Biophysical Reviews (2018) 10:527–534
https://doi.org/10.1007/s12551-017-0353-8

REVIEW

Recent progress on understanding the mechanisms of amyloid nucleation

Eri Chatani[1] · Naoki Yamamoto[1]

Received: 2 October 2017 / Accepted: 15 November 2017 / Published online: 6 December 2017

Abstract

Amyloid fibrils are supramolecular protein assemblies with a fibrous morphology and cross-β structure. The formation of amyloid fibrils typically follows a nucleation-dependent polymerization mechanism, in which a one-step nucleation scheme has widely been accepted. However, a variety of oligomers have been identified in early stages of fibrillation, and a nucleated conformational conversion (NCC) mechanism, in which oligomers serve as a precursor of amyloid nucleation and convert to amyloid nuclei, has been proposed. This development has raised the need to consider more complicated multi-step nucleation processes in addition to the simplest one-step process, and evidence for the direct involvement of oligomers as nucleation precursors has been obtained both experimentally and theoretically. Interestingly, the NCC mechanism has some analogy with the two-step nucleation mechanism proposed for inorganic and organic crystals and protein crystals, although a more dramatic conformational conversion of proteins should be considered in amyloid nucleation. Clarifying the properties of the nucleation precursors of amyloid fibrils in detail, in comparison with those of crystals, will allow a better understanding of the nucleation of amyloid fibrils and pave the way to develop techniques to regulate it.

Keywords Amyloid fibril · Nucleation · Oligomer · Precursor · Nucleated conformational conversion

Introduction

Self-assembly is a hallmark of proteins that is responsible for the organization of complex and hierarchical structures in biological systems. The most foundational type of the protein self-assembly is intramolecular folding, which is followed by the formation of more massive structures, such as multi-subunit globular proteins, filaments, motors, virus capsids, among others. The amyloid fibril (Dobson 2003) is regarded as another type of supramolecular structure resulting from protein self-assembly. This structure was originally discovered as a form of pathological deposits associated with more than 40 serious human diseases (Sipe et al. 2014). It standardly adopts a very ordered "cross-β" structure, in which β-strands are stacked perpendicularly to the longer axis of each fibril (Riek and Eisenberg 2016).

Recent studies have suggested that a variety of proteins and peptides have the potency to form amyloid fibrils or amyloid-like structures and that there are several kinds of amyloid fibrils possessing functions in a wide variety of organisms, including humans (Fowler et al. 2007; Maury 2009). In light of this background, clarifying molecular mechanisms underlying the organization of the amyloid structure is an important focus of research not only due to its close involvement with pathologies, but also because the structure is one of the most intrinsic types of self-assemblies of proteins. In this review, we discuss recent views on the mechanisms of amyloid fibril assembly.

The formation of amyloid fibrils is typically described by a nucleation-dependent polymerization mechanism, which comprises nucleation and elongation, and is often considered as a kind of crystallization (Crespo et al. 2012; Yoshimura et al. 2012; So et al. 2016). Here, we focus on the nucleation process, which is important as a primary step in amyloid

This article is part of a Special Issue on 'Biomolecules to Bionanomachines—Fumio Arisaka 70th Birthday' edited by Damien Hall, Junichi Takagi and Haruki Nakamura.

✉ Eri Chatani
chatani@crystal.kobe-u.ac.jp

Naoki Yamamoto
yamamoto@phoenix.kobe-u.ac.jp

[1] Graduate School of Science, Kobe University, 1-1 Rokkodai, Nada, Kobe, Hyogo 657-8501, Japan

formation. We summarize recent progress on our understanding of how protein molecules assemble into nuclei, the smallest structural unit that can facilitate growth into amyloid fibrils. We first describe a conventional mechanism in which the nucleus is assumed to be a thermodynamically unfavorable species, based on a concept that is analogous to classical nucleation theory (CNT) established in the field of crystallography. Then we will introduce multi-step nucleation schemes that have recently attached attraction, in which the formation of intermediates prior to amyloid nucleation plays an important role. Finally, we introduce various theoretical and experimental reports on the identification and characterization of the nuclei intermediates and discuss the roles of these intermediate species on the generation of the ordered cross-β structure.

Nucleation-dependent polymerization of amyloid fibrils

When the increase in the mass of amyloid fibrils is tracked during the fibrillation reaction, a sigmoid-shaped profile is typically observed (Fig. 1). The time period showing the mass increment of amyloid fibrils is referred to as the elongation phase, and the induction period before the elongation is called the lag phase. The shape of the fibrillation profile is predominantly determined by the rates of nucleation and elongation and, indeed, a previous work on theoretical simulation demonstrated that the fibrillation curve shapes could be approximately reproduced using a crystallization-like model that included only two parameters for nucleation and growth rates (Crespo et al. 2012). In this model, a sigmoidal shape was obtained when nucleation was remarkably slower than growth, with the shape becoming hyperbolic as the nucleation-to-growth ratio increased to more than 0.5. This result suggests that nucleation acts as a rate-limiting step in the production of amyloid fibrils, although an exception, called down-hill fibrillation, has been reported for transthyretin, in which no nuclei are required for fibril formation (Johnson et al. 2012). Under the nucleation-dependent polymerization mechanism, the seeding of fragmented amyloid fibrils into protein solutions effectively accelerates the formation of fibrils by skipping the nucleation process (Jarrett and Lansbury 1993).

