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Solvent-Driven Dynamical Cross-Over in the Phenylalanine Side-Chain from the Hydrophobic Core of Amyloid Fibrils Detected by ^2H NMR Relaxation

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Abstract

Aromatic residues are important markers of dynamical changes in proteins' hydrophobic cores. In this work we investigated the dynamics of the F19 side-chain in the core of amyloid fibrils across a wide temperature range of 300 to 140 K. We utilized solid-state ^2H NMR relaxation to demonstrate the presence of a solvent-driven dynamical cross-over between different motional regimes, often also referred to as the dynamical transition. In particular, the dynamics are dominated by small-angle fluctuations at low temperatures and by π -flips of the aromatic ring at high temperatures. The cross-over temperature is more than 43 degrees lower for the hydrated state of the fibrils compared to the dry state, indicating that interactions with water facilitate π -flips. Further, cross-over temperatures are shown to be very sensitive to polymorphic states of the fibrils, such as the 2-fold and 3-fold symmetric morphologies of the wild-type protein as well as D23N mutant protofibrils. We speculate that these differences can be attributed, at least partially, to enhanced interactions with water in the 3-fold polymorph, which has been shown to have a water-accessible cavity. Combined with previous studies of methyl group dynamics, the results highlight the presence of multiple dynamics modes in the core of the fibrils, which was originally believed to be quite rigid.

Graphical Abstract

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Supporting Information

SI1. TEM procedure and examples of images. SI2. Effects of distribution of conformers. SI3. Details of experimental results and fits. SI4. Packing densities calculations. SI5. Inter-chain contacts around F19 side-chain. This material is available free of charge at <http://pubs.acs.org>.

INTRODUCTION

It is well known that protein dynamics occur in a multimodal hierarchical fashion due to complex energy landscapes.^{1–4} The dynamical transitions in globular proteins are characterized by the onset of slow anharmonic larger-amplitude motions on a finite time-scale when the protein is heated from cryogenic temperatures to about the 240–200 K temperature range.^{1, 2, 5–11} The slow anharmonic motions are believed to be important for the emergence of biological activities such as enzymatic catalysis and functionally important biomolecular interactions.^{8, 12, 13} One of the distinguishing features in the onset of these biologically relevant anharmonic motions is their dependence on solvation as well as involvement of concerted fluctuations between different groups.^{1, 5–7, 10, 13–20} More localized dynamical cross-overs from one motional mode to another do not require hydration and usually occur at lower temperatures.^{1–3, 21}

Dynamical transitions are observed as cross-over behavior in spectroscopic functions such as, for example, mean square displacement measured by inelastic neutron scattering or NMR relaxation.^{2, 3} They do not represent abrupt changes in protein structure, but rather refer to gradual relative enhancement of various dynamical modes present in the protein.¹ Each spectroscopic observable is sensitive to a particular range of time scales, and thus the choice of the spectroscopic observable determines accessible time scales of motions. In this study ²H NMR relaxation at high field (17.6 T), which is most sensitive to motions in the sub-nanosecond to microsecond timescale range, is used to observe dynamical changes of a phenylalanine side-chain as a function of temperature.

The goal of this work is to investigate whether the solvent-dependent dynamical changes are present in the amyloid fibril systems. As fibrils are comprised of misfolded proteins, it is not a priori clear if the dynamical transitions exist in these systems, and, if so, how different their features are to what is observed for globular proteins. Further, as polymorphism is inherent to the fibrils and is likely related to their neurotoxic behavior,²² it is important to assess the changes in the properties of the dynamical transitions between different polymorphs. Two main types of quaternary structures have been reported for the mature fibrils comprised of A β _{1–40}: the 2-fold and 3-fold symmetric structures (Figure 1).^{23, 24} The basic secondary structure is similar in both polymorphs, comprising a well-structured hydrophobic core with parallel β -sheet and an unstructured N-terminal.²³ Among multiple mutations that have been recently found to be associated with an early onset of the disease,

the D23N mutation²⁵ stands out from a structural standpoint because the mutant protein is capable of forming metastable protofibrils²⁶ with antiparallel β -sheets.^{27, 28}

