Molecular Basis of Amyloid Polymorphism: Multiple Misfolding Pathways

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Protein misfolding and amyloid formation is implicated in numerous debilitating human diseases such as Alzheimer’s, Parkinson’s and Prion diseases [1]. More than 40 different types of polypeptides including intrinsically disordered as well as natively folded proteins were shown to be associated with amyloid diseases. Prion protein is unique in that the natively folded protein is able to form infectious aggregates with distinct molecular conformations (prion strains), which are proposed to underlie different disease phenotypes [2,3]. A central theme in the prion hypothesis is that the prion strain is encoded in the primary sequence, specifying amyloid conformation and disease phenotype. Mutations of the protein may induce different prion strains and cause distinct disease phenotypes.

Recent studies have suggested that the prion-like mechanism is applicable to other amyloid diseases that also manifest diverse disease phenotypes [2,4]. It is intriguing that natively folded amyloidogenic proteins can form amyloid with distinct morphologies and molecular conformations depending on aggregation conditions, and the structural diversity may be linked to the phenotype variations of amyloid diseases [2]. Elucidation of the multiple amyloid formation processes leading to distinct amyloid conformations is, therefore, of critical importance in identifying therapeutic targets for the fatal human diseases.

Amyloid formation is a complex process involving a series of steps where several intermediate states are populated (Figure 1). Amyloid formation of a natively folded protein can proceed via multiple misfolding and aggregation pathways, which may lead to diverse amyloid conformations [5,6]. The multiple misfolding pathways and polymorphism of amyloid imply that amyloid formation can result from different patterns of inter-residue interactions. The amyloids with distinct molecular conformations (amyloid strains) may have different toxic activities related to the phenotype diversity of amyloid diseases. Understanding the multiple misfolding and amyloid formation pathways is essential to unraveling molecular mechanism of amyloid polymorphism and phenotype diversity.

In this hypothesis, the kinetic folding intermediate of the amyloidogenic proteins has an inherent aggregation propensity, and the pathogenic mutations alter the rugged landscape for the intermediate state and amyloid.

In order to elucidate the complicated amyloid formation pathway, each step of the conformational transitions needs to be examined, requiring structural characterization of the initial conformational transition and the final product, amyloid (Figure 2). Previous biophysical studies showed that a natively folded protein must undergo a local and/or global unfolding transition to intermediate states in the misfolding pathway [1]. The partly unfolded intermediate is also believed to adopt a dynamic conformational ensemble [7]. The conformers present in the conformational ensemble may self-assemble into amyloid with distinct molecular conformations (Figure 1). Pathogenic mutations may facilitate a common misfolding pathway by lowering the energy barrier and/or by stabilizing a common aggregation competent conformer in the ensemble. In this hypothesis, amyloids derived from mutant forms of the protein would have similar amyloid conformations. On the other hand, the mutations may stabilize a different conformer in the aggregation-prone ensemble, leading to distinct amyloid conformations (Figure 1). Comprehensive structural studies of misfolding and amyloid formation for wild-type and various pathogenic mutant forms would be required to test the amyloid strain hypothesis.

The structural studies of the initial transition from the native state to (partly) unfolded intermediate and the end product amyloid (Figure 2) have, however, been challenging due to the millisecond time scale conformational fluctuation of the amyloidogenic intermediate state and the non-crystalline nature of amyloid. Recent advances in biophysical studies showed that a natively folded protein must...

Figure 1: A plausible schematic diagram of the free-energy landscape for protein folding (black) and amyloid formation (red) and the potential effect of mutation.

Figure 2: Molecular mechanism of amyloid formation and the biophysical techniques to elucidate the molecular basis of amyloid polymorphism.
biophysical techniques made it possible to investigate the complex problems at atomic resolution. For example, relaxation dispersion solution NMR spectroscopy provided the first atomic-resolution structure of misfolding intermediate state [8]. Molecular dynamics simulations and single-molecule spectroscopy will also play critical roles in exploring mechanistic details of misfolding trajectory for a single-molecule protein [9,10]. In addition, high-resolution structures of insoluble amyloid have begun to be revealed by X-ray crystallography of amyloid-like microcrystals formed from small peptides [11,12] and by solid-state NMR spectroscopy [13-15]. The structural information derived by the multiple biophysical techniques combined with protein engineering will provide unprecedented detailed insights into the misfolding and amyloid formation pathways of the natively folded protein, leading to better understanding of amyloid diversity and amyloid strain hypothesis.

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