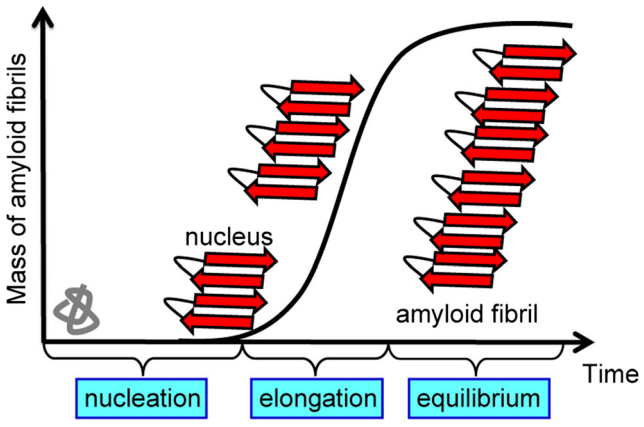


Fig. 1 Representative illustration of the kinetics of amyloid fibril formation that follows the nucleation-dependent polymerization mechanism. The three phases, i.e. nucleation, elongation, and equilibrium, are labeled

The initial formation of nuclei in a reaction space where there are still no amyloid fibrils present is specifically designated primary nucleation, while the term secondary nucleation is used to refer to nucleation that is induced by the presence of amyloid fibrils. With global fittings of experimental data using more complicated kinetic models, it has been demonstrated that not only primary nucleation but also secondary nucleation, elongation and fragmentation after the formation of amyloid fibrils play important roles in determining the length of the lag phase (Cohen et al. 2012; Arosio et al. 2015; Meisl et al. 2016). While the latter three molecular processes occur in fibril-dependent manners only after some amount of fibrils have been generated (Arosio et al. 2015), primary nucleation is viewed as the initialization process of amyloid formation, which will be the main focus in this review.

One-step schemes of amyloid nucleation

Kinetic and thermodynamic analyses play an important role in clarifying in detail the mechanisms of primary nucleation. Such analyses have been widely performed to elucidate the mechanism of protein aggregation, and historical highlights are reviewed by Morris et al. (2009). Above all, the earliest study on actin polymerization, performed by Oosawa and Kasai (1962), offered valuable insights and still serves as the foundation for the mechanism of the nucleation of amyloid fibrils. Based on the cooperative feature of actin polymerization kinetics, these authors proposed that this reaction involves a nucleation process occurring in supersaturated solutions, which was formulated as the formation of helical polymers by subsequent monomer addition. If the number of monomers required for helix closure is assumed to be four, for example, a helical trimer is defined as a nucleus in this model. While aggregates smaller than a tetramer are less stable relative to monomers and the helical trimer is the least stable of aggregates, the helical tetramer formed by attaching the fourth monomer to the helical trimer acquires stability by contacting both ends of the helix, which leads to a positive cooperativity. A similar scheme was later proposed for the polymerization of deoxyhemoglobin S by Hofrichter et al. (1974); based on their observation of a delay period prior to the onset

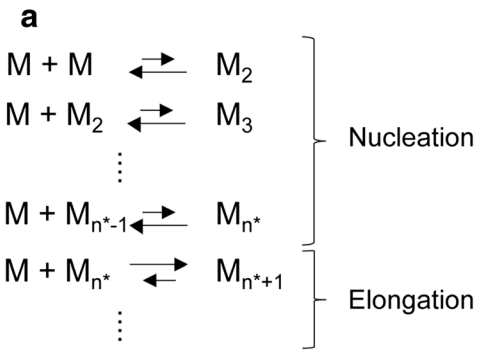
of polymerization, they suggested that this reaction is governed by a nucleation process. Furthermore, they theoretically explained the nucleation and subsequent polymerization processes with reference to a homogeneous nucleation theory developed for vapor condensation. These theories have served as useful guidelines for the development of kinetic and thermodynamic analyses of amyloid nucleation. A representative example of schemes currently used for describing fibril formation is shown in Fig. 2a (Ferrone 1999).

In the above-mentioned formulation described as the sequential addition of protein molecules, nuclei are characterized as a thermodynamically unfavorable species with the highest Gibbs energy along the reaction coordinate. This energetic feature is analogous to the view of CNT, the most standard theoretical model established for understanding nucleation of crystals (Erdemir et al. 2009; Gebauer et al. 2014). In the original CNT, the shape of nuclei formed through the association of crystallizing substrates is assumed to be that of a sphere, and nucleation is governed by the balance between the increased interfacial free energy and the decreased bulk free energy, which gives rise to the nucleation barrier (Fig. 2b). The radius of the nucleus at the maximum energy barrier (r_c) is called the critical size. During initial steps of association with nucleus size below r_c, the nucleus is thermodynamically unstable with positive total free energy. The total free energy decreases above r_c due to the dominance of the bulk free energy, leading to crystal formation. In recent studies, CNT and a corrected CNT (CCNT) have been used to describe fibrillation nucleation of amyloid fibrils (Kashchiev and Auer 2010). Furthermore, a theoretical model called the atomistic nucleation theory (ANT) has been developed to treat details in the shape and size of nucleus in a molecular level (Cabriolu et al. 2010).