Our previous studies focused on hydrophobic core methyl groups and indicated extensive motions persisting down to temperatures of 200-190K for many methyl side chains.^{29, 30} However, the dynamics of methyl groups in the core remained invariant between the dry and hydrated fibrils, unlike the dynamics at the methyl groups of M35 which defines the contacts between the cross- β subunits.^{29, 30} The M35 side-chain has been shown to undergo a solvent-driven transition.²⁹

Phenylalanine residues have been implicated as important markers of dynamical transitions,^{7, 31} as motions of bulky aromatic groups often require accommodations of the entire core.^{32–35} We have recently observed a solvent-driven dynamical transition for a phenylalanine residue in the core of globular villin headpiece subdomain (HP36) that did not entirely disappear in the dry protein, but rather showed a transition temperature which was 80° higher.⁷ Thus, to address the question of the presence of the dynamical transition in the fibrils it is necessary to probe aromatic residues. F19's location inside the core (Figure 1), makes it a key target for such studies. We utilize deuterium NMR relaxation measurements to show that the fibrils indeed undergo the dynamical transition in the native 2-fold and 3-fold symmetric polymorphs, as well as in the mutant D23N protofibrils. We compare the behavior among the different polymorphs and contrast them with the behavior of phenylalanine in a globular protein.

MATERIALS AND METHODS

Preparation of A β _{1–40} peptide and D23N mutant

The native and D23N mutant proteins were prepared using solid-state peptide synthesis (performed by Thermofisher Scientific Co, Rockford, IL). The native sequence is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV.

Fluorenylmethyloxycarbonyl (Fmoc) *L*-Phenylalanine-ring-d₅ was purchased from Cambridge Isotopes Laboratories (Andover, MA). The peptides were purified by reversed-phase HPLC and their identity and purity (>97% level for all peptides) was confirmed by mass spectrometry and reversed-phase HPLC. The resulting peptides had isotopic labels in only one residue, F19.

Preparation of the fibrils

The fibrils of the wild-type A β were prepared as described³⁰ and based on protocols established by Tycko's laboratory.^{23, 24} The main difference in preparation between the 2-fold and 3-fold symmetric polymorphs is in the agitation/sonication patterns of the seeds: for the two-fold polymorph, sonication was performed every three to four hours and the growth occurred under agitated conditions for 4 days. For the 3-fold variant, sonication was performed one time at 24 hours with the growth occurring under quiescent conditions for 4 days. The bulk fibrils were pelleted by centrifuging at 300,000 *g* for 7–9 hours. Fibril pellets were re-suspended in deionized water, rapidly frozen with liquid nitrogen, and lyophilized. Preparation of the D23N protofibrils with anti-parallel β -sheet structure utilized a two-step

seeding/filtration cycle that takes advantage of the differences in fibril formation rate between the parallel and anti-parallel structures.²⁸ The resulting morphologies were confirmed with transmission electron microscopy (TEM) imaging, examples of which are given in Supporting Information (SI1). The samples were packed in 5 mm NMR tubes (cut to 21 mm length) using Teflon tape to center the sample volume in the coil of the NMR probe. The amount of material packed varied from 14 to 25 mg.

The hydration state corresponding to water content of 200% was achieved by exposing lyophilized powder to water vapor in a sealed chamber at 25°C until the water content reached saturating levels corresponding to about 30–40% by weight, followed by pipetting the remaining water using deuterium depleted H₂O and equilibrating for 24–48 hours at room temperature.

NMR Spectroscopy

²H T_{1Z} (longitudinal relaxation) measurements under static conditions were performed at 17.6 T field strength using the inversion recovery sequence for relaxation times below about 200 ms and saturation recovery sequence for longer times. The multiple-echo (QCPMG) detection scheme was used for signal enhancement.³⁶ The durations of 90° pulses were between 2.0–3.0 μs. Ten to fifteen QCPMG echoes were collected with 50 μs pulse spacing. The number of scans ranged from 16 to 4096 depending on the signal to noise ratio in each sample, as well as the precision of the data needed to define the non-exponential magnetization build-up curves. Seven to nine relaxation delays were collected. ²H QCPMG spectra were processed with 1 kHz exponential line broadening. Temperature calibration procedure: temperature calibration was performed by recording static lead nitrate line shapes³⁷ and using the freezing point of D₂O, 3.8°C, as the fixed point for the calibration.

