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Possible Existence of α -Sheets in the Amyloid Fibrils Formed by a TTR_{105–115} Mutant

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Abstract

Herein, we combine several methods to characterize the fibrils formed by a TTR_{105–115} mutant in which Leu111 is replaced by the unnatural amino acid aspartic acid 4-methyl ester. We find that this mutant peptide exhibits significantly different aggregation behavior than the wild-type peptide: (1) it forms fibrils with a much faster rate, (2) its fibrils lack the long-range helical twists observed in TTR_{105–115} fibrils, (3) its fibrils exhibit a giant far-UV circular dichroism signal, and (4) its fibrils give rise to an unusual amide I' band consisting of four distinct and sharp peaks. Based on these results and also several previous computational studies, we hypothesize that the fibrils formed by this TTR mutant peptide contain both β - and α -sheets.

Graphical Abstract

INTRODUCTION

Amyloids formed by the transthyretin (TTR) protein have been linked to various diseases, including Senile Systemic Amyloidosis, Familial Amyloid Polyneuropathy, Familial

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Notes

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Methods and materials, additional FTIR, ATR-FTIR, 2D IR, UV-Vis, and CD spectra, AFM and TEM images.

Amyloid Cardiomyopathy, and Central Nervous System Selective Amyloidosis.^{1–3} As such, numerous studies have focused on TTR amyloid fibrils, ranging from structure determination to elucidation of the underlying fibrilization mechanism.^{4–9} In particular, an 11-residue segment of TTR, TTR_{105–115} (sequence: YTIAALLSPYS where the numbering is consistent with the full-length protein) has been widely used as a model system in this regard, as this segment is known to form ‘cross- β ’ amyloid fibrils similar to those formed by the full-length protein.^{10,11} Recently, Griffin, Dobson, and co-workers have characterized the atomic structure of TTR_{105–115} fibrils,¹¹ using a combination of structural techniques, including magic angle spinning nuclear magnetic resonance (NMR), cryoelectron microscopy (cryo-EM), X-ray diffraction, scanning transmission electron microscopy (STEM), and atomic force microscopy (AFM). They found that these fibrils are formed from four, six, or eight two-sheet proto-filaments which are aligned in a head-to-tail manner, with each protofilament consisting of parallel, in-register β -sheets that stack in an antiparallel fashion (Figure 1). In addition, their structure models suggested that structured water exists in the fibril core¹¹ which initially motivated us to introduce a site-specific infrared (IR) probe into the sequence of TTR_{105–115} and use both linear and nonlinear IR spectroscopic techniques to assess the dynamics of such confined or structured water, a topic that has attracted considerable attention in the past few years.¹² Specifically, we mutated Leu111 to aspartic acid 4-methyl ester (D_M), which is a well-established protein IR hydration probe.¹³ The choice of this mutation is based on the fibril structure (Figure 1 and Figure S1 in SI), which shows that 50% Leu111 sidechains are buried in a water-inaccessible region and the rest are exposed to confined water. Much to our surprise, our spectroscopic results suggest that this TTR_{105–115} mutant (hereafter referred to as TTR-111D_M) forms fibrils that are distinctly different from those formed by the wild-type peptide, as the former consist of both β - and α -sheets.

