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Do amyloid structures formed by *Staphylococcus aureus* phenol-soluble modulins have a biological function?

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ABSTRACT

Phenol-soluble modulins (PSMs) are alpha-helical, amphipathic peptides that have multiple functions in staphylococcal physiology and virulence. Recent research has suggested that PSMs form amyloid fibrils and amyloids are involved in PSM-mediated phenotypes such as cytolysis and biofilm stability. While we observed PSM amyloid formation using electron microscopy and dye assays, there were no apparent differences in the production of extracellular fibrous material between a PSM-deficient strain and the isogenic wild-type strain. Furthermore, we detected no correlation between cytolytic or pro-inflammatory activities with the propensity of PSM derivatives to form amyloids. In addition, we propose a model based on our finding of non-specific attachment of PSMs to DNA, which we here report results in resistance to DNase digestion, explaining previous findings on PSM-mediated biofilm stability without the necessity to assume amyloid involvement. Collectively, our results indicate that PSM amyloid formation may not be of major relevance for known key biological functions of PSMs. Intriguingly, however, we found that amyloid-forming capacity of PSMalpha3 allows almost no amino acid exchanges, suggesting importance of amyloid formation in possibly yet unknown functions of PSMs.

1. Introduction

Phenol-soluble modulins (PSMs) have first been described in *Staphylococcus epidermidis* by the group of Seymour Klebanoff, who coined the name for a group of three pro-inflammatory peptides he named PSMα, PSMβ, and PSMγ, which participated into the phenol phase during hot phenol extraction (Mehlin et al., 1999). One of those peptides, PSMγ, is identical to the *S. epidermidis* δ-toxin, a homologue of the well-known *Staphylococcus aureus* δ-toxin.

In the following years, we performed systematic investigations to analyze the PSM composition of staphylococcal pathogens. Most staphylococci appear to produce PSMs, as judged by chromatographic and mass spectrometric (MS) analyses, with the pattern being characteristic for, but different in every species (Rautenberg et al., 2011). Notably, a complete analysis of the PSM pattern of a given species requires purification efforts in addition to high-pressure liquid chromatography (HPLC)/MS analyses. To date, the list of PSMs in *S. epidermidis* has been completed (Otto, 2009; Vuong et al., 2004; Yao et al., 2005); and we analyzed the PSMs produced by *S. aureus* and *Staphylococcus haemolyticus* (Da et al., 2017; Wang et al., 2007).

S. aureus is by far the most important pathogenic species among the staphylococci. It is a widespread and leading human pathogen, causing many thousands of deaths per year in the U.S. alone. *S. aureus* causes a wide spectrum of diseases, ranging from chronic diseases such as atopic dermatitis and other skin diseases, to acute and often fatal conditions such as sepsis (Lowy, 1998). Together with *S. epidermidis*, *S. aureus* is also a premier cause of infections associated with indwelling medical devices, in which the propensity to form biofilms plays a major role for pathogenesis (Otto, 2008).

PSMs have multiple functions in staphylococcal physiology and pathogenesis (Cheung et al., 2014a). They have been implicated in major types of staphylococcal infections, including skin, blood, and bone infections (Cassat et al., 2013; Peschel and Otto, 2013; Wang et al., 2007). Many PSMs, in particular those of the smaller α-type (~20–25 amino acids, as opposed to β-type PSMs, ~44–45 amino acids), lyse leukocytes and erythrocytes, among other cell types, and

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thus have a major function in acute *S. aureus* infection (Peschel and Otto, 2013; Rasigade et al., 2013; Wang et al., 2007). All PSMs structure biofilms and cause dissemination of biofilm-associated infection to the bloodstream and organs in vivo (Periasamy et al., 2012; Wang et al., 2011). In addition, there is recent evidence for the δ-toxin representing a major factor exacerbating atopic dermatitis (Nakamura et al., 2013). Finally, PSMs activate the formyl peptide receptor 2 (FPR2) (Kretschmer et al., 2010), and in an indirect fashion the toll-like receptor 2 (TLR2) (Hanzelmann et al., 2016), an interaction probably serving for the recognition of staphylococcal invaders by innate host defense, with one of the most important consequences being the stimulation of neutrophil chemotaxis.

Amyloids are aggregates of peptides or proteins that stick together to form fibrils. In humans, they are most infamous to cause neurodegenerative disorders and other diseases, a process known as amyloidosis (Dobson, 2003; Jucker and Walker, 2013). In bacteria, amyloids are now being recognized to have beneficial functions that contribute to normal bacterial physiology, which may include pathogenesis (Romero and Kolter, 2014). For example, they form curli in *Escherichia coli* and other Enterobacteriaceae (Evans and Chapman, 2014). Furthermore, most biofilms produce extracellular material that is in an amyloid form (Taglialegna et al., 2016). Finally, several human receptors appear to be stimulated strongly by amyloid ligands (Ye and Sun, 2015), including FPR2 (Tiffany et al., 2001).