Line shape measurements with the quadrupolar echo acquisition scheme³⁸ were performed at 17.6 T with an eight-step phase cycle and a delay of 31 μs between the 90° pulses. The number of scans varied between 40·1024 and 250·1024, which was determined by the amount of the sample and whether it was in the dry or hydrated state. The delay between the scans was set to at least 1.5 times the effective relaxation time at each temperature to optimize data collection for this low sensitivity samples. Each spectrum was collected over 24–48 hours.

Theory and Choice of Model

²H relaxation measurements are most sensitive to fast motions in the pico- to nanosecond range. Static deuteron NMR line shape and relaxation measurements are dominated by a single-particle mechanism: the interaction of the nuclear electric quadrupole moment with the electric field gradient at the site of the nucleus.³⁸ The main mechanism of relaxation is usually considered to be 180° flips of the aromatic ring.^{7, 39–49} Of note is the work of Gall and Opella⁴⁸ which demonstrated that phenylalanine residues have a distribution of ring-flip rates.

The general equation for the spin-lattice relaxation rate $1/T_1$ is given by^{38, 50}

$$\frac{1}{T_1} = \frac{C_q^2}{3} (J_1(\omega_0) + 4J_2(2\omega_0)) \quad (1)$$

where ω_0 is the Larmor frequency, J_1 and J_2 are spectral density functions, and C_q refers to the quadrupole coupling constant (units are Hz) in the absence of motion. J_1 and J_2 are dependent on the timescales and types of underlying motional processes as well as on the crystallite orientations. While analytical expressions can be obtained for several simple cases of models of the spectral density,⁵¹ for situations where more than one motional mode is expected, simulations are usually employed to calculate the spectral densities.

When the dynamics across a wide temperature range are investigated, it is necessary to consider other possible mechanisms besides the π -flips, such as fast rotational diffusion of the ring for high temperatures, or small angle fluctuations of the phenyl axis at low temperatures. For example, for phenylalanine rings inside the hydrophobic core of globular villin headpiece subdomain (HP36), it was necessary to employ a two-mode motional model consisting of π -flips and small-angle fluctuations of the phenyl axis.⁷ A similar model has also been employed for phenylene rings of glassy polymers.^{52, 53} The π -flips involve fluctuations around the χ_2 dihedral angle, while the small-angle fluctuations can involve both the χ_2 and χ_1 dihedral angles. For simplicity in our modeling we restrict small-angle fluctuations to the χ_2 angle only. Based on the temperature-dependence of the relaxation data, both processes (i.e., π -flips and small angle fluctuations) are thermally activated, and thus the potential has the form depicted in Figure 2. Small-angle jumps contribute comparably to the relaxation process independent of their exact geometry with a caveat that for motions that involve a change in the direction of the $C^\beta - C^\gamma$ axis, the D^S deuteron contributes to the relaxation on the same timescale as the other phenyl ring deuterons. The motion in the potential shown in Figure 2 can, in principle, follow two different scenarios: i) the restricted diffusion case, in which the large-angle mode is coupled to the small-angle mode as described by Kramers' escape theory.⁵⁴ In this limit, both large- and small-angle fluctuations are described by a single diffusion constant. ii) The large-angle fluctuations occur in the strong collision limit and there are two independent rate constants of large- and small-angle fluctuations. The strong collision limit implies that large-angle jumps can occur from any angle position inside the first well into any angle position inside the second well. Contrary to the case of polymers, the field-dependent data for phenylalanine residues in HP36 suggested the strong-collision limit for the protein. Further, Hattori et al.³⁴ and Wagner³² also suggested that large-angle ring-flips occur in the strong collision limit. For this reason we have invoked the strong-collision limit for the simulations of the dynamics in the fibrils. The qualitative results are not dependent on the choice of this limit.

