New and Notable

Amyloid Fibrils: the Eighth Wonder of the World in Protein Folding and Aggregation

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Protein folding, misfolding, and aggregation are among the central topics of molecular biology in the postgenome era. Increasing evidence indicates that protein aggregation plays a vital role in a variety of biological processes. Specifically, fibrillar aggregates, i.e., filamentous assemblies of peptides and proteins that possess a common cross-β sheet structure, have been found in a wide range of organisms, from bacteria to mammals, and have been shown to possess different physiological functions. However, the extensive research on fibrillar aggregates has primarily been driven by the fact that some of these aggregates, known as amyloid fibrils, are found as deposits in the tissues and organs of patients diagnosed with various amyloid-associated diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, prion disease, type-2 diabetes, etc. (1). These maladies can be sporadic, inherited, or even infectious, and misfolded protein aggregates are often considered biomedical markers of these diseases. Despite great interest, a full understanding of the causes of these diseases and the roles of protein oligomers and fibrils has not yet been achieved. According to one hypothesis, an abnormally high local protein concentration in multivesicular bodies results in protein aggregation. These prefibrillar aggregates cause cell death and the release of toxic species into the extracellular space. In this regard, the formation of mature fibrils is considered a defense mechanism, which nature uses to clear these toxic oligomers from the cell media. Therefore, prefibrillar oligomers rather than mature fibrils are thought to be responsible for the progression of neurodegenerative diseases.

Significant knowledge regarding the in vitro formation and structure of amyloid fibrils has been accumulated over the past 60 years. Based on kinetic studies of fibrillation, several mechanisms for this process have been proposed. It is well accepted that fibrillation begins with a thermodynamically unfavorable nucleation step, followed by the rapid elongation of fibrils (2). An understanding of the nucleation mechanism is crucial for developing inhibitors for the limiting stage of the pathological process. Thus far, the proposed nucleation models have been primarily verified by correlations between the fibrillation lag-time or the rate of fibril accumulation and protein concentrations or other experimental conditions, although direct detection of the fibrillation nucleus has been also reported (3). According to the thermodynamic nucleus model for homogeneous nucleation, the nucleus is defined as a metastable species, the formation of which determines the lag-phase of fibrillation.

At first glance, various proteins form fibrillar aggregates with similar morphologies (elongated, unbranched) and a common cross-β sheet structure, despite differences in the proteins' primary sequences, native structures, and physiological activities. However, a more detailed analysis reveals a great variety exhibiting subtle changes in the shape, size, and structure of amyloid fibrils, as well as the fibrillation kinetics, depending on the protein sequence (2). In a recent *Biophysical Journal* article (4), a collaboration between three well-established laboratories in the field, headed by Raleigh, Zanni, and Serrano, demonstrated that mutations cause a great deal of variation in the fibrillation of islet amyloid polypeptides (IAPPs or Amylin). IAPP is a pancreatic hormone that is cosecreted with insulin and forms islet amyloid in type-2 diabetes and leads to β-cell dysfunction and cell death. The formation of islet amyloid is also thought to significantly contribute to the failure of islet cell transplants. Despite its importance, islet amyloid formation is not yet well understood. The authors investigated the role of the C-terminus of IAPP, particularly the interaction of Tyr-37 with His-18, during amyloid formation to evaluate a number of hypothetical mechanisms reported in the literature for early oligomerization of IAPP.

Previous NMR studies proposed the formation of a head-to-tail IAPP dimer generated by stacking of the side chains of His-18 and Tyr-37. However, this model was developed based on an IAPP variant with a free C-terminus at low pH. New data reported by Tu et al. (4) indicate that even if such interactions occur in the physiologically relevant amidated form of IAPP (at physiological pH), they do not promote amyloid formation. Seeding experiments were used to investigate the structural similarity and dissimilarity between fibrils prepared from different IAPP variants, including those with an amide group at the C-terminus replaced with a carboxylate and His-18 replaced with the neutral residues Gln or Leu. The obtained results illustrated the importance of the C-terminus and strongly suggest the presence of differences in the fibril structure formed by amidated and unamidated IAPP. Thus, one may wonder that simply replacing an amide group at the C-terminus with a carboxylate would be more effective than replacing His-18 with residues that differ significantly in shape and polarity (4).

It has been well documented in the literature that changing the state of

Submitted January 6, 2014, and accepted for publication February 4, 2014.

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Editor: David Eliezer.

