Formation and properties of amyloid fibrils of prion protein

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Abstract

Amyloid fibrils formed from prion protein (PrP) are associated with prion diseases. In this review we discuss a number of extrinsic and intrinsic experimental factors related to the formation of PrP amyloid fibrils *in vitro*. We first examined the effects of ultrasonic power on the induction of amyloid fibrillation from PrP. The most important conclusion drawn from the results is that an applied ultrasonic power of approximately 2 W enhanced the nucleation of amyloid fibrils efficiently but that more powerful ultrasonication led to retardation of growth. We also reviewed evidence on the amyloidogenic regions of PrP based on peptide screening throughout the polypeptide sequence. These results showed that helix 2 (H2) peptides of PrP were capable of both the fibrillation and propagation of straight, long fibrils. Moreover, the conformation of preformed H2 fibrils changed reversibly depending on the pH of the solution, implying that interactions between side-chains modulated the conformation of amyloid fibrils. The evidence discussed in this review relates specifically to PrP but may be relevant to other amyloidogenic proteins.

Keywords Amyloid fibrils · Prion diseases · Ultrasonication · Amyloidogenic region · Conformational change of amyloid fibrils

Introduction: prion diseases and amyloid fibrils

Prion diseases, or transmissible spongiform encephalopathies, are a family of fatal neurodegenerative disorders that affect both humans and animals. They include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease, and kuru in humans, bovine spongiform encephalopathy in cattle, and scrapie in sheep and goats (Aguzzi and Polymenidou 2004; Prusiner 1998). Variant CJD in humans is caused by the same

prion strain as that causing bovine spongiform encephalopathy in cattle, and with the identification of this relationship a major health issue emerged, with a focus on the propagation of the scrapie form of prion protein (PrP^{Sc}) (Coaling et al. 1996). Chronic wasting disease has also been found capable of crossing the species barrier, spreading from both farm and free-range elk and deer into free-range cattle (Mathiason et al. 2006). The pathogenesis of prion diseases is due to the conformational rearrangement of the cellular isoform of prion protein (PrP^C) to PrP^{Sc} in the brain (Aguzzi and Polymenidou 2004; Prusiner 1998; Weissmann 2004). PrP^C consists of three α -helices [Helix 1 (H1), Helix 2 (H2), and Helix 3 (H3)] and a short antiparallel β -sheet [Strand 1 (S1) and Strand 2 (S2)] (Riek et al. 1996). A single disulfide bond connects H2 and H3 at cysteine residues 179 and 214, respectively. Although PrP^C is monomeric and has a rich α -helical structure, the PrP^{Sc} conformer is characterized by an increased proportion of the β -sheet structure, partial resistance to proteinase K digestion, and a propensity to aggregate into amyloid fibrils or plaques (Prusiner 1998). A growing body of evidence supports the protein-only hypothesis, which proposes that PrP is itself the infectious pathogen (Legname et al. 2004; Wang et al. 2010).

Although the various amyloid-forming proteins have different primary structures, amyloid fibrils themselves have common characteristics, i.e., Congo red binds to all amyloid fibrils, with the fibrils visualized as an apple-green birefringence under

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polarized light microscope. Thioflavin T (ThT) also binds specifically to amyloid fibrils and exhibits fluorescence at 485 nm when it is excited at this wavelength (Fig. 1) (Naiki et al. 2016). The kinetics of amyloid fibrillation can be monitored by using ThT fluorescence, in which a lag phase for nucleation is usually seen at the initial step in the absence of seeds, but in the presence of seeds, amyloid fibrils rapidly form without a lag phase (Fig. 1). Amyloid fibrils are generally 10–20 nm in diameter and several micrometers in length, and they form a cross- β structure in which β -strands are arranged perpendicular to the fibril axis (Naiki et al. 2016; Riek and Eisenberg 2016). Amyloid fibrillation is currently considered to be a genetic property of polypeptides, since a number of sequentially unrelated proteins and peptides form amyloid fibrils (Dobson 2003). In the case of *Escherichia coli*, curli amyloid fibrils of CsgA, the major subunit of curlin, aid in the formation of biofilms (Chapman et al. 2002), in which the amyloid fibrillation of CsgA is definitely controlled by a biological molecule. The results of genome-wide searches for amyloidogenic proteins, termed the amyloome, imply that most proteins have the potential to form amyloid fibrils in vivo (Ciriyam et al. 2013).

