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Amyloid fibril formation by macrophage migration inhibitory factor ☆

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Abstract

We demonstrate herein that human macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine expressed in the brain and not previously considered to be amyloidogenic, forms amyloid fibrils similar to those derived from the disease associated amyloidogenic proteins β-amyloid and α-synuclein. Acid denaturing conditions were found to readily induce MIF to undergo amyloid fibril formation. MIF aggregates to form amyloid-like structures with a morphology that is highly dependent on pH. The mechanism of MIF amyloid formation was probed by electron microscopy, turbidity, Thioflavin T binding, circular dichroism spectroscopy, and analytical ultracentrifugation. The fibrillar structures formed by MIF bind Congo red and exhibit the characteristic green birefringence under polarized light. These results are consistent with the notion that amyloid fibril formation is not an exclusive property of a select group of amyloidogenic proteins, and contribute to a better understanding of the factors which govern protein conformational changes and amyloid fibril formation in vivo.

Keywords: Amyloid; Amyloid fibrils; Alzheimer's disease; Acid denaturation; Macrophage; Migration Inhibitory Factor; Cytokine; P53; Apoptosis; Sedimentation velocity; Electron microscopy

Amyloidogenic proteins undergo a conformational change either prior to or coincident with their self-assembly into highly ordered fibrils that have a characteristic cross β-structure [1]. The presence of amyloid fibrils surrounding dead neurons in the brain is a hallmark of certain neurodegenerative conditions, including Alzheimer's disease, Parkinson's disease, and Prion diseases. Amyloid formation in tissues can also be a pathological sequelae of many chronic inflammatory diseases [2,3]. Electron microscopic examination of the amyloid fibrils that form in vivo reveals long and unbranching filaments that are typically 10 nm in diameter. These fibrils often are detected in vivo by their ability to bind to the dye Congo red, which produces a characteristic green birefringence when illuminated by a polarized light source. Approximately 20 human proteins have been demonstrated to form amyloid in vivo, and several of these have been linked by genetic evidence with neurodegeneration and/or organ dysfunction [4]. A comparison of the primary sequence or tertiary structure of the 20 amyloidogenic proteins that occur in vivo has revealed no clear homology. Nevertheless, these amyloidogenic proteins are each capable of forming highly ordered fibrils of similar structure as discerned by X-ray fibril diffraction, electron, and atomic force microscopy [5]. The ability of these structurally and functionally diverse proteins to form amyloid fibrils with a common structure [6] is puzzling, and has been explained by the apparent tendency of these proteins to adopt a common, alternative β-sheet rich conformation (amyloidogenic intermediate(s)) that facilitates conversion into a cross-β amyloid structure

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[4,7]. In the case of the "structured" amyloidogenic proteins transthyretin [8] and lysozyme [9] for instance, the formation of amyloidogenic intermediate(s) has been shown to occur via partial denaturation of the native protein. In the case of "unstructured" amyloidogenic proteins such as β-amyloid, and α-synuclein, amyloid fibril formation appears to proceed via partial folding and linked self-assembly [7]. Recent evidence that several non-amyloidogenic proteins can convert into amyloid under the appropriate conditions nevertheless suggests that amyloid fibril formation is a generic property of many proteins [3,4].

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that is highly expressed in many tissues and disease states [10]. Its cellular actions include glucocorticoid counter-regulation [11], sustained MAP kinase activation [12], inhibition of p53-dependent growth arrest [13,14], and control of Jab1 transcriptional effects [15]. There is a significant level of baseline MIF expression in the neurons of the hippocampus as well as in other regions of the brain, and pro-inflammatory stimuli lead to a marked upregulation of neuronal MIF mRNA and protein [16]. MIF's function in the brain is not understood, but its intrinsic tautomerase activity has suggested a possible role in the detoxification of oxidized catecholamines [17]. Interestingly, MIF also has been isolated in association with the Alzheimer's disease, β-amyloid protein [18], which is the main constituent of the fibrils in Alzheimer's disease plaques, thereby supporting an emerging theory of a pro-inflammatory etiology for this neurodegenerative disease.