This type of potential leads to two different rate constants for each of the two processes k^{small} and k^{large} , the temperature dependence of which is governed by the Arrhenius law:

$k(E_a^{large}, T) = k_0^{large} e^{-E_a^{large}/T}$ and $k(E_a^{small}, T) = k_0^{small} e^{-E_a^{small}/T}$. The strong collision limit implies that the large-angle jumps can occur from any angle position inside the first well into any angle position inside the second well. Therefore, the following overall jump

pattern applies: $\alpha \Leftrightarrow \pi$, $0 \Leftrightarrow \pi$, $\alpha + \pi \Leftrightarrow 0$, $\alpha + \pi \Leftrightarrow \alpha$, where α is the angle of small amplitude fluctuations. The large-angle flips occur between sites 1–3 and 1–4 with an equal probability; this also holds for the 2–3 and 2–4 pairs. The small-angle jumps with the amplitude α occur between sites 1–2 and 3–4. The change in the χ_2 angle for transitions 1–3 and 2–4 in Figure 2 is exactly 180° due to the symmetry of the ring. The extension of the model in the presence of distributions of conformers is presented in SI2.

Modeling Procedure

In order to simulate the jumps between the four sites in Figure 2, one has to define the angle between the C-D bond and $C^\beta - C^\gamma$ axis as well as the angles of rotation around the $C^\beta - C^\gamma$ axis for each of the sites. We assume that the principal axis system of the quadrupolar tensor for each deuteron is aligned along the position of the C-D bond. The angle between the C-D bond and $C^\beta - C^\gamma$ axis remains the same for all four exchange sites. In order to specify the angle of rotation around the $C^\beta - C^\gamma$ axis (the χ_2 rotation), only one parameter is required because the rotation angles between sites 1–2 and 3–4 are equal and the transitions 1–3 and 2–4 are exactly 180° . For example, if we model small-angle jumps by the amplitude of α , this is the only parameter that needs to be specified. The rotations around the χ_2 angle do not involve the ζ (para) deuteron. Thus, only the D^δ and D^ϵ deuterons participate in the jumps. The value of the quadrupolar coupling constant C_q , asymmetry parameter η of the tensor, and the angles between either the C^δ -D or C^ϵ -D bonds and the $C^\beta - C^\gamma$ axis (i.e., deviations from the ideal ring geometry) were taken as: $C_q = 180$ kHz and $\eta = 0$, and the angle of 59.2 – 59.3° . The errors in fitted model parameters were determined by the inverse covariance matrix method and errors in transition temperatures were obtained by propagation from errors in the model parameters. Line shapes were simulated using the same approach as the one used for modeling relaxation. Simulated inter-scan delays were matched to experimental conditions, as T_1 values for the typical distributions in these samples range several orders of magnitude between individual conformers. Thus, in the experiment the conformers are effectively weighted by different extent of partial relaxation. All simulations were performed using the EXPRESS program.⁵⁵ Further details are available in SI2.

RESULTS AND DISCUSSION

All of the fibril samples were labeled with deuterium at a single residue - F19, with the ring- d_5 labeling pattern. We analyzed the following four fibril samples: hydrated wild-type fibrils of $A\beta_{1-40}$ in the 3-fold symmetric morphology (3fwet), lyophilized wild-type fibrils in the 3-fold symmetric morphology (3fdry), hydrated fibrils of the wild-type $A\beta_{1-40}$ in the 2-fold symmetric morphology (2fwet), and hydrated protofibrils of D23N mutant (D23N). The morphologies of the samples were confirmed by TEM (SI1) based on the previously published TEM analysis by Tycko and co-workers.^{23, 24, 28}

Longitudinal relaxation measurements point to the existence of the dynamical transition

Relaxation measurements were performed over a broad temperature range from the physiological temperature of 310 K down to cryogenic conditions to determine the motional mechanisms and assess the presence of the dynamical transitions. The multiple echo

acquisition scheme (QCPMG) was utilized for signal enhancement^{36, 56} and examples of spectra with the use of this scheme are shown in Figure 3.

