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Microbial Amyloids—functions and interactions within the host

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Abstract

The aggregation of proteins into amyloid fibers is a common characteristic of many neurodegenerative disorders including Alzheimer's, Parkinson's, and prion diseases. Amyloid formation was originally characterized in these systems and is traditionally viewed as a consequence of protein misfolding and aggregation. An emerging field of study brings functional amyloids, like those produced by bacteria, into the scientific mainstream, and demonstrates a ubiquitous role for amyloids in living systems. This review aims to summarize what is known about the bacterial amyloids and their interactions within various host environments.

Introduction

Amyloids were first described in 1854 as deposits in human tissue that resembled carbohydrates starches when stained with iodine (Latin: *amylum*). In the decades that followed, many devastating diseases were found to be characterized by the formation of amyloid deposits throughout the body [1,2]. Amyloids isolated from patients with a variety of disorders are known to be biochemically similar. Recent advances have revealed that the amyloid structure is a general folding motif involved in a broad range of biological systems throughout all domains of life.

Amyloids form remarkably stable polymeric fibrils with a central diameter of 3–12 nm, composed of folded β -sheets stacked perpendicular to the fibril axis [3]. Fibrillation is initiated by self-aggregation of protein monomers into oligomers, which accumulate over time and nucleate the self-assembly cascade of fibril polymerization characterized by the stacking of parallel or anti-parallel β -sheet secondary structure; this transition can be detected by circular dichroism (CD) spectroscopy. Hydrogen bonding between adjacent β -sheets provides additional fiber stability. Once formed, amyloid fibers are robust and can resist disassembly by enzymatic or chemical digestion. They show characteristic FTIR and X-ray diffraction patterns, and bind the amyloid specific dyes Congo Red and Thioflavin T to produce measurable shifts in the absorbance and excitation/emission spectra, respectively, relative to non-amyloid structures. [4]. The amyloid fold can be adopted by a variety of proteins with varying primary sequence. The structural similarity of amyloids has led to the isolation of several antibodies (A11, W01, and W02) that are used to detect various oligomeric and fibril conformations [5–8]. Discovery of the “amyloids” - a classification of proteins which readily form amyloids under biologically relevant conditions- has improved

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our understanding of the propensity of many proteins to form amyloids that contribute to misfolding and disease [9].

Chapman *et al.* proposed the concept of functional amyloids in their ground breaking 2002 *Science* article. Their work characterized the curli fimbriae produced in *Escherichia coli* biofilms as being biochemically similar to disease-associated amyloid structures. Using curli as a model, common methods and assays have been developed to identify and study microbial amyloids and the environmental forces that affect amyloid formation. Bacterial systems provide us with a simplified model for studying the conserved mechanisms of amyloid formation, degradation, and function.

What defines amyloids as “functional” are the rigid mechanisms controlling the formation and degradation of oligomers and fibrils such that they serve a useful purpose for the organism [10]. Many of the systems controlling bacterial amyloid formation have been characterized in recent years (Table 1). Amyloids have even been detected in naturally-occurring bacterial populations of *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Actinobacteria*, and *Firmicutes* [11–13]. In this review, we describe a collection of exciting new research at the intersection of microbial functional amyloids and their impact on the human host.

The Many Roles of Functional Amyloids

Functional amyloids contribute to numerous aspects of bacterial growth and survival [1].

Functional amyloids in the bacterial lifecycle and biofilm development

Biofilms are communities of surface-associated microbes encased in a protective matrix of polysaccharides, DNA, and proteins including amyloid fibrils. Amyloids’ inherent resistance to protease digestion and denaturation helps them reinforce and shield biofilms from harsh environmental stresses [14]. The production of curli greatly contributes to biofilm formation [15,16]. Bacteria producing curli fibers are often coated in exopolysaccharides and create flocculates that help to establish biofilms [17]. Curli have also been implicated in surface adhesion, immune evasion, and pathogenesis [18]. Curli-like systems have been described in numerous enteric bacterial species, and recently interspecies complementation between non-homologous *E. coli*, *Salmonella*, *Citrobacter*, and *Shewanella* curli subunits has been demonstrated [19]. Beyond curli systems, bacterial amyloids produced by gram-negative organisms that contribute to biofilm formation, like FapC in *Pseudomonas fluorescens*, continue to be elucidated [20].