The high molecular weight and insolubility of amyloid fibrils prevent the application of conventional structural analyses, such as solution nuclear magnetic resonance (NMR) and X-ray crystallography, to obtain high-resolution structural information. By contrast, the one-dimensional (1-D) crystal-like repeat in the amyloid fibrils provides a structural framework for polymorphisms using magic angle spinning solid-state NMR methods (Tycko and Wickner 2013), in which β -strands of the amyloid structure are stacked in parallel and in register (Paravastu et al. 2008; Van Melckebeke et al. 2010). In addition, an orthogonal Greek-key topology stabilized by a steric zipper conformation was observed in pathogenic amyloid fibrils formed by α -synuclein (α -syn) (Tuttle et al. 2016). By contrast, X-ray crystallography of a microcrystal revealed that both parallel and antiparallel extended β -strands were formed in several kinds of amyloidogenic peptides (Eisenberg and Sawaya 2017; Nelson et al. 2005; Sawaya et al. 2007). The amyloidogenic peptides formed a pair of β -sheets, with the facing side-chains interdigitated in amyloid steric zipper that was extremely highly ordered. However, the structure of the full-length PrP^{Sc} remains unknown. It is essential to obtain detailed structural information of PrP amyloid fibrils or PrP^{Sc} using newly developed methods, such as those that integrate the use of an X-ray free electron laser (XFEL) (Kameda et al. 2017) and cryo-electron microscope (Fitzpatrick et al. 2017).

Ultrasonic power and strength to induce amyloid fibrillation of PrP

Significant efforts have focused on the development of a cell-free conversion system that could reconstruct the infectious

Fig. 1 Kinetics of the formation of amyloid fibrils monitored by thioflavin T (ThT) fluorescence. ThT exhibits enhanced fluorescence at 485 nm only when it is excited at 445 nm after binding specifically to the amyloid fibrils. In the absence of seeds, the kinetics of amyloid fibril formation shows a sigmoidal curve (solid line). The initial lag phase represents nucleation of amyloid fibrils, and the following growth phase represents an elongation of amyloid fibrils. In the presence of seeds, amyloid fibrils rapidly form by seeding without a lag phase (dashed line), indicating that nucleation is a rate-limiting step in amyloid fibrillation

PrP^{Sc} from recombinant PrP (Bocharova et al. 2005; Castilla et al. 2005; Kirby et al. 2003). The use of ultrasonication has been successfully applied for the amplification of PrP^{Sc} in vitro, which is known as the protein misfolding cyclic amplification (PMCA) method (Castilla et al. 2005; Saborio et al. 2001). During ultrasonication the population of PrP^{Sc} aggregates, which catalyze the formation of PrP^{Sc}, can be increased by breaking large PrP^{Sc} aggregates into smaller units. Using this technique, ultrasensitive PrP^{Sc} detection has been achieved in urine (Moda et al. 2014) and blood (Concha-Marambio et al. 2016) from patients with variant CJD with 100% sensitivity and specificity, using only a few microliters of sample volume. PMCA methods have also been used for the detection of A β oligomers in cerebrospinal fluid, which can provide a sensitive biochemical diagnosis of Alzheimer's disease (Salvadores et al. 2014). In addition, the agitation-based real-time quaking-induced conversion (RT-QuIC) method has been applied to the diagnosis of human prion diseases because of its high sensitivity (Atarashi et al. 2011; Schmitz et al. 2016).

Ultrasonication accelerates nucleation of the amyloid fibrils of several proteins, including β_2 -microglobulin (β_2 -m) (Adachi et al. 2015; So et al. 2011), α -syn (Yagi et al. 2015), lysozyme (Nitani et al. 2017), and others (Muta et al. 2014; Stathopoulos et al. 2004). It is commonly known that ultrasonication triggers the formation of amyloid fibrils. In this review we discuss the evidence relating to the adequate levels of ultrasonic power required for the stable amplification of amyloid fibrils. A first point to realize is that the ultrasonic power in solution is considerably less than the real output

electric power (Kimura et al. 1996; Koda et al. 2003). The conversion efficiency of electrical power into ultrasonic energy not only depends on the types of instruments but also on the conditions of the oscillator. Several methods are available to estimate the amount of ultrasonic power that enters into a sonochemical reaction (Kimura et al. 1996; Koda et al. 2003). We estimated the ultrasonic power and strength in a sample solution using calorimetry and potassium iodide (KI) oxidation as measurement principles, respectively (Yamaguchi et al. 2012). The range of ultrasonic power estimated by the calorimetry method was between 0.3 and 2.7 W depending on the position of the ultrasonic stage at a frequency of 17–20 kHz, although the output power was approximately 550 W. Sonochemical oxidation of KI was caused by a microscopic transient reaction field at several thousand Kelvin and several hundred atmospheres (Koda et al. 2003; Suslick 1986), which were generated by the quasi-adiabatic collapse of the cavitation bubbles in solution (Fig. 2a). Importantly, the efficiency of sonochemical KI oxidation was in good agreement with the ultrasonic power obtained by the calorimetry method, indicating that the microscopic transient increase in temperature leads to a macroscopic equilibrium increase in solution temperature.

