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Biochimica et Biophysica Acta 1753 (2005) 92 – 99
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
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Review

From chance to frequent encounters: Origins of β2-microglobulin fibrillogenesis

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Received 15 June 2005; received in revised form 6 September 2005; accepted 7 September 2005
Available online 22 September 2005

Abstract

It is generally accepted that amyloid formation requires partial, but not complete unfolding of a polypeptide chain. Amyloid formation by β-2 microglobulin (β2m), however, readily occurs under strongly native conditions provided that there is exposure to specific transition metal cations. In this review, we discuss transition metal catalyzed conformational changes in several amyloidogenic systems including prion protein, Alzheimer's and Parkinson's diseases. For some systems, including β2m from dialysis related amyloidosis (DRA), catalysis overcomes an entropic barrier to protein aggregation. Recent data suggest that β2m samples conformations that are under thermodynamic control, resulting in local or partial unfolding under native conditions. Furthermore, exposure to transition metal cations stabilizes these partially unfolded states and promotes the formation of small oligomers, whose structures are simultaneously near-native and amyloid-like. By serving as a tether, Cu^{2+} enables the encounter of amyloidogenic conformations to occur on time scales which are significantly more rapid than would occur between freely diffusing monomeric protein. Once amyloid formation occurs, the requirement for Cu^{2+} is lost. We assert that β2m amyloid fiber formation at neutral pH may be facilitated by rearrangements catalyzed by the transient and pair wise tethering of β2m at the blood/dialysate interface present during therapeutic hemodialysis.

Keywords: Amyloid; Hemodialysis; β-2 microglobulin; Protein folding

1. Protein folding, misfolding and pathological misassembly

It is well known that proteins fold and adopt well-defined three-dimensional structures required for function [1]. Studies aimed at understanding protein folding have characterized the folding process as traversing a funnel-shaped multidimensional landscape [2]. This includes the captivating aspect that most proteins fold, without assistance, to a unique structure representing the energetically most stable conformation [3]. The funnel-shaped landscape of protein folding allows proteins to access a broad ensemble of conformational states. Since conformational sampling occurs under thermodynamic control, even a solution of stably folded protein will transiently adopt both partially and incorrectly or misfolded states [2,4]. The assembly of these states into insoluble aggregates is associated with an increasing number of human diseases. Notable examples include Alzheimer's disease, Creutzfeldt–Jakob disease, dialysis related amyloidosis (DRA), and type II diabetes. Although each disease has a different clinical presentation, a central component is the formation and deposition of aggregated protein in cells and tissues, resulting in loss of function, physical obstruction, and/or gain of toxic function leading to cell death [5–7].

Although amyloid precursor proteins differ significantly in secondary and tertiary structure, all amyloid fibers share a common cross-β structure. In the cross-β structure, β-strands are aligned perpendicular to the long axis of the fiber. These strands are arranged into sheets in which the backbone hydrogen bonding runs parallel to the fiber axis [8–10]. The formation of similar structures from vastly different protein precursors strongly suggests a common mechanism for all amyloid assembly. As all amyloid fibers are β-sheet rich in structure, global rearrangements and conformational changes are often required prior to or concomitant with assembly. The

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1570-9639/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.bbapap.2005.09.002

tightly packed environment of a native protein, however, prohibits such changes from readily occurring. Therefore, experiments have suggested amyloid formation most likely occurs from the partially structured or non-natives states naturally sampled by proteins [11]. This has lead to partial unfolding of globular proteins as an accepted prerequisite to amyloid assembly.

A variety of protein features are emerging as central to the assembly of disparate primary sequences into closely similar fibrillar structures. These include the presence of polyglutamine rich repeats such as those observed in the huntingtin protein of Huntington's disease [12], and charge neutralization [13]. In our own work, we recently identified that β2m from DRA is a Cu^{2+} specific binding protein [14] and this binding can promote amyloid assembly under near physiological solution conditions [15]. Divalent cation interaction, particularly with Cu^{2+}, is emerging as a feature of several amyloid systems. To date, divalent cation interactions have been implicated in formation or alteration of ordered aggregates in a number of different diseases including: Alzheimer's, Creutzfeldt–Jakob, light chain amyloidosis, Parkinson's, and DRA [16–20]. In each of these diseases, Cu^{2+} associates with a different and unrelated protein resulting in aggregates and amyloid. Thus, it is of critical importance to identify the structural basis of transition metal cation associated conformational changes resulting in assembly.