The α -sheet structure was first proposed by Pauling and Corey as a secondary structural element of proteins.¹⁴ As indicated (Figure S2 in SI), the structure of an ideal α -sheet is similar to that of an ideal β -sheet as both have the same meridional repeat distance (4.75 Å) and the same average hydrogen bond distance (2.3 Å); however, they differ in the orientation of their respective backbone NH and CO groups. In the β -sheet structure, the NH and CO groups alternate on either side of the sheet, while all the NH (CO) groups in an α -sheet are aligned on one side of the peptide backbone pleat.^{14–16} Therefore, each NH/CO group in an α -sheet would form two hydrogen-bonds (H-bonds) with two CO/NH groups on a neighboring strand.¹⁵ Comparably, β -sheets are more stable than α -sheets, which lead Pauling and Corey to reject the α -sheet structure as an “important” configuration in proteins.^{14,17} Indeed, very few α -sheet structures have been found in native proteins,¹⁵ although they can be induced to form in short peptides consisting of alternating D- and L-amino acids.^{18,19} Interestingly, however, α -sheet conformations are frequently observed in molecular dynamics (MD) simulations of amyloid-forming proteins, including TTR, β_2 -microglobulin, lysozyme, the prion protein, and polyglutamine, particularly in the regions of the protein which are considered the most amyloidogenic experimentally.^{15,16,20–22} In this regard, the α -sheet has been postulated to be a common intermediate state in the formation of amyloid fibrils and, also, the underlying structure of the toxic amyloid oligomers.¹⁵ Nevertheless, to the best of our knowledge, this structure has never been observed experimentally to exist in

either transiently populated amyloid oligomers or mature fibrils, making it an elusive target for further studies. Thus, the experimental confirmation of this structure in amyloid fibrils will open up new avenues for providing a better understanding of the biological role of α -sheets.

RESULTS AND DISCUSSION

As shown (Figure S3), like the wild type TTR_{105–115} peptide,¹¹ TTR-111D_M readily aggregates and forms fibrils at concentrations of a few millimolar. However, the amide I' (amide I in D₂O) bands of these peptides, when aggregated, show significant differences (Figure 2). The amide I band, which arises mainly from the backbone C=O stretching vibrations, is a well-established IR reporter of protein conformations. This is because the amide I band of a polypeptide manifests the underlying vibrational coupling between the individual amide units, an interaction depending on its secondary structure.²³ For example, the amide I' band of amyloid fibrils consisting of either parallel or antiparallel β -sheets is characterized by a strong and narrow peak located at a frequency of less than 1630 cm⁻¹, whereas fibrils composed of antiparallel β -sheets also give rise to a weak amide I' peak at a frequency of 1680 cm⁻¹ or higher.^{23–25} Therefore, the FTIR results in Figure 2 provide the first evidence indicating that the Leu111 to D_M mutation in TTR_{105–115} leads to a change in the underlying fibril structures. Consistent with the fibril structures of Fitzpatrick *et al.*,¹¹ the amide I' band of aggregated TTR_{105–115} peptides shows characteristics of parallel β -sheets,^{26–28} as it is dominated by a pair of narrow peaks located at ~1625 (strong) and ~1665 cm⁻¹ (weak), respectively. Further two-dimensional infrared (2D IR) measurements show that a cross-peak at (ω_{pump} , ω_{probe}) = (1665 cm⁻¹, 1625 cm⁻¹) is developed at early waiting times (Figure S4 in SI), indicating that these peaks arise from the same fibrillar species and not from heterogeneity in the sample.

In comparison (Figure 2), the FTIR spectrum of an aggregated TTR-111D_M sample in the amide I' region contains more resolvable spectral features. As expected, the ester carbonyl of the D_M sidechain gives rise to an additional IR band outside the amide I' profile, which can be well fit by a Gaussian function with a peak frequency of 1744.8 cm⁻¹ and a width (i.e., FWHM) of 7.2 cm⁻¹ (Figure S5 in SI). According to the study of Pazos *et al.*,¹³ this frequency indicates that the ester carbonyls in the fibrils are situated in a dehydrated environment, an unexpected result. Based on the fibril structures of TTR_{105–115} (Figure 1 and Figure S1 in SI), 50% of the sidechains at position 111 would face towards the hydrated core of the fibril and, hence, should produce an IR band at a frequency of 1730 cm⁻¹ or lower due to H-bonding interactions with water. To verify that the fibrils produced by both the wild-type and mutant TTR_{105–115} peptides indeed contain confined water, we measured the FTIR spectra of the respective fibril samples in the form of a dry film. As shown (Figure S6 in SI), the data clearly show that even for fibril samples that have been dried on the surface of a Ge crystal under vacuum for at least 24 hours, the D₂O IR band¹² at ~2575 cm⁻¹ is still present. This result indicates that fibrils formed by both the wild-type and mutant TTR peptides can host trapped water, as initially suggested.¹¹ Thus, the fact that the D_M sidechain in the TTR-111D_M fibrils is not located in a hydrated environment suggests that the corresponding Leu111 to D_M mutation changes the fibril structure(s). A more convincing evidence in support of this notion comes from the amide I' band of the mutant sample,