Amyloid fibrillation experiments of full-length mouse prion protein (mPrP) have been performed at various positions on the ultrasonic stage (Fig. 2b) (Yamaguchi et al. 2012). We used an exponential curve to examine the correlation between nucleation time and ultrasonic power, since the nucleation time cannot be negative at very high applied power (Fig. 2c). The nucleation of the amyloid fibrils was remarkably accelerated depending on the ultrasonic power. Nucleus formation requires a series of association steps of monomers that are thermodynamically unfavorable. Cavitation accompanies vigorous agitation, which increases the probability of the proper association of monomers. In addition, a temporary increase in protein concentration around the surface of the cavitation bubbles (Nakajima et al. 2016) and a transient microscopic field with a temperature of several thousand Kelvin can efficiently trigger the nucleation of amyloid seeds (Fig. 2a). By contrast, horn-type ultrasonicators can frequently be used for the production of amyloid seeds because the actual power (>10 W) of such ultrasonicators is much stronger than that of water bath-type ultrasonicators (Kimura et al. 1996). In this study, the ultrasonic power of about 2.0 W was suitable for the formation of rigid amyloid fibrils under the ultrasonic conditions (i.e., ultrasonication for 30 s every 9 min) (Yamaguchi et al. 2012), and an ultrasonic power of > 2.0 W induced the fragmentation of amyloid fibrils. Umemoto et al. (2014) performed high-throughput analyses of ultrasonication-forced amyloid fibrillation using a Handai amyloid burst inducer (HANABI). With the HANABI system, a 96-well microplate was moved in the *x*–*y* axes in sequence to average the ultrasonic energy, following which variations in the KI oxidation

rate decreased. Thus, the observed variations in the KI oxidation rate had represented the basic performance of the HANABI system based on intrinsic variations in the simple chemical reaction and mechanical instability of ultrasonic irradiation. By contrast, the agitation method has also been employed to induce fibrillation in vitro (Bocharova et al. 2005; Makarava et al. 2009), but the calorimetry and KI oxidation methods cannot be applied to monitor the efficiency of agitation. Although the effects of agitation may be similar to those of ultrasonication, the cavitation bubbles which lead to the breakdown of supersaturation and ultimately to fibrillation are only generated under ultrasonication. In their comparison of high-speed stirring agitation at 1200 rpm and ultrasonication, Nakajima et al. (2017) observed that ultrasonication significantly accelerated the fibrillation reaction.

The amyloid fibrillation of β 2-m was also accelerated by ultrasonication, depending on the ultrasonic power (Adachi et al. 2015; So et al. 2011; Umemoto et al. 2014). Thus, amyloid fibrillation is generally enhanced under ultrasonic conditions where cavitation bubbles are formed efficiently, but amyloid fibrils may not be formed under weak ultrasonic conditions. Nakajima et al. (2016) reported that the nucleation reaction was significantly enhanced at an acoustic wave of a frequency of 29 kHz, increasing in a pressure-dependent manner by three orders of magnitude at the optimum acoustical condition. Thus, the adequate ultrasonic power needed to induce amyloid fibrillation should be determined; otherwise, the reproducibility of amyloid fibrillation experiments, including PMCA experiments, may be poor. The calorimetry and KI oxidation methods used here as well as the acoustic techniques will be useful for this purpose.

