Mammalian prion amyloid formation in bacteria

Bruno Macedo, a,b, Yraima Cordeiro, b and Salvador Ventura a

aInstitut de Biotecnologia i de Biomedicina and Departament de Bioquimica i Biologia Molecular, Universitat Autonoma de Barcelona, Bellaterra (Barcelona), Spain;
bFaculdade de Farmacia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

ABSTRACT. Mammalian prion proteins (PrPs) that cause transmissible spongiform encephalopathies are misfolded conformations of the host cellular PrP. The misfolded form, the scrapie PrP (PrPSc), can aggregate into amyloid fibrils that progressively accumulate in the brain, evolving to a pathological phenotype. A particular characteristic of PrPSc is to be found as different strains, related to the diversity of conformational states it can adopt. Prion strains are responsible for the multiple phenotypes observed in prion diseases, presenting different incubation times and diverse deposition profiles in the brain. PrP biochemical properties are also strain-dependent, such as different digestion pattern after proteolysis and different stability. Although they have long been studied, strain formation is still a major unsolved issue in prion biology. The recreation of strain-specific conformational features is of fundamental importance to study this unique pathogenic phenomenon. In our recent paper, we described that murine PrP, when expressed in bacteria, forms amyloid inclusion bodies that possess different strain-like characteristics, depending on the PrP construct. Here, we present an extra-view of these data and propose that bacteria might become a successful model to generate preparative amounts of prion strain-specific assemblies for high-resolution structural analysis as well as for addressing the determinants of infectivity and transmissibility.

KEYWORDS. amyloid, bacteria, inclusion body, prion, recombinant, strain, transmission

BACKGROUND

Prions are misfolded, self-perpetuating and infectious entities derived from proteins natively expressed in the host. Once formed, prions can propagate in an auto-catalytic manner, recruiting the normal cellular protein to fold into its abnormal conformation.1,2 Mammalian prion diseases, also termed transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders that affect humans and other mammals. Despite they have...
been described since the late 18th century, the recognition that these diseases were caused by infectious proteins became clear only in the early 1980s, largely due to the work of Stanley Prusiner. Prusiner’s studies were fueled by the work from Tikvah Alper on the infectious-agent resistance to extreme conditions, such as ionizing radiation; and also by the initial proposal of the protein-only hypothesis by Griffith in the mid 1960s. Nowadays, a prion-like behavior is also attributed to other proteins involved in neurodegenerative diseases, including Alzheimer’s and Parkinson’s diseases, besides the classical prion itself (TSEs).

Therefore, prion pathogenesis seems to be more general than previously thought, and it will likely pass from being the exception to become the general case in protein misfolding diseases (PMDs).

Despite diverging in primary sequence and structure, many misfolded proteins can aggregate into highly-ordered amyloid fibrils leading to the pathological condition called amyloidosis. The amyloid state of a protein can convert the cellular form of a protein with the same or very similar primary sequence into its amyloid conformation, ultimately leading to its intracellular aggregation and cell-to-cell transmission. The progressive accumulation of the aberrant protein in the neuronal tissue is the hallmark of the neurodegenerative PMDs.

One peculiar characteristic of mammalian prion proteins (PrPs) is the formation of strains, which have been related to the multiple conformations that could be adopted by the pathogenic scrapie PrP (PrPSc). Prion strains are responsible for the wide spectrum of phenotypes observed for prion diseases. PrPSc strain isolates, when inoculated into hosts of their respective species, cause disease with different features, i.e. the length of incubation time, the pattern of protein deposition in the brain, and the extension of the histopathological damage. Once it became evident that the strain properties of PrPSc were encoded by the particular conformation of the aggregated state, many efforts were devoted to recreate prions in vitro, and to study their biochemical and physical characteristics, e.g. their mechanism of aggregation, their structure and their resistance to PK-digestion. A first advance was made when recombinant C-terminal murine prion protein (mPrP89-230) produced in Escherichia coli (E. coli) was polymerized into amyloid aggregates in vitro and then inoculated intrace-rebrally into transgenic mice expressing PrP89-230. The mice developed neurologic dysfunction and the prion protein in their brain extracts showed resistance to protease digestion. These brain extracts were also able to transmit the disease to other mice but with low levels of infectivity. Many subsequent works showed that highly infectious synthetic prions could not be generated in vitro without the addition of cofactors. In fact, PrP is considered a ‘promiscuous’ protein because of its ability to bind to different classes of macromolecules, such as nucleic acids (NAs), lipids, glycosaminoglycans, or even metallic ions; all these cofactors have been shown to trigger changes in PrP conformation leading to aggregation and toxicity. It appears that the minimal components necessary to facilitate PrP in vitro conversion and promote de novo prion formation with higher degree of infectivity are polyanions, mainly nucleic acid (NAs) molecules or lipids. The challenging task of creating recombinant prions that hold strain-like conformational characteristics and infectivity responds to the difficulty in reproducing an in vitro environment with proper facilitating factors, such as molecular crowding, the presence of chaperones and proteases, continuous synthesis of the protein in the ribosome or the presence of interacting NAs or lipids. In this context, modeling prion amyloid formation inside simple organisms might provide a system to recapitulate prion protein aggregation under more biologically relevant conditions.

