Published in final edited form as:

J Phys Chem B. 2015 July 09; 119(27): 8521–8525. doi:10.1021/acs.jpcb.5b04779.

Rapid Filament Supramolecular Chirality Reversal of HET-s (218–289) Prion Fibrils Driven by pH Elevation

Maruda Shanmugasundaram^a, Dmitry Kurouski^a, William Wan^b, Gerald Stubbs^b, Rina K. Dukor^c, Laurence A. Nafie^{c,d}, Igor K. Lednev^{a,*}

^aDepartment of Chemistry, University at Albany, State University of New York, 1400 Washington Avenue, Albany, New York 12222, USA

^bDepartment of Biological Sciences and Center for Structural Biology, Vanderbilt University, Nashville, Tennessee 37235, USA

^cBioTools Inc., 17546 Bee Line Hwy, Jupiter, Florida 33458, USA

^dDepartment of Chemistry, Syracuse University, 1-014 Center for Science and Technology, Syracuse, New York 13244, USA

Abstract

Amyloid fibril polymorphism is not well understood despite its potential importance for biological activity and associated toxicity. Controlling the polymorphism of mature fibrils including their morphology and supramolecular chirality by post-fibrillation changes in local environment is the subject of this study. Specifically, the effect of pH on the stability and dynamics of HET-s (218–289) prion fibrils has been determined through the use of vibrational circular dichroism (VCD), deep UV resonance Raman and fluorescence spectroscopies. It was found that a change in solution pH causes deprotonation of Asp and Glu amino acid residues on the surface of HET-s (218–289) prion fibrils and triggers rapid transformation of one supramolecular chiral polymorph into another. This process involves changes in higher-order arrangements like lateral filament and fibril association and their supramolecular chirality, while the fibril cross- β core remains intact. This work suggests a hypothetical mechanism for HET-s (218–289) prion fibril refolding and proposes that the interconversion between fibril polymorphs driven by the solution environment change is a general property of amyloid fibrils.

Graphical Abstract

* Corresponding Author : Dr. Igor K. Lednev, ilednev@albany.edu Telephone 518-591-8863 Fax 518-442-3462.

^cPresent Addresses D.K.: Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, USA.

^fPresent Addresses W.W.: Structural and Computational Biology Unit, European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany.

The authors declare no competing financial interests.

Supporting Information. Additional information on AFM analysis and the role of ionic strength in Trp fluorescence intensity. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

Disaggregation; pH Effect; Filament Twist; Chirality; Morphology; Infrared Vibrational Circular Dichroism; Fluorescence; Deep-UV Resonance Raman spectroscopy

INTRODUCTION

Protein misfolding and aggregation can often lead to the formation of highly-organized β -sheet protein aggregates known as amyloid fibrils.¹ Although fibrils possess a typical cross- β core, their morphology varies significantly depending on the aggregation conditions.^{2,3} It was recently demonstrated by the use of vibrational circular dichroism (VCD) that at the initial stage of protein aggregation and filament (simplest single cross- β -core linear structure) formation, pH defines the sense of filament chirality (left or right twist along filament axis) and the resulting morphology of fibrils of insulin, lysozyme, apo- α -lactalbumin, HET-s (218–289) prion protein from *Podospora anserina* and a short peptide fragment of transthyretin, TTR (105–115).^{4,5} At pH above 2.3, both insulin and lysozyme aggregate to form multi-filament left-twisted fibrils with normal VCD (*vide infra*), whereas at pH below 2.3 flat, tape-like fibrils are formed with reversed-signed VCD. On the other hand, HET-s (218–289) and TTR (105–115) form left twisted fibrils at pH 2.0 and below with normal VCD and flat tape-like fibrils above pH 2.0 with reversed VCD. This finding indicated that pH-controlled filament chirality (reported by the normal and reversed signage of VCD) and fibril polymorphism may be a general phenomenon related to all amyloid protein fibrils.⁵