which consists of four resolvable, narrow peaks, at ~ 1616 , ~ 1652 , ~ 1662 , and ~ 1672 cm^{-1} , respectively (Figure 2). The peaks at 1616 and 1652 cm^{-1} likely correspond to those observed in TTR_{105–115} fibrils,²⁶ with a modest shift toward lower wavenumbers. This similarity suggests that the fibrils formed by TTR-111D_M contain parallel β -sheets. The question is then what produces the other two spectral features.

One possibility is that the 1662 and 1672 cm^{-1} peaks arise from non-aggregated peptides and residual trifluoroacetic acid (TFA) in the fibril sample, respectively. This is because the amide I' band of a disordered peptide is centered around 1650 cm^{-1} and TFA, which was used in the peptide synthesis and purification, exhibits a strong IR signal at 1672 cm^{-1} . To test this possibility, we followed the aggregation/fibrillization process of the TTR-111D_M peptide by measuring its FTIR spectra as a function of time. As shown (Figure 3), the amide I' band obtained at 'zero time' is dominated by a broad feature centered at ~ 1650 cm^{-1} , which directly rules out the possibility that the 1672 cm^{-1} peak originates from TFA. Furthermore, all of the fine spectral features are developed concomitantly, at the expense of the broad 1650 cm^{-1} feature, which, therefore, argues against the idea that the 1662 cm^{-1} peak corresponds to non-aggregated peptides. This argument is further corroborated by the ester carbonyl signal of the D_M sidechain. If a significant amount of peptide monomers were still present, one would expect the presence of a broad peak at 1725 cm^{-1} , as that observed at 'time zero', in the spectrum collected at 315 minutes, which is not observed (Figure 3 inset). In fact, the fairly narrow bandwidth of the ester carbonyl band of the fully aggregated sample provides strong evidence indicating that the D_M sidechains in the fibrils sample a rather homogenous environment. In support of this picture, the 2D IR spectrum of the ester carbonyl band at $T = 0$ shows little, if any, elongation (Figure S7 in SI). Thus, taken together these results indicate that the four narrow amide I' peaks of TTR-111D_M originate from the underlying fibril structure, rather than from any sample heterogeneity. Moreover, the time dependence of the amide I' band of TTR-111D_M (Figure 3) indicates that its fibrils are formed within ~ 5 hrs, which is much faster than the fibrilization rate of the wild-type peptide measured under similar experimental conditions.¹¹

Theoretical calculations have predicted that the amide I' frequency of the α -sheet is different from its β -sheet counterpart. For example, the density functional theoretical study of Torii²⁹ indicated that the α -sheet conformation of a glycine dipeptide gives rise to a strong amide I' peak between $1670 - 1690$ cm^{-1} and a less intense one between $1630 - 1650$ cm^{-1} . Similarly, the quantum mechanics calculation of Huo and coworkers³⁰ on the amide I band of an alanine dipeptide predicted that the α -sheet conformation would exhibit a strong peak at 1653 cm^{-1} , with two smaller ones at 1623 and 1681 cm^{-1} . Therefore, based on these theoretical predictions, a more plausible interpretation of the atypical amide I' band shape of the TTR-111D_M fibrils is that it contains contributions from both β - and α -sheets. Because of the narrowness of the ester carbonyl vibrational band, it is unlikely that the aggregated TTR-111D_M sample is composed of two types of fibrils, with one consisting of α -sheets and the other of β -sheets. Therefore, we hypothesize that the fibrils are assembled from protofibrils consisting of both β - and α -sheets. To further validate this notion, we carried out 2D IR measurements on TTR-111D_M fibrils. Should those 4 amide I' bands indeed share the same structural origin, we expect to observe 2D IR cross-peaks developed between them due to either vibrational coupling or energy transfer. As shown

