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Dynamics of the Proline-Rich C-Terminus of Huntingtin Exon-1 Fibrils

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Abstract

Intrinsically disordered protein domains are not only found in soluble proteins but can also be part of large protein complexes or protein aggregates. For example, several amyloid fibrils have intrinsically disordered domains framing a rigid β -sheet rich core. These disordered domains can often be observed using solution NMR methods in combination with modest magic angle spinning and without perdeuteration. But how can these regions be detected using solution NMR methods when they are part of a fibril that is not tumbling isotropically in solution? Here we addressed this question by investigating the dynamic C-terminus of huntingtin exon-1 (HTT_{ex1}) fibrils that are important in Huntington's Disease. We assigned the most dynamic regions of the C-terminus of three HTT_{ex1} variants. Based on this assignment, we measured site-specific residual chemical shifts, peak intensities, and R_1 , R_2' and $R_{1\rho}$ ^{15}N relaxation rates. In addition, we determined the residual ^1H - ^{15}N dipolar couplings of this region. Our results show that the dipolar couplings are averaged to a very high degree resulting in an order parameter that is essentially zero. Together, our data show that the C-terminus of HTT_{ex1} is intrinsically disordered and undergoes motions in the high picosecond to low nanosecond range.

TOC Graphic

Introduction

The importance of intrinsically disordered protein domains (IDDs) is increasingly recognized, and new methods are needed to study the dynamics and residual structure of these domains. While solution NMR has emerged as one of the most important tools to study these domains in soluble proteins, IDDs are more difficult to study when part of a structure too large to be detected using solution NMR.

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One example of such as IDD is found in fibrillar aggregates formed by huntingtin exon-1 (HTT_{ex1}) important in Huntington's Disease (HD). HD is a heritable neurodegenerative disease that is caused by a CAG expansion in exon-1 of the huntingtin gene. This CAG expansion leads to an elongated poly-glutamine (polyQ) region in the huntingtin protein, which increases its propensity to aggregate.¹ HTT_{ex1}, which has been shown to be dominant in cellular huntingtin inclusions in HD and to exist as an alternative splice variant,² becomes especially aggregation prone when the polyQ expansion exceeds 36 glutamine residues,³ which coincides with the repeat length above which HD is observed.⁴

HTT_{ex1} exhibits a highly heterogeneous structure, as shown by circular dichroism (CD), electron paramagnetic resonance (EPR), and solid-state nuclear magnetic resonance (NMR) spectroscopy.^{5–10} The polyQ domain forms the static core of the fibril, whereas the proline-rich C-terminus is highly dynamic and in a mixed random coil, polyproline II (PPII) helical conformation.^{5,11} The N-terminus of HTT_{ex1} (N17) has helical propensity in the soluble^{12,13} and fibrillar state.⁶ These findings led us to propose a bottle brush model of HTT_{ex1} fibrils where the polyQ domain forms the core and the dynamic, proline-rich domain the bristles of the brush.⁵

Similar heterogeneous systems with static, β -sheet-rich cores and highly flexible domains have been observed for several pathologic amyloid fibrils (e.g. α -synuclein¹⁴ and hPrP¹⁵) as well as functional amyloids (e.g. HET-s,¹⁶ Orb2^{17,18}). The role of many of the disordered domains found in conjunction with a cross- β fibril core is unclear. However, in the case of HTT_{ex1} the dynamics of the flanking sequences and the C-terminus in particular correlates with the toxicity of recombinant fibrils.^{9,19} This correlation suggests that the interaction of other cellular components with the C-terminal bristles of the bottle brush might be important for understanding HTT_{ex1} toxicity. Therefore, site-specific assignment and site-specific dynamics are required to measure interactions of the dynamic C-terminus with binding partners.

We and others have shown that a combination of solid-state and solution NMR techniques allows us to selectively detect IDDs as part of amyloid fibrils,^{14,16,17,20–22} membrane proteins,²³ protein-DNA complexes,²⁴ and chemotaxis receptors.²⁵ We recently showed that site-specific assignments of dynamic amyloid flanking sequences can be obtained using triple resonance NMR experiments developed for protein backbone assignments in solution.¹⁸ But how dynamic do regions have to be to allow ¹H detected methods to work at moderate MAS frequencies and in the absence of perdeuteration? To answer this question, we not only assigned the most dynamic regions of HTT_{ex1} fibrils, but also measured site-specific relaxation rates and residual dipolar couplings. These measurements allow us to present a detailed model of this dynamic domain.