In our laboratory, we had observed for many years that PSMs have a strong tendency to aggregate, which makes working with them often extremely troublesome. More recently, reports have been published describing that PSM produce amyloids. The Boles group published that several *S. aureus* PSMs produce amyloids and that they contribute to *S. aureus* biofilm stability (Schwartz et al., 2012). Recently, Tayeb-Fligelman et al. reported that PSMα3, an extremely cytolytic PSM (Cheung et al., 2012; Wang et al., 2007), produces a novel amyloid structure, which the authors claimed is linked to PSM function, most notably cytolysis (Tayeb-Fligelman et al., 2017).

Here, we analyzed all *S. aureus* PSMs for their propensity to form amyloid structures using transmission electron microscopy (TEM) as well as with an amyloid-staining dye. In addition, we critically revisited the claims that PSM amyloid formation is associated with PSM function. In particular, we analyzed whether there is a correlation between amyloid formation and PSM function using an alanine screen peptide bank of PSMα3. Finally, we propose a model explaining the observed biofilm stability-mediating properties of PSMs by attachment of PSMs to extracellular DNA, thus without the necessity to assume amyloid formation.

2. Materials and methods

2.1. Bacterial strains

Strain LAC (pulsed-field type USA300) and isogenic deletion mutants produced in this strain were used in the present study. In strain LAC Δ*psmα*Δ*psmβ*Δ*hld,* the *psmα* and *psmβ* operons are deleted in their entirety and translation of the *hld* (δ-toxin) gene is abolished by mutation of the start codon (not to interfere with the regulatory function of RNAIII, in which the gene is embedded).

2.2. Peptides

All PSM peptides were synthesized by commercial vendors at > 95% purity in their N-formylated forms and quality-checked by the Protein Chemistry Section, Research Technologies Branch, NIAID.

2.3. Transmission electron microscopy (TEM)

For analysis of amyloid formation by TEM, cells were prepared as published by Schwartz et al. (Schwartz et al., 2012). Cells were grown in TSB with 0.5% glucose for 48 h in a flow cell culture system as previously described (Periasamy et al., 2012). Cells were harvested from the glass surface of the flow cells and treated with fixative (2% paraformaldehyde in 0.1 M sodium phosphate buffer), after which tubes were sealed. For negative staining by TEM, five µl of sample was adsorbed on glow-discharged 200 mesh formvar-coated copper grids. Following a quick wash with water, the grids were stained with 2% uranyl acetate. The grids were imaged using a Hamamatsu camera (Advanced Microscopy Techniques) on a Hitachi 7500 TEM instrument at 80 kV.

2.4. Thioflavin T (ThT) assay for amyloid formation

S. aureus PSM solutions were prepared by diluting 1:100 with water from a 10 mg/ml stock in DMSO. PSM samples were incubated with filtered ThT at a final concentration of 0.2 mM.

PSMα3 and derivatives in powder form were treated with trifuoroacetic acid/hexafluoroisopropanol (1:1), dried in a chemical hood for 2 days, followed by further drying in a rotary vacuum concentrator for 2 h. Then, peptides were dissolved in 10 mM sodium phosphate buffer (pH 8.0) containing 150 mM NaCl. After sonication for 10 min, undissolved material was removed by centrifugation at 10,000 rpm for 5 min in a table top microcentrifuge. Alternatively, PSMs were diluted from 10 mg/ml stocks in DMSO in the same buffer and treated accordingly.

Thioflavin T (ThT) was purchased from AnaSpec (SensoLyte Thioflavin T β-Amyloid (1–42) Aggregation Kit). In each reaction, 200 µM peptide was mixed with 50 µM ThT in 10 mM sodium phosphate buffer (pH 8.0) and 150 mM NaCl. The fluorescence of ThT was scanned from 450 nm to 700 nm with excitation at 438 nm at the indicated times using a Tecan Safire multimode microtiter plate reader every 5 min at 37 °C.

2.5. DNase digestion experiment

For testing whether PSMs protect DNA from digestion by DNase, ~300 ng each of two different PCR fragments of staphylococcal DNA were incubated for 5 min with a mixture of all seven *S. aureus* PSMs, after which DNase was added and samples were incubated for 15 min. The final concentrations of PSMs in the incubation mixture were 0.1 mg/ml for each PSM, and the final concentration of DNase was 0.5 Kunitz units/ml. Then, samples were loaded on an 0.8% agarose gel, which was run for 45 min at 180 V and stained using ethidium bromide.

3. Results and discussion

3.1. Analysis of PSM amyloid fibril formation by electron microscopy and amyloid-specific dyes

In 2012, Schwartz et al. published the first study showing amyloid formation by PSMs (Schwartz et al., 2012). These authors used a mixture of all *S. aureus* PSMs at 0.1 mg/ml each or PSMα1 alone, and showed fibril formation using TEM and increased absorption of the amyloid-specific dye thioflavin T (ThT) compared to controls. For the ThT experiments, they used a 1:100 dilution from stock solutions made at 10 mg/ml in dimethylsulfoxide (DMSO), which we also routinely do in our laboratory to avoid the aggregation of PSMs that is frequently observed in concentrated aqueous stocks. For consistency, we used this method for both the ThT and TEM experiments.