(Figure 4), the 2D IR spectrum obtained with $\langle XXXX \rangle$ polarization configuration at $T = 0$ shows cross-peaks between the 1662 and 1672 cm^{-1} bands. In addition, 2D IR spectra measured with $\langle XXY Y \rangle$ polarization configuration show enhanced cross-peaks between the 1652 cm^{-1} and two higher-frequency bands and those obtained at longer waiting times indicate the development of cross-peaks between the 1616 cm^{-1} and other three bands (Figure S8 in SI). Taken together, these 2D IR results provide strong evidence indicating that the unique amide I' band shape of the TTR-111D_M fibrils does not arise from sample heterogeneity, but instead supporting the hypothesis that it manifests the difference in the underlying backbone-backbone H-bond arrangements.

The protein amide A band mainly arises from the backbone N-D stretching vibrations (in D₂O). Therefore, the difference between the backbone-backbone H-bonding patterns of the β - and α -sheets is expected to affect not only their amide I' bands, as observed (Figure 2), but also their amide A vibrations. Indeed, the aggregated TTR₁₀₅₋₁₁₅ and TTR-111D_M samples show distinct difference in their amide A bands, as seen from ATR-FTIR spectroscopic measurements (Figure S6 in SI). This provides additional evidence supporting the notion that the underlying fibrillar structures of these two peptides are not identical.

Further supporting evidence comes from the far-UV circular dichroism (CD) spectra of TTR₁₀₅₋₁₁₅ and TTR-111D_M. As indicated (Figure 5), the CD spectrum of an aggregated TTR₁₀₅₋₁₁₅ sample is similar to that observed previously.³¹ However, in comparison the CD spectrum of an aggregated TTR-111D_M sample is not only different in shape from that of TTR₁₀₅₋₁₁₅ but also exhibits a giant CD signal at 205 nm. This is an amazing result, as, to the best of our knowledge, such a giant CD signal has never been observed in a peptide or protein system. It is well known that certain amino acids, such as tryptophan, can produce additional CD signals (i.e., a CD couplet) in the far-UV spectral region due to exciton couplings.^{32,33} Therefore, the giant CD signal observed for the TTR-111D_M aggregates is most likely due to additional contributions from the D_M sidechains, as methyl acetate, a model compound for the sidechain of D_M, exhibits a strong far-UV absorption band, peaked at ~205 nm in water (Figure S9 in SI). Since the magnitude of an exciton CD couplet is sensitively dependent on the relative positions (i.e., distance and orientation) of the two coupled chromophores,³⁴ the greatly enhanced CD signal at 205 nm suggests that in the fibrils, the D_M sidechains from two in-register sheets must be in close proximity. This agrees with the linear and 2D IR results showing that the ester group of D_M is not only buried in a dehydrated environment but also experiences little, if any, heterogeneity. In support of this picture, the far UV CD spectrum of TTR-111D_M monomers only show typical spectral features associated with unstructured peptides (Figure S10 in SI). It is also possible that the giant CD signal is due to exciton couplings involving both electronic transitions arising from the peptide backbone and the D_M sidechains. Yet, another possible scenario is that the underlying fibril structure supports a supramolecular chirality, leading to a large increase in the CD signal, as has been observed for chiral helical stacks.³⁵⁻³⁸ Irrespective of the interpretation, these CD spectra once again indicate that TTR-111D_M form fibrils with a distinct structure.