In many instances, heterologous protein expression in bacteria results high intracellular protein concentration, where the aggregation pathway tend to dominate over the folding one, and thus insoluble deposits of the recombinant protein are formed. Different studies showed that amyloidogenic proteins, when expressed in bacteria, form inclusion bodies with common structural and functional features with the
highly ordered aggregated amyloids found in the original host species.\textsuperscript{24,25}

The first characterization of the amyloid properties of the bacterial IBs formed by a prionic protein was that of the fungal HET-S prion protein.\textsuperscript{26} Transmission electron microscopy (TEM) combined with solid-state NMR spectroscopy revealed that \textit{E. coli} IBs of the HET-s prion forming domain consist of fibrillar structures that are almost identical to the molecular structure of HET-s amyloid fibrils assembled \textit{in vitro}; and these HET-s IBs also showed prion infectivity when transfected into the natural host.\textsuperscript{26} Another report provided clear evidence that the bacterial cytoplasm can support the formation of infectious prion aggregates, as shown for the NM-Sup35 yeast protein.\textsuperscript{27} The NM-Sup35 bacterial IBs where later shown to possess amyloid-like properties. The infectivity of these aggregates was modulated by the environmental conditions in which they were formed.\textsuperscript{28} Importantly, the NM-Sup35 prion conformation can be propagated in bacteria for over a hundred generations, even when the cells can no longer produce the protein that serves as the trigger for the initial conversion.\textsuperscript{19} Seminal work from Giraldo’s group showed that N-terminal domain of a bacterial protein, RepA, could form amyloid-like fibrils within bacteria upon interaction with defined double-stranded DNA sequences.\textsuperscript{29} This protein, when modified, generated a prion-like disease in bacteria that can be transmitted from mother to daughter cells.\textsuperscript{30} More recently, this group showed that this bacterial prionoid can seed amyloid aggregation of mutants from the same protein,\textsuperscript{31} and characterized the bacterial nucleoid as the initial site of assembly of these prion-like aggregates.\textsuperscript{32} This bacterial amyloidosis, shared some characteristics with mammalian prion diseases, mainly transmissibility and the presence of phenotypically distinct strains.

Altogether, those observations provided solid evidences that the IBs molecular structure can resemble the architecture of amyloid fibrils, in such a way that both the prion strain phenomenon and the infectious nature, which are dependent on the specific protein conformational state, seem to be conserved in these morphologically different aggregates deposits. A remaining question is whether, like their fungal counterparts, mammalian prions could form amyloid intracellular aggregates when expressed in bacteria. We have answered this question in our recent paper\textsuperscript{33} where we selected two mammalian prion constructs, the wild-type murine PrP encompassing residues 23-231 (PrP\textsuperscript{WT}) and the C-terminal domain of murine PrP (PrP\textsuperscript{90-231}), and expressed them in bacteria. As expected, recombinant PrPs accumulate in the insoluble bacterial fraction as IBs. We purified and studied the conformational characteristics of the PrPs IBs, as well as their biochemical strain-like properties. Our recent study provides the first demonstration that PrPs from mice can form amyloids inside bacterial IBs with common characteristics to PrP amyloids formed \textit{in vitro} or even the amyloid PrP\textsuperscript{Sc} \textit{in vivo}. Moreover, although possessing similar secondary structure, PrP\textsuperscript{WT} IBs and PrP\textsuperscript{90-231} IBs exhibit conformational diversity, as they bind to amyloid dyes (Congo red and thioflavin T) at different levels, possess different morphology, different stability against urea-induced unfolding and also different resistance to proteinase K digestion. It is known that amyloid spread requires a high degree of sequential and conformational specificity; only amyloids with sequence identity can form cross-\(\beta\)-sheet interactions able to be incorporated into the amyloid fibrils. This sequential/conformational specificity is probably responsible for the species barrier between two different prions. However, the rules dictating the species barriers as well as effects of the environmental conditions on that barrier are still not clear. In our study, we show that the PrP\textsuperscript{WT} amyloid IBs are able to seed \textit{in vitro} the amyloid polymerization of purified recombinant PrP\textsuperscript{WT}, reducing the lag-phase of the sigmoidal reaction. But, interestingly, when we tried to seed the same reaction with the PrP\textsuperscript{90-231} IBs, we could not accelerate the polymerization, which indicates that, despite the fact that the 2 PrP forms share 70\% of the sequence, the lack of sequential identity at the N-terminus and/or the different structural properties of the aggregates, preclude the establishment of appropriate protein-protein contacts. Finally, the conformational differences among the PrPs IBs result in different
toxicity of the 2 PrP IBs resistant cores after PK-digestion when added to neuroblastoma-cultured cells. We believe that the intracellular aggregates retain strain-like features, a characteristic unique for prion proteins (from yeast and mammals). We show that some of these features, such as different migration pattern after digestion with proteinase-K, and different stability are conserved after IBs extraction (Fig. 1).

