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Structure and Intermolecular Dynamics of Aggregates Populated during Amyloid Fibril Formation Studied by Hydrogen/Deuterium Exchange

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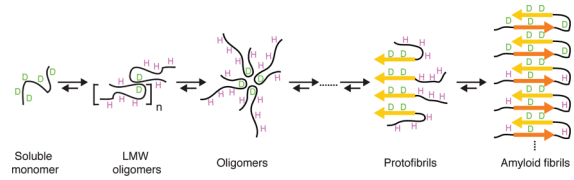

The aggregation of proteins into amyloid fibrils is a complex and fascinating process associated with debilitating clinical disorders such as Alzheimer's and Parkinson's diseases. The process of aggregation involves a series of steps during which many intermediate aggregation states are populated. Recent evidence points to these intermediate states as the toxic moieties primarily responsible for cell damage or cell death, which are critical steps in the origin and progression of these disorders. To understand the molecular basis of these diseases, it is crucial to investigate and define the details of the aggregation process, and to achieve this objective, researchers need the tools to characterize the structure and kinetics of interconversion of the various species present during amyloid fibril formation. Hydrogen−deuterium (HD) exchange experiments are based on solvent accessibilities and provide one means by which this kind of information may be acquired. In this Account, we describe research based on HD exchange processes that is directed toward better understanding the dynamics and structural reorganizations involved in the formation of amyloid fibrils.

Amide hydrogens that normally undergo rapid exchange with solvent hydrogens experience much slower exchange when involved in H-bonded structures or when sterically inaccessible to the solvent. The rates of exchange can be monitored by replacing some hydrogens with deuterons. When peptide and protein molecules assemble into amyloid fibrils, the fibrils contain a core region based on repetitive arrays of β-sheets oriented parallel to the fibril axis. HD experiments have been applied extensively to map such structures in different amyloid fibril systems. By an extension of this approach, we have observed that HD exchange can be governed by a mechanism through which molecules making up the fibrils are continuously dissolving and reforming, revealing that amyloid fibrils are not static but dynamic structures. Under such circumstances, the kinetic parameters that define this "recycling" behavior can be determined, and they contain information that could be of significant value in the design of therapeutic strategies directed against amyloid-related diseases. More recently, to gain insights into the variety of intermediates that are thought to be involved in the aggregation process, we have applied a kinetic pulse labeling HD experiment that is able to characterize such species even if they are only transiently populated. Using this approach, we have been able to obtain structural insights into the different aggregates populated during the process of amyloid fibril formation as well as kinetic and mechanistic information on the structural reorganizations that take place during aggregation.

HD exchange experiments, when carefully designed, constitute powerful tools for mapping the core structures of amyloid fibrils, for investigating the recycling of fibril components, and for characterizing the various types of structural reorganization that occur during aggregation. Such information is invaluable for understanding and addressing the molecular origins of the increasingly common and highly debilitating diseases associated with protein misfolding and aggregation.

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TABLE 1. HD Experiments To Study Protein Aggregation[a]

aggregate component	aggregation state	disaggregation and quenching buffer	technique used to evaluate HD
Aβ40	fibrils[16] and protofibrils[28]	50:50:0.2 H_2O/ACN/FA	MS
Aβ42	fibrils[18]	99.9:0.1 DMSO-d_6/TFA-d_1	NMR
Aβ42	fibrils[24]	80:20 HFIP/D_2O at pH* 2.6	NMR
Aβ40	early aggregates[30]	2.5:22.5:71.2:3.8 H_2O/D_2O/DMSO/DCA	MS
α-synuclein	fibrils[22]	4 M GdmSCN at pH 2.3	MS
106−126 prion protein fragment	fibrils[21]	95:4.5:0.5 DMSO-d_6/D_2O/DCA-d_2 at pH* 5	NMR
Het-S prion	fibrils[23]	99.9:0.1 DMSO-d_6/TFA-d_1	NMR
human prion protein	fibrils[25]	7.2 M GdnHCl at pH 2.5	MS
β2-microglobulin	fibrils[16] and immature filaments[26]	95:5 DMSO-d_6/D_2O at pH* 5 adjusted with DCA-d_2	NMR
PI3-SH3	fibrils[19] and aggregates populated during fibril formation[20]	95:5 DMSO-d_6/D_2O at pH* 4.2 adjusted with DCA-d_2	NMR and MS

[a] Abbreviations: MS, mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; H_2O, water; ACN, acetonitrile; FA, formic acid; DMSO-d_6, deuterated dimethyl sulfoxide; D_2O, deuterium oxide; DCA-d_2, deuterated dichloroacetate; GdmSCN, guanidinium thiocyanate; HFIP, hexafluoroisopropanol; GdnHCl, guanidinium hydrochloride.