The most direct evidence indicating that the fibril structures of the wild-type and mutant aggregates are dissimilar comes from the AFM images of individual fibrils formed by

TTR_{105–115} and TTR-111D_M. It is apparent that the dimension perpendicular to the long fibril axis (hereafter referred to as **d**) is larger for the TTR-111D_M fibrils than for the TTR_{105–115} fibrils (Figure 6). A more quantitative assessment further substantiates this notion as it indicates that the height (**h**) of the TTR_{105–115} fibrils is about 10–12 nm, which is on the same order as measured previously,¹¹ whereas the TTR-111D_M fibrils have a **h** of about 4–6 nm and a **d** about 25–45 nm (Figure 6). This aspect ratio seems to suggest that the TTR-111D_M fibrils adopt a ribbon-like structure; however, the fact that these fibrils contain confined water, even under dry conditions (Figure S6 in SI), argues against this picture. Moreover, a two-dimensional fast Fourier transform (2D-FFT) analysis of individual fibrils reveals, as shown (Figure S11 in SI), that the wild-type fibrils have an intrinsic twist along the long fibril axis, with a periodicity of ~83 nm, a value similar to that reported in other studies for TTR_{105–115} (95 ± 10 nm).¹¹ On the contrary, the corresponding 2D-FFT analysis of the TTR-111D_M fibrils indicates that they have no detectable twists or pitches (Figure S11 in SI). In this regard, the morphology of the TTR-111D_M fibrils resembles that of tape-like fibrils observed in various amyloid aggregates.³⁹ For example, the AFM image of a tape-like fibril formed by apo- α -lactalbumin shows that it has a **h** of ~5 nm and a **d** of ~26 nm and exhibits no detectable twists along the fibril axis.⁴⁰

Hayward and Milner-White have predicted through molecular dynamics simulations that parallel α -sheets tend to assemble into nanotube-like structures.⁴¹ Therefore, to provide further information on the morphology of TTR-111D_M fibrils, we examined them using transmission electron microscopy (TEM). As indicated (Figure 7 and Figure S12 in SI), the TEM images also shows that this mutant peptide assembles into fibrils that lack the long-range helical twists observed in TTR_{105–115} fibrils,¹¹ which have a cross-over distance of ~95 nm. Thus, these results suggest that the TTR-111D_M peptide forms either non-twisting fibrils or fibrils with a twist that is not detectable by the current method, hence consistent with the AFM result. A closer examination of the TEM images (Figure S12 in SI) reveal, however, that the fibril width (9 ± 2 nm) is ~3–5 times smaller than that determined via AFM measurements. One likely interpretation of this discrepancy is that the experimental conditions used in TEM measurements (e.g., staining and vacuum) lead to disassembly of thicker fibrils and convert them into thinner ones. Another possibility is that the procedures used to prepare AFM samples promote thicker fibril formation.⁴² Irrespective of the underlying cause of this discrepancy, the fact that both types of images show that the TTR-111D_M fibrils do not exhibit the commonly observed, long-range helical twists suggests that conversion of a thinner fibril to a thicker one is through lateral association of protofilaments without braiding, a mechanism that has been earlier proposed to explain the formation of flat, tape-like fibrils.⁴⁰

To ensure that the non-twisting fibril morphology observed in both AFM and TEM measurements is not due to their special experimental conditions, we used the induced circular dichroism (ICD) method⁴³ to further characterize the twist of TTR-111D_M fibrils in solution. The ICD method uses the induced CD signal of an amyloid-bound dye, for example thioflavin T (ThT), to characterize the supramolecular chirality of amyloid fibrils. It has been demonstrated that fibrils with distinct helical twists can display a strong ICD signal in the absorption spectrum region of the dye.^{39,43} As shown (Figure S13 in SI), in the

presence of TTR-111D_M peptide, the ThT fluorescence intensity is significantly increased, indicating of ThT binding to fibrils; however, the same solution does not show any appreciable ICD signals (Figure S13 in SI). In agreement with Lokszejn *et al.*⁴³ fibrils with periodic twists such as an A β _{16–22} derivative, A β _{16–22}F19K*,⁴⁴ was also found to have an observable ICD signal (Figure S14 in SI). Thus, this result corroborates the notion that TTR-111D_M forms fibrils without long-range twists.