It was recently demonstrated that besides the determination of fibril morphology and filament supramolecular chirality at the stage of protein aggregation, a small change in pH causes drastic morphological changes in mature insulin fibrils.⁶ Here VCD revealed that as a

result of these morphological changes filament supramolecular chirality was reversed.⁶ Separately it was reported that changes in electrostatic interactions lead to significant morphological changes of mature insulin fibrils grown at pH 2.0 if the solution pH is elevated above pH 6.0.⁷ It was also shown that morphology and structure of apo- α -lactalbumin fibrils can be controllably changed by small alteration of solution temperature and ionic strength.⁸ The question to ask is how general are these intrinsic instabilities of mature protein fibrils and whether changes in filament supramolecular chirality is the underlying cause of pH driven transformations of fibril morphology and fibril polymorphism as recently suggested.⁵

MATERIALS AND METHODS

Preparation of HET-s (218–289) Prion Fibrils

The procedure for HET-s (218–289) prion fibril formation is described in detail elsewhere.⁹ Briefly, HET-s (218–289) prion protein was overexpressed in *E. coli* cell culture and the inclusion bodies were purified and solubilized in denaturing conditions. HET-s (218–289) prion protein was then purified by Ni-NTA chromatography and eluted by exchanging to low pH buffer under the same denaturing conditions. Then the peptide was desalted using a Sephadex G-25 superfine column. Fibril formation was then induced by incubating the peptide in 20 mM citric acid at pH 2.0 at room temperature. Fibril formation was confirmed by solution turbidity and negative stain electron microscopy and used for further measurements.

Atomic Force Microscopy

HET-s (218–289) prion fibrils grown in 20 mM citric acid (pH adjusted to 2.0 with HCl) were separated from supernatant by centrifugation and redispersed in pH 2.0 citric acid solution. The redispersed fibril solution was diluted 200X with pH 2.0 citric acid solution, whereas pH 6.0 HET-s (218–289) prion fibril solution was diluted 200X with distilled water (pH ~6.0). Then the fibrils were deposited on freshly cleaved mica; the surface was gently washed with pH 2.0 citric acid solution or distilled water, respectively and dried under nitrogen flow. AFM imaging was performed using a MFP3D™ Bio Asylum Research Microscope (Asylum Research, CA, USA) in AC mode with Olympus AC240TS cantilevers.

Igor Pro 6.2.1.0 software was used to prepare images. For the population density distribution analysis, the width of 50 different fibrils or filaments were measured using Igor Pro 6.2.1.0 software and plotted together using the Kernel Density Estimate¹⁰ program. One width measurement was made per fibril and the probability distribution plots (Figure 1, c) were normalized such that area under the curve is the same for both curves.

Vibrational Circular Dichroism (VCD) and Infra-red (IR) Spectroscopy

VCD and IR spectra were measured at BioTools, Inc., Jupiter, FL using a ChiralIR-2X Fourier transform VCD (FT-VCD) spectrometer equipped with an MCT detector and the DualPEM option for enhanced VCD baseline stability. For each measurement, ~10 μ L of fibril sample was placed in a BioCell (BioTools, Inc.) with CaF₂ windows and a 6- μ m path

length. During measurements the BioCell was rotated at a constant velocity about the IR beam axis using SyncRoCell S2 (BioTools, Inc.) to eliminate cell and possible sample birefringence. VCD and IR spectra were acquired for 2–8 hours at 8 cm^{-1} spectral resolution. Spectral baselines for VCD and IR were determined from measurements of the corresponding solvent in the same BioCell for the same length of time as the sample measurements. All subsequent data processing leading to final spectra were carried out in GRAMS/AI 7.0 (Thermo Galactic, Salem, NH, USA).

Deep UltraViolet Resonance Raman (DUVRR) Spectroscopy

Two aliquots of pH 2.0 HET-s (218–289) prion fibril solutions were centrifuged and redispersed separately in pH 2.0 citric acid solution and distilled water (pH ~6.0). The samples were then washed with pH 2.0 citric acid solution or distilled water, respectively, once again before measuring their DUVRR spectra. DUVRR spectra were measured using a home-built Raman spectrometer¹¹ with an excitation wavelength of 199.7 nm. A continuously spinning NMR tube was used for holding the samples with a magnetic stirrer inside. Each spectrum was the average of forty independent thirty second accumulations. GRAMS/AI 7.01 software (Thermo Galactic, Salem, NH, USA) was used for all DUVRR data treatment. The spectrum of Teflon was used for calibrating the spectra. The contributions of the NMR tube, solvent and oxygen were subtracted and baseline irregularities due to Rayleigh scattering were corrected.