Introduction

The deposition of misfolded peptides and proteins as amyloid fibrils is the hallmark of many disorders such as Alzheimer's, Parkinson's and Huntington's diseases, and type II diabetes.[1] The formation of amyloid fibrils involves a multistep process that is thought to start with the aggregation of monomeric protein molecules into transient oligomeric species that can grow and reorganize into characteristic long, unbranched, and often twisted fibrillar structures.[1] Amyloid diseases are characterized by the accumulation in a variety of tissues and organs of such fibrillar species, which were initially thought to be the direct cause of pathogenesis.[2] But in some disorders, notably neurodegenerative conditions such as Alzheimer's disease, the correlation between the quantities of amyloid fibrils present in the deposits and the severity of the disease is relatively weak.[3] Over the past decade, however, data have emerged suggesting that oligomeric, prefibrillar assemblies rather than mature fibrils are the species responsible for the origin and progression of these diseases as a result of their intrinsic cellular toxicity.[4−6] Moreover, recent data suggest that fibrils could represent a source of toxic oligomeric species that give rise to disease.[7] A crucial aspect to understand misfolding disorders is therefore to characterize the different species present during aggregation, their mechanism of formation and their kinetics of interconversion.

The mature fibrils are the easiest to characterize because of their long-lived nature and high degree of order. Thus, X-ray fiber diffraction data have revealed that significant regions of peptide and protein molecules within amyloid fibrils adopt a cross β-sheet structure, comprised of β-strands running approximately perpendicular to the fibril axis and with the backbone hydrogen bonds linking the β-strands into sheets being oriented essentially parallel to the fibril axis.[8] Solid-state nuclear magnetic resonance (NMR)[9] and electron paramagnetic resonance (EPR)[10] techniques are providing residue-specific information on the regions of the peptide and protein molecules that are organized into the β-strands, turns, and loops that make up the complete amyloid fibrils. Together with evidence from other biochemical or biophysical measurements, these approaches provide great promise for detailed structural models of amyloid fibrils. Smaller aggregates, often denoted as oligomeric or prefibrillar species are, however, much more difficult to characterize because they are often transient and short-lived, usually highly heterogeneous, and frequently present at low concentrations. Despite these problems, several techniques such as mass spectrometry,[11] photoinduced cross-linking of unmodified proteins (PICUP),[12] two-color single-molecule fluorescence spectroscopy,[13] pyrene labeling,[14] and ion-mobility mass spectrometry (IM-MS)[15] have provided information on their molecular nature.

An approach that is proving greatly valuable in providing insights into structural, mechanistic, and dynamic aspects of amyloid fibril formation involves the exploitation of hydrogen−deuterium exchange (HD) experiments.[16−20] These experiments are generally based on the measurement of the solvent accessibilities of the amide hydrogens within these structures. In the absence of structure, amide hydrogens undergo rapid exchange with the hydrogen atoms of solvent water, but if they are involved in hydrogen bonds or if they are inaccessible to solvent as a result of being incorporated within stable elements of structure, they experience much slower exchange. Since the core structures of amyloid fibrils are stabilized by networks of hydrogen bonds, HD experiments are an extremely powerful tool for mapping the core structures of amyloid fibrils[16−18,21−25] (Table 1). The experimental approach used in such studies has recently been reviewed,[26,27] and we refer the reader to these articles for further information. These types of experiments have also been used to define the most protected regions of intermediates in

the aggregation process, such as clusters of stabilized Aβ40 protofibrils[28] and immature filaments of β_2-microglobulin.[29]

In this Account, we focus, on recent studies in our laboratories that are designed to probe the intermolecular dynamics of amyloid fibrils and to investigate the structures of the variety of species populated during the aggregation process through which fibrils are generated. In these initial studies, we have focused on the SH3 domain of the α-subunit of bovine phosphatidylinositol-3′-kinase (PI3-SH3). Although this protein is not involved in any known disease, it forms highly ordered fibrils *in vitro*, particularly at low pH.[31] These fibrils and their precursors show structural and cytotoxic properties that are closely similar to those observed in many depositional disorders.[4] PI3-SH3 is therefore a powerful model of the generic process of amyloid formation, and the experimental procedures developed in studies of this model system should be widely applicable to other peptide and protein systems, including those directly associated with disease.

