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Structural mechanism for modulation of functional amyloid and biofilm formation by Staphylococcal Bap protein switch

Junfeng Ma[1], Xiang Cheng[1], Zhonghe Xu[1], Yikan Zhang[1], Jaione Valle[2], Shilong Fan[1], Xiaobing Zuo[3], Iñigo Lasa[2] & Xianyang Fang[1,*]

Abstract

The Staphylococcal Bap proteins sense environmental signals (such as pH, [Ca^{2+}]) to build amyloid scaffold biofilm matrices via unknown mechanisms. We here report the crystal structure of the aggregation-prone region of *Staphylococcus aureus* Bap which adopts a dumbbell-shaped fold. The middle module (MM) connecting the N-terminal and C-terminal lobes consists of a tandem of novel double-Ca^{2+}-binding motifs involved in cooperative interaction networks, which undergoes Ca^{2+}-dependent order–disorder conformational switches. The N-terminal lobe is sufficient to mediate amyloid aggregation through liquid–liquid phase separation and maturation, and subsequent biofilm formation under acidic conditions. Such processes are promoted by disordered MM at low [Ca^{2+}] but inhibited by ordered MM stabilized by Ca^{2+} binding, with inhibition efficiency depending on structural integrity of the interaction networks. These studies illustrate a novel protein switch in pathogenic bacteria and provide insights into the mechanistic understanding of Bap proteins in modulation of functional amyloid and biofilm formation, which could be implemented in the anti-biofilm drug design.

Keywords biofilm associated protein; calcium-binding protein; functional amyloid; liquid-liquid phase separation; order-disorder conformational switches
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Introduction

Amyloids are insoluble, highly structured protein aggregates (Eisenberg & Sawaya, 2017) best known for their association with debilitating human diseases such as amyotrophic lateral sclerosis (ALS) or Alzheimer's disease (AD) (Chiti & Dobson, 2017). Recently, the amyloids have also been found to perform physiological functions from prokaryotes to humans (Pham *et al,* 2014). In bacteria, functional amyloids are fulfilling diverse roles relevant for bacterial growth and survival in the environment, including as part of the extracellular biopolymer matrix that ties the bacteria together into multicellular communities called biofilm (Van Gerven *et al,* 2018). Biofilm formation provides bacteria with many advantages such as high tolerance to harsh environments and increased recalcitrance to antimicrobial agents (Santos *et al,* 2018), thus extremely difficult to eradicate and poses significant challenges to the healthcare system today (Bjarnsholt *et al,* 2013; Santos *et al,* 2018).

Biofilm-associated proteins (Baps) are a group of bacterial surface proteins mainly involved in intercellular adhesion and mediating biofilm development (Lasa & Penades, 2006; Latasa *et al,* 2006). Its first member is identified in bovine *Staphylococcus aureus* which is a large multidomain protein of 2,276 amino acids (aa) (Cucarella *et al,* 2001) (Fig 1A), of which region B contains three putative EF-hand motifs that may regulate Bap functionality upon Ca^{2+} binding (Arrizubieta *et al,* 2004). Although *bap* gene has never been found in *S. aureus* human isolates, bap ortholog genes are present in the core genomes of several coagulase-negative staphylococcal species that are frequent colonizers of human skin (Tormo *et al,* 2005). Moreover, functionally related proteins homologous to Bap exist in many phylogenetically unrelated Gram-positive and Gram-negative bacteria (Lasa & Penades, 2006), suggesting its functional importance in a diverse group of bacteria.

Recent results indicate that the N-terminal of Bap is proteolytically processed, released, and form amyloid-like aggregates able to induce bacterial biofilm formation under acidic pHs and low concentrations of Ca^{2+} ([Ca^{2+}]). Restoring pH to neutrality reverses the amyloids formed at acidic buffer; addition of Ca^{2+} in the millimolar range inhibits biofilm formation despite acidification of the medium (Taglialegna *et al,* 2016). These findings suggest that staphylococcal Baps can sense the bacterial environment through pH and [Ca^{2+}] changes and modulate biofilm formation in an amyloid-dependent way. However, how Ca^{2+} and pH signals reconcile to regulate Bap-mediated amyloid and biofilm formation is largely unknown.

1 Beijing Advanced Innovation Center for Structural Biology, School of Life Sciences, Tsinghua University, Beijing, China
2 Laboratory of Microbial Pathogenesis, Navarrabiomed-Universidad Pública de Navarra-Departamento de Salud, IDISNA, Pamplona, Spain
3 X-ray Science Division, Argonne National Laboratory, Lemont, IL, USA
*Corresponding author. Tel: +86 10 62771071; E-mail: fangxy@mail.tsinghua.edu.cn