Methods

Protein Production and Fibril Formation

Uniformly labeled ¹³C,¹⁵N-HTT_{ex1} Q46 was expressed¹⁰ and purified²⁷ as described earlier except that this time we used a BioRad NGC Scout FPLC system for protein

isolation. The C-terminally His-tagged HTT_{ex1}HT and HTT_{ex1}Pro constructs were expressed and purified as previously described.^{5,10,26}

Fibril formation was instigated by addition of 1% (V/V) unlabeled HTT_{ex1}(Q46) seeds to the eluted protein. Samples were incubated with seeds for ~36 hours at 4 °C in 10 mM/100 mM NaCl tris buffer, pH = 7.4 and harvested by ultra-centrifugation with a Beckman Coulter Optima TLX using a Beckman TLA 100.3 rotor at 40,000 rpm for 40 minutes at 4 °C. Pellets were washed with 0.1% trifluoroacetic acid (TFA) and ultra-centrifuged again at 40,000 rpm for 40 minutes at 4 °C. Supernatant was reduced to 100 µL for loading the rotor. Electron microscopy (EM) images were taken to ensure fibril quality using a JEOL JEM-1400 Electron Microscope at 100 kV. EM grids were prepared as previously described.¹⁰ A 1.6 mm Agilent rotor was loaded by centrifugation of HTT_{ex1}(Q46) fibrils directly into the MAS rotor at 10,400 rpm and 4 °C in a Sorvall Surespin 630 rotor for 40 minutes using a home-built packing tool.

NMR spectroscopy

All NMR experiments were measured on a 14.1 T Agilent DD2 solid-state NMR spectrometer (¹H = 599.78 MHz; ¹³C = 150.83 MHz; ¹⁵N = 60.78 MHz) equipped with a T3 1.6 mm probe. A MAS frequency of 12 kHz was used if not noted otherwise, with 200 kHz and 50 kHz pulses on ¹H and ¹⁵N, respectively. Temperature was maintained at 0 °C.

The HSQC, HNCA, and HNcoCA spectra were recorded as described previously with an additional BIRD filter with a water relaxation delay of 260 ms for improved water suppression.¹⁸

R_1 rates were measured using the pulse sequence shown in Figure 1A.²⁷ The values of τ and γ were 2.2 and 260 ms, respectively. ¹H GARP decoupling with an rf-field strength of 5 kHz was applied during the relaxation delay framed by two 1 ms 10 kHz ¹H spin lock pulses for water suppression. ¹⁵N WALTZ decoupling with an rf-field strength of 5 kHz was used during acquisition. R'_2 rates were measured using the pulse sequence shown in Figure 1B.²⁸ The delays were the same as for the R_1 experiments with the additional CPMG delay δ of 0.5 ms, and the spin lock pulses for water suppression lasting for 3 ms with an rf-field strength of 40 kHz. $R_{1\rho}$ was measured with the pulse sequence shown in Figure 1C, which is very similar to the R'_2 experiment, except that the CPMG spin echoes were replaced with a 6 kHz spin lock pulse on ¹⁵N and 2.5 kHz WALTZ decoupling on ¹H. An additional Z-filter of $\zeta=20$ ms was added before the spin lock. Residual dipolar couplings were measured using the ¹H-¹⁵N REDOR pulse sequence shown in Figure 1D.²⁹ This experiment was equivalent to the $R_{1\rho}$ experiment, except that the MAS frequency was 12.5 kHz ($\tau_r=80$ µs), and the spin lock was replaced with ¹H-¹⁵N REDOR recoupling in which the rf-field strength of the ¹H 180° pulses was 50 kHz to reduce sample heating. S_0 was recorded without ¹H 180° pulses during the REDOR recouplings. R_1 , R'_2 , $R_{1\rho}$ and ¹H-¹⁵N REDOR experiments were recorded with spectral widths of 10 kHz and 3 kHz for ¹H and ¹⁵N, respectively. 128 acquisitions were co-added for each of the 60 indirect increments. The ¹H-¹³C 2D INEPT HETCOR experiments were recorded as described previously.⁵ Data were processed using NMRPipe and NMRDraw and plotted with custom scripts written with Python and NMRglue.³⁰

Data analysis

Resonance assignment and peak integration were done using the program CARA. Relaxation curves were fit using custom scripts written in Python.