HD Exchange Protocol

Pioneering HD exchange studies designed to map the core structure of amyloid fibrils[16,17] constitute the basis of the protocols described in this Account. We have, however, used these general strategies to study both equilibrium[19] and kinetic[20] aspects of protein aggregation, and in addition, we have combined the results of two complementary techniques for monitoring the HD exchange process, namely, NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS)[32] (Table 1). Thus, for example, $^{1}H-^{15}N$ heteronuclear single quantum coherence (HSQC) NMR spectra under optimal conditions contain resolvable signals for each amide hydrogen in the polypeptide sequence, and when the protein is immersed in D_2O solvent, analysis of the deuterium content at a specific site is based simply on the decrease in intensity or absence of signal of the hydrogen (H) that has exchanged with a deuterium (D). MS analysis of the deuterium content of a protein, however, is based on the fact that the masses of a proton and a deuteron differ by one unit; thus, the replacement of hydrogens by deuterons in the polypeptide chain will be reflected by an increase in the total mass of the protein. Moreover, if different populations of protein molecules exchange to different degrees, MS will reflect this behavior by the appearance of different peaks in the mass spectrum.

Amyloid fibrils and their relatives are too large and often too heterogeneous for direct analysis by either solution NMR or conventional ESI-MS. An important step forward was the recognition that amyloid fibrils could be dissolved. It is essential, of course, to preserve the isotopic distribution in this dissociation step, and this objective can be achieved by using a solvent that dissolves the fibrils efficiently but in which HD exchange of even exposed amide hydrogens is very slow. Several solvents have been described to achieve this aim (Table 1), and in our studies we have used a mixture of 95% DMSO-d_6 and 5% D_2O, adjusted to pH* 4.2 with dichloroacetic acid-d_3.[17] With this approach, NMR measurements allow the HD occupancy of individually defined amide hydrogens to be obtained on a residue-specific basis, averaged over the distribution of protein molecules, and ESI-MS allows detection and characterization of distinct populations of protein molecules within a given sample that have different degrees of exchange. By combination of the two techniques, therefore, it is possible to obtain structural information concerning distinct species even when they exist simultaneously in solution. This approach was first used to characterize the distribution of intermediates populated at different times during lysozyme folding,[32] but we have found that it is equally applicable for dissecting the process of protein aggregation.

Studying Intermolecular Dynamics within Amyloid Fibrils by HD Exchange

HD exchange experiments can provide important information about the intermolecular dynamics of the molecular components of amyloid fibrils.[19] Such experiments are carried out in a similar manner to those for mapping the core structures of the fibrils[16−18,21−25] but employing extended labeling times, Δt_{label}, of up to several weeks (Figure 1). We have used this approach to probe the nature of PI3-SH3 molecules within amyloid fibrils. Analysis of the NMR data reveals that longer Δt_{label} times lead to a decrease in the extent of protection, although the overall protection profile is similar at the different time points studied (Figure 2A). Residues at the C-terminus were found to have exchanged to a significantly greater degree than the average of the other residues, but overall the data indicate that the degree of exchange of each of the residues in the sequence is very similar. This finding is contrary to the expectation that exchange results from the differential exposure to solvent of residues within the fibrils, and therefore implies that a different mechanism other than simple exposure is responsible for the HD exchange behavior.