Proposal of multi-step schemes of amyloid nucleation

According to the fundamental type of nucleation schemes mentioned above, the formation of nuclei is achieved in one step by crossing a single positive energy barrier, and since intermediate states are considered to be thermodynamically unfavorable, they barely accumulated. This hypothesis of one-step nucleation without any stable intermediates has been conventionally assumed in nucleation-dependent polymerization. Yet, at the same time, a wide variety of oligomer-like early aggregates have been identified in early stages of fibrillation reactions (Young et al. 2017). These aggregates are highly diverse in terms of secondary structure, morphology and functional properties, such as toxicity and seeding ability. Relationships between these aggregates and the formation of amyloid fibrils have often become a subject of debate (Breydo and Uversky 2015).

The direct involvement of early aggregates in the nucleation processes was first raised by Serio et al. in a yeast prion protein, Sup35 (Kelly 2000; Serio et al. 2000). In this study, the authors investigated the effects of protein concentration on the lag time and observed—unexpectedly—a small dependence on concentration. This is a conundrum for the conventional model, according to which the nucleation rate should vary exponentially with the number of monomers constituting a stable nucleus. Furthermore, the authors also reported that the amount of oligomers formed in the early stages correlated well with the fibrillation rate. These observations led to the notion that nuclei formation occurred by conformational rearrangements of proteins within an oligomer, i.e. a nucleated-conformational-conversion (NCC) model, the schematic illustration of which is shown in Fig. 3. Slightly before the above proposal was published, Lomakin et al. proposed that nucleation occurs within the micelles during the formation of



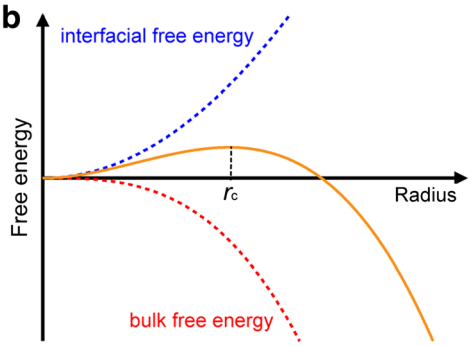


Fig. 2 **a** Basic reaction model commonly used for describing the formation of amyloid fibrils. M and M_n are a monomer and a polymer of length n, respectively, and polymers are formed by subsequent monomer addition. All reaction steps are represented as equilibrium processes, and nucleation, i.e. polymerization steps up to forming a nucleus (M_{n*}), is thermodynamically unfavorable. Conversely, subsequent elongation steps are considered to be thermodynamically favorable. **b** Free energy diagram of nucleation as predicted by the classical nucleation theory (CNT). In CNT, the shape of nuclei is modeled as a sphere, and free energy is drawn as a function of the radius of nucleus. The nucleation barrier (orange solid line) is described as the sum of interfacial free energy (blue dashed line) and bulk free energy (red dashed line). The radius at the maximum energy barrier (r_c), is labeled as the critical size

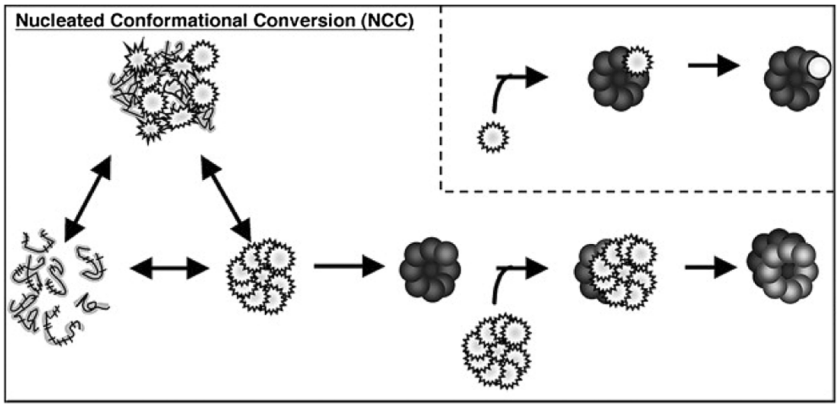


Fig. 3 Model of nucleated conformational conversion (NCC), which was originally proposed from the experimental observations of the fibrillation of Sup35, a yeast prion protein. Reprinted from Serio et al. (2000), with permission. Smooth and jagged circles represent structural units of nuclei and oligomers, respectively. Distorted jagged circles and jagged lines denote conformational heterogeneity of proteins before nucleation. In this model, nuclei are formed through conformational rearrangements of protein molecules within oligomers. This figure also represents that, in parallel with the typical templated assembly of monomers (top right panel), the oligomer species undergoes conformational conversion upon association with preformed nuclei in the step of elongation, a discussion of which is beyond the scope of this review

amyloid-β (Aβ) fibrils at low pH, based on their observation that the initial rate of elongation became independent above a certain concentration of Aβ (Lomakin et al. 1996), which appears to resemble the NCC mechanism. The NCC model has also been verified through theoretical and computational studies (Auer et al. 2007, 2012; Baftizadeh et al. 2012; Saric et al. 2014, 2016; Hsieh et al. 2017). The concept of a multi-step nucleation via a cascade of oligomer-like intermediate states has been incorporated into the conventional one-step nucleation model, and the effect of NCC on the fibrillation time course has been successfully evaluated in a theoretical study (Garcia et al. 2014).