Human MIF is encoded by a unique gene, and its three-dimensional crystal structure is that of a homotrimer. Each monomer consists of 114 amino acid residues and has a molecular weight of 12,343 Da. As revealed by X-ray crystallography [19,20], the tertiary structure of MIF defines a novel protein fold, which is characterized by the packing of an extended 4-stranded β-sheet and two antiparallel α-helices (Fig. 1A). Three subunits interact via contacts between the β-sheets and wrap completely around to form a symmetrical trimer of a unique α/β structure with a solvent-exposed central channel (Fig. 1B). Although MIF crystallizes as a trimer [19], experimental studies employing NMR spectroscopy [21], size-exclusion chromatography [22], chemical cross-linking [23,24], and analytical ultracentrifugation support the existence of dimeric and monomeric forms in solution [24].

We have observed that partial acid denaturation of recombinant MIF is sufficient to induce amyloid fibril formation. We considered that investigation of the mechanism by which MIF converts from its normally folded, solution form into amyloid fibrils may contribute to a better understanding of the factors which govern protein conformational changes and amyloid fibril formation in vivo. A closer definition of the physicochemical properties of MIF also adds to our comprehension of this mediator's role in immunopathology and neurodegenerative processes.

Material and methods

Protein expression and purification. Recombinant human MIF was expressed in *Escherichia coli* and purified to homogeneity by two successive chromatographic steps as described previously [25]. Buffers used for acid denaturation were 0.05 M phosphate, 0.05 M acetate, and 0.05 M glycine/HCl in the presence of 0.15 M NaCl.

Evaluating secondary structural changes by far-UV circular dichroism. Circular dichroism (CD) spectroscopy was used to evaluate the secondary structural requirements for MIF amyloid fibril formation. The far-UV CD spectra of MIF as a function of pH were recorded on an Aviv Model SF202 spectrometer (25 °C). MIF solutions at a concentration where aggregation does not occur (0.1 and 0.02 mg/mL) and at the desired pH (50 mM acetate or phosphate buffer, 100 mM NaCl) were prepared by dilution of a 0.7–1.0 mg/mL stock solution (10 mM phosphate, 100 mM KCl, and 1 mM EDTA). The CD studies carried out at 0.02 and 0.1 mg/mL were performed using 0.1 cm quartz cuvettes. A step size of 0.2 nm, an averaging time of 3 s, and an average of 15 scans were recorded to generate the data reported in units of mean residue ellipticity. The far- and near-UV CD data were smoothed using a Stineman function (KalidaGraph software), which reduced the noise without perturbing the data.

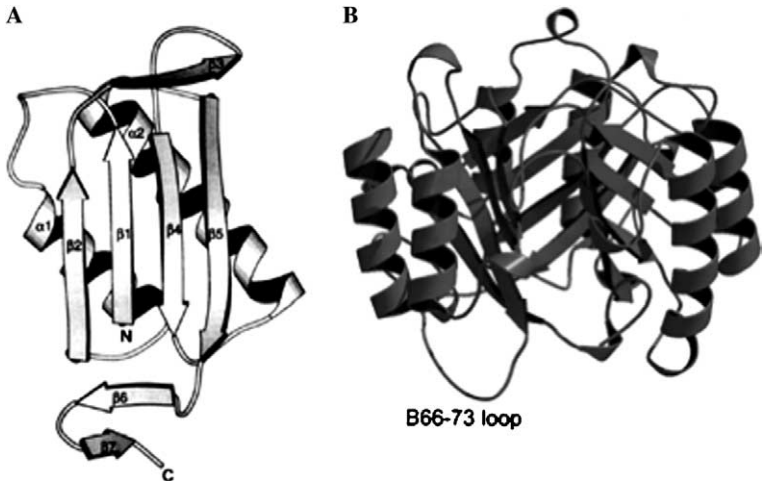

Fig. 1. Ribbon diagram of human macrophage migration inhibitory factor (MIF) monomer (A), and trimer (B).