We observed ThT absorption over control, indicative of amyloid formation, for the mix of all PSMs at 0.1 mg/ml, similar to the result reported by Schwartz et al. (Schwartz et al., 2012) (Fig. 1A). However, for the single PSMs, there was ThT absorption over control only for PSMα1 among the α-type PSMs (PSMα1-4, δ-toxin) (Fig. 1B) − the only single PSM for which this was also reported by Schwartz et al. (Schwartz et al., 2012). Interestingly, we found the most pronounced

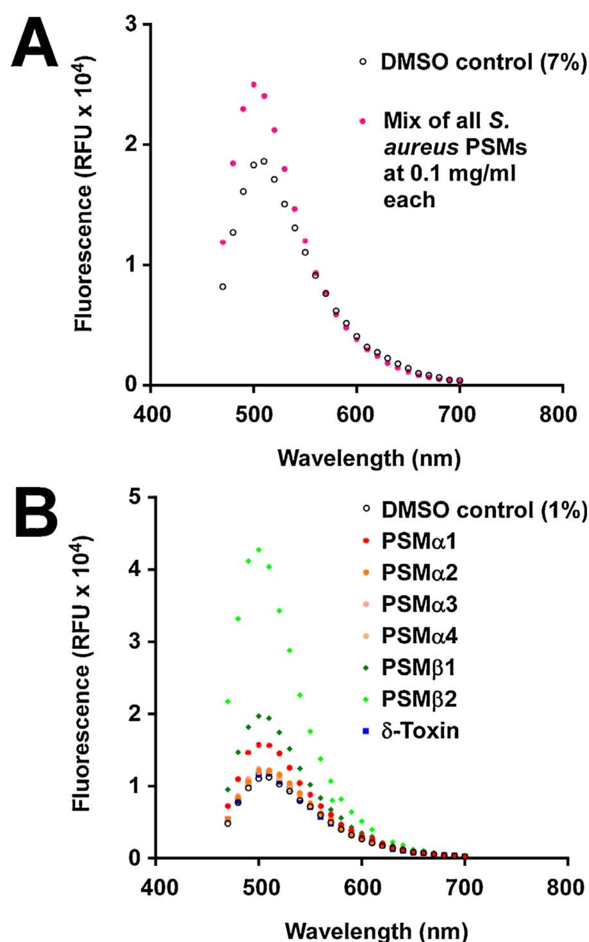

Fig. 1. Amyloid formation of *S. aureus* PSMs. Synthetic PSMs were analyzed for the propensity to form amyloids using a ThT assay. PSMs were measured in a mix of 0.1 mg/ml each (A) or singly at 0.1 mg/ml (B) upon incubation for 48 h.

amyloid formation as indicated by ThT absorption with the two PSMβ peptides, PSMβ1 and PSMβ2 (Fig. 1B).

In the TEM experiments, we found that fibrils are in general extremely difficult to detect. However, we were able to detect structures that can be interpreted as fibrils in the PSMβ1 sample (Fig. 2A), which is consistent with our ThT results. Collectively, these results are in general accordance with the notion that PSMs may form amyloid structures.

3.2. PSM amyloid formation and biofilms

PSMs have been shown to structure biofilms and detach biofilm clusters, an effect which ultimately leads to more extensive and compact biofilm formation in mutants deficient in all or some PSMs, results in accordance with in-vivo findings during biofilm-associated infection (Otto, 2013; Periasamy et al., 2012; Wang et al., 2011). The Boles group described that the in-vitro biofilm in a mutant devoid of PSMα and PSMβ peptides has decreased capacity to withstand enzymatic matrix degradation or environmental impact, a phenomenon they explained by PSM amyloid formation (Schwartz and Boles, 2013; Schwartz et al., 2012). While predictions on in-vivo biofilm formation capacity on the basis of the PSM amyloid model thus are difficult to align with previous in-vivo findings, a PSM amyloid-dependent mechanism that leads to biofilm stabilization is of great interest and may affect in-vivo situations that have not yet been investigated for PSM dependence. Furthermore, structuring by non-aggregated PSMs and amyloid-forming capacity of PSMs may be observed under different conditions, as suggested by Schwartz et al. (Schwartz et al., 2012), and may both affect the biofilm phenotype. Here, we revisited whether PSM amyloids are formed by *S. aureus*, and propose a possible alternative cause for the observed PSM-dependent stabilization of biofilms.