0006-3495/14/04/1433/3 $2.00

http://dx.doi.org/10.1016/j.bpj.2014.02.007

disulfide bonds in proteins by reduction or methylation results in significant variations in the aggregation kinetics, as well as the structure and morphology of the resultant fibrils. It is thought that disulfide bonds limit the manner in which a protein can aggregate into a fibril via steric restraint. From this perspective, a recent study of disulfide bond behavior during insulin fibrillation revealed somewhat unexpected results (5). All three disulfide bonds of native insulin remained intact during the aggregation process, withstanding scrambling. Moreover, the predominant *gauche-gauche-gauche* (g-g-g) conformation of the C-C-S-S-C-C segment is identical for all disulfide bonds in fibrils and the native protein, as evident from Raman spectroscopic measurements. The possibility of preserving disulfide bonds in their original conformation despite significant structural rearrangements of the protein during the fibrillation process was illustrated by all-atom molecular-dynamic simulations.

In addition to the protein sequence, variations in aggregation conditions often result in significant changes in fibrils, a phenomenon known as "fibril polymorphism". This display of functional and structural heterogeneity in amyloid fibrils is particularly important because different polymorphs often exhibit different biological activity and associated toxicity. Surprisingly small changes in conditions can generate different fibril polymorphs from the same protein. For example, two amyloid states with distinct morphologies were prepared under identical solvent conditions but with different shaking modes using the same pool of highly pure, full-length Syrian hamster recombinant prion protein (6). The cross-β cores of these polymorphs exhibited similar crystalline-like structures, but the interstrand hydrogen bonds were of different strengths.

According to a 2012 article in *Biophysical Journal* (7), an increase in the solution pH by only one unit during the aggregation process results in significant variations in the morphology of insulin fibrils. Tapelike individual and binary fibrils form at pH 1.5 whereas insulin fibrils with a strong left-handed twist dominate at pH 2.5. Unexpectedly, these distinct fibril polymorphs displayed opposing supramolecular chiralities, as determined by vibrational circular dichroism (VCD) measurements (7). VCD supramolecular chirality is correlated with the apparent fibril handedness and provides a sense of chirality from a deeper level of chiral organization, at the protofilament level of the fibril structure. Specifically, normal VCD fibrils have a left-handed twist, whereas reversed VCD fibrils are flat-like aggregates with no obvious helical twist, as evident from atomic force and scanning electron microscopy.

Although the vast majority of accumulated knowledge is associated with either bulk (overall) fibrils or fibril core structures, the fibril surface is expected to play a significant role in amyloid biological activity and toxicity. Tip-enhanced Raman spectroscopy is uniquely suitable for structural characterization of the fibril surface. The surfaces of two insulin fibril polymorphs with tapelike and left-handed twist morphology (as mentioned above) were observed by tip-enhanced Raman spectroscopy to differ substantially in both amino-acid composition and protein secondary structure (8). The amounts of Tyr, Pro, and His differ, as does the number of carboxyl groups on the respective surfaces, whereas the amounts of Phe and of positively charged amino and imino groups remain similar. In addition, the surfaces of protofilaments, the precursors of mature fibrils, exhibit substantial differences with respect to mature fibrils.

It is well accepted that amyloid fibrils are extraordinarily stable and represent the most energetically favorable form of proteins. Typically, harsh denaturing conditions are required to dis-integrate fibrils prepared from full-length proteins, and further fibrillation is not expected to occur under these conditions. We recently discovered that mild temperature and salinity variations result in substantial melting of the fibril core of mature apo-α-lactalbumin fibrils and spontaneous refolding into a different fibril polymorph (9). This spontaneous refolding, which occurs without the disintegration of individual fibrils, has potential disease relevance and is of fundamental significance to biology in general as a "new type of protein folding-aggregation phenomenon" (9). Understanding the mechanism of this process could allow regulation of the biological activity of fibrils and their associated toxicity. Spontaneous refolding is not limited to a single amyloid system or one type of solution change. A small pH change can initiate the spontaneous transformation of insulin fibrils from one polymorph to another, resulting in switching of fibril supramolecular chirality along with both morphological and structural changes (10).

This brief commentary merely scratches the surface of the broad and intriguing field of amyloid fibrillation. The main goal of this commentary is to illustrate the great variety of factors affecting amyloid morphology, properties, and formation mechanisms. Inspired by recent advances in the field, as exemplified by the exciting work of Tu et al. (4), who demonstrated the significant consequences of Amylin sequence variations, researchers have found many solution factors that affect in vitro fibrillation. Although significant knowledge about amyloid fibrils has accumulated over the past 60 years, further study is required to improve our understanding of the biophysics of the fibrillation process to predict fibril structure and properties based on the underlying protein sequence and aggregation conditions. The great importance of amyloid fibrils for biology and medicine and the multitude of fibril variations in response to changes in conditions and protein sequences make us wonder.

This project is supported by award No. R01AG033719 from the National Institutes of Health.

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