All of the experimental results support the notion that the Leu111 to D_M mutation in TTR_{105–115} significantly changes the fibrilization energy landscape of this short peptide, resulting in fibrils of different structure and morphology. However, the spectroscopic methods used in this study do not allow us to assess the detailed molecular arrangements of the peptide molecules in the fibrils. Nevertheless, based on the IR data, we speculate that the protofibrils formed by TTR-111D_M could have a structure as depicted in Figure 8, where each sheet is a mix of α - and β -sheet characters with the D_M sidechains packed between two sheets and hence in a dehydrated environment. While further studies using high-resolution structural techniques are required to validate this model, it is nevertheless consistent with the study of Armen *et al.*,²⁰ which showed, via MD simulations, that at low pH, an α -sheet intermediate is formed in the 105–115 region of the full-length TTR monomeric protein. Furthermore, the authors postulated that the α -sheet may be an important pathological conformation in neurodegenerative amyloidosis. Incidentally, the D_M mutation used in the current study is similar to that (i.e., Leu111 to Met) found in the full-length TTR aggregates associated with a Danish variant of familial amyloid cardiomyopathy.⁴⁵ Therefore, it would be interesting to explore, in future studies, whether TTR-111D_M aggregates exhibit a stronger toxicity toward human cells.

CONCLUSIONS

In summary, we have employed a combination of biophysical techniques to characterize the structural characteristics of the fibrils formed by TTR_{105–115} and a Leu111 to D_M mutant (TTR-111D_M). We found that the TTR-111D_M fibrils exhibit quite unique spectroscopic and morphological properties: (1) their D_M sidechains give rise to a single and narrow IR band at $\sim 1745\text{ cm}^{-1}$, signifying a rather homogenous fibril population; (2) their amide I' band consists of a new pair of strongly coupled peaks; (3) they produce a giant far-UV CD signal; and (4) they have no detectable pitches, or twists, along the long fibril axis. Taken together, these results not only indicate that the fibrils formed by TTR-111D_M are structurally different from those formed by the wild-type peptide, but are also, according to previous simulations studies, best explained by a view that the TTR-111D_M fibrils consist of both β - and α -sheets. However, it is worth noting that IR spectroscopy is unable to provide a full atomic view about the fibril structure in question and, hence, we cannot rule out other interpretations of our spectroscopic results. Nevertheless, because of the elusiveness of α -sheet and its potential role in amyloid diseases, we hope that our work will inspire further structural studies on the TTR-111D_M fibrils, using computational and high-resolution structural methods.

EXPERIMENTAL SECTION

Sample Preparation

The details about peptide synthesis and purification are given in the Supporting Information. All peptide samples were prepared initially by dissolving the corresponding lyophilized peptide in solvent composed of 10% acetonitrile (ACN) and 90% D₂O (pD 2) with a final monomer concentration of ~4 mM. Procedures for fibril formation are described in the Supporting Information.

Atomic Force Microscopy Measurements

AFM samples were prepared by placing ~5 μ L of the aggregated peptide sample in question directly on a clean mica sheet/strip, which was then rinsed with 200 μ L of Millipore water and was subsequently dried using N₂ gas. AFM images were obtained on a Bruker Dimension Icon Atomic Force Microscope and were processed using the free Gwyddion software.

Transmission Electron Microscopy Measurements

TEM samples were prepared by placing ~3 μ L of an aggregated peptide solution on a thin carbon film (deposited on a copper grid) which was initially made hydrophilic through glow discharging. After 1 minute, the solution was gently blotted with filter paper and then ~3 μ L of a 1% uranyl acetate solution (pH 4.5) was added to stain the sample. The resultant solution was then blotted, which was followed by another round of staining using the same procedure. TEM image was obtained with a JEOL 1010 transmission electron microscope operated at 80 KV, equipped with a Hamamatsu digital camera (250,000 magnification) and the images were processed using the free AMT Advantage Image Capture software.