Correlation times τ_c were calculated from the experimentally determined values for R_1 , R_2 , and S . According to Schanda & Ernst,³¹ R_1 can then be written as

$$R_1 = \left(\frac{\delta_D}{4}\right)^2 (J(\omega_1 - \omega_S) + 3J(\omega_S) + 6J(\omega_1 + \omega_S)) + \delta_{CSA}^2 \frac{3}{4} J(\omega_S)$$

where $\delta_D/2\pi$ is the NH dipolar coupling of 22.954 Hz, ω_S the ^{15}N Larmor frequency, ω_I the ^1H Larmor frequency, and $\delta_{CSA}/2\pi$ the chemical shift anisotropy of backbone ^{15}N of 6867 Hz. In a single-timescale, model-free approach under MAS, the spectral density function is described as

$$J(\omega) = \frac{2}{5} (1 - S^2) \frac{\tau_c}{1 + (\omega\tau_c)^2}$$

Similarly, the transverse relaxation rate can be described as

$$R_2 = \left(\frac{\delta_D}{4}\right)^2 \left(\frac{4}{3} J(\omega_r) + \frac{2}{3} J(2\omega_r) + \frac{1}{2} J(\omega_I - \omega_S) + 3J(\omega_I) + \frac{3}{2} J(\omega_S) + 3J(\omega_I + \omega_S) \right) + \delta_{CSA}^2 \left(\frac{1}{4} J(\omega_r) + \frac{1}{8} J(2\omega_r) + \frac{3}{8} J(\omega_S) \right)$$

where ω_r is the MAS frequency. R_1 and R_2 were solved for τ_c using the program Mathematica to estimate correlation time of the motion.

We calculated R_2 from the experimental values of R_1 and $R_{1\rho}$ using the following equation

$$R_2 = [R_{1\rho} - R_1 \cos^2(\theta)] / \sin^2(\theta)$$

where θ is the angle between the effective ^{15}N rf-field and the static magnetic field.³²

Results

How many residues of HTT_{ex1} Q46 fibrils are so dynamic that they can be detected using ^1H detected, J-coupling based, triple resonance spectroscopy? To answer this question, we prepared HTT_{ex1} Q46 fibrils at 4 °C in the presence of 1% (V/V) HTT_{ex1} seeds (see Materials and Methods) and acquired the HSQC (heteronuclear single-quantum correlation) spectrum shown in Figure 2. The lack of a gradient coil in our 1.6 mm Agilent MAS probe required a BIRD (bilinear rotational decoupling) filter element preceding the HSQC experiment to achieve water suppression rather than the pulsed-field gradient based methods

used in solution-state NMR spectroscopy. The HSQC spectrum of uniformly labeled ^1H , ^{13}C , ^{15}N -HTT_{ex1} fibrils has 12 well-resolved amide cross peaks with ^1H and ^{15}N linewidths as low as 22 Hz and 24 Hz, respectively. The intensities of the cross peaks vary, with some resonances being well defined and intense and others broadened and barely detectable. The relatively narrow distribution of amide proton shifts around 8.5 ppm indicates intrinsic disorder in this domain.

To determine the origins of these signals, we recorded HNCA and HNcoCa spectra similar to our previous work on the flexible domains of fibrils formed by the functional amyloid Orb2A.¹⁸ As can be seen from the strip plots of the HNCA and HNcoCa in Figure 3, the signal to noise of these spectra is good, which allowed for the resonance assignment of most resonances detected in the HSQC in Figure 2. G102 was assigned based on its unique chemical shift and the fact that there is only one Gly residue in HTT_{ex1}.

The assigned residues are all located at the end of the C-terminus of HTT_{ex1}, termed C12, that follows the second polyP stretch P10 (see Figure 4A). To evaluate the (residual) secondary structure of this region, we calculated the secondary $\text{C}\alpha$ chemical shift ($\delta\text{C}\alpha$), the difference between the measured $\text{C}\alpha$ chemical shift and the random coil chemical shift of the same residue type.³³ As can be seen from Figure 4B, the absolute value of $\delta\text{C}\alpha$ is close to or below 1 ppm for most of the residues, indicative of a random coil structure. The larger $\delta\text{C}\alpha$ values observed for E108 and R112 can be explained by the fact that these residues precede Pro residues, known to lower the $\text{C}\alpha$ shift by up to 2 ppm.³⁴ Although prolines could not be detected using amide-proton based spectroscopy, we could observe the $\text{C}\alpha$ chemical shifts of P103 and P109 as $\text{C}\alpha$ -1 of Ala104 and Leu110 in the HNcoCA spectrum. In both cases, the chemical shift of these prolines is about 63 ppm, which is compatible with the Pro type B we described earlier to be in a random coil conformation.⁵

Because intrinsically disordered domains are defined as much by their residual secondary structure as by their dynamics, we characterized the dynamics of the C12 region in more detail. Figure 4C shows the sequence-dependent NH peak intensities of the HSQC spectrum in Figure 2. The intensity increases gradually from G102 toward the C-terminus. This increase suggests an increase in mobility resulting in a decrease of R_2 relaxation rates, making the HSQC experiment more efficient. To confirm this interpretation, we directly measured the R_1 and R_2' relaxation rates of the assigned residues.

