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Danielsen, Heidi Nolsøe; Hansen, Susan Hove; Herbst, Florian-Alexander; Nielsen, Per Halkjær; Dueholm, Morten Simonsen

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Direct identification of amyloids in biofilms by label-free quantitative LC-MS combined with formic acid treatment

H. N. Danielsen, S. H. Hansen, F.-A. Herbst, P. H. Nielsen, M. S. Dueholm*

Aalborg University, Department of Chemistry and Bioscience, Fredrik Bajers Vej 7H, 9220 Aalborg, Denmark, *md@bio.aau.dk

Keywords: Amyloid; label-free quantitative LC-MS; biofilm; extracellular polymeric substances

Introduction

Extracellular polymeric substances (EPS) determine the structural and functional properties of biofilms. Detailed knowledge of the key EPS components may therefore provide the theoretical background required for the development of new biofilm control strategies.

Amyloids represent a fascinating class of highly ordered fibrillar protein polymers, which are characterized by a cross-β quaternary structure and the ability to self-assemble from their monomeric counterparts in a nucleation-dependent process (Fandrich, 2007). Although, amyloids were first discovered as malicious entities associated with protein misfolding disorders, it is now well-recognized that amyloids are also used for a wide range of beneficial purposes by organisms from all domains of life due to their exceptional properties (Dueholm *et al.*, 2013a; Blanco *et al.*, 2012).

Histological staining of biofilms with amyloid-specific dyes and amyloid conformation-specific antibodies has revealed that functional amyloids are widespread among microorganisms in biofilms that originate from nature and engineered systems (Larsen *et al.*, 2007). They are therefore believed to play important roles in biofilm ecology. Despite their omnipresence in biofilms, few amyloids have been purified from biofilm-associated bacteria and investigated in depth. However, the few amyloids which have been studied so far have already provided an astonishing demonstration of how amyloids are exploited in biofilms with roles ranging from fimbriae and other cell appendages for adhesion and biofilm formation to structural components of cell envelopes and spore coats (Dueholm *et al.*, 2013a; Blanco *et al.*, 2012). They may even be used as cytotoxins (Maji *et al.*, 2009) and as reservoirs for quorum-sensing signaling molecules (Seviour *et al.*, 2015).

The identification of novel functional amyloids is key to understand the many roles of amyloids in biofilm structure and function. Isolation of amyloids is, unfortunately, not a straightforward task. The insolubility and extreme stability of most functional amyloids exclude them from traditional protein analyses such as SDS-PAGE (Dueholm *et al.*, 2013b). Many functional amyloids are furthermore highly adhesive and therefore bind to pipette tips and other consumables. Large sample volumes and high productivity of amyloids are therefore required for successful purification. We here demonstrate a novel label-free proteomics technique that allow the direct identification of functional amyloid candidates based on the structural stability in the presence of increasing formic acid concentration.

Material and Methods

The method is described in Figure 1.1. Biofilm samples are resuspended in buffer and lysed by freeze/thaw cycles or sonication. The samples are then divided into aliquots, which are lyophilized and treated with concentration of formic acid ranging from 0-100%. The samples are lyophilized again and subjected to a short run SDS-PAGE, yielding a narrow gel band containing all proteins. The protein bands are excised and in-gel digested with trypsin. They are then analysed by label-free quantitative LC-MS. Amyloid proteins require a high concentration of formic acid to depolymerize and they are therefore only observed in samples treated with high concentration of formic acid.

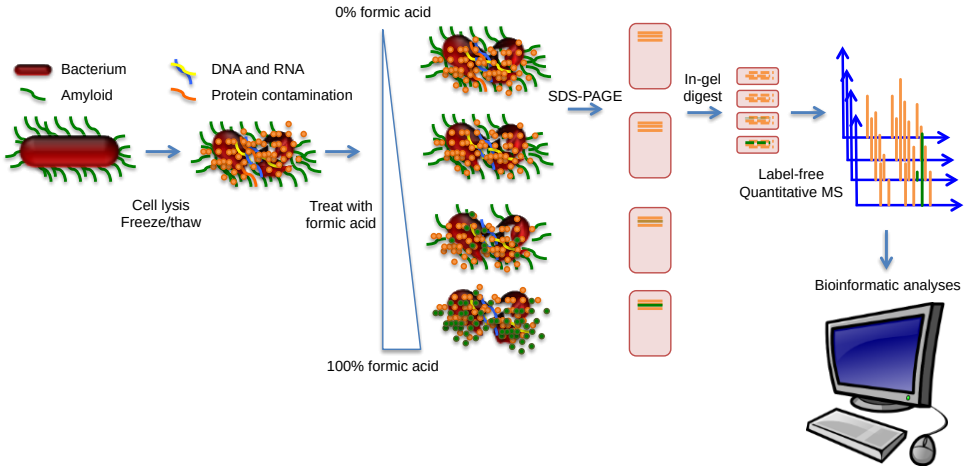

Figure 1.1: Direct identification of amyloids by label-free quantitative LC-MS and formic acid treatment.

Results and Conclusions

The new method was evaluated using the well-described functional amyloid model systems, *Escherichia coli* curli (Chapman *et al.*, 2002) and Functional amyloids of *Pseudomonas* (Fap) (Dueholm *et al.*, 2010). The method showed amyloid-specific profiles for the major (CsgA) and minor (CsgB) curli subunit as well as for the major (FapC) and minor (FapB) Fap subunits. No other proteins showed similar profiles. The method was also evaluated on the TasA system of *Bacillus subtilis* (Romero *et al.*, 2010). We where here able to identify TasA, however, we also observed multiple false positives, which we believe originate from amyloid coated spores, which are first lysed during the formic acid treatment. Finally, we analysed a complex sample (biofilm from a wastewater treatment plant). Again, we were able to identify amyloid candidates. These will be the focus of future studies. The possibility to directly identify amyloid proteins in complex samples is a game changer in the field of functional amyloids. We believe that it will be used to uncover many new amyloid systems in the future, which will have implications in technical and medical settings.

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