Tryptophan Fluorescence

For fluorescence measurements, redispersed pH 2.0 HET-s (218–289) prion fibrils were diluted 200X in the appropriate solvents. For time dependence assay, they were diluted directly in pH 2.0 citric acid solution and distilled water (pH 6.0). For the pH dependence assay, 20 mM citric acid solution of different pH ranging from 2.0 to 5.0, adjusted by NaOH, was used to directly dilute the pH 2.0 fibrils. Since the same fibril sample was used as source for measuring fluorescence under different conditions, concentration remained the same between samples used in every experiment.

All fluorescence spectra were measured with a Fluorolog®-3 spectrofluorometer (HORIBA Jobin Yvon, Edison, NJ, USA). An excitation wavelength of 295 nm was used in all cases to selectively excite tryptophan. Each reported spectra except Figure 4,a is the average of six independently measured spectra, and their standard deviations are indicated by the error bars. The spectra shown in Figure 4,a were averaged from three acquisitions. FluorEssence™ software (HORIBA Jobin Yvon, Edison, NJ, USA) was used to collect all data and GRAMS/AI 7.01 (Thermo Galactic, Salem, NH, USA) was used to treat the data.

RESULTS AND DISCUSSION

Filament Chirality Reversal and Morphological Rearrangements with pH

Here we investigate the effect of pH on the stability of HET-s (218–289) prion fibrils. We find that left- twisted HET-s (218–289) prion fibrils formed at pH 2.0 unwind (multi-filament de-braiding) and fragment to some degree as a result of solution pH elevation to pH 6.0 (Figure 1, a and b). Previously we have reported VCD spectra of HET-s (218–289) prion

fibrils formed at pH 2.0, 3.3 and 3.9.⁵ Fibrils grown at pH 2.0 show VCD and morphology the same as reported here, and fibrils formed at pH 3.3 and 3.9 showed weak reversed VCD. In the present study, we investigate only the properties of HET-s (218–289) prion fibrils grown at pH 2.0 that were subsequently studied at pH 2.0 and at the elevated pH of 6.0.

Using AFM we have investigated the topologies of HET-s (218–289) prion fibrils grown at pH 2.0 and found two major populations. One exhibits a well-defined left-handed twist and has a width of around 58 nm and several microns in length, assigned to mature fibrils. The second can be assigned to thinner shorter fibrils having a width of ~19 nm (Figure 1, c) and are less than one micron in length. After the pH was elevated to 6.0 there were no longer left-twisted fibrils, but rather only short (50–700 nm) fibrils with a width of ~19 nm and no detectable twists were present. This indicates that left-twisted fibrils unwind and partially fragment forming shorter, thinner fibrils, possibly single cross- β -core filaments.

VCD is a unique spectroscopic technique that has a high sensitivity to filament supramolecular chirality in fibrils.^{4–6, 13, 14} One can suspect that pH-driven unwinding of left-twisted HET-s (218–289) prion fibrils might cause significant changes in their supramolecular chirality. This is confirmed by VCD observations. Left-twisted HET-s (218–289) prion fibrils grown at pH 2.0 exhibit a ‘normal’ VCD spectrum with a (+ + – + +) band pattern (Figure 2, b). Previously it was reported that left-twisted fibrils are formed by braiding of proto-fibrils and filaments that have the same left-twist handedness.¹⁴ Remarkably, after the solution pH was elevated to pH 6.0 the signs of the VCD of HET-s (218–289) prion fibrils are reversed indicating a reversal of the filament supramolecular chirality (Figure 2, b). This indicates that intertwined left-twisted HET-s (218–289) prion fibrils at pH 2.0 not only unwound but also reversed the handedness of their filament chirality and became right-twisted to almost the same degree. The right-handed twist, however, is not revealed by AFM (Figure S1, ESI) which indicates that, as previously concluded,⁵ individual filament chirality lies below the detection limit of AFM microscopy but is clearly detectable with VCD.¹⁴ In addition, we found that the changes observed by VCD are irreversible. When the solution pH of the newly formed pH 6.0 ‘filaments’ was brought back to 2.0, no noticeable changes in the VCD spectra were observed even after 24 hours of exposure (data not shown). This indicates that supramolecular chirality of newly formed pH 6.0 ‘filaments’ does not change if pH is lowered to 2.0.