Evidence for the structural conversion of oligomer-like aggregates to amyloid structures

The NCC model has emphasized the importance of early aggregates as a key molecular species in nucleation and has driven researchers to determine the nature of such aggregates that serve as nucleation precursors. A growing number of studies have revealed the structures of oligomer-like early aggregates using various analytical techniques, including solid-state nuclear magnetic resonance (NMR) (Kamihira et al. 2000; Chimon et al. 2007; Potapov et al. 2015), X-ray crystallography (Laganowsky et al. 2012), hydrogen/deuterium (H/D) exchange combined with mass spectrometry (Eghiaian et al. 2007; Paslawski et al. 2014), photo-induced cross-linking (Bitan and Teplow 2004), immunological assays (Kayed et al. 2003), among others. However, there is insufficient evidence to date to show that these oligomers actually play an on-pathway role in the formation of amyloid nuclei, largely due to the obstacles encountered when attempting to apply these techniques to time-resolved in situ tracking.

Several powerful approaches have been introduced to obtain experimental clues on the potency of oligomers, such as nucleation precursors. Good examples of such methods include small-angle X-ray scattering (SAXS). Inspired by the traditional use of these techniques to monitor the crystallization of synthesized polymers, in the past few decades researchers have applied SAXS to analyses of protein aggregates (Groenning et al. 2009). Vestergaard and coworkers, one of the pioneer groups studying fibrillation using SAXS, first clarified the structure of the oligomeric species of insulin by performing time-lapse elucidation of the changes in the fractions and conformations of monomers, oligomers and mature amyloid fibrils during the fibrillation process (Vestergaard et al. 2007). Based on the observation that the elongation rate of the mature amyloid fibrils was proportional to the amount of oligomers, they suggested that the oligomers act as a direct precursor of the formation of mature amyloid fibrils. They also clarified the oligomer species of α-synuclein as another example of nucleation precursors, shedding light on the molecular mechanisms of how protein aggregation is initialized in the time range of the nucleation phase (Nors Pedersen et al. 2015).

We also have recently investigated the formation of oligomer-like prefibrillar intermediates in another pathway of insulin fibrillation (Chatani et al. 2014). One of the difficulties in detailed analyses of oligomers is the low population and transient stability. To deal with this issue, we identified a new pathway under high salt concentration combined with high temperature, where almost all proteins within the reaction mixture were transformed to long-lived and on-pathway prefibrillar intermediates. The process of the formation of the prefibrillar intermediates and a subsequent fibrillation

were further investigated by time-resolved SAXS measurements combined with thioflavin T fluorescence, Fourier-transform infrared and light scattering studies. It was revealed that insulin molecules associated into rod-like prefibrillar intermediates in the very early time range of the reaction, and that after their further coalescing to form larger clusters, a conformational conversion towards mature amyloid fibrils occurred (Chatani et al. 2015) (Fig. 4).

Several new techniques have also provided important evidence for the structural transitions of oligomers to β-sheet-rich fibrils. Bleiholder et al. (2011) utilized ion mobility-mass spectrometry for clarifying the fibrillation pathway of several kinds of oligopeptides. In this study, a specific aggregation state was first mass selected from a distribution of oligomers, and then its conformation was analyzed in terms of collision cross-section. A series of measurements as a function of oligomer size revealed that fibril nuclei are produced via the formation of small oligomers that do not possess clear secondary structures. In the case of Aβ aggregation, Lee et al. (2011) developed methodology of oligomer sensing by combining $A\beta_{1-40}$ with two consecutive cysteine residues (CC-$A\beta_{1-40}$) and a covalently bound fluorescent probe as a latent fluorophore. In this system, the probe became fluorescent when the Cys-Cys motifs of the CC-$A\beta_{1-40}$ peptides came close by the formation of oligomers to create tetra-Cys binding sites for the fluorophore binding. By quantifying the amount of the oligomers formed during the reaction with this system, the authors showed that the rate of fibril formation depended on the amount of oligomers, thereby providing support for the NCC mechanism. Recently, Pavlova et al. (2016) tracked the early aggregation processes of a tau variant by probing the dynamics of hydration water and side-chain mobility with Overhauser dynamic nuclear polarization-enhanced NMR relaxometry and electron spin resonance (ESR) spectroscopy, respectively. A unique strategy was performed in this study, with two kinds of electron paramagnetic resonance spin labels utilized to identify how oligomers assemble into mature fibrils in solution. First, the oligomeric aggregates of tau proteins labeled with two different spin labels were separately prepared. After aliquots of these two types of spin-labeled oligomers were mixed to form mature amyloid fibrils, the extent of spin-dilution was examined on the basis of ESR spectral shape. The results revealed that the oligomers converted to amyloid fibrils without any dissociation into monomeric units.

Roles of precursors in the formation of amyloid nuclei

The observation of various cases of amyloid nucleation via oligomer-like early aggregates has led to the view that, in addition to the classical one-step mechanism, the multi-step scheme is also an important and universal mechanism of nucleation. Curiously, in recent years a revisiting of CNT has become an active issue in crystallography studies on a wide spectrum of inorganic and organic compounds, as well as proteins (Erdemir et al. 2009; Vekilov 2010; Gebauer et al. 2014; Sosso et al. 2016). Evidence for the formation of amorphous-like clusters during nucleation has been obtained using more recently developed and improved observation techniques, such as time-resolved in situ transmission electron microscopy (Loh et al. 2017), single-molecule real-time transmission electron microscopy (Harano et al. 2012), confocal scanning laser microscopy (Vivares et al. 2005), SAXS (Chattopadhyay et al. 2005; Sauter et al. 2015), depolarized oblique illumination dark-field microscopy (Maes et al. 2015), and the use of colloidal particles as a model of atoms (Zhang and Liu 2007). These experimental analyses have provided clear evidence of the presence of prenucleation clusters, emphasizing the two-step nucleation as an important pathway of crystallization.