To gain further insight into the nature of the mechanism that leads to these observations, the samples investigated by NMR were also analyzed by ESI-MS in order to probe the relative populations of PI3-SH3 molecules with different degrees of exchange. Mass spectra of each fibril sample obtained after different labeling times, Δt_{label}, show in each case two extremely well-resolved peaks (Figure 2B), a result that indi-

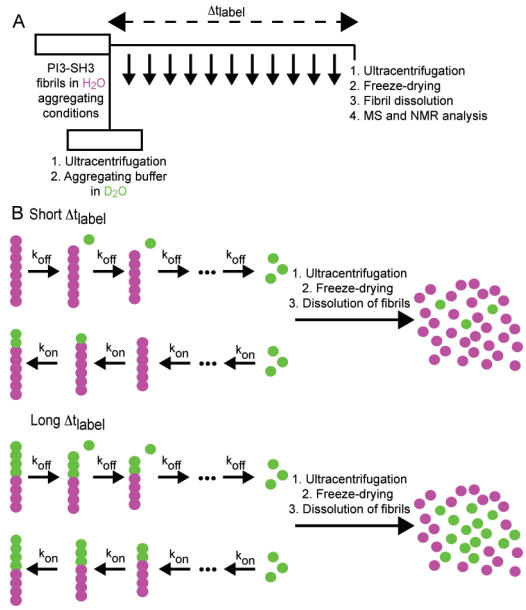

FIGURE 1. Schematic description of the equilibrium HD experiment designed to study molecular recycling: (A) Protonated amyloid fibrils are exposed to deuterated buffer for varying periods of time, Δt_{label}. After the labeling pulse, the solution is ultracentrifuged to remove any soluble protein, and the pelleted fibrils are freeze-dried to quench exchange. Amyloid fibrils are later solubilized into monomers by transfer to a DMSO solution and analyzed by NMR and ESI-MS. (B) Schematic representation of the recycling mechanism for a distribution of amyloid fibrils at different times of exchange. At a given time point, the dissociation of a molecule from a fibril (above) is counteracted by the reassociation of that molecule in another fibril (below). Following fibril dissolution, two populations of molecules are found in solution, those corresponding to the population of molecules that has not yet dissociated from the fibrils (pink circles), and those representing the population that has dissociated from the fibrils and then been reincorporated after complete exchange (green circles).

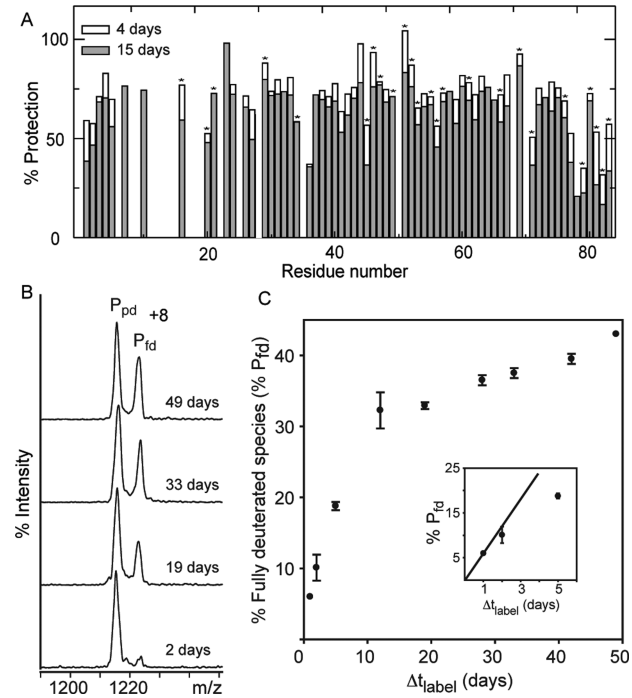

FIGURE 2. Molecular recycling within PI3-SH3 amyloid fibrils using the protocol described in Figure 1: (A) Protection profile for PI3-SH3 amyloid fibrils obtained by NMR after 4 (white) and 15 (gray) days of Δt_{label}. The bars represent the percentage of protection for each residue. A value of 0% protection for a particular residue indicates that its amide backbone hydrogen has undergone complete HD exchange during the labeling time, Δt_{label}, and is thus located in a region of structure that does not provide any measurable protection from solvent interactions. A value of 100% indicates that the amide hydrogen has not exchanged at all, and it is thus located in a region of structure able to offer a high degree of protection. An asterisk above a bar indicates a residue whose resonance is not fully resolved; the absence of a bar indicates that the resonance of the residue is not detectable except for residues 50, 70, and 84, which are prolines. (B) ESI-MS (8+ charge state) showing the relative populations of the peaks corresponding to partially deuterated, P_{pd}, and fully deuterated, P_{fd}, species after exchange for different Δt_{label}. The spectral intensities are all plotted relative to the same intensity of the P_{pd} 8+ charge state. (C) Plot of the relative fraction of P_{fd} molecules in the sample as a function of Δt_{label}. Error bars are standard deviations resulting from duplicate samples. Insert shows how the initial slope of the plot is used to get an estimate of k_{off}. Adapted from ref 19.