Figure 1A illustrates the pulse sequence we used for measuring ^{15}N R_1 rates,²⁷ which includes an additional BIRD filter for improved water suppression. We measured ^{15}N relaxation up to 1.2 s, which could be fit well to mono-exponential decay rates, as illustrated for residue A106 in Figure 5A (Supporting Figure S1 includes the relaxation curves of all assigned residues). We determined R_1 rates between 1.4 s^{-1} and 2.2 s^{-1} , with the highest rates for G102, *i.e.* the beginning of the C12 region, and from E107 to H111 (Figure 5B).

We used the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence shown in Figure 1B to measure ^{15}N R_2' rates of the residues in the C12 region of HTT_{ex1}.²⁸ The decay under the CPMG sequence was measured for up to 160 ms, leading to a mono-exponential decay of the peak intensity, as illustrated in blue for residue A106 shown in Figure 6A (Supporting

Figure S2 includes the relaxation curves of all assigned residues). The ^{15}N R'_2 rates decrease from a value of $12.89 \pm 1.62 \text{ s}^{-1}$ for residue G102 to $R'_2 = 4.40 \pm 0.43 \text{ s}^{-1}$ for residue R112, indicative of an increase in dynamics toward the C-terminus (Figure 6B).

A problem with estimating R_2 using a CPMG-type experiment in the solid state is that coherent dephasing from incompletely averaged anisotropic interactions can lead to an overestimation of the relaxation rate. $R_{1\rho}$ measurements were shown to be much less affected by this problem while often containing the same information.^{31,32,35} To test if the CPMG pulse sequence leads to systematically higher rates compared to $R_{1\rho}$ in our case, we measured $R_{1\rho}$ relaxation rates using the pulse sequence shown in Figure 1C. The $R_{1\rho}$ relaxation curve for A106 (Figure 6A) overlaps very well with the R'_2 curve of the same residue, and the resulting rate constants are, within error, the same. (Supporting Figure S3 includes the R'_2 relaxation curves of all assigned residues). To directly compare $R_{1\rho}$ with R'_2 , we converted $R_{1\rho}$ to R_2 (see Methods section). The resulting R_2 was essentially the same as $R_{1\rho}$ for all residues under the conditions used.³² Figure 6B shows that R_2 and R'_2 are, within error, the same for each residue.

The fact that we can apply J-based, ^1H detected NMR experiments under moderate MAS frequencies in the absence of perdeuteration suggests an almost complete averaging of the dipolar couplings in the C12 region, a surprising result due to the absence of isotropic molecular tumbling in HTT_{ex1} fibrils compared to molecules in solution. To measure the extent of the residual dipolar couplings that are not averaged by molecular motion, we used the ^1H - ^{15}N rotational echo double resonance (REDOR) pulse sequence shown in Figure 1D, adapted from Schanda and co-workers.²⁹ Measuring residual dipolar couplings this way also allows us to determine the order parameter independent of the relaxation rates.³¹ In contrast to the original use of the ^1H - ^{15}N REDOR experiment in rigid, perdeuterated solids, scaling of the dipolar couplings for improved detection was not necessary, and ^1H 180° REDOR pulses were applied every half a rotor period for up to a total of 1000 rotor periods (80 ms at 12.5 kHz MAS). The REDOR dephasing curve for residue A104 is shown in Figure 7A. The whole set of REDOR dephasing curves is shown in Supporting Figure S4. We fit the REDOR curves to a series of Bessel functions to estimate the residual dipolar couplings.³⁶ Figure 7B shows these for each of the assigned residues. The residual dipolar couplings continuously decrease from about 350 Hz for residue G102 to 17 Hz at residue R112, with the exception of V105. The order parameter can be calculated as follows $S = \delta_{\text{exp}}/\delta_{\text{rigid}}$ where $\delta_{\text{rigid}} = \delta_{\text{NH}}/(2\pi) = 22954 \text{ Hz}$ and δ_{exp} is the measured dipolar coupling.³¹ This order parameter is close to zero for all assigned residues, indicating fast motions that lead to substantial reorientation of the NH bond vector for all residues in C12.