Staphylococcus aureus produces small peptides called phenol soluble modulins (PSMs) that were recently shown to form amyloid fibers in biofilms (Fig. 1). The presence of PSM fibrils correlates to a robust biofilm phenotype that resists dispersal by surfactants and mechanical disruption [21]. Interestingly, soluble PSM species have been shown to contribute to dispersal of established biofilms [22]. Autoaggregation of PSMs into fibers abrogates the biofilm dispersal associated with addition of soluble peptides [21]. These findings demonstrate that amyloid formation may regulate PSM activity in the microenvironment of the biofilm.

The spore-forming filamentous bacterium *Streptomyces coelicolor* uses amyloids called “chaplins” to complete its lifecycle progression in biofilms growing at the air-liquid interface [23]. The chaplin family consists of eight amphipathic proteins (ChpA-H) which function to break the surface tension at the air-liquid interface, allowing hyphae to complete sporulation. The chaplins also form extremely hydrophobic spore coats that are thought to aid in efficient wind dispersal [24].

Bacillus subtilis and its close pathogenic relative *Bacillus anthracis*, produce a spore-coat protein called TasA [25,26]. TasA, which forms amyloid structures in *B. subtilis* biofilms, is encoded on an operon with an anchor protein, TapA, and the bifunctional secretion machinery SipW [27,28]. TasA and SipW have been characterized in *B. subtilis* as necessary for the formation of pellicle biofilms on the air-liquid interface [29].

Functional amyloids as toxin repositories

Amyloids can aggregate into toxic oligomers, which cause damage to lipid membranes [30]. These toxic oligomers also promote amyloid fibril formation by acting as nucleators for aggregation [4]. Some bacteria can parlay the toxicity associated with amyloid formation (Fig. 2). Microcin E492 (Mcc) is a small bactericidal peptide produced by *Klebsiella pneumoniae*. Mcc monomers and oligomers create cytotoxic pores that induce cell lysis in niche-occupying enteric bacteria like *Enterobacteriaceae* [31]. Mcc oligomers can aggregate into amyloid fibers, effectively sequestering them as inert fibril structures [32]. Recent studies described environmental triggers, like pH and the ionic dissociation of salt, that induced fiber formation accompanied by a loss of toxic oligomeric species. Changing environmental conditions to favor fiber dissociation (high pH or low salt concentration) trigger Mcc amyloids to disassociate back into cytotoxic oligomers [31].

Listeria monocytogenes can escape from phagolysosome engulfment by creating cytolytic pores with the listeriolysin O (LLO) toxin. It has recently been shown that LLO aggregates under alkaline conditions to form fibrous structures that bind the amyloid-specific dyes ThT and Congo red *in vitro*. While LLO fibrils lost their toxicity, cytolytic dimers were detected in acidic conditions similar to the phagolysosome [33].

Toxic amyloid oligomers are also utilized by plant pathogens. Harpins produced by the gram-negative bacterium *Xanthomonas axonopodis* contribute to pathogenesis of plant tissue. Amyloid-like fibrils are also formed by analogous proteins expressed from pathogenic *Erwinia* and *Pseudomonas* species [34].

Microbial amyloids and the host

Bacterial and disease-associated amyloids are structurally similar, and the interconnections between host immune response and microbial amyloids are beginning to emerge. Using curli again as a model, we will describe some of these interactions.

Bacterial amyloids and the host immune system

The innate immune response detects foreign bodies through leukocytes, like macrophage and neutrophils, and initiates a signaling cascade resulting in inflammation and recruitment of immune factors to clear bacteria and debris from the site of infection [35]. Curli and the major curlin subunit (CsgA) produced by *E. coli* and *Salmonella* species are recognized by Toll-like receptors (TLR) in macrophage and microglia as microbial-associated molecular patterns (MAMPs) [36]. This interaction induces IL-8 production, recruiting neutrophils that stimulate the host inflammatory response. The ability of CsgA to adopt the amyloid fold is important for IL-8 induction, as mutated CsgA does not promote a strong IL-8 response [37]. Both TLR1 and TLR2 act cooperatively to sense CsgA and curliated *E. coli* [38]. Immune response to curli through TLR2 activation is similar to the proinflammatory effects elicited by host amyloids, like β -amyloid toxic oligomers [36].