cates unambiguously that two distinct isotopically labeled populations of PI3-SH3 are present within the fibrils. Analysis of the data shows that the lower mass peak represents a population of partially deuterated molecules with a set of backbone amides that are highly resistant to exchange. The mass of the other peak indicates the presence of a population of molecules in which almost complete exchange has occurred and is therefore denoted fully deuterated. Importantly, as Δt_{label} increases, the intensity of the fully deuterated species increases relative to that of the partially deuterated species (Figure 2B,C).

These data suggest a mechanism of exchange in which protein molecules dissociate from the fibrils with a rate constant k_{off} and, once in solution, hydrogen exchange takes place with a rate constant k_{ex}. The protein molecules are then reincorporated into the fibrils, with a rate constant k_{on} (Figure 1B). Assuming that $k_{ex} \gg k_{on}$, two peaks will then be observed by ESI-MS: one partially deuterated population that results from molecules that have not yet dissociated from the fibrils, and a second fully deuterated population that represents those molecules that have dissociated from the fibrils, and then been reincorporated after essentially complete exchange. On this model, in the initial stages of the exchange reaction all the molecules that dissociate from the fibrils will undergo isotope exchange. The initial slope of a plot of the population of fully deuterated species against the exchange time represents an estimate of k_{off} (Figure 2C).

In summary, this form of HD experiment has shown that molecules within an ensemble of amyloid fibrils are in continuous dynamic equilibrium between the fibrils and the solution. Data consistent with this mechanism have also been observed for fibrils formed by the 40 residue amyloid-β peptide, Aβ40.[16] The ability to observe the recycling behavior of amyloid fibrils provides an opportunity for obtaining quantitative estimates of the rate constants that determine fibril dissolution and hence allows the factors that determine such properties to be investigated. These factors can be of fundamental significance in understanding a range of important aspects of amyloid phenomena including differences between the behavior of variants of peptides or proteins that relate to their different roles in the disease process. For example, amyloid fibrils formed by the Aβ variants of 40 and 42 residues, Aβ40 and Aβ42, have different recycling properties, which has important implications in defining the properties of the amyloid fibrils formed by each variant and therefore in the design of therapeutic strategies (L. Sánchez, S. Madurga, T. Pukala, M. Vilaseca, C. López-Iglesias, C. V. Robinson, E. Giralt, N. Carulla; manuscript in preparation). Amyloid fibrils have been suggested to be a potential reservoir of toxic oligomers.[7] In this context, an important implication of the recycling process is the nature of the recycling species. The recycling process is also likely to influence the production and behavior of novel biomaterials where the readiness or resistance of the fibrils to dissolve is likely to be a key factor in determining their value for a given application. Thus, amyloid fibrils have been shown to be useful in the formulation of long-acting drugs that rely on the slow release of peptide molecules, which primarily occurred from the fibril ends.[33]

HD Exchange Studies on the Kinetics and Mechanism of Peptide and Protein Aggregation

The HD exchange experiments described so far have been extremely valuable for characterizing "long-lived" species associated with peptide and protein aggregation. To gain insights into transient forms of protein aggregates, including oligomeric species that are the most likely origin of toxicity in amyloid disease, we have applied a kinetic pulse-labeling strategy that enables transient species populated during aggregation to be probed[20] (Figure 3). In designing such a procedure, we were inspired by pulse-labeling HD experiments widely used to detect and characterize intermediates that are populated during protein folding.[32]

To discover the value of this type of pulse-labeling experiment for characterizing the process of aggregation, we have