The separate measurement of the order parameter and relaxation rates allows us to estimate the correlation time of the motions using a single-timescale, model-free approach under MAS.^{29,31} Using the order parameters determined via the REDOR experiment described above, we could estimate τ_c from both the experimental R_1 and R'_2 rates (see Supporting Figure S5). The one physical realistic solution for $\tau_c^{R_2}$ is in between the two solutions for $\tau_c^{R_1}$. Generally, the correlation time is estimated to be in the 500 ps to 9 ns range. The slight

difference between the correlation time calculated from R_1 and R_2' can be either due to systematic errors in the relaxation rates or the simple, single-timescale model-free model used here. We plan to expand the set of relaxation measurements, measure R_1 rates at different magnetic fields, repeat our data on perdeuterated samples, and use different models to improve the characterization of this domain in future studies.

Our previous studies on HTT_{ex1} were performed on a C-terminally His-tagged construct ($\text{HTT}_{\text{ex1}}\text{HT}$).^{5,10} To test how the C12 region was affected by the His-tag, we collected HSQC, HNCA, and HNcoCA spectra of $\text{HTT}_{\text{ex1}}\text{HT}$. Figure 8A shows the HSQC recorded on $\text{HTT}_{\text{ex1}}\text{HT}$. The assignment showed that only the C12 region was detected in these experiments, very similar to HTT_{ex1} without His-tag. The NH peaks of the His-tag could be assigned in addition to the residues in the C12 region. The secondary chemical shifts δCa of this sample are similar to HTT_{ex1} without the His-tag, and the additional His residues are compatible with a random coil conformation, except for the C-terminal His (Figure 8B).

The NH peak intensities in Figure 8C show a general increase in intensity toward the C-terminus, with G102 having the lowest intensity and H119 almost twice the intensity compared to the other residues, which might be due to additional conformational freedom at the very C-terminus of the protein.

$\text{HTT}_{\text{ex1}}\text{Pro}$ is another HTT_{ex1} mutant affecting the C12 region. This construct, which was shown not to bind to the PHP1 and PHP2 antibodies due to the absence of the required epitope, has the L17 region replaced with 17 prolines and all prolines in the C12 region replaced with Gly.²⁶ The INEPT-HETCOR spectrum of this mutant confirms the changes to the C-terminus via the absence of the ProB peak, a strong Gly signal, weaker Leu signals, and more well-defined Glu peaks (see comparison of the INEPT HETCOR spectra of the three HTT_{ex1} variants in Supporting Figure S6). Figure 9A shows the HSQC spectrum of $\text{HTT}_{\text{ex1}}\text{Pro}$. The assignment of the residues in this spectrum using HNCA and HNcoCA spectra again showed that all assigned peaks are located in the C12 region. In contrast to $\text{HTT}_{\text{ex1}}\text{HT}$, we were neither able to assign the individual His residues of the His-tag nor the Gly113 preceding the His-tag. The δCa values shown in Figure 9B are all close to zero due to the absence of Pro in the C12 region, with the exception of R112. The HSQC peak intensities shown in Figure 9C are different than those of HTT_{ex1} and $\text{HTT}_{\text{ex1}}\text{HT}$. After an initial increase in intensity from G102 to A104, the intensity decreases toward the C-terminus, rendering the His-tag barely detectable.

The data on the two HTT_{ex1} variants show that the C12 region is very dynamic irrespective of the presence of the His-tag or prolines in the sequence. Nevertheless, we are able to detect changes in chemical shift and dynamics in this region.

Discussion

Previously, we and others have shown that the Pro-rich C-terminus of HTT_{ex1} fibrils is dynamic in contrast to the rigid polyQ core of the HTT_{ex1} fibrils.^{5,8,11} Here, we go a step further by showing that the C12 region of HTT can be assigned using ^1H detected, triple resonance, out an and back style solution NMR experiments in the absence of perdeuteration

or without high MAS frequencies. But how is this possible in the absence of isotropic tumbling that it is usually required for solution NMR methods to work? To answer this question, we analyzed peak intensities, secondary chemical shifts of these residues, and measured site-specific relaxation rates and residual dipolar couplings.

