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Dynamic domains of amyloid fibrils can be site-specifically assigned with proton detected 3D NMR spectroscopy

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Abstract

Several amyloid fibrils have cores framed by highly dynamic, intrinsically disordered, domains that can play important roles for function and toxicity. To study these domains in detail using solid-state NMR spectroscopy, site-specific resonance assignments are required. Although the rapid dynamics of these domains lead to considerable averaging of orientation-dependent NMR interactions and thereby line-narrowing, the proton line-widths observed in these samples is far larger than what is regularly observed in solution. Here, we show that it is nevertheless possible to record 3D HNCQ, HNCA, and HNcoCA spectra on these intrinsically disordered domains and to obtain site-specific assignments.

Keywords

Amyloid fibrils; Intrinsically disordered domains; Resonance assignment; Solid-state NMR

Many amyloid fibrils have disordered dynamic domains framing a relatively static β -sheet rich amyloid core (Siemer et al. 2006; Heise et al. 2005; Tompa 2009). The conformational space and residual structure of these dynamic domains is of interest since they are on the surface of the fibril and might play important roles in the toxicity of pathological amyloids or the function of functional amyloids (Isas et al. 2015). Although attached to a relatively immobile amyloid core, these dynamic domains exhibit a surprising degree of motions that narrow ^1H lines to well below 100 Hz (Siemer et al. 2006). As a result J -coupling based ^1H – ^{13}C and ^1H – ^{15}N correlation spectroscopy becomes possible and the amino acid compositions of the dynamic domains of several amyloid fibrils have been reported (Siemer et al. 2006; Isas et al. 2015; Raveendra et al. 2013; Helmus et al. 2010; Loquet et al. 2009). Site-specific assignments of these dynamic domains are more challenging because of the limited ^1H resolution compared to proteins in solution and the signal loss that occurs during the multiple coherence transfers in three-dimensional (3D) ^1H – ^{15}N – ^{13}C spectra typically acquired for protein resonance assignment in the liquid-state. In the following, we show that it is nevertheless possible to use 3D liquid-state NMR ^1H – ^{15}N – ^{13}C “out and back” type

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pulse sequences on the dynamic domains of an amyloid fibril. These spectra allow the site specific assignment of many residues found in the dynamic domain and, consequently, a more stringent analysis of the residual structure found in these domains.

Figure 1a shows an ^1H - ^{15}N HSQC and Fig. 1b an HNCO spectrum recorded on fully protonated and uniformly ^{15}N - ^{13}C labeled amyloid fibrils formed by the A isoform of the functional amyloid Orb2 (Orb2A) (Majumdar et al. 2012). Orb2A fibrils were fully hydrated and prepared as described in the Supplementary Material. The spectra were recorded on an Agilent DD2 600 MHz spectrometer in a 1.6 mm magic angle spinning (MAS) probe operating at 12 kHz MAS and 25 °C. Considering that Orb2A has 551 residues, the HSQC spectrum only shows a small portion of the protein with varying intensity and linewidth. The 1D slices on the peak at $\delta^1\text{H} = 8.01$ ppm and $\delta^{15}\text{N} = 123.9$ ppm in Fig. 1a illustrate the quality of this spectrum. The ^1H linewidth of ~60-Hz is well above what is regularly obtained for small globular proteins in solution (<10-Hz) (Cavanagh et al. 1995) and still larger than what was found for highly perdeuterated, crystalline proteins in the solid-state (~25 Hz) (Agarwal et al. 2006). The same is true for the ^{15}N linewidth of about 40 Hz. We found that higher temperatures changed the efficiency but had little effect on the linewidth of our HSQC spectra. Higher MAS frequencies had no effect on the spectra besides the resulting change in temperature (see Figure S1).

We are confident that the signal observed in these experiments originates from dynamic regions of the fibril and not free monomers or oligomers based on the following reasons: (1) the signal-to-noise ratio of the HSQC recorded in about 1 h is good, (2) we washed our fibrils multiple times before packing the NMR rotor, (3) our EM images do not show any Orb2A oligomers, and (4) monomers and oligomers would likely have more highly dynamic residues than the small number of peaks observed in the HSQC.