To analyze whether PSM fibrils are formed by *S. aureus*, Schwartz et al. used the comparison of a Δ*psmα*Δ*psmβ* mutant with the corresponding *S. aureus* laboratory wild-type strain (SH1000) parent. In their publication, they present a TEM picture taken with the wild-type strain that shows fibrous material around cells, while the picture of the mutant cells does not reveal such material (Schwartz et al., 2012). In their original publication (Schwartz et al., 2012), these authors showed that such fibril formation can only be observed in a specific medium (PNG), while in their follow-up publication they observed PSM fibrils also in the more commonly used tryptic soy broth containing 0.5% of additional glucose (TSBg), and at more extensive levels after adding extracellular DNA (eDNA) (Schwartz et al., 2016). The related, PSM amyloid-dependent biofilm-stabilizing effects were observed in experiments, in which biofilms were grown for different times, for 30 h, 3 d, or 5 d (Schwartz et al., 2016; Schwartz et al., 2012). In our experiments, we used TSBg and a biofilm growth time of 2 d. We chose those conditions because they represent conditions commonly used by staphylococcal biofilm researchers, and it is certainly of great interest whether under those commonly used in-vitro conditions, the phenomenon of PSM amyloid formation contributes to biofilm effects. According to the general findings by Schwartz et al. (Schwartz et al., 2016; Schwartz et al., 2012), PSM amyloids should form under those conditions.

To verify whether extracellular fibrous material produced by *S. aureus* under biofilm-forming conditions is due to PSMs, we used the total PSM deletion strain (Δ*psmα*Δ*psmβ*Δ*hld*), which we had produced in the background of the clinically relevant strain LAC (USA300) (Joo et al., 2011), and the isogenic wild-type strain, and analyzed the cells using TEM. We found that in both the preparations made from the wild-type or the mutant strain, fibrous material can be frequently found, independently of the presence of PSMs (Fig. 2B).

These results indicate that the fibrous material around *S. aureus* cells is not primarily composed of PSMs. This raises the question of why Schwartz et al. described that material to be predominantly PSMs (Schwartz et al., 2012). Given that our previous studies showed that PSMs are produced in extremely high amounts (Chatterjee et al., 2013; Wang et al., 2007) and that PSMs have also recently been shown to interact with the *S. aureus* cell surface (Kizaki et al., 2016), it is easily conceivable with their mass spectrometric analysis (Schwartz et al., 2012), which predominantly analyses proteinogenic molecules, Schwartz et al. overlooked the main, backbone macromolecules of those fibrils − possibly teichoic acids, exopolysaccharide, or eDNA (Otto, 2008), and misinterpreted merely attached PSMs as the predominant component of those fibrils. This would also explain results in their follow-up paper on eDNA-mediated PSM amyloid formation, in which they showed dramatically increased fibril formation in PNG as compared to TSBg media despite comparable PSM production (Schwartz et al., 2016), inasmuch as they report that eDNA levels in PNG are considerably higher due to increased lysis.Thus, we hypothesized that Schwartz et al. may have misinterpreted PSM attachment to eDNA as fibril formation by PSMs themselves. While those authors proposed a model in which eDNA serves as a “seed” for PSM fibrils by possibly increasing the local concentrations of PSMs around the DNA molecule (Fig. 3A), their results on PSM stability and formation may well be explained by the non-specific attachment of PSMs to eDNA, which we demonstrated in a previous publication (Joo et al., 2016) (Fig. 3B). Attachment of PSMs to DNA may also explain the increase of absorption in the ThT assays with PSMα1 and eDNA that Schwartz et al. reported (Schwartz et al., 2016). Therefore, we analyzed whether PSMs can protect DNA from degradation. We found that a mixture of all PSMs indeed protects DNA from degradation by DNAse. (Fig. 4). Of note, we ruled out a sequence-specific effect by using two different DNA fragments. These findings are in accordance with a model, in which PSMs