G102 is the first residue detectable in our HSQC experiment because the polyP region cannot be detected using amide proton-based spectroscopy. On the other hand, the dynamics of G102 were insufficient to obtain peaks in the HNCA and HNcoCA spectra, indicating that its reduced flexibility limits detection in our approach. This is probably why we could not assign any residues from the L17 region, which is likely to be less dynamic than the C12 region. Nevertheless, the L17 region is still much more mobile than the polyQ domain that forms the core of the HTT_{ex1} fibrils and can, therefore, be detected using straight-through INEPT-HETCOR 2D spectra (as seen in Supporting Figure S6), which are more forgiving to transverse ^1H and ^{13}C relaxation. Our R'_2 and residual dipolar coupling data support that G102 is the least flexible of the assigned residues. The increase in intensity of the NH cross peaks toward the C-terminus indicates an increase of motions toward the C-terminus. This conclusion is supported by both the R'_2 rates and residual dipolar couplings, both of which decrease toward the C-terminus. Furthermore, these conclusions are compatible with EPR data showing that residue 111 is the most dynamic in HTT_{ex1} HT fibrils¹¹ and with our bottle brush model of the HTT_{ex1} fibrils.⁵ In this model, the Pro-rich C-termini form entropic bristles that protrude from the static polyQ core of the fibrils, which allows for maximal motility at the end of the C-terminus.

Interestingly, our R_I rates do not show a clear trend (Figure 5). Generally, the ^{15}N R_I rates we measured are large compared to what has been observed in solid-state NMR on globular proteins^{29,37} and more comparable to ^{15}N R_I rates observed in solution NMR of intrinsically disordered or denatured proteins,^{38,39} indicating a highly dynamic and disordered nature of this region. At R_I rates this high, an error due to ^{15}N - ^{15}N spin diffusion is no concern.³¹ However, a systematic error of up to 20% due to H_2O proton-spin saturation similar to what is observed in solution NMR of larger proteins cannot be ruled out.^{40,41}

Measuring R'_2 rates using CPMG-type experiments in solid-state NMR is difficult because of coherent dephasing from incompletely averaged anisotropic interactions even at very high MAS frequencies and with perdeuteration. The fact that we can observe narrow ^1H lines in our HSQC spectrum in the absence of perdeuteration suggests that we have favorable conditions to measure R'_2 in which dipolar couplings are averaged to a very high degree and the motions are on much faster time scales than the MAS frequency.³¹ This is confirmed by the R_{Ip} measurement that, although less affected by coherent dephasing,³⁵ resulted in essentially the same relaxation rates. The R'_2 rates are generally similar to what has been observed in globular proteins using solid-state NMR as well as intrinsically disordered proteins in solution.^{32,35,38} Both R'_2 and R_{Ip} decrease continuously towards the C-terminus compatible with a continuous increase in dynamics.

Because it can be challenging to determine both the correlation time of the motion and the order parameter from relaxation rates alone, additional measurements of S as the scaling of anisotropic interactions like the NH dipolar coupling is a useful method to characterize

dynamics in solid-state NMR. Our measurements of the residual NH couplings confirmed that the dipolar couplings in the C12 region are averaged from their theoretical value of 22,954 Hz to only 350–17 Hz. The resulting order parameters are very close to zero, showing that this domain is dynamically disordered. The low order parameter in the C12 region is also compatible with our observation that the secondary chemical shifts $\delta C\alpha$ are very close to their random coil values. Together these data indicate that the motions of the C12 region of HTT_{ex1} are fast and lead to substantial reorientation of the NH bond vector (at least close to a semi angle θ_0 of 90° in a diffusion on a cone model in which

$S^2 = [\cos \theta_0 (1 + \cos \theta_0)/2]^2$). However, the fact that we were able to measure residual dipolar couplings that decrease toward the C-terminus also shows that the detected residues are not free in solution and still part of the amyloid fibril. Using the order parameter for the NH dipolar coupling measurements, we estimated the motional correlation time from both the R_1 and R'_2 measurements, which are in the high ps to low ns range. In summary, our data support a model in which the C12 region of HTT_{ex1} is intrinsically disordered and undergoes fast and motions, explaining why they seem to be almost in solution from an NMR perspective.