UV-Vis Absorption and Fluorescence Measurements

UV-Vis absorption spectra were collected on a Jasco V-650 UV-Vis spectrophotometer using a 1.0 cm quartz cuvette at room temperature. Fluorescence spectra were taken on Jobin Yvon Horiba Fluorolog 3.10 fluorometer.

Circular Dichroism Measurements

Circular dichroism (CD) spectra were collected on an Aviv CD 62A DS spectrometer with an averaging time of 20 s. For fibril samples, the solution was held between two CaF₂ windows separated by a 6 μ m spacer; whereas for monomer samples, a regular quartz CD cuvette with a pathlength of 1 mm was used. For all presented CD spectra, a background spectrum measured with only the corresponding solvent but otherwise under the same conditions has been subtracted.

Fourier Transform Infrared Spectroscopic Measurements

Fourier Transform Infrared (FTIR) spectra were collected on a Nicolet 6700 FTIR spectrometer with a spectral resolution of 1 cm⁻¹ and an average of 256 scans, using a sample holder made with two CaF₂ windows and a 50 μ m Teflon spacer. Attenuated total reflectance Fourier transform IR (ATR-FTIR) spectra were collected using a Horizon ATR

accessory (Harrick Scientific Products, Inc.) for the Nicolet 6700 spectrometer. The aggregated peptide sample in question was first placed onto the germanium (Ge) crystal and was then dried under vacuum for at least 24 hours before measurement.

Two-dimensional Infrared Measurements

2D IR spectra were collected on a homebuilt 2D IR setup that has been described in detail previously.⁴⁶ Briefly, three femtosecond IR pulses (k_1 , k_2 , and k_3 , respectively) were focused onto the sample, which was sandwiched between two CaF_2 windows separated by a 25 μm Teflon spacer, in a box-CARS geometry to generate the desired third-order response. This response was then heterodyned with a local oscillator (LO) pulse, and the combined signal was further dispersed by a monochromator and detected with a 64-pixel liquid nitrogen-cooled IR array detector (Infrared Associates, FL). By scanning the time delay (τ) at 2 fs steps between the k_1 and k_2 pulses from -3.5 to 4.0 ps, the rephrasing and nonrephrasing data were measured and added up to obtain the absorptive 2D spectrum for a fixed delay time (T) between k_1/k_2 and k_3 pulses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A cartoon representation of the TTR₁₀₅₋₁₁₅ protofibril structure determined by Fitzpatrick *et al.*,¹¹ showing the orientation of Leu111 sidechains (red sticks).

Figure 2.
Comparison of the normalized FTIR spectra of TTR₁₀₅₋₁₁₅ and TTR-111D_M fibrils, as indicated.

Figure 3. Time-dependent FTIR spectra of TTR-111D_M, from 0 to 315 minutes with an interval of 45 minutes. Shown in the inset are the corresponding spectra in the ester vibrational band region.

Figure 4. 2D IR spectrum of TTR-111D_M fibrils at waiting time $T=0$, with red squares highlighting cross-peaks between the 1662 and 1672 cm^{-1} bands.

Figure 5.

CD spectra of TTR₁₀₅₋₁₁₅ and TTR-111D_M fibrils, measured using a sample holder with a pathlength of 6 μm . The mean residue ellipticity was calculated using the peptide monomer concentration (4.0 mM).

Figure 6.

AFM images of fibrils formed by TTR₁₀₅₋₁₁₅ (left) and TTR-111D_M (right). The width of the TTR-111D_M fibril at the indicated positions is given.

Figure 7.
A TEM image of fibrils formed by TTR-111D_M.

Figure 8.

A proposed structural model, indicating the possible location of the D_M sidechains (red sticks) as well as the α -sheet (dashed line) and β -sheet (solid line) segments in the protofibril of TTR-111 D_M fibrils.