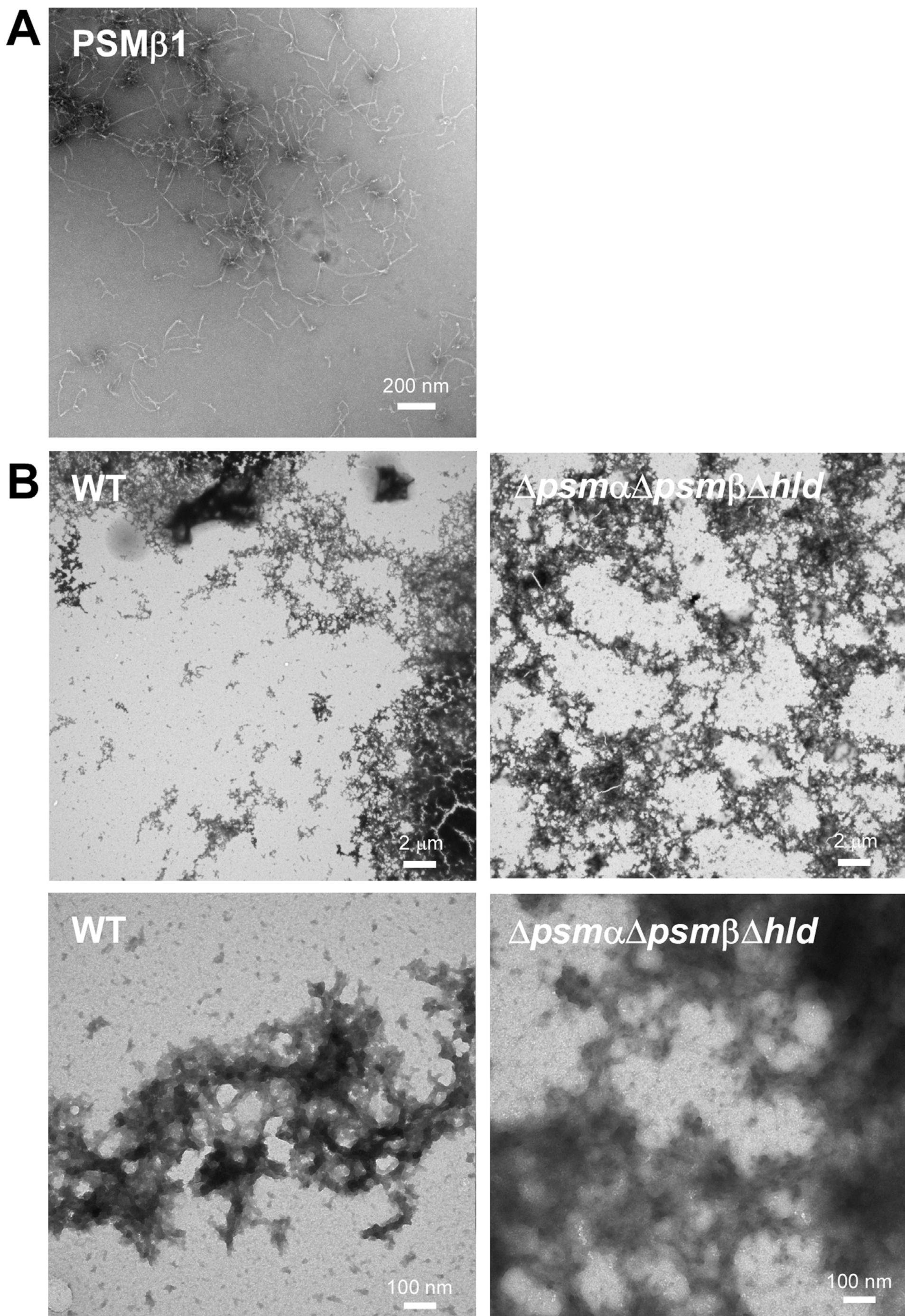

Fig. 2. Transmission electron microscopy (TEM) of PSMs and PSM-producing *S. aureus*. (A) Synthetic PSMs at 0.1 mg/ml incubated for 48 h in water were analyzed by TEM as described in methods. Shown is the fibrous material detected with PSMβ1. (B) *S. aureus* LAC (USA300) wild-type cells and cells of the isogenic PSM-deficient *ΔpsmαΔpsmβΔhld* mutant strain, prepared as described in methods, were analyzed by TEM. The pictures shown are at two different magnifications.

structure biofilms, as we have shown previously (Periasamy et al., 2012; Wang et al., 2011), while they also protect pre-formed biofilms from enzymatic degradation, as noted by Schwartz et al. (Schwartz et al., 2012) (Fig. 3B). However, this model does not include the formation of functional PSM amyloids to explain the observed biofilm phenotypes.

3.3. PSM amyloid formation and cytolysis

The cytolytic activity of PSMs is arguably their most important function for the pathogenesis of acute infections. Notably, PSMs contribute to the lysis of neutrophils after they have ingested *S. aureus* cells (Surewaard et al., 2013), making PSMs key players in the bacterial defense against elimination by innate host defense. PSMα3 is the most cytolytic PSM produced by *S. aureus* (Cheung et al., 2012; Wang et al., 2007), which is why it has been in the focus of most investigation on PSMs, including the mechanism by which they cause disease and structure-function relationship studies (Cheung et al., 2014b; Peschel and Otto, 2013).

While both our results and those by Schwartz et al. (Schwartz et al., 2012) attribute only a comparatively minor amyloid-forming capacity to PSMα3, PSMα3 has recently moved into the center of interest regarding PSM amyloid formation. This is because in a recent publication, Tayeb-Fligelman et al. presented a high-resolution crystal structure of an amyloid fibril formed by PSMα3 (Tayeb-Fligelman et al., 2017). While the structure revealed the typical characteristics of β-sheet containing amyloid fibrils, it also showed what the authors described as a novel, “cross-α” amyloid-like architecture. Notably, the authors