The relaxation and residual dipolar coupling data show that the dynamic C12 region of HTT_{ex1} fibrils are, from an NMR point of view, an interesting chimera between solid protein and a protein in solution (see Figure 10). On the one hand, HTT_{ex1} fibrils are not tumbling isotropically in solution, yet the C12 region is so dynamic that homo- and heteronuclear dipolar couplings are averaged to a very high degree. This allows the use of relatively standard solution-state NMR pulse sequences, including CPMG R'_2 measurements that suffer from large systematic errors on structured and less dynamic proteins in the solid-state, even at very high MAS frequencies or with perdeuteration.³¹ On the other hand, we were able to directly observe residual dipolar couplings using dipolar recoupling under MAS similar to less dynamic proteins in the solid-state. For proteins in solution the measurement of residual dipolar couplings usually requires the alignment of the protein with respect to the static magnetic field.⁴²

Our comparison of three different HTT_{ex1} C-terminal variants shows that the high mobility in this region does not depend on the presence of a His-tag or the presence of the Pro in this region. This is supported by the observation that the His tag is more dynamic in the case of $HTT_{ex1}HT$ but less in $HTT_{ex1}Pro$. Therefore, the location of this region at the outer tip of the Pro-rich seems to be more important for its high mobility. Although the majority of the Pro-rich domain is in a poly-proline II helical conformation, as shown by its Pro chemical shifts,^{5,7} it is nevertheless a highly dynamic structure due to the absence of stabilizing hydrogen bonds.⁴³ This high flexibility makes it possible to detect the Pro-rich domain in our INEPT-HETCOR experiments and allows the C12 region at its tip to be detected using the HSQC based experiments used in this study.

The results from the all Pro sample clarify the assignment of our original INEPT-HETCOR data.⁵ Pro B is from those Pro that have non-Pro residues as neighbors. Likewise, the Gln resonances observed in these spectra result from the Gln in L17 and not the end of the polyQ

domain. There is very little evidence for signals from the N17 region, which was shown relatively less mobile by EPR and solid-state NMR.^{6,11}

Our spectra of the three C-terminal HTT_{ex1} variants show that we are able to detect structural changes in these regions due to mutations and potentially detect the interaction of binding partners to this region using the approach presented in this study. This is important because the dynamic C-terminus has been identified to be the site that distinguishes different HTT_{ex1} fibril polymorphs.⁹ Furthermore, the C-terminus of HTT_{ex1} has been shown to be an important epitope for HTT_{ex1} specific antibodies²⁶ and intrabodies⁴⁴ that can reduce HTT toxicity. The Pro-rich C-terminus of HTT_{ex1} is also also a major site of functional protein-protein interactions involving HTT_{ex1},⁴⁵ including profilin^{46,47} and membrane associated proteins containing SH3 domains.^{48,49}

Conclusions

We assigned the most dynamic domains of HTT_{ex1} using ¹H detected J-based triple resonance experiments, moderate MAS frequencies, and fully protonated protein. All assigned residues are located in the C12 region, the very C-terminus of the protein. The assignment allowed us to record residue specific ¹⁵N R₁, R₂, and R_{1ρ} rates. In addition, we measured residual dipolar couplings of this region. The Cα chemical shifts, relaxation rates, and residual dipolar couplings show a) that the C12 region is in a random coil conformation, with order parameters that are very close to 0, and b) that it undergoes motions in the high picosecond to low nanosecond range. Besides providing a site specific description of the dynamics of the C12 region of HTT_{ex1}, we demonstrate that it is not only possible to assign intrinsically disordered domains in the solid-state but also study their dynamics in detail similarly to proteins in solution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

IDD	intrinsically disordered protein domain
HD	Huntington's disease
HTT	huntingtin
HTT_{ex1}	huntingtin exon-1
polyQ	polyglutamine
NMR	nuclear magnetic resonance

EPR	electron paramagnetic resonance
PPII	polyproline II
MAS	magic angle spinning
BIRD	bilinear rotational decoupling
HSQC	heteronuclear single-quantum correlation
CPMG	Carr-Purcell-Meiboom-Gill
INEPT	insensitive nuclei enhanced by polarization transfer
HETCOR	heteronuclear correlation
rf	radio frequency

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Pulse sequences used to measure relaxation rates and residual dipolar couplings of dynamic domains A) The pulse sequence used to measure ^{15}N R_1 includes a BIRD filter for water suppression and GARP ^1H decoupling during the relaxation delay, T. B) Pulse sequence used to measure $R'_{2\text{,}}$ C) Pulse sequence used to measure $R_{1\rho}$. D) ^1H - ^{15}N REDOR based pulse sequence used to measure δ_{HN} . All pulse sequence include a BIRD filter for additional water suppression.

Figure 2:

The HSQC spectrum of HTT_{ex1} fibrils shows 12 resolved amide cross peaks. Spectrum was recorded at 0 °C, 12 kHz MAS, and in 4 h of measurement time. The ^1H and ^{15}N linewidth in the absence of a window function is illustrated for residue Ala 106.