2. Transition metal cations in amyloid disease

Protein interactions with transition metal cations have long been the subject of investigation, particularly in the neurodegenerative amyloids [21,22]. In vitro and in vivo studies have shown that transitions metal cations can initiate or modulate aggregation assembly through a variety of complex mechanisms. For example, divalent cations such as Cu^{2+} can give rise to one or more interrelated effects, such as inducing structure in unstructured regions, free radical mediated oxidation, and stabilization of partially and globally unfolded conformations (Fig. 1). The involvement of Cu^{2+} in amyloid assembly can be further divided into two categories. The first are Cu^{2+} interactions with amyloid precursor proteins as part of an in vivo function. These include amyloid β peptide (Aβ) of Alzheimer's disease and prion protein (PrP) from the spongiform encephalopathies [21–23]. The second category involves opportunistic interactions with Cu^{2+}, where such interactions are regarded as strictly pathological. This category includes β2m from DRA, α-synuclein of Parkinson's disease, and immunoglobulin light chains of light chain amyloidosis [14,18,24]. The interaction of Cu^{2+} with these precursor proteins promote aggregation, however, a functional role for these interactions has not yet been identified.

Amyloidogenic proteins in both categories bind divalent cations and undergo similar modifications resulting in inter-

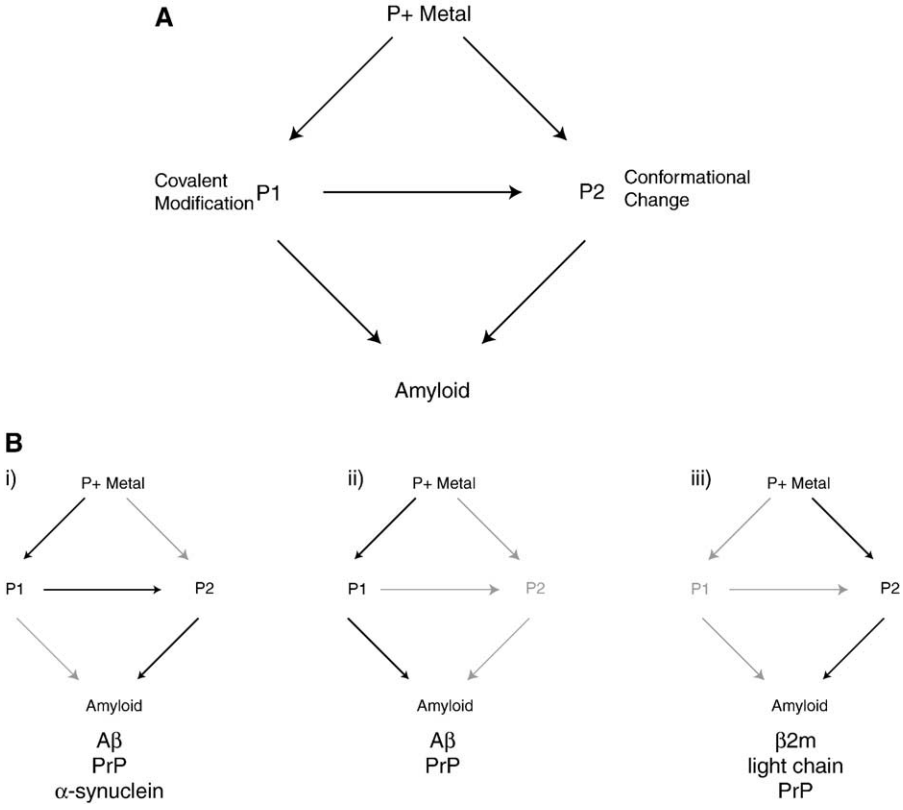

Fig. 1. (A) Simplified model of metal ion associated protein modifications leading to aggregation. Interactions of protein (**P**) and divalent metal, particularly Cu^{2+}, can result in covalent (**P1**) and conformational (**P2**) modifications. The most common covalent modification is protein oxidation as a result of the Cu^{2+} redox chemistry. Cu^{2+} induced conformational changes include the formation of well-defined substructures in unstructured regions and protein oligomerization. (B) Three pathways of metal ion associated modifications promoting amyloidosis are highlighted in black. Amyloidogenic proteins that undergo the indicated modifications to form aggregates are listed below the mechanism.