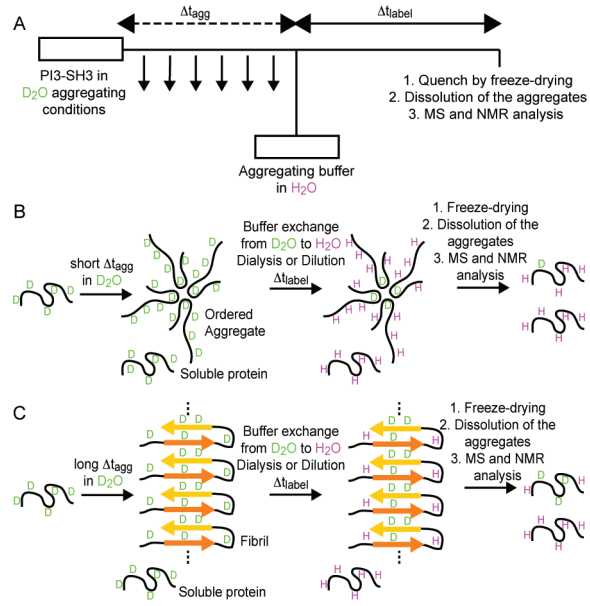

FIGURE 3. Schematic description of the pulse-labeling HD exchange experiment developed to study protein aggregation: (A) Soluble protein is incubated under aggregation conditions in a deuterium-based buffer. After a variable aggregation time, Δt_{agg}, labeling takes place for a fixed period of time, Δt_{label}, using protonated aggregation buffer. The magnitude of Δt_{label} is chosen so that only unprotected amide deuterons will exchange significantly with the solvent. After the labeling pulse, freeze-drying is used to quench exchange. Different samples are prepared at defined Δt_{agg}, which are later solubilized into monomers by transfer to a DMSO solution and analyzed by NMR and ESI-MS. The figure illustrates hypothetical scenarios when the protein is left to aggregate for (B) a short Δt_{agg} and (C) a long Δt_{agg}. Adapted from ref 20.

studied the events occurring during the aggregation of PI3-SH3 at pH 1.5, conditions that favor protofibrillar-like intermediates, over a period of 21 days.[20] ESI-MS analysis reveals that aggregation occurs as a multistate process with multiple species appearing, coexisting, and decaying with time (Figure 4A). Species coexisting for significant periods of time, described as early (from 0 to 4 days), intermediate (between 6 and 10 days), and late (after 13 days) aggregation times, are grouped together and are indicated, respectively, by a green, blue, and orange band in Figure 4A. Of particular interest is the conformation specific antibody named A11, which interacts with soluble oligomers from many types of peptides and proteins, regardless of sequence.[5] In agreement with the MS classification, A11 recognizes the PI3-SH3 intermediate species populated between 6 and 10 days, but not their precursors or the subsequent mature fibrils (Figure 4B). NMR spectroscopy of samples identical to those used for the ESI-MS analysis provides us with the average fractional exchange on a residue-by-residue basis. After multilinear regression analysis using the populations obtained by ESI-MS and the ave-