Fibril Secondary Structure is Preserved During the Chiral Transformations

While VCD shows a complete reversal in the signage, IR spectra of both pH 2.0 and pH 6.0 HET-s (218–289) prion fibrils are nearly identical, which suggests that their secondary structure does not change as a result of fibril debraiding and partial fragmentation.¹⁵ The decrease in the intensities of ~1736 and 1720 cm^{-1} bands, which correspond to side chain carboxyl groups of Asp and Glu, is attributed to deprotonation.¹⁶ HET-s (218–289) peptide has 4 Asp and 5 Glu residues respectively (Asp 220, 230, 258, 288 and Glu 234, 235, 265, 272, 280). We also utilized deep UV resonance Raman (DUVRR) spectroscopy, which is highly sensitive to the fibril secondary structure.^{17–19} The typical protein DUVRR spectrum is dominated by amide bands, which characterize the polypeptide backbone conformation, and aromatic amino acid bands, which report on their local environment.¹⁹ The DUVRR

spectra of both pH 2.0 and 6.0 HET-s (218–289) prion fibrils show sharp, intense Amide I and II bands as well as high intensity of the C α -H band that are indicative of an extended β -sheet conformation (Figure 3). It was found that the positions and relative intensities of all bands remained the same, which shows that the secondary cross- β core structure of HET-s (218–289) prion fibrils do not change upon reversal of the filament supramolecular chirality. The fact that the core structure remains intact during pH elevation indicates that all observed changes are associated with the non-core region of the HET-s (218–289) prion fibrils.

Proposed Mechanism for Fibril Spontaneous Refolding

To determine the time scale of the observed prion fibril unwinding, tryptophan fluorescence was measured to reveal changes in Trp environment with respect to interactions with nearby residues or the solvent.²⁰ VCD and DUVRR measurements are time-consuming and currently unsuitable to study fast processes whereas fluorescence was found to be a fast and suitable technique. HET-s (218–289) peptide has only one Trp residue.²¹ Trp intrinsic fluorescence intensity was monitored during pH-driven prion fibril disintegration, and it was found that after 30 seconds of pH elevation the intensity of Trp fluorescence increased almost two-fold (Figure 4, a) and thereafter remained constant (Figure S2). Since the hydrophobic²¹ core structure of HET-s (218–289) prion fibrils remain the same upon fibril (but not filament) disintegration (unwinding), large changes in Trp fluorescence indicate that Trp residues are located close to or on the surface of fibrils, which are normally solvent accessible. Besides, the peak maxima of ~350 nm show a hydrophilic local environment. Although there is no compelling proof that VCD and Trp fluorescence changes are simultaneous, such a rapid pH-driven disintegration of HET-s (218–289) prion fibrils suggests a possible electrostatic repulsion between filaments in multi-filament fibrils, which can be initiated by the deprotonation of some amino acid side chain residues. Similar handedness inversion behavior in certain helical peptides in response to side chain substitution as well as other external stimuli is well-documented. We also demonstrated that the observed changes in Trp fluorescence intensity is independent of changes in ionic strength associated with the pH elevation (Figure S3).