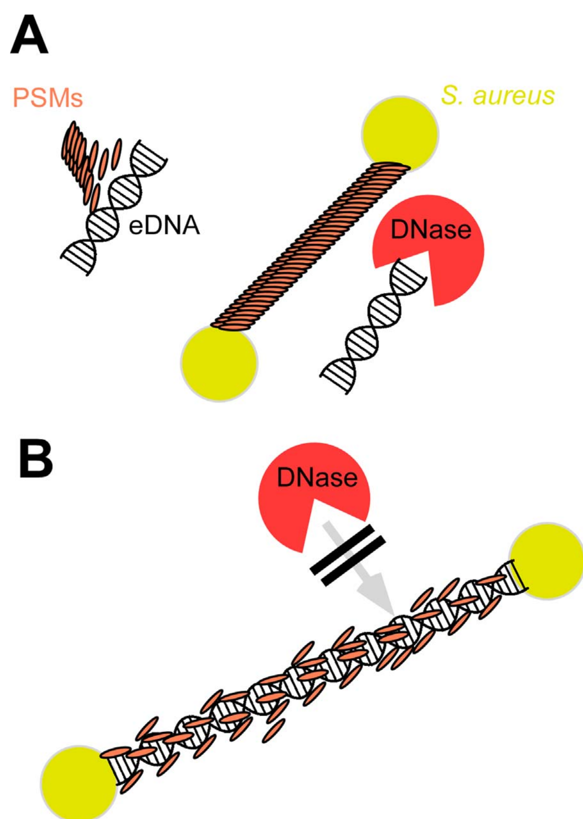

Fig. 3. Comparison of models explaining PSM-mediated resistance of biofilms to enzymatic digestion. (A) Model based on PSM amyloid-mediated cellular connections. DNA triggers PSM amyloid formation by an unknown mechanism (Schwartz et al., 2016). PSM amyloids lead to intercellular connections in biofilms that are not susceptible to enzymatic digestion of other biofilm matrix macromolecules such as eDNA (Schwartz et al., 2012). (B) Model based on PSM attachment-mediated resistance of biofilm matrix molecules to enzymatic digestion. PSMs attach to biofilm matrix macromolecules such as eDNA. Attachment leads to increased resistance towards enzymatic digestion.

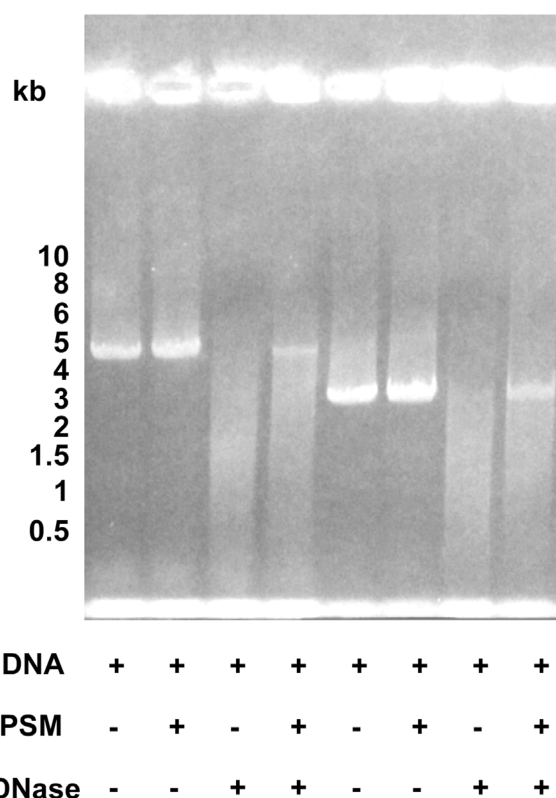

Fig. 4. Protection of DNA from DNase-mediated degradation by PSM attachment. Two different DNA PCR fragments (of ~3 or 5 kb in size, respectively) were digested with DNase (0.5 Kunitz units/ml) either with or without addition of PSMs (each *S. aureus* PSM at 0.1 mg/ml) at room temperature for 15 min.

claimed that the specific PSMα3 structure they report generally underlies PSMα3 biological function. The evidence the authors present in that regard is an analysis of the correlation between amyloid formation as measured by ThT assays and cytotoxicity using three PSMα3 derivatives. Two derivatives had reduced cytotoxicity and ThT absorption, while one had maintained cytotoxicity and showed ThT absorption indicative of amyloid formation. Furthermore, they showed that Tween 80 inhibited cytotoxicity and argued that this is due to the fact that Tween 80 disrupts amyloids. However, that Tween 80 inhibits cytotoxicity of PSMα3 could be due to many mechanisms. Thus, the evidence that Tayeb-Fligelman et al. showed for PSM amyloid formation being correlated to cytolysis is very limited. Furthermore, the authors did not address, as claimed, whether cytotoxicity is due to their specific – versus a more general – amyloid structure. Finally, given widely accepted models of how pore-forming peptides work (Huang, 2006; Lohner and Blondelle, 2005; Oren and Shai, 1998; Park and Hahm, 2005), it is very hard to imagine how an assembled amyloid structure would form a cytolytic pore. While the authors note that the mechanism of amyloid-mediated toxicity in general is unclear and there are carpet models of antimicrobial peptide pore-forming activity (Taylor et al., 1996), we are not aware of models explaining how a large assembled structure such as an amyloid would form a pore in a bacterial membrane.