Magnetization build-up curves at chosen spikelet positions indicate non-exponential behavior (Figure 4).

Therefore, relaxation times T_1^{eff} were obtained from magnetization build-up curves $M(t)$ at chosen spikelet positions using an empirical stretched-exponential function:⁵⁷

$$M(t) - M(\infty) = (M(0) - M(\infty)) e^{-(t/T_1^{eff})^\beta} \quad (2)$$

in which $M(t)$ is the signal intensity, T_1^{eff} is the effective relaxation time, and β is the parameter that reflects the degree of non-exponentiality, $0 < \beta \leq 1$. β less than 1 corresponds to a non-exponential behavior. Note that for saturation recovery measurements $M(0) = 0$. The choice of the stretched exponential function is dictated primarily by its simplicity and use in polymer studies.^{58, 59} Full analysis of the experimental build-up curves, as well as effects of non-exponentiality are presented in the Supporting Information (SI3).

Our main discussion is focused on the analysis of the relaxation times. First, we observed that there are significant differences between the wet and the dry state of F19 in the 3-fold symmetric morphology. (Figure 5A). This difference is most pronounced at high temperatures at which the relaxation times are significantly smaller in the hydrated state. There are also significant differences between the different polymorphs of the wild-type A β and the D23N protofibrils in the hydrated state (Figure 5B). Again, the differences are most pronounced at high temperatures and the 3fwet polymorph displays the shortest relaxation times, while the 2fwet has the longest times and the D23N protofibrils are intermediate between the two native polymorphs. At high temperatures the dominant relaxation mechanism is expected to be the π -flips,^{7, 39–49} thus these difference in the relaxation times report on the underlying differences in the rate constants of these motions.

For all types of samples, there is a change in the slope of the T_1^{eff} curve, indicating that there are two thermally activated motional mechanisms present with different values of the activation energies, E_a^{large} and E_a^{small} . The two underlying motional modes are the π -flips dominating the relaxation at high temperatures and small angle fluctuations dominating at low temperatures, as elaborated in the Theory and Choice of Model section.

The analysis of the non-exponentiality of the magnetization build-up curves supports the notion of a distribution of conformers distinguished by different values of E_a^{large} and E_a^{small} (SI2). The distribution is taken as Gaussian with the parameters of the central values of the distribution $\langle E_a^{large} \rangle$ and $\langle E_a^{small} \rangle$, and the widths of σ^{large} and σ^{small} . The fitted parameters are shown in Table 1.

The cross-over effect between the two motional mechanisms is often defined as the dynamical transition, as elaborated in the Introduction. The cross-over results from the change of dominance in the relaxation mechanisms and does not imply any sudden changes in the protein. Without fitting the data to the model, it is already evident from the T_1^{eff} curves that the 3fdry fibrils undergo the cross-over at a higher temperature than the 3fwet fibrils (Figure 5A). Thus, the observed transition is clearly solvent-driven.

High-temperature quadrupolar echo line shapes

Line-shapes obtained with the quadrupolar echo detection scheme at three high temperatures (Figure 6) provided additional information regarding μs -ms time scale dynamics. First, they have allowed for the determination of the upper limit of the amplitude of small angle fluctuations $\alpha/2$, which is determined to be around 5° (Figure 7A). The value of C_q of 180 kHz determines the widths of the shoulder region. This value was taken based on the pattern of the low temperature QCPMG-detected line shape (Figure 3, 145 K). Small-angle fluctuations have an effect of narrowing this part of the spectrum. An angle of larger than 5° causes excessive narrowing which is inconsistent with the experimental data. Further, the distance between the two inner horns of the powder pattern is reflective of the exact values for the angles between the C-D bonds and phenyl axis (Figure 7A). An ideal ring geometry is 60° . The data for all samples points to slight deviations from the ideal geometry with angles of about 59.2 – 59.3° .