Mice injected with curli fibers develop curli-dependent increases in expression of nitric oxide synthase, nitric oxide, and decreases in blood pressure [39]. Curliated *E. coli* and *Salmonella* spp. induce the contact-phase immune response, resulting in fever, pain, and

hypotension triggered by bradykinin release in a murine sepsis model [40–42]. The contact system is an enzymatic cascade activated when circulating sensors in blood come in contact with surfaces like bacterial membranes, triggering the production of antimicrobial peptides [43]. The contact system also recognizes misfolded proteins produced during systemic amyloidosis [44]. Curli-producing *E. coli* are able to bind and sequester several contact system complexes causing a release of pro-inflammatory signals like bradykinin. Finally, it has also been observed that sera of patients with *E. coli* bacteremia often contains anti-CsgA antibodies [42].

Host antimicrobial peptides

Neutrophils recruited to infection sites produce antimicrobial peptides like LL-37 [45]. The human cathelicidin LL-37 is an antimicrobial peptide cleavage product that can create pores in bacterial membranes and is protective against bacterial infection in the urinary tract [46,47]. LL-37, along with another host immune factor, Serum amyloid A (SAA) and its peptide derivatives were shown to be protective against pathogenic strains of *E. coli* and *Salmonella* [48]. Recent work has also shown that curli expression in uropathogenic *E. coli* increases resistance to LL-37 [49]. The authors demonstrate that LL-37 interacts with CsgA and can inhibit fibril formation *in vitro*, thus likely interfering with biofilm formation. Interestingly, LL-37 has also been shown to exhibit amyloid-like properties [50–53].

The term *amyloidosis* refers to a variety of medical conditions wherein amyloid proteins cause harm as they are deposited in organs or tissues. It has shown that exogenously added amyloid proteins, including curli from *E. coli*, promote secondary amyloidosis disease in mice by aggregation a cleavage product SAA, amyloid protein A (AA) [54]. Thus, the mere presence of bacteria or inflammation caused by bacteria may contribute to the deleterious effects of amyloid disease.

Microbial amyloids facilitate pathogenesis in the host

Curli are often associated with pathogenicity in enteric gammaproteobacteria [37,42,49,55]. The production of curli in biofilms protects bacteria from clearance by immune factors or other antimicrobials [49]. Curli also bind to host extracellular matrix (ECM) components, like fibronectin [40] and laminin [56], promoting surface adhesion and internalization into host cells [57,58].

Mycobacterium tuberculosis produce amyloid-forming proteins which also bind to the ECM component, laminin [59]. In 2007, Alteri and colleagues described a pilin structure isolated from *Mycobacterium tuberculosis* biofilms that displayed many of the biophysical and morphological characteristics of amyloids, and bound to host extracellular matrix component, laminin, [59,60]. Although the *Mycobacterium tuberculosis* pili (MTP) protein has not been studied for its role in infection, the authors did show that sera from TB patients contain antibodies reactive to MTP, which is a strong indication that it exerts similar immunogenic effects when present in human hosts [59].

When they are not aggregated in amyloid fibrils during biofilm growth (Fig. 1), *S. aureus* PSMs are detected as formylated and non-formylated peptides and derivatives that interact with the host immune system. *S. aureus* is sensed by macrophages, triggering an inflammatory immune response which recruits neutrophils [61]. PSMs are described to be the major neutrophils chemoattractants in *S. aureus* supernatants [62]. PSMs are strong activators of neutrophil formyl-peptide receptor 2 (FPR2), and FPR1 to a lesser extent, even in the absence of N-terminal formylation [63,64]. Interestingly, FPR2 binds a wide variety of amyloid-like ligands [65], including LL-37, serum amyloid A [66], brain amyloid precursor [67], and annexin 1 [68], indicating a link between immune response to PSMs and

other amyloids. PSMs produced by *Staphylococcus epidermidis* have also been shown to bind TLR2 receptors [69], and more recently Wang et al. demonstrated IL-8 expression in neutrophils exposed to synthetic *S. aureus* PSMs and strains expressing PSMs endogenously [62].