In the case of the two-step nucleation mechanism of crystals, the feature of prenucleation clusters is often depicted as highly condensed and liquid-like particles of solutes, inside of which the formation of crystalline nucleus would be facilitated by increasing chances of interatomic or intermolecular interactions (Erdemir et al. 2009; Vekilov and Vorontsova 2014).

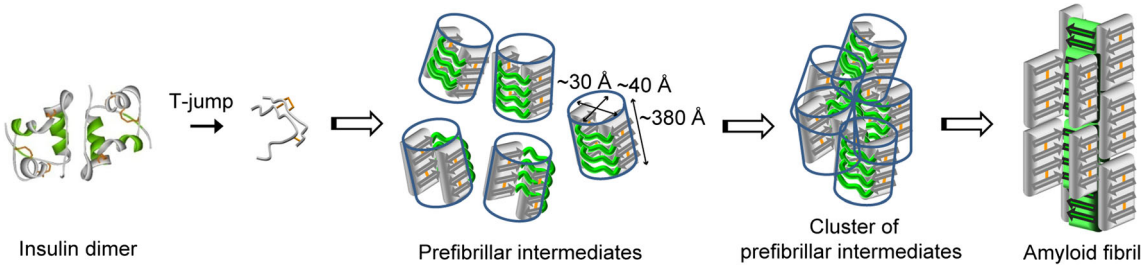


Fig. 4 Scheme of insulin fibrillation deduced at a high concentration of NaCl and high temperature, constructed based on the publications of Chatani et al. (2014, 2015). In this reaction, rod-like shaped prefibrillar intermediates having a partial β-sheet structure (gray regions) are initially formed. They appear to further coalesce to form clusters, inside of which an additional β-structure is formed (green regions), leading to the formation of mature amyloid fibrils. The radii and length of the prefibrillar intermediate are values estimated from small-angle X-ray scattering (SAXS) measurements

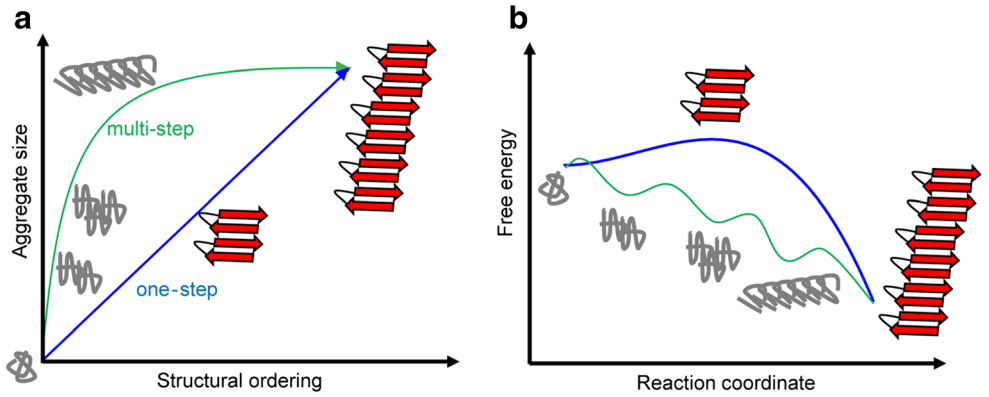


Fig. 5 **a** Schematic illustration of one-step and multi-step amyloid nucleation represented in the plane of aggregate size and structural ordering. In the case of one-step nucleation, the pathway is shown by a blue line that proceeds diagonally, indicating that the evolution in aggregate size and structural ordering proceed cooperatively. In the case of multi-step nucleation, on the other hand, aggregation precedes structural ordering, and thereby less-ordered oligomers are formed in the process of nucleation. A possible pathway of the multi-step nucleation process is shown by a green line, and multiple pathways would be considered depending on the balance of the aggregate size and structural ordering. **b** The corresponding free energy diagrams of the one-step and multi-step nucleation processes. It should be noted that the energy level of each species as well as the height of energy barriers among them are only provisional and would vary depending on environmental conditions and physical properties of proteins

Although it is unclear how similar the prenucleation clusters of crystals are to the oligomer-like aggregates of amyloid fibrils, the crucial role of intermolecular interactions among peptides inside the oligomer has been revealed by the computer simulations of amyloid nucleation performed by Saric et al. (2014). These authors used a simple coarse-grained model of an amyloidogenic peptide whose propensity for oligomer formation was controlled by changing the strength of nonspecific attractions and then simulating the occurrence of the two-step nucleation in amyloid fibril formation. As a result, it was demonstrated that oligomers not only help peptides meet each other but also offer an environment that facilitates the conversion of peptides into the β-structure. Furthermore, importantly, the classical one-step nucleation did not occur at physiological low concentrations of peptides. In light of an experimental observation that dense liquid-like clusters were present in undersaturated protein solutions (Vekilov and Vorontsova 2014), multi-step nucleation is implicated to occur in the fibril formation under physiological conditions.