The line shape also provided an independent control on the parameters of the distribution of the π -flips mode. The examples of simulated curves in Figure 7B correspond to the parameters of the distribution determined based on relaxation data. Good agreement is obtained in terms of the overall height of the inner horns to the shoulder region, which is the most sensitive to the parameters of the π -flips mode. We note that at high temperatures many conformers of the distribution have π -flip rates close to the fast limit for all polymorphs, i.e. ring-flip rate constants are much larger than C_q . Therefore, the line shapes in this regime are less sensitive to the ring-flip rate constants compared to the relaxation data. For the 3fwet fibrils there are some deviations between the experimental and simulated line shapes, suggesting potentially an additional mode not accounted for in the present model. Inclusion of motions around the χ_1 angle does not improve the quality of fit.

Comparative discussion of the features of the dynamical transitions

One can choose from several approaches to define the cross-over temperature. For example, one choice is to define it as the temperature of the maximum second derivative of the experimental $\ln T_1^{\text{eff}}$ curve:

$$T_c = \text{argmax} \left| \frac{d^2 \ln T_1^{\text{eff}}}{(d(1/T))^2} \right|. \quad (3)$$

This definition coincides with the visual interpretation of the most pronounced changes in the slope. An alternative definition of the cross-over temperature T_c^* is the temperature at which the individual contribution from the two relaxation mechanisms are identical:

$$\frac{1}{T_1^{\text{eff large}}(T_c^*)} = \frac{1}{T_1^{\text{eff small}}(T_c^*)} \quad (4)$$

$1/T_1^{\text{eff large}}$ and $1/T_1^{\text{eff small}}$ are the projected relaxation rates for the two mechanisms considered in isolation. T_c equals to T_c^* when there are single conformers for each of the mechanisms and the overall relaxation rate is the sum of the relaxation rates due to the two individual mechanisms. However, deviations from the simple additive behavior and the presence of a relatively wide distribution of conformers causes T_c and T_c^* to differ significantly from each other, as is the case for our systems (Table 1). As there are no sudden changes in the dynamics of the protein, the choice of the definition is dictated by the nature of comparison one wants to conduct. We will focus the current discussion primarily on the comparison of T_c values. Regardless of which definition is used, the comparison between different samples and states (e.g., wet vs dry) is not affected.

The temperatures of the cross-over (Table 1) are clearly dependent both on the hydration state and the morphology. The dry state of 3-fold symmetric fibrils has a T_c of 262 K, while in the wet state this value is 219 K. For the 2-fold symmetric morphology in the wet state T_c is much higher than for the 3-fold symmetric morphology, 263K, while the D23N protofibrils show an intermediate value of 235 K. The differences in the transition temperatures are driven largely by the distribution parameters for the π -flips mode (SI2).

The central values of the activation energy distributions for the π -flips are between 38 and 48 kJ/mol. The width of the distributions of the activation energies is the widest for the 3fdry sample (8.1 kJ/mol), and they are comparable in the hydrated samples (4.8–6.8 kJ/mol range). The central values of the distributions for the small-angle fluctuations are in 7.5–10.5 kJ/mol range and the widths of these distributions are all similar at around 2 kJ/mol.

The presence of the dynamical transition in F19 in the core of amyloid fibrils combined with the data for methyl groups of the M35 side-chain,²⁹ which does not point toward the core (Figure 1), supports the notion that fibrils, in general, are capable of undergoing dynamical transitions. M35 undergoes a cross-over from the dominance of fast methyl rotations at low temperatures to slower diffusive motions of the methyl axis at higher temperatures. The transition was not observed for the dry fibrils or a model hydrophobic system.^{29, 60}

These transitions are also solvent-driven at the phenylalanine sites, as there is a clear difference between the hydrated and the dry states. In particular, the value of T_c is higher by 43° in the dry fibrils. The dependence of the dynamics on hydration indicates that there are motional modes in the core which are coupled to the solvent. These modes were not detected when only the core methyl groups were probed in the fibrils,^{29, 30} thus indicating that phenylalanine core residues are sensing additional motions, which likely involve slower concerted fluctuations of the core. We note that the overall structures are believed to be very