Conclusion

Bacteria utilize amyloids as structural materials, adhesions, toxins, and protection against host defenses. The growing list of characterized bacterial amyloid systems include: the curli fibers of enteric gram-negative bacteria [18]; amphipathic chaplins of *Streptomyces coelicolor* [11,12,23]; the TasA/TapA system in *Bacillus subtilis* [27,28]; Microcin 492 in *Klebsiella pneumoniae* [32]; FapC in *Pseudomonas fluorescens* [20]; pili in *Mycobacterium tuberculosis* [40–42,59]; and most recently, the phenol soluble modulins in *Staphylococcus aureus* [21] (Table 1). These works suggest that functional amyloid formation is a widespread phenomenon utilized by a diversity of microbes. Current research has only begun to elucidate the interactions of bacterial amyloids as a contributing factor of infection and pathology within their hosts. A more detailed understanding of these bacterial systems will likely give insights into host-microbe interactions, microbial physiology and perhaps even protein misfolding diseases. It will be fascinating to follow future research that connects amyloid aggregation within the host and its microbial residents.

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Highlights

- Numerous examples of functional amyloids are emerging in diverse bacterial species.
- Bacterial functional amyloids facilitate a multitude of interactions with the host.
- Functions of bacterial amyloids include roles in: biofilm development, toxin storage, sporulation, and modulation of the immune response.

Figure 1. *S. aureus* phenol soluble modulins form amyloid fibers in biofilms

TEM micrograph of a *Staphylococcus aureus* cell after five days of biofilm growth. The extracellular fibers observed have amyloid properties and consist of small peptides called phenol soluble modulins (PSMs). Bar length indicates 500 nm.

Figure 2. Microcin E492 (Mcc) is an antimicrobial peptide that forms amyloid fibrils under certain environmental conditions

Mcc displays cytotoxicity when associated into oligomeric species that create in the outer membranes of niche-occupying bacteria [70]. They are also known to trigger apoptosis in diseased human cell lines [71]. When aggregated into amyloid fibrils, Mcc loses the antimicrobial activity associated with smaller oligomeric and monomeric species. Varying the pH and salt concentration of the surrounding environment can trigger this association/disassociation [31]. It is likely that this post-translational control mechanism serves to regulate the growth-phase dependant level of antimicrobial activity while preserving peptide stocks in non-toxic amyloid repositories [72]. Oligomeric Mcc resembles the transient oligomers formed by disease-associated amyloids, like amyloid-beta, which also induces antimicrobial pore formation at the cell membrane [50].

Table 1

Examples of known bacterial amyloids and their functions.
 Characterized Bacterial Amyloid Systems.

Organism	Amyloid Protein(s)	Amyloid function/characteristics
<i>Escherichia coli</i>	Curli (CsgA)	Biofilm component; Adhesion to surfaces
<i>Salmonella ssp.</i>	Curli/Tafi (CsgA)	Biofilm component; Adhesion to surfaces
<i>Mycobacterium tuberculosis</i>	Mtp	pili formation; binding to laminin
<i>Klebsiella pneumoniae</i>	MccE492	amyloid formation proposed to regulate MccE492 antimicrobial activity
<i>Pseudomonas fluorescens</i>	FapC	Biofilm component
<i>Streptomyces coelicolor</i>	Chaplins (ChpA-H)	Spore surface protein Biofilm component; amyloid formation proposed to regulate PSM
<i>Staphylococcus aureus</i>	Phenol Soluble Modulins	biofilm dispersal activity
<i>Bacillus subtilis</i>	TasA	Biofilm component; spore surface protein
<i>Xanthomonas axonopodis</i>	Harpins (HpaG)	amyloid formation proposed to regulate HpaG cytotoxic activity