We recently identified two types of prefibrillar aggregates during the fibrillation of an insulin-derived peptide; one of these acted as a nucleation precursor, and the other did not (N. Yamamoto et al., submitted). This observation cannot be explained based on the assumption that oligomers possess uniform interior densities, under which the frequency of nucleation per oligomers should linearly correlate with the oligomer size, as proposed by Saric et al. (2014). Interestingly, these authors have recently proposed a more sophisticated view that the nucleation process is controlled by the internal structure of oligomers, based on updated coarse-grained computer simulations (Saric et al. 2016). Briefly, in this more recent study, the authors allowed a conformational conversion of protein molecules within the oligomer undergoing nucleation by newly defining the β-sheet propensity of peptides. As a result, they found that the nucleation rate was not governed by the size of the oligomer, but rather by the portion of peptides inside the oligomer that actively participates in the conformational conversion, in particular for the case of low β-sheet propensity. This result as well as our experimental observation imply that intermolecular arrangements of protein molecules inside the oligomer play an important role for the execution of conformational conversion to generate amyloid nuclei, while this is not a prerequisite for the case of crystallization. Future investigations on the structural characteristics of precursors of amyloid nucleation as well as the monitoring of structural conversions inside the precursors will advance our understanding of a more unified mechanism on their conformational conversions.

Conclusions

We have addressed recent views of the mechanisms of amyloid nucleation by summarizing the one-step and the multi-step schemes based on the literature and our own recent studies. We have especially focused on the NCC mechanism involved in the multi-step nucleation, and recent developments in experimental and theoretical studies have been outlined. An interesting and important aspect of the NCC mechanism is that it has some analogy with the two-step nucleation of protein crystals and inorganic and organic crystals. Oligomers that serve as nucleation precursors in amyloid fibrillations may share many similarities with prenucleation clusters found in crystallization, although more dramatic intramolecular conformational conversions should be involved in the case of amyloid nucleation. Investigations to identify the structural

properties of oligomer-like early aggregates of amyloids in contradistinction to those of prenucleation clusters of crystals as well as the introduction of techniques applied to the crystal studies will provide valuable clues for elucidating details of the molecular mechanisms of how amyloid nuclei are formed.

A comprehensive interpretation of the one-step and the multi-step nucleation processes has recently been proposed in several papers (Auer et al. 2007; Vekilov 2010; Saric et al. 2016; Sosso et al. 2016), which is represented as an illustrative diagram shown in Fig. 5. In this diagram, the nucleation pathway is represented in the plane of aggregate size and structural ordering of protein molecules. If proteins or peptides are rich in hydrophobic amino acid residues, they will have a high potency to interact intermolecularly to form oligomers. In this case, the structural ordering tends to occur after oligomer formation and, consequently, multi-step nucleation involving the formation of oligomers will become a dominant pathway (green lines in Fig. 5a, b). If both of size and structure order are developed simultaneously, the pathway will become one-step (blue lines in Fig. 5a, b).

According to the above diagram, the balance between intermolecular aggregation and structural ordering, which would depend on the physical properties of proteins and peptides and environmental conditions, will dictate the pathway of nucleation. Given that distinct types of amyloid structures, also called amyloid polymorphisms, are formed dependent on the fibrillation pathways (Chatani and Goto 2005; Tycko 2014), the structural differences of the oligomers may be the cause of the structural diversity of amyloid fibrils leading to different pathologies. A large number of oligomer species have been identified, but it is difficult to determine whether they are on-pathway species working as nucleation precursors or dead-end species in terms of structures. Identifying the structural differences between the nucleation precursors and dead-end oligomers is needed to improve our understanding of amyloid nucleation. Elucidation of nucleation mechanisms on the basis of oligomer-like early aggregates will advance our understanding of not only the nature of protein self-assembly, but also of the molecular mechanisms underlying the pathology of amyloidoses.

Acknowledgements We wish to express our congratulations to Professor Fumio Arisaka on his 70th birthday, and we thank the editors for the great opportunity to participate in this special issue. We thank Dr. Hiroshi Imamura (Ritsumeikan Universiy) for his helpful comments and discussions on the earlier draft of this article. This work was supported by JSPS KAKENHI Grant Numbers JP16H04778, JP16H00772, JP16K17783, and JP17H06352.

Compliance with ethical standards

Conflicts of interest Eri Chatani declares that she has no conflicts of interest. Naoki Yamamoto declares that he has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Arosio P, Knowles TP, Linse S (2015) On the lag phase in amyloid fibril formation. Phys Chem Chem Phys 17:7606–7618

Auer S, Dobson CM, Vendruscolo M (2007) Characterization of the nucleation barriers for protein aggregation and amyloid formation. HFSP J 1:137–146

Auer S, Ricchiuto P, Kashchiev D (2012) Two-step nucleation of amyloid fibrils: omnipresent or not? J Mol Biol 422:723–730

Baftizadeh F, Biarnes X, Pietrucci F, Affinito F, Laio A (2012) Multidimensional view of amyloid fibril nucleation in atomistic detail. J Am Chem Soc 134:3886–3894

Bitan G, Teplow DB (2004) Rapid photochemical cross-linking–a new tool for studies of metastable, amyloidogenic protein assemblies. Acc Chem Res 37:357–364

Bleiholder C, Dupuis NF, Wyttenbach T, Bowers MT (2011) Ion mobility-mass spectrometry reveals a conformational conversion from random assembly to β-sheet in amyloid fibril formation. Nat Chem 3:172–177

Breydo L, Uversky VN (2015) Structural, morphological, and functional diversity of amyloid oligomers. FEBS Lett 589:2640–2648