β -sheet core region of PrP amyloid fibrils

To understand the molecular pathogenesis of prion diseases, it is essential to reveal the amyloidogenic region of PrP^{Sc}. Historically, the PrP region within residues around 90–140 has been known to be important for the conversion of PrP^C to PrP^{Sc} (Gasset et al. 1992; Muramoto et al. 1996; Tagliavini et al. 1993). The formation of amyloid fibrils has been reported for many kinds of synthetic peptides derived from a PrP sequence of residues 90–145 (Jones and Surewicz 2005; Natalello et al. 2008), under nonphysiological pH or in the presence of organic solvents (Kuwata et al. 2003; Nguyen et al. 1995; Salmona et al. 1999). The most well-characterized peptide is PrP106–126, in which the amyloid core consists of the middle region of the peptide (residues 111–123) (Kuwata et al. 2003). Govaerts et al. (2004) proposed a structural model for PrP^{Sc} in which residues 89–175 formed the left-handed β -helix that associated into trimers, constructed based on electron microscopy images of 2-D

Fig. 2 Ultrasonication-induced nucleation of amyloid fibrils. **a** Upon quasi-adiabatic collapse of the cavitation bubbles, temperature and pressure inside the cavity were increased up to several thousand Kelvin and several hundred atmospheric pressure, respectively, and temperature around the cavity (gas–liquid interface) increased up to several thousand Kelvin. Sonochemical oxidation of potassium iodide (KI) was caused by these transient microscopic fields, and the calorimetry method monitored an increase in the temperature of bulk water. Nucleation of amyloid fibrils could be efficiently generated around the cavitation bubbles during

ultrasonication. **b** The formation of mouse prion protein (mPrP) amyloid fibrils at various positions of the ultrasonic stage. ThT fluorescence values at ultrasonic powers of >2.0 W (red), 1.0–2.0 W (black), and <1.0 W (blue). **c** Correlation between the nucleation time of amyloid fibrils and the ultrasonic power as estimated by the calorimetry method. Each bar represents the standard deviation (SD) of the nucleation times and ultrasonic power at each position. The curve was fitted using an exponential curve

crystals, whereas H2 and H3 of the C-terminal region remained in their native α -helical conformation.

Meanwhile, a deletion mutant of PrP ($\Delta 23$ –88 and $\Delta 141$ –176), which consists of 106 amino acid residues, showed conversion to PrP^{Sc} in transgenic mice and formed β -sheet-rich aggregates (Baskakov et al. 2000; Muramoto et al. 1996). This result implies that the core region of PrP^{Sc} contains the C-terminal α -helical regions of PrP^C. Lu et al. performed a hydrogen–deuterium exchange coupled with a mass spectrometry (HXMS) analysis to examine the structure of amyloid fibrils of human PrP90–231 (Lu et al. 2007). These authors mapped the β -sheet core region onto the C-terminal region (starting at around residue 169), which corresponds to H2, a major part of H3, and the loop between these two helices in the native structure of PrP. Hydrogen bonding was not detected in the N-terminal region of PrP amyloid fibrils, which argues against the left-handed β -helix model (Govaerts et al. 2004). Site-directed spin labeling and electron spin resonance spectroscopy also showed that the core region of human PrP

amyloid fibril maps to C-terminal residues from approximately 160–220 and that these residues form single-molecule layers that stack on top of one another in parallel (Cobb et al. 2007). In addition, HXMS showed that brain-derived PrP^{Sc} formed a β -sheet and short turn or loop conformations throughout the entire region of PrP (Smirnovas et al. 2011).

We investigated the amyloidogenic properties of mPrP using a series of fragmented peptides that corresponded to the secondary structural elements of mPrP (Yamaguchi et al. 2008). Only two peptides, located in the H2 (residues 171–193) or H3 (residues 199–226) regions of mPrP, which were relatively hydrophobic (Kyte and Doolittle 1982), formed amyloid-like fibrils (Fig. 3a). However, the morphologies of the H2 and H3 fibrils were significantly different in that H2 fibrils were straight and long and H3 fibrils were twisted and relatively short (Yamaguchi et al. 2008). Studies with various acylphosphatase mutants have demonstrated that the hydrophobicity and β -sheet propensity of key regions, as well as the net charge of the protein, are critical factors for aggregation

(Chiti et al. 2003). Intriguingly, the H2 peptide was predicted to have a high β -sheet propensity among mPrP (Fig. 3a) (Rost et al. 1994), although this region formed an α -helix in native mPrP (Riek et al. 1996). The hydrophobicity and β -sheet propensities of the H2 peptide can promote the formation of β -sheet-rich amyloid fibrils. By contrast, although H3 peptide is hydrophobic, this region is predicted to form α -helix conformation (Fig. 3a), so it may be difficult to form β -sheet-rich amyloid fibrils rather than the H2 peptide. Several algorithms, including PASTA 2.0 (Walsh et al. 2014) and TANGO (Fernandez-Escamilla et al. 2004), suggested that the amyloidogenic region of mPrP, i.e., the H2 region of mPrP, had a high propensity to aggregate (Fig. 3b). PASTA 2.0 showed a score of > 0.06 , which was comparable to the value of the amyloidogenic A β 1–40 peptide (Trovato et al. 2006) and those of the core regions of other amyloidogenic proteins (Chen et al. 2012; Trovato et al. 2006). Furthermore, the cross-reaction efficiently occurred between H2 fibrils and full-length mPrP (Yamaguchi et al. 2008). The H2 fibrils may have internal structures similar to those of mPrP fibrils. Taken together, these results suggest that the H2 region in mPrP rather than the H3 region has a greater potential for the formation of amyloid fibrils and could propagate β -sheet conformation by itself to the rest of the molecule, which corresponds to a proposed amyloid structure based on HXMS analysis (Lu et al. 2007) and site-directed spin labeling (Cobb et al. 2007).