similar in the dry and wet states based on the chemical shifts measured with magic-angle spinning solid-state NMR.²⁴ Further, crude packing densities calculated as the number of atoms in a sphere with 8 Å radius is roughly the same for all polymorphs (SI4). If one focuses on hydrophobic inter-chain contacts that are known to be important in defining the quaternary structure of the fibrils,^{27, 28, 61} it appears that the contacts involving the F19 side-chain are different between the 2-fold and 3-fold symmetric polymorphs, while the D23N side-chain is similar to the 3-fold symmetric native polymorph in this respect (SI5). In particular, in the 3-fold and D23N polymorphs the ring of F19 is between L34 and I32 side-chains, while in the 2-fold polymorph it is aligned directly with the L34 side-chain. These structural differences may contribute to the observed differences in the dynamics between the polymorphs.

As hydration is very important in the enhancement of the π -flip motions and shifting the cross-over to higher temperatures, it is possible that an additional factor contributing to the observed differences among the polymorphs is the effective extent of hydration sensed by the phenylalanine ring. In other words, at the same total bulk hydration level, transient interactions of the core with water can be polymorph-dependent. Hong and coworkers⁶² have recently identified possibilities of several pools of water in the fibrils that can also interconvert between each-other. Thus, it is possible that differences in these pools of water for each polymorph modify core-water interactions and lead to differences in slow concerted motions of the core. In particular, the water-accessible cavity, found for the 3-fold polymorph, can indirectly lead to enhanced water-core interactions.

One of the main differences between the globular HP36 and the fibrils, is that the transition temperatures T_c for phenylalanines in all of the hydrated samples are significantly higher than the value of 175 K found for HP36.⁷ Note, that in the original work only the value of T_c^* was reported, and we calculated here the value of T_c for F58 residues in HP36 for consistent comparison. Another difference in the dynamics between the case of the hydrated fibrils and HP36 is that in the latter the central values $\langle E_a^{large} \rangle$ are smaller at 21–25 kJ/mol and the widths of the distributions σ^{large} are also much smaller at 2 kJ/mol. Thus, in general motions are more restricted in the core of the fibrils compared to HP36. The combination of more restricted motions and larger distribution width leads to higher transition temperatures in the fibrils.

It is also interesting that the dynamical transition temperature T_c (calculated in this work from the original data according to Eq. 3) was the same, around 221 K, for methyl groups in M35 in both the 3fwet and 2fwet fibrils.²⁹ In contrast, there are clear differences in the cross-over temperatures for the two native polymorphs at the F19 site, with the 3-fold morphology at 219 K, but the 2-fold one at 263 K. Thus, the additional slower, larger-amplitude modes induced by the solvent do not necessarily have the same temperature of onset at all sites and are sensitive to the polymorphic state of the fibrils. For the M35 site, slow diffusive motion of the methyl axis was shown to have higher activation energy (i.e., more restricted) in the 2fwet fibrils compared to the 3fwet fibrils, while D23N protofibrils display a value similar to the 3-fold polymorph. Overall, the results may suggest a trend that in the 3fwet fibrils interactions with additional water pools or differences in the amount and

properties of the peptide-bound water may cause enhanced dynamics at some sites of the fibrils.

We note that the studies of cytochrome P450cam by elastic incoherent neutron scattering did not show hydration dependence for the dynamics of the aromatic side-chains in the nanosecond range,³ which was likely due to the fact the π -flips occurred on a much slower time scale. This emphasizes the need to use multiple spectroscopic techniques that cover a broad range of time scales in order to discern the details of the complex hierarchical dynamics in proteins.

Conclusion

Our results have demonstrated that amyloid fibrils undergo solvent-driven dynamical changes in similarity to what has been observed for globular proteins. There are clear differences in the underlying dynamics and cross-over temperatures among the polymorphs, however for all investigated polymorphs the transition temperature was found to be significantly higher compared to what was observed for the globular HP36. Combined with previous results on methyl group dynamics, this study underlines the fact that the core of the amyloid fibrils is not rigid as previously thought but rather participates in a number of dynamical modes that are sensitive to local environments created by different polymorphs as well as to complexities of interactions with water.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A) Ribbon diagram corresponding to the monomeric unit of the wild-type Aβ₁₋₄₀ in the 3-fold symmetric polymorph, highlighting key residues in the C-terminal domain. B) Quaternary structures of the 2-fold (2LMN.pdb) and 3-fold symmetric polymorph (2LMP.pdb) of Aβ₁₋₄₀ as well as monomeric D23N mutant protofibrils (2LNQ.pdb) F19 side-chains are in red and M35 side-chains are in black.