Cabriolu R, Kashchiev D, Auer S (2010) Atomistic theory of amyloid fibril nucleation. J Chem Phys 133:225101

Chatani E, Goto Y (2005) Structural stability of amyloid fibrils of β_2-microglobulin in comparison with its native fold. Biochim Biophys Acta 1753:64–75

Chatani E, Imamura H, Yamamoto N, Kato M (2014) Stepwise organization of the β-structure identifies key regions essential for the propagation and cytotoxicity of insulin amyloid fibrils. J Biol Chem 289:10399–10410

Chatani E, Inoue R, Imamura H, Sugiyama M, Kato M, Yamamoto M, Nishida K, Kanaya T (2015) Early aggregation preceding the nucleation of insulin amyloid fibrils as monitored by small angle X-ray scattering. Sci Rep 5:15485

Chattopadhyay S, Erdemir D, Evans JMB, Ilavsky J, Amenitsch H, Segre CU, Myerson AS (2005) SAXS study of the nucleation of glycine crystals from a supersaturated solution. Cryst Growth Des 5:523–527

Chimon S, Shaibat MA, Jones CR, Calero DC, Aizezi B, Ishii Y (2007) Evidence of fibril-like β-sheet structures in a neurotoxic amyloid intermediate of Alzheimer's β-amyloid. Nat Struct Mol Biol 14:1157–1164

Cohen SI, Vendruscolo M, Dobson CM, Knowles TP (2012) From macroscopic measurements to microscopic mechanisms of protein aggregation. J Mol Biol 421:160–171

Crespo R, Rocha FA, Damas AM, Martins PM (2012) A generic crystallization-like model that describes the kinetics of amyloid fibril formation. J Biol Chem 287:30585–30594

Dobson CM (2003) Protein folding and misfolding. Nature 426:884–890

Eghiaian F, Daubenfeld T, Quenet Y, van Audenhaege M, Bouin AP, van der Rest G, Grosclaude J, Rezaei H (2007) Diversity in prion protein oligomerization pathways results from domain expansion as revealed by hydrogen/deuterium exchange and disulfide linkage. Proc Natl Acad Sci USA104:7414–7419

Erdemir D, Lee AY, Myerson AS (2009) Nucleation of crystals from solution: classical and two-step models. Acc Chem Res 42:621–629

Ferrone F (1999) Analysis of protein aggregation kinetics. Methods Enzymol 309:256–274

Fowler DM, Koulov AV, Balch WE, Kelly JW (2007) Functional amyloid—from bacteria to humans. Trends Biochem Sci 32:217–224

Garcia GA, Cohen SI, Dobson CM, Knowles TP (2014) Nucleation-conversion-polymerization reactions of biological macromolecules with prenucleation clusters. Phys Rev E Stat Nonlinear Soft Matter Phys 89:032712

Gebauer D, Kellermeier M, Gale JD, Bergstrom L, Colfen H (2014) Pre-nucleation clusters as solute precursors in crystallisation. Chem Soc Rev 43:2348–2371

Groenning M, Frokjaer S, Vestergaard B (2009) Formation mechanism of insulin fibrils and structural aspects of the insulin fibrillation process. Curr Protein Pept Sci 10:509–528

Harano K, Homma T, Niimi Y, Koshino M, Suenaga K, Leibler L, Nakamura E (2012) Heterogeneous nucleation of organic crystals mediated by single-molecule templates. Nat Mater 11:877–881

Hofrichter J, Ross PD, Eaton WA (1974) Kinetics and mechanism of deoxyhemoglobin S gelation: a new approach to understanding sickle cell disease. Proc Natl Acad Sci USA 71:4864–4868

Hsieh MC, Lynn DG, Grover MA (2017) Kinetic model for two-step nucleation of peptide assembly. J Phys Chem B 121:7401–7411

Jarrett JT, Lansbury PT Jr (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 73:1055–1058

Johnson SM, Connelly S, Fearns C, Powers ET, Kelly JW (2012) The transthyretin amyloidoses: from delineating the molecular mechanism of aggregation linked to pathology to a regulatory-agency-approved drug. J Mol Biol 421:185–203

Kamihira M, Naito A, Tuzi S, Nosaka AY, Saito H (2000) Conformational transitions and fibrillation mechanism of human calcitonin as studied by high-resolution solid-state ^{13}C NMR. Protein Sci 9:867–877

Kashchiev D, Auer S (2010) Nucleation of amyloid fibrils. J Chem Phys 132:215101

Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486–489

Kelly JW (2000) Mechanisms of amyloidogenesis. Nat Struct Biol 7: 824–826

Laganowsky A, Liu C, Sawaya MR, Whitelegge JP, Park J, Zhao M, Pensalfini A, Soriaga AB, Landau M, Teng PK, Cascio D, Glabe C, Eisenberg D (2012) Atomic view of a toxic amyloid small oligomer. Science 335:1228–1231

Lee J, Culyba EK, Powers ET, Kelly JW (2011) Amyloid-β forms fibrils by nucleated conformational conversion of oligomers. Nat Chem Biol 7:602–609

Loh ND, Sen S, Bosman M, Tan SF, Zhong J, Nijhuis CA, Kral P, Matsudaira P, Mirsaidov U (2017) Multistep nucleation of nanocrystals in aqueous solution. Nat Chem 9:77–82