To more systematically analyze whether PSM amyloid formation is necessary for cytotoxicity, we used the general approach of Tayeb-Fligelman et al., but a more extensive array of PSMα3 derivatives. Namely, we used a PSMα3 alanine screen peptide bank (containing 20 derivative peptides) to test for a correlation between cytotoxicity and amyloid formation. We previously reported the cytolytic activities of those derivatives toward human neutrophils and erythrocytes (Cheung et al., 2014b). We now tested amyloid formation using the ThT assay. We specifically ordered dedicated peptide derivatives for this assay and refrained from preparing stock solutions in DMSO, to avoid a potential amyloid-reducing function of DMSO. However, for consistency with the other experiments in this study, we also included an experiment based on PSM stock solutions prepared in DMSO.

The results of the PSMα3 alanine screen peptide bank obtained in the ThT amyloid assay were striking. Only PSMα3 itself and one derivative, PSMα3 V4A (a conservative exchange), showed amyloid formation, while no other derivative did. In the DMSO-containing samples, results were overall similar, but amyloid formation was generally reduced, as expected (Fig. 5; Table 1). This suggests that the three-dimensional structure of PSMα3 that underlies the propensity to form amyloid fibrils does not allow considerable changes. Importantly, given that among the peptide derivatives that showed no amyloid formation, many maintain considerable cytotoxicity, this correlative analysis indicated that amyloid formation by PSMα3 does not underlie its cytolytic function. In our analysis, we followed the protocol by Tayeb-Fligelman et al. and removed precipitated material before analyzing amyloid formation. Finally, in our analysis of the propensity of *S. aureus* PSMs to form amyloids (Fig. 1B), those that were most active in that regard − PSMβ1 and PSMβ2–are reportedly not or only barely cytolytic (Cheung et al., 2012; Wang et al., 2007), further confirming that amyloid formation is not linked PSM cytotoxicity.

3.4. PSM amyloid formation and FPR2 activation

Amyloid structures have also been reported to activate human immune receptors, including the PSM receptor, FPR2 (Tiffany et al., 2001; Ye and Sun, 2015), raising the question of whether PSM amyloid