Figure 2.

A) The phenylalanine side chain with the deuteron labeling pattern marked in orange. B) Motional model for the fluctuations around the χ_2 dihedral angle in the phenylalanine side-chains. The diagram displays sites' connectivities according to the four-site strong collision model, illustrated for one of the C^δ -D bonds. The large-angle flips occur between sites 1–3 and 1–4 with an equal probability; this also holds for the 2–3 and 2–4 pairs. The small-angle jumps with the amplitude α occur between sites 1–2 and 3–4. C) Potential of the model, in which E_a^{large} and E_a^{small} are the activation energies for the large-angle ring flips and small-angle fluctuations, respectively.

Figure 3. Examples of QCPMG spectra corresponding to the largest relaxation delay in the longitudinal relaxation time measurements for the F19-ring-d₅ side-chain in the 3fwet fibrils.

Figure 4.

Examples of longitudinal relaxation build-up curves $M(t)$ at 17.6 T magnetic field strength for F19 in the 3-fold symmetric polymorph in the hydrated state corresponding to 60 kHz spikelets. A) Inversion recovery curve at 295 K. B) Saturation recovery curve at 220 K. Dashed lines correspond to the best fit mono-exponential decays and solid lines to the best fit stretched-exponential function defined in Eq. (2). Intensity is shown in arbitrary units.

Figure 5.

Plots of T_1^{eff} as a function of $1000/T$ at 17.6 T. A) 3-fold symmetric wet (red) and dry (black) states. B) Hydrated states in 3-fold symmetric (red triangles) wild-type fibrils, 2-fold symmetric wild-type fibrils (blue circles), and D23N protofibrils (green squares). The data are shown for the $\pm 60 \text{ kHz}$ spikelets of QCPMG spectra. Solid-lines represent the fits to the full model and the values of T_c are calculated according to Eq. (3).

Figure 6.

Quadrupolar echo line shapes. Experimental line shapes collected at the three indicated temperatures using the quadrupolar echo detection scheme. The narrow peak at the center of the spectra is the signal from HOD.

Figure 7.

A) Experimental quadrupolar echo line shape for the 2fwet sample at 295 K overlaid with simulations, in which specified values of the small angle fluctuations $\alpha/2$ were used. Small angle fluctuations were modeled around the χ_2 angle. The marked distance between the “shoulder” region is most sensitive to the angle of small fluctuations, and the distance between the inner horns permits quantification of deviations from the ideal ring geometry. B) Experimental line shapes at 295 K (black) for all samples are overlaid with corresponding simulated line shapes (blue) obtained using the two-mode motional model, which includes the effects of the distributions in the activation energies specified in Table 1.

Table 1

Fitted parameters of the model for F19-ring-d₅ fluctuations.

polymorph	$\ln k_0^{large}, s^{-1}$	$\langle E_a^{large} \rangle, kJ/mol$	$\sigma_{large}, kJ/mol$	$\ln k_0^{small}, s^{-1}$	$\langle E_a^{small} \rangle, kJ/mol$	$\sigma_{small}, kJ/mol$	T^*, K	T_c, K
3f wet	31.6±1.5	38.4±2.5	5.2±0.4	22.5±0.7	8.2±0.6	2.2±0.3	245±3	219±3
3f dry	30.4±1.4	41.0±2.7	8.1±0.5	22.1±0.8	7.5±0.8	2.0±0.3	290±3	262±3
2f wet	31.8±1.5	45.1±2.7	6.8±0.4	23.8±0.8	10.5±1.1	1.7±0.3	301±4	263±3
D23N wet	35.1±2.2	47.8±3.5	4.8±0.5	23.2±0.8	9.2±1.1	2.0±0.4	266±5	235±4