HET-s (218–289) peptide has 4 Asp and 5 Glu residues²¹ with side-chain pK_a values of 3.9 $\pm$ 0.1 and 4.2 $\pm$ 0.1 respectively.^{23–25} One can expect that if fibril unwinding took place close to the pH of their pK_a, this would directly indicate that deprotonation of Asp and Glu amino acid residues causes large changes in the charges on the filament surfaces, which consequently leads to their repulsion. We titrated HET-s (218–289) prion fibrils from pH 2.0 to pH 6.0 monitoring the change in Trp fluorescence (Figure 4, b). It was found that Trp fluorescence intensity remained steady from pH 2.0 to 3.9, but increased dramatically when the pH was raised to 4.0. This transition occurs in the same range of pH as the pK_a of Asp and Glu amino acid residues, and it is well-known that Trp fluorescence can be quenched by nearby protonated acidic amino acids like Asp and Glu.^{26, 27} The fact that Asp288 is present next to Trp287 in HET-s (218–289) peptide leads us to suspect that its deprotonation at pH ~4.0 causes unquenching of Trp287 fluorescence. This is further supported by the IR spectral evidence for different protonation states of Asp and Glu residues (Figure 2). Trp fluorescence might also be self-quenched by Tyr or Phe, but self-quenching alone does not explain the transition observed at pH ~4.0. Hence, we can hypothesize that the deprotonation

causes the loss of quenching, with possible contribution from the loss of self-quenching due to the fibril structural/conformational rearrangements.

CONCLUSIONS

Herein we explored the stability of HET-s (218–289) prion fibrils and found that a left-twisted multi-filament fibril polymorph formed at pH 2.0 unwinds and partially fragments as a result of a sudden solution pH increase to pH 6.0. VCD reveals that at the level of individual filament twist chirality, the filaments of the majority of HET-s (218–289) prion fibrils at pH 2.0 have left-twisted chirality that results in a normal VCD pattern while at pH 6.0 have nearly mirror-image VCD implying net right-twisted filament chirality. Application of atomic force microscopy (AFM) and Trp fluorescence spectroscopy revealed that fibril transformation after raising the pH from 2.0 to 6.0 is very rapid and occurs within 30 seconds. Using deep UV resonance Raman (DUVRR) and infra-red (IR) spectroscopy we have found that there are no changes in the secondary structure of fibrils with increase in solution pH, indicating that changes in morphology and fibril supramolecular chirality do not affect fibril cross- β core. The remarkable similarity of the VCD, aside from opposite signs of all bands, indicates that the filament helical chirality is changed to nearly the same degree of twist but opposite sense (left-handed to right-handed) with no other changes to the filament structure, otherwise the relative intensities of the VCD bands would be significantly different. The observed chiral and morphological transformations of HET-s (218–289) prion fibrils are triggered by deprotonation of Asp and Glu amino acid residues, which leads to the changes in electrostatic interactions along their surfaces, and causes repulsion between fibril filaments. The significant reduction in the thickness of the fibrils at pH 6.0 indicates that the AFM observed fibrils may be individual fibril filaments. These findings further challenge the concept of the extraordinary stability of amyloid fibrils, indicating that they are indeed “alive”,⁸ and suggest that pH-controlled transformations of mature fibrils might be a general phenomenon.

Supplementary Material

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ACKNOWLEDGMENT

We thank Marketa Pazderkova for valuable discussion regarding interpretation of IR spectra. This work was supported by the National Science Foundation under Grants No. CHE-1152752 (I.K.L.) and IIP-0945484 (SBIR phase II to R.K.D. and L.A.N.), and the National Institutes of Health under Grants AG002132 (G.S.) and F31-AG040947 (W.W.).

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Figure 1. AFM images of HET-s (218–289) prion fibrils at pH 2.0 (a) and pH 6.0 (b). Scale bar: 100 nm. Distributions of width (c) of HET-s (218–289) prion fibrils grown at pH 2.0 and pH 6.0 determined by Kernel density function analysis.¹⁰ Area under the curve is the same for both curves.

Figure 2.
IR (a) and VCD (b) spectra of HET-s (218–289) prion fibrils at pH 2.0 and 6.0.

Figure 3.
DUVRR spectra of HET-s (218–289) prion fibrils at pH 2.0 and pH 6.0.

Figure 4.

Trp fluorescence emission of HET-s (218–289) prion fibrils (a) at pH 2.0 and pH 6.0 and (b) peak fluorescence intensity between pH 2.0 and 5.0, recorded with 295 nm excitation. Error bars indicate standard deviation.