Figure 3:
 3D HNCA and HNcoCA spectra allow the sequential assignment of most of the signals detected in the HSQC spectrum of HTT_{ex1} . Strip plots of HNCA and HNcoCA spectra at the same ^{15}N and ^1H frequency are shown in red and blue, respectively. The assignments of the α resonances for residues A104 to E108 are indicated.

Figure 4:

All assigned residues are at the end of the Pro-rich C-terminus of HTT_{ex1} and have chemical shifts compatible with a random coil conformation. A) Regions of HTT_{ex1} . The location of the amphiphilic N-terminus (N17) is indicated in orange, the central polyQ domain in blue, and the C-terminus in green. The Pro-rich C-terminus has two polyP regions (P11 and P10) that are separated by a Pro and Gln-rich region (L17), and ends in a region termed C12. B) Secondary C_α chemical shift of the assigned residues. Most values are relatively small, compatible with a random coil conformation. Exceptions are residues with a C-terminal Pro neighbor, which is known to lower the C_α shift. C) NH peak intensities of the HSQC spectrum shown in Figure 2. The NH peaks become more intense toward the C-terminus, indicating an increase in dynamics.

Figure 5:

^{15}N R_1 relaxation rates of HTT_{ex1} C-terminus show small differences between individual residues. A) Example of the ^{15}N relaxation data obtained for residue A106. The integrated ^1H - ^{15}N peak intensity is shown with blue dots. The error bar of the intensity lies within these dots. The best fit to a mono-exponential decay curve is shown in red. The good fit of the data illustrates the mono-exponential nature of the data. B) Site-specific ^{15}N R_1 rates of the C-terminus of HTT_{ex1} . Error bars reflect the standard error of the fit.

Figure 6:

The ^{15}N R'_2 and $R_{1\rho}$ of the HTT_{ex1} C12 region are very similar and decrease toward the C-terminus. A) Example of the ^{15}N relaxation data obtained for residue A106. The integrated ^1H - ^{15}N peak intensity is shown with circles. The error bar of the intensity lies within these circles. The best fit to a mono-exponential decay curve is shown with a solid line. B) Site-specific ^{15}N R'_2 rates (blue) and the R_2 rates calculated from the measured $R_{1\rho}$ (magenta) of the C-terminus of HTT_{ex1} . The relaxation rates measured using both methods are essentially the same and show a general decrease of the rates toward the C-terminus. Error bars reflect the standard error of the fit.

Figure 7:

The residual ^1H - ^{15}N dipolar couplings decrease toward the C-terminus of C12. A) ^1H - ^{15}N REDOR dephasing curve S/S_0 measured for residue A104. S is the difference between the integrated ^1H - ^{15}N peak intensity of the REDOR dephased spectrum (S) and the same experiments without the ^1H refocusing pulses (S_0). S/S_0 values depending on the number of REDOR cycles are shown as black dots, and the best fit to a series of Bessel functions is shown as a solid black line. B) Site-specific residual dipolar couplings δ_{HN} of the C-terminus of HTT_{ex1} . A general decrease of the residual couplings is observed toward the C-terminus. Error bars reflect the standard deviation of the fit.

Figure 8:

The C12 region is similarly mobile in the presence of a C-terminal His-tag. A) HSQC spectrum of $\text{HTT}_{\text{ex1}}\text{HT}$, assignments are indicated. B) Secondary chemical shift $\Delta\delta C_{\alpha}$. The low values for residues not preceding Pro indicate a random coil structure of this region that continues through the His-tag. C) NH peak intensity of the HSQC spectrum in A showing a general increase in intensity towards the C-terminus.

Figure 9:

Dynamics of the C12 region of $\text{HTT}_{\text{ex1}}\text{Pro}$. A) HSQC spectrum of $\text{HTT}_{\text{ex1}}\text{Pro}$, assignments are indicated. B) Secondary chemical shift δC_α . Except for R112, all values are compatible with a random coil conformation. C) NH peak intensity of the HSQC spectrum in A showing an increase in intensity until A104 followed by a decrease toward the C-terminus.

Figure 10: Model of the C-terminal Pro-rich bristles of HTT_{ex1} . The pure polyP regions P_{11} and P_{10} form polyproline II helices which are interrupted by the L_{17} linker region. The C_{12} region at the tip of the bristle is free to undergo motions that lead to substantial reorientations of the NIH bond vectors with a correlation time in the high ps to ns range.