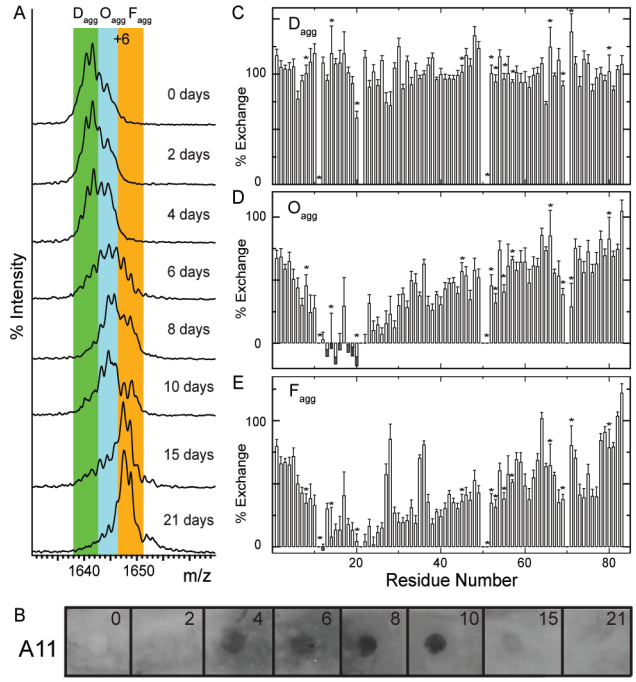

FIGURE 4. Characterization of PI3-SH3 aggregates populated during the process of amyloid fibril formation using the strategy described in Figure 3. Analysis of PI3-SH3 pulse labeled samples obtained at different Δt_{agg} times while being incubated under protofibrillar conditions. (A) ESI mass spectra (+6 charge state) showing the relative populations of D_{agg} (green band), O_{agg} (blue band), and F_{agg} (orange band) species at the indicated Δt_{agg} times. (B) Kinetics of oligomer-specific immunoreactivity. At the times indicated, aliquots were applied to a nitrocellulose membrane and probed with the A11 antibody, which is specific for oligomeric amyloid species. (C−E) Exchange profile for aggregates populated under protofibrillar conditions: (C) D_{agg}; (D) O_{agg}; and (E) F_{agg}. The exchange profiles are obtained by multilinear regression analysis combining the NMR data of samples prepared after Δt_{agg} equal to 0, 2, 6, 10, 13, and 15 days and the distribution of populations obtained by ESI-MS. The bars represent the percentage of exchange for each residue. A value of 100% exchange for a particular residue indicates that its amide backbone hydrogen has undergone complete HD exchange during the labeling time, Δt_{label}, and is thus located in a region of structure that does not provide any measurable protection from solvent interactions. A value of 0% indicates that the amide hydrogen has not exchanged at all and it is thus located in a region of structure able to offer a high degree of protection. An asterisk above a bar indicates a residue whose resonance is not fully resolved; the absence of a bar indicates that the resonance of the residue is not detectable except for residues 50, 70, and 84, which are prolines. Adapted from ref 20.

rage residue-specific information gained from NMR data, we derived the exchange profiles for each of the three groups of species (Figure 4C−E). These profiles reveal that protein molecules corresponding to the first set of peaks (green band in Figure 4A) have exchanged almost completely (Figure 4C), indicating that these species represent aggregates lacking persistent structure, and we denote them disordered aggregates, D_{agg}. The exchange profiles for the second set of species (blue band in Figure 4A) reveal that a stretch of residues between Tyr12 and Ile22 are highly protected from exchange while the amides of the remainder of the residues are increasingly unprotected as their positions in the sequence become more distant from this most protected region (Figure 4D). These results, discussed in more detail below, indicate that the initially disordered aggregates have evolved to form conformations where some residues are protected significantly, and residues Tyr12 to Ile22 are persistently structured.

The third set of species (orange band in Figure 4A) is attributed to mature fibrils, F_{agg}, since these peaks correspond to species where amides are highly protected and electron micrographs show the presence of well-defined fibrils.[20] Its deconvoluted exchange profile reveals that the most protected region again comprises residues Tyr12 to Leu26, as with the intermediates, but that further structural consolidation and reorganization has taken place (Figure 4E). Thus, in the fibrillar state, additional regions of the protein have become more highly protected (e.g., residues Tyr73 to Ile77). Importantly, however, several residues have become more accessible to solvent (e.g., residues Gly27 and Glu28, Gly35 and Ser36, and Gly64) compared with the intermediate species (Figure 4D). It is particularly interesting that the residues that become more exposed in the fibrillar state are polar residues or glycines, implying that within the amyloid fibril structure, these are located in surface or turn positions. Although we cannot rule out the possibility that the intermediate species form off-pathway to fibril formation, the fact that the most protected residues in the intermediate species are also the most protected ones in the fibrils suggests that these intermediates are likely to be species that progress to fibril formation. In addition, the exposure of polar residues in the fibrils will reduce the overall hydrophobicity of the surface, a factor likely to contribute, along with reduced surface-to-volume ratios, to the decrease in toxicity of the fibrils relative to their precursors.[14,34]

In summary, the results for PI3-SH3 reveal that the pulse-labeling experiment is an extremely powerful method for obtaining information on the mechanism of aggregation and on the structural reorganizations that are an inherent part of the process of amyloid formation. Indeed, the present data provide residue-specific information that is consistent with the nucleated conformational conversion (NCC) mechanism of aggregation.[35] On this model, a group of monomers present in solution initially coalesces to form amorphous oligomers. These oligomers then convert into more highly organized oligomers and then fibrils rich in β-sheet structure. Interestingly, computer simulations of the early stages of amyloid forma-

tion reveal that the structural reorganization of the oligomers formed rapidly by hydrophobic collapse results in the formation of ordered oligomeric species with a greater proportion of exposed hydrophobic residues, a process that is likely to be a major determinant of their toxic properties.[14,34] Up to the present time, however, a description at the molecular level of such a structural reorganization has remained elusive because of the challenge in describing the early stages of aggregation by experiment; nevertheless, methodologies for this purpose are emerging,[13] and we can be optimistic that further progress will be made. The pulse-labeling experiment analyzed by ESI-MS and NMR can, however, provide direct experimental evidence at the molecular level of the structural reorganizations occurring during aggregation, therefore representing a significant advance in understanding the nature and origin of amyloid diseases.

