with local S. aureus infections. Edema occurs through kinin release, unclottable fibrinogen generation impedes induction of the host-defense coagulation against pathogens, and collagen degradation destroys tissues, thereby facilitating S. aureus invasion into the circulation and its dissemination. Therefore, staphopains may be novel targets for drug development for therapy of infectious diseases caused by S. aureus.

Ethical approval

Ethical approval is not required.

Conflict of interest

There is no conflict of interest in this review

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