In addition, the mechanisms of protein misfolding into amyloid aggregates were examined using peptides comprising the hairpin formed by the H2 and H3 region (Adrover et al. 2010). The isolated H2H3 fragment retained its secondary and tertiary structure and formed amyloid fibrils similar to those formed from the full-length PrP. Moreover, using deuterium exchange experiments with NMR, we revealed that the S1–

H1–S2 segment at the N-terminus was preferentially unfolded, whereas the H2–H3 segment at the C terminus remained marginally stable (Honda et al. 2014). The initial population of the partially unfolded state of PrP was well correlated with the rate of the β -rich oligomer formation. Thus, the specific conformation of the partially unfolded state, which accumulates as a late folding intermediate (Honda et al. 2015), is considered to provide crucial insights into the mechanisms of oligomerization and pathogenic conversion (Honda et al. 2014, 2015.).

Intriguingly, the C terminus of the H2 and the H2–H3 loop undergo slow exchange dynamics (Kuwata et al. 2002, 2004). A metastable state of the PrP^C was characterized by using high-pressure NMR (Kuwata et al. 2002), and a Carr–Purcell–Meiboom–Gill relaxation dispersion experiment revealed that a slow fluctuation on a time scale of microseconds to milliseconds occurs at the corresponding regions, indicating conformational rearrangements between the native and the metastable high-energy states (Kay et al. 2004; Kuwata et al. 2004). The E196K mutant at the C terminus of the H2 causes a rapidly progressive dementia and ataxia (Peoc'h et al. 2000) and could be expected to convert into the β -sheet structure by destabilizing salt bridges between E196, E156, and K194. The anti-prion compound GN8 was then designed to fit into a “pocket” created by residues undergoing the slow conformational rearrangements and that directly bound to these residues (Kuwata et al. 2007). Since GN8 connects distant regions (N159 and E196) in the PrP sequence by hydrogen bonds, large conformational shifts may be prohibited, and thus intermediate (PrP^{*}) and further amyloid fibrils or PrP^{Sc} formation may be also blocked. A number of PrP mutations causing inherited human prion disease are also located within the H2 and H3 segments of the C-terminal region (Fig. 3b) (Prusiner 1998). Thus, once the native structure is destabilized under

Fig. 3 Primary structure analyses of mouse prion protein (mPrP). The secondary structural elements determined on the basis of the nuclear magnetic resonance structure (PDB ID 1AG2), octameric repeat, and hydrophobic cluster are shown schematically at the top of the figure. **a** Hydrophobic profile of mPrP was predicted by the Kyte and Doolittle method (Kyte and Doolittle 1982). The amino acid sequence of mPrP is shown and colored red (helix), green (sheet), or black (random),

depending on the secondary structure predicted by the PHD algorithms (Rost et al. 1994). **b** Prediction of aggregation propensities of mPrP. The β -aggregation propensities predicted by PASTA 2.0 (black) (Walsh et al. 2014) and TANGO (red) (Fernandez-Escamilla et al. 2004) are plotted against the residue number of mPrP. PrP mutations causing inherited human prion disease are shown by solid black circles

conditions that the region of H2 may trigger generic folding, it may form a β -sheet conformation instead of an α -helix.