Future Perspectives

HD experiments are widely used in studies of protein structure and dynamics, and progressive improvements in MS and NMR techniques are taking place that will undoubtedly increase the power of its application to studies of protein misfolding and aggregation. Improving HD analysis by MS entails obtaining protection data at near single-residue resolution, for example, quenching in a DMSO-based buffer and using gas-phase fragmentation protocols that avoid problems with scrambling of the label.[36−39] Improving HD analysis by NMR involves the development of more sensitive spectrometers and probes and the design of faster and more efficient NMR experiments.[40] More generally, the complex nature of amyloid formation means that increased emphasis on multidisciplinary approaches will be of great importance, and a combination of the HD methodologies described here with other approaches, for example, single-molecule studies,[13] theoretical simulations,[34] and biochemical techniques,[5] will result in increasingly powerful methods for obtaining detailed models of the amyloid assembly process.

The structure of aggregates populated during amyloid fibril formation and the recycling of molecular species have been probed with a model system, the PI3-SH3, using carefully designed HD exchange experiments. The time is now ripe for application of such HD exchange approaches to other peptide and protein systems, particularly those associated with important medical disorders, and this approach will undoubtedly enhance our understanding of the molecular events underlying aggregation and their relationship to the origins of protein misfolding diseases. Moreover, as well as examining the aggregates in isolation, the methods allow exploration of the effects of other molecular species on the aggregating system; extension of these approaches to address such issues will undoubtedly have significant biological and pharmaceutical importance.

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BIOGRAPHICAL INFORMATION

Natàlia Carulla received her B.Sc. in Organic Chemistry in 1996 from the University of Barcelona. In 2001, she obtained her Ph.D. at the University of Minnesota. The same year, she moved to the University of Cambridge for postdoctoral research. In 2004, she returned to Barcelona at the Institute for Research in Biomedicine, first as a postdoctoral fellow and since 2006 with a tenure-track ICREA research position. Her research interests focus on characterizing the process of amyloid fibril formation with particular emphasis on the amyloid β (Aβ) protein associated with Alzheimer's disease.

Min Zhou obtained her B.Sc. and M.Sc. in Biochemistry from Nanjing University in China. In 2001, she moved to the University of Cambridge where she first obtained her Ph.D. degree in 2005 and later worked as a postdoctoral fellow. In 2009, she was appointed manager of the Mass Spectrometry Facility in the Chemistry Department of the University of Cambridge and is particularly involved in collaborative research programs to characterize noncovalent protein complexes and to explore the application of mass spectrometry in proteomics.

Ernest Giralt received his Ph.D. in 1974 from the University of Barcelona. After postdoctoral work at the University of Montpellier, he returned to the University of Barcelona as an Assistant Professor, and in 1986, he was promoted to Full Professor. In 2000, he moved to the Institute for Research in Biomedicine as Director of the Chemistry and Molecular Pharmacology Programme. His major research interests lie in the fields of peptide synthesis, molecular recognition, and structure determination, in particular using NMR spectroscopy.

Carol V. Robinson earned her Ph.D. from the University of Cambridge in 1982, and in 1995, she was awarded a Royal Society University Research Fellowship to work in the Centre for Molecular Sciences at the University of Oxford. In 2001, she was appointed Professor of Mass Spectrometry in the Department of Chemistry at the University of Cambridge, and in 2009, she returned to Oxford as Dr. Lee's Professor of Chemistry. Her current research interests lie in determining protein interaction networks and more generally in studying the properties of protein complexes in the gas phase.

Christopher M. Dobson received his doctorate from the University of Oxford in 1976, and in 1977, he moved to Harvard University as an Assistant Professor of Chemistry. In 1980, he returned to the University of Oxford first as a Lecturer and later as Professor and Director of the Oxford Centre for Molecular Sciences. In 2001, he moved to the University of Cambridge as John Humphrey Professor of Chemical and Structural Biology. His research interests focus on understanding the consequences of protein misfolding particularly in terms of its relationship to human disease.

FOOTNOTES

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