Lomakin A, Chung DS, Benedek GB, Kirschner DA, Teplow DB (1996) On the nucleation and growth of amyloid β-protein fibrils: detection of nuclei and quantitation of rate constants. Proc Natl Acad Sci USA 93:1125–1129

Maes D, Vorontsova MA, Potenza MA, Sanvito T, Sleutel M, Giglio M, Vekilov PG (2015) Do protein crystals nucleate within dense liquid clusters? Acta Crystallogr F Struct Biol Commun 71:815–822

Maury CP (2009) The emerging concept of functional amyloid. J Intern Med 265:329–334

Meisl G, Kirkegaard JB, Arosio P, Michaels TC, Vendruscolo M, Dobson CM, Linse S, Knowles TP (2016) Molecular mechanisms of protein aggregation from global fitting of kinetic models. Nat Protoc 11: 252–272

Morris AM, Watzky MA, Finke RG (2009) Protein aggregation kinetics, mechanism, and curve-fitting: a review of the literature. Biochim Biophys Acta 1794:375–397

Nors Pedersen M, Fodera V, Horvath I, van Maarschalkerweerd A, Norgaard Toft K, Weise C, Almqvist F, Wolf-Watz M, Wittung-Stafshede P, Vestergaard B (2015) Direct correlation between ligand-induced α-synuclein oligomers and amyloid-like fibril growth. Sci Rep 5:10422

Oosawa F, Kasai M (1962) A theory of linear and helical aggregations of macromolecules. J Mol Biol 4:10–21

Paslawski W, Mysling S, Thomsen K, Jorgensen TJ, Otzen DE (2014) Co-existence of two different α-synuclein oligomers with different core structures determined by hydrogen/deuterium exchange mass spectrometry. Angew Chem Int Ed Engl 53:7560–7563

Pavlova A, Cheng CY, Kinnebrew M, Lew J, Dahlquist FW, Han S (2016) Protein structural and surface water rearrangement constitute major events in the earliest aggregation stages of tau. Proc Natl Acad Sci USA 113:E127–E136

Potapov A, Yau WM, Ghirlando R, Thurber KR, Tycko R (2015) Successive stages of amyloid-β self-assembly characterized by solid-state nuclear magnetic resonance with dynamic nuclear polarization. J Am Chem Soc 137:8294–8307

Riek R, Eisenberg DS (2016) The activities of amyloids from a structural perspective. Nature 539:227–235

Saric A, Chebaro YC, Knowles TP, Frenkel D (2014) Crucial role of nonspecific interactions in amyloid nucleation. Proc Natl Acad Sci USA 111:17869–17874

Saric A, Michaels TCT, Zaccone A, Knowles TPJ, Frenkel D (2016) Kinetics of spontaneous filament nucleation via oligomers: insights from theory and simulation. J Chem Phys 145:211926

Sauter A, Roosen-Runge F, Zhang F, Lotze G, Jacobs RM, Schreiber F (2015) Real-time observation of nonclassical protein crystallization kinetics. J Am Chem Soc 137:1485–1491

Serio TR, Cashikar AG, Kowal AS, Sawicki GJ, Moslehi JJ, Serpell L, Arnsdorf MF, Lindquist SL (2000) Nucleated conformational conversion and the replication of conformational information by a prion determinant. Science 289:1317–1321

Sipe JD, Benson MD, Buxbaum JN, Ikeda S, Merlini G, Saraiva MJ, Westermark P (2014) Nomenclature 2014: amyloid fibril proteins and clinical classification of the amyloidosis. Int J Exp Clin Invest 21:221–224

So M, Hall D, Goto Y (2016) Revisiting supersaturation as a factor determining amyloid fibrillation. Curr Opin Struct Biol 36:32–39

Sosso GC, Chen J, Cox SJ, Fitzner M, Pedevilla P, Zen A, Michaelides A (2016) Crystal nucleation in liquids: open questions and future challenges in molecular dynamics simulations. Chem Rev 116:7078–7116

Tycko R (2014) Physical and structural basis for polymorphism in amyloid fibrils. Protein Sci 23:1528–1539

Vekilov PG (2010) The two-step mechanism of nucleation of crystals in solution. Nano 2:2346–2357

Vekilov PG, Vorontsova MA (2014) Nucleation precursors in protein crystallization. Acta Crystallogr F Struct Biol Commun 70:271–282

Vestergaard B, Groenning M, Roessle M, Kastrup JS, van de Weert M, Flink JM, Frokjaer S, Gajhede M, Svergun DI (2007) A helical structural nucleus is the primary elongating unit of insulin amyloid fibrils. PLoS Biol 5:e134

Vivares D, Kaler EW, Lenhoff AM (2005) Quantitative imaging by confocal scanning fluorescence microscopy of protein crystallization via liquid-liquid phase separation. Acta Crystallogr D Biol Crystallogr 61:819–825

Yoshimura Y, Lin Y, Yagi H, Lee YH, Kitayama H, Sakurai K, So M, Ogi H, Naiki H, Goto Y (2012) Distinguishing crystal-like amyloid fibrils and glass-like amorphous aggregates from their kinetics of formation. Proc Natl Acad Sci USA 109:14446–14451

Young LM, Ashcroft AE, Radford SE (2017) Small molecule probes of protein aggregation. Curr Opin Chem Biol 39:90–99

Zhang TH, Liu XY (2007) How does a transient amorphous precursor template crystallization. J Am Chem Soc 129:13520–13526