APPLICATIONS OF BACTERIALLY GENERATED MAMMALIAN PRION AMYLOIDS

There are several proposals for the structure of infectious PrPSc, but to date there is still no consensus about the 3D high-resolution conformation of the scrapie PrP.34-36 One of the main challenges is to obtain homogeneous material from infected brain in sufficient amount for the structural studies. Besides, there are also safety limitations for the study of bona fide infectious mammalian prions. Although there are obvious differences in post-translational changes in the prokaryotic and eukaryotic cytoplasm, the generated recombinant prion proteins might become a valuable source of homogeneous but conformationally different assemblies produced with high yield for future transmissibility and structural studies. Besides, our bacteria-generated mammalian PrPs might be useful as seeds for amplification of strain-specific prions and their subsequent study, in protocols such as PMCA (protein misfolding cyclic amplification) and RT-QuIC (real time quaking induced conversion).37,38 Our prion-forming system can also be used for the screening of drugs that inhibit aggregation and amyloid formation, as already proposed for other amyloid proteins.23

CONCLUSION AND PERSPECTIVES

Our previous work provided clear experimental evidence that yeast prions can form amyloids in prokaryotes that can be transmitted to naïve yeasts that will gain the prion-state, confirming infectivity.28 Recently, we demonstrated that modeling mammalian PrP amyloids inside bacteria might be a useful tool to understand the conformational variability of prions. We showed that the IBs obtained upon expression of mammalian PrP in bacteria contain seeding competent amyloid-like structures. We need to get now a more detailed picture about the neurotoxic activity of these IBs. It remains to be confirmed in vivo if we are recapitulating a real prion strain phenomenon. We are performing in vivo experiments to check if PrP IBs can trigger the conversion of host PrP into an aggregated state that retains the PK-resistance and the stability profiles of the seed. Our next study will answer whether bacteria-produced mammalian PrP IBs have only amyloid nature or are indeed infectious to mammals and can be considered bona fide prions (Fig. 1). For that purpose, PrP IBs will be administered to healthy transgenic mice by intracerebral injection and the animals will be evaluated for disease progression. Clinical and histopathological parameters will be followed to identify prion disease characteristics. If clinical symptoms of TSE appear, disease-incubation time and western blot analysis of brain-derived material will be assessed to see whether they correlate with strain-like properties of different inoculated IBs.

Overall, despite speculative, the possibility that mammalian prion amyloid assemblies generated in bacteria would display transmissible and infectious properties in mammalian hosts is worth to be explored, since it would open a myriad of novel opportunities to study the structure and biology of these intriguing proteins.

ABBREVIATIONS

| Abbreviation | Description |
|--------------|-------------|
| IB           | inclusion body |
| PrP          | prion protein |
| TSE          | transmissible spongiform encephalopathy |

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.
FIGURE 1. Mammalian PrP IBs formation in bacteria and their characterization by different techniques. Two constructs of murine PrP were selected (1) and E. coli cells were transformed with plasmids harboring either PrP<sup>WT</sup> or PrP<sup>90-231</sup> cDNAs (2). Induction of recombinant expression of both PrPs resulted in their accumulation in the insoluble bacterial fraction, known as IBs, which were purified upon appropriated cell lysis and extensive washing (3). SDS-PAGE analysis revealed that both PrP IBs are constituted mainly by the recombinant mammalian PrPs (4). Transmission electron microscopy, binding to amyloid-specific dyes and FTIR spectroscopy analysis of isolated PrP IBs revealed that mammalian PrPs IBs possess an amyloid-like nature (5). Furthermore, digestion with proteinase-K (PK), seeding of the amyloid polymerization reaction and stability studies (6), indicate that these IBs may display prion strain-like properties. It remains to be investigated whether the isolated mammalian PrPs IBs behave as true infectious entities upon intracerebral injection in mice. Accordingly, clinical and histopathological features, as well as transmissibility will be followed to confirm the development of prion disease characteristics in animal models.
FUNDING

This work was supported by Ministry of Education of Brazil [CAPES process number: 99999.002869/2014-04 to the fellow student B. M]; and by Ministerio de Economía y Competitividad, Spain [BFU2013-44763-P to S.V.]; by ICREA [ICREA Academia 2009 to S.V.] and by FAPERJ, CNPq and INBEB from Brazil to Y.C.

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