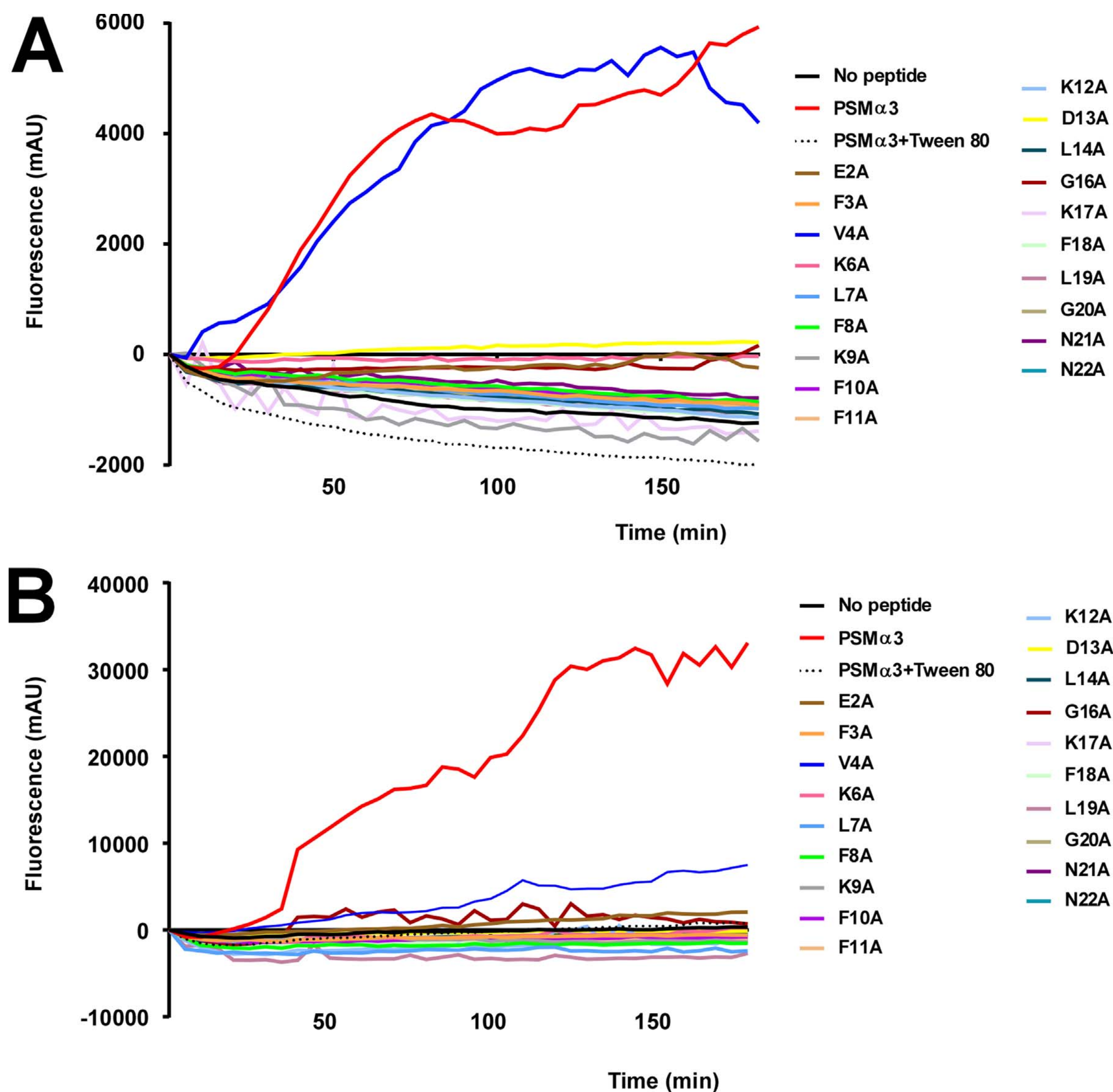

Fig. 5. Thioflavin T assay for amyloid formation in a PSMα3 alanine screen peptide bank. PSMα3 and derivatives representing an alanine screen peptide bank were prepared at 0.1 mg/ml from powder (A) or from 10 mg/ml stocks in DMSO (B) by dissolving in water. Non-dissolved material was removed by centrifugation. Amyloid formation was measured by adding ThT and determining fluorescence over 3 h. Curves are derived from the measurement of three replicates and show the mean.

formation is necessary for FPR2 activation. Considering our previous analyses of pro-inflammatory functions of PSMα3 derivatives, with calcium flux and CD11b expression as readouts of FPR2-dependent neutrophil priming and activation (Cheung et al., 2014b), we did not find a correlation with amyloid formation as determined in this study. Many PSMα3 derivatives that did not form amyloids in the ThT test maintained pro-inflammatory activities in the range of those observed with PSMα3. These findings suggest that also FPR2 activation by PSMs is not dependent on PSM amyloids, but the question of the structural components of PSMs that are needed for FPR2 activation certainly awaits further investigation.

4. Conclusions

In this study, we critically reviewed the published evidence underlining the notions that (i) PSMs form amyloids, and (ii) amyloid formation has a role in main biological functions attributed to PSMs. In addition, we performed a set of experiments aimed to verify some of the previous findings, as well as to evaluate an alternative model for PSM-dependent biofilm stability that may explain the observed phenotype without assuming amyloid formation. While our experimental results generally confirm that PSMs can aggregate to form amyloid-like structures, our findings do not support that amyloid formation is a prerequisite for cytolysis. As for biofilm stability, our results describing attachment of PSMs to DNA and the resulting resistance of DNA to degradation by DNase may explain biofilm-related phenotypes previously associated with PSM amyloid formation, but without the necessity for PSM amyloids. According to this model, the media- and condition-dependent observations in terms of biofilm stability reported by Schwartz et al. may simply be due to the observed variations in eDNA presence, with eDNA having the well-established role of an in-vitro biofilm matrix component rather than the assumed “seed” for PSM amyloid formation. Generally and in accordance with recent studies on PSM-cell surface interaction (Kizaki et al., 2016), these findings indicate that the binding of the commonly abundantly present PSMs to cell surface macromolecules may have important consequences for staphylococcal physiology. It will be interesting to investigate in further studies whether PSMs also bind in similar fashion to other biofilm matrix molecules and whether the attachment of PSMs to macromolecules such as DNA occurs in an ordered fashion. Finally, our findings demonstrating that the amyloid-forming capacity of PSMalpha3 allows almost no amino acid exchanges, in contrast to other

Table 1
Analysis of correlation between PSM amyloid formation, cytolysis, and receptor-mediated pro-inflammatory activities.

	Neutrophil lysis[a]	Hemolysis[a]	Ca Flux in neutrophils[a]	CD11b expression in neutrophils[a]	Amyloid formation (ThT)[c]	Amyloid formation (ThT) cont. 1% DMSO[c]
PSMα3	100[b]	100	100[b]	100	100	100
E2A	68	95	105	57	13	2
F3A	77	72	84	87	6	0
V4A	90	78	64	97	104	14
K6A	16	80	32	90	17	0
L7A	37	52	113	99	6	0
F8A	80	68	72	90	8	0
K9A	92	91	108	94	0	0
F10A	79	74	75	85	8	0
F11A	73	86	107	107	7	0
K12A	18	40	39	50	4	0
D13A	89	87	97	75	22	0
L14A	70	74	98	102	4	0
L15A	76	68	103	96	ND[d]	ND[d]
G16A	85	95	100	90	15	3
K17A	14	66	99	110	0	0
F18A	87	87	101	103	3	0
L19A	81	70	110	99	4	0
G20A	79	92	64	103	5	0
N21A	89	86	30	46	9	0
N22A	91	88	35	62	7	0

[a] Data are from Cheung et al. (2014a, b).
[b] Raw data were compared to the value obtained for PSMα3, which was set to 100%.
[c] Raw data obtained at 90 min after incubation with ThT were compared to the value obtained for PSMα3, which was set to 100%. 0% was set for the control value achieved without peptide. Negative percentage values were set to 0.
[d] Sample could not be measured due to high background fluorescence.

phenotypes, suggests a possibly yet unknown role for PSM amyloids.

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