Conformational change of amyloid fibrils

In general, amyloid fibrils are highly ordered supramolecular complexes that are likely to be stiffer than most functional biological filaments (Knowles et al. 2007). Intriguingly, the H2 peptide of PrP formed two types of amyloid fibrils; one was highly ordered fibrils formed at pH 2.9 (named pH 2.9 fibrils) (Fig. 4a, left), and the other was slightly disordered fibrils formed at pH 7.5 (named pH 7.5 fibrils) (Fig. 4a, right) (Yamaguchi et al. 2013). The formation of amyloid fibrils is basically sensitive to the net charge of proteins or peptides. The net charge of the H2 peptide decreases around the isotropic point (pI) of 8.6, at which pI aggregates formed, but ordered amyloid fibrils were formed far from its pI by taking advantage of moderate repulsion (Lopez de la Paz et al. 2002; Yamaguchi et al. 2008). By contrast, the circular dichroism (CD) spectra of the pH 2.9 fibrils showed a minimum at 207 nm (Fig. 4b, solid black line); these spectra were rarely seen in the β -sheet conformation of the globular protein, except for α -helix or random conformations, but they were occasionally seen in highly ordered β -sheet conformation of amyloid fibrils (Rufo et al. 2014; Yamaguchi et al. 2013). The H2 peptide may form a β -strand–turn– β -strand conformation in pH 2.9 fibrils, as shown by the Fourier transform infrared (FT-IR) spectrum (Fig. 4c, solid black line).

Then, a pH-jump experiment from 2.9 to 7.5 was performed using the pH 2.9 fibrils (Fig. 4a) (Yamaguchi et al.

2013). The conformation of pH 2.9 fibrils converted to that of pH 7.5-like fibrils readily, but the conformation of pH 7.5-like fibrils was definitely distinct from that of pH 7.5 fibrils that spontaneously formed at pH 7.5, as confirmed by CD spectra (Fig. 4b) and FT-IR spectra (Fig. 4c). Moreover, pH 7.5-like fibrils almost reverted to the pH 2.9 fibrils when the solution pH was restored to 2.9. Kuroski et al. (2012) reported pH-induced conformational changes in insulin amyloid fibrils monitored by vibrational CD and fluid-cell atomic force microscope, and a temperature-induced structural rearrangement also has been reported for mPrP fibrils (Bocharova et al. 2006). In the high-pressure experiment, amyloid fibrils of β 2-m showed a structural reorganization into more tightly packed ones (Chatani et al. 2005), implying that amyloid fibrils are not tightly packed and that they contain a large number of cavities at atmospheric pressure. Knowles et al. (2007) proposed that amyloid fibrils were predominantly determined by the hydrogen bond network in the extended intermolecular β -sheet architecture of peptide backbones, but the packing between the side-chains may not be optimized. Hence, it is possible that interactions between the side-chains modulate fibril conformation when the solution pH changes, unless amyloid fibrils dissolve into the monomeric unit. By contrast, both amyloid fibrillation and crystallization occur after a long period of nucleation and propagate rapidly (Adachi et al. 2015; Yoshimura et al. 2012). In the supersaturated state, the solute concentration is slightly above the solubility, and seeding is essential to break the supersaturation state in both the propagation of amyloid fibrils and crystallization. The multiple prion strains may be propagated by a mechanism similar to that used for the various morphologies

Fig. 4 Conformational change in H2 fibrils by pH jump. **a** pH 2.9 fibrils (left) reversibly changed to pH 7.5-like fibrils (middle), in which two conformations were separated by a relatively low energy barrier, by shifting the solution pH from 2.9 to 7.5. However, pH 7.5-like fibrils did not change to pH 7.5 fibrils (right), in which these two conformations were separated by a high energy barrier. **b**, **c** Circular dichroism spectra (**b**) and Fourier transform infrared spectra (**c**) exhibited the secondary structural changes of pH 2.9 fibrils depending on the solution pH

of crystals. Conformational varieties of the amyloid fibrils explain the physical background in the generation of a new prion strain (Li et al. 2010; Petkova et al. 2005; Tanaka et al. 2004).

In conclusion, we have reviewed (1) the effects of ultrasonic power on the amyloid fibrillation of PrP, (2) the β -sheet core region of PrP amyloid fibrils, and (3) the effect of pH on the super-quaternary structure of amyloid fibrils formed by PrP. Applying these techniques, the formation and properties of amyloid fibrils of PrP have been revealed, and the process of PrP aggregation can be controlled definitely, such as by using ultrasonication after proper calibration of the ultrasonic power. Although these studies seem to be unique and focused on the PrP, the physiological properties of amyloid fibrils might also be applied for those formed from other proteins. Various methods employed in biophysics and protein science would provide insight into the molecular mechanism of amyloid fibrillation of PrP as well as of other amyloidogenic proteins.

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Compliance with ethical standards

Conflict of interest Kei-ichi Yamaguchi declares that he has no conflict of interest. Kazuo Kuwata declares that he has no conflict of interest.

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

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