Despite the limited resolution, we were able to record a 3D HNCO experiment (Kay et al. 2011; Grzesiek and Bax 1992) on this sample. In the absence of a gradient coil in our MAS probe, we obtained excellent water suppression using x and y ^1H purge pulses (Muhandiram and Kay 1994) as illustrated in Figure S2. As can be seen from Fig. 2b, the signal-to-noise ratio for this HNCO experiment is ~67:1, which given the acquisition time of about 70 h, is excellent.

Due to the larger chemical shift dispersion of C^α chemical shifts, HNCA and HNcoCA experiments (Kay et al. 2011; Grzesiek and Bax 1992) (see pulse sequences in Figure S2) are better suited for site-specific backbone assignments. The HNCA experiment gives cross peaks that correlate the amide ^1H and ^{15}N to the C^α of the same and preceding residue. The HNcoCA experiment, by contrast, only shows the peak corresponding to the C^α of the preceding residue. The combination of the two experiments allows the backbone assignment of all $\text{H}_i^{\text{N}} - \text{N}_i^{\text{H}} - \text{C}_i^\alpha$ resonances. Although these experiments are less efficient than the HNCO experiment, we were still able to record both experiments on the same sample as can be seen from Fig. 2.

The signal-to-noise ratios of the C_i^α peak in the HNCA spectrum and the C_{i-1}^α peak in the HNcoCA spectrum which correspond to the NH peak highlighted in Fig. 1a are 23:1 and

18:1 respectively. Using these spectra, we sequentially connected many of the cross peaks in Fig. 1a. We then identified amino acid types using the 2D ^{13}C - ^{13}C adiabatic TOBSY spectrum shown in Figure S3. The $C_i^\alpha - C_{i-1}^\alpha$ connectivities were confirmed using the $H_i^N - N_i^H - C_{i-1}'$ resonances from the HNCO spectrum and the $C_i' - C_i^\alpha$ peaks from the ^{13}C - ^{13}C CTUC-COSY (Chen et al. 2006) spectrum shown in Figure S2. The assignment was further facilitated by (a) the fact that there are relatively few peaks in these spectra, (b) that the HNCA and HNcoCA spectra are dominated by very strong Gly peaks that have the same C_i^α and C_{i-1}^α shift indicating a poly-Gly structure, and (c) identifying a serine-threonine pair that is unique in the sequence of Orb2A (S160-T161). We consequently determined the sequential assignment of many of the cross peaks in Fig. 1a and identified them as belonging to the sequence highlighted in red in Fig. 3a. The Gly repeats framing the assigned sequence are very likely part of this dynamic domain, resulting in the intense Gly peaks seen in Figs. 1a and 2. Due to their repetitive nature, these Gly residues could not be assigned with certainty. The same is true for the intense peak used for the 1D slices in Fig. 1a, which is likely D173 based on its side-chain assignment and its N-terminal Gly neighbor. Other cross peaks in the HSQC spectrum in Fig. 1a were not assigned because they did not have corresponding peaks in all 3D experiments.

This assignment is compatible with the assumption that Gly and Ser rich regions have a high propensity to be intrinsically disordered (Krieger et al. 2003), and this region is likely to form a flexible linker between the N-terminal domain that has been shown to be important for amyloid formation (Majumdar et al. 2012) and the RNA recognition motifs (RRMs) at the C-terminus of the protein. The relatively small C^α secondary shifts of the assigned residues indicate that the most dynamic domain of Orb2A has relatively little residual structure (see Fig. 3b). The fact that a small region of Orb2A gives signal in our amide ^1H detected 2D and 3D experiments does not mean that these are the only dynamic domains in the protein, but that other regions are not dynamic enough to be detected using this approach. Some of these less dynamic residues give signals in the adiabatic TOBSY spectrum of Figure S3 but no corresponding signal in the 3D spectra.

How dynamic are the assigned regions when compared to globular proteins in solids and solution? The fact that repeated INEPT transfers work for residues in the most dynamic regions indicates that dipolar couplings are motionally averaged to a degree that coherent dipolar dephasing is minimal. Therefore, we estimate the order parameter $S = \delta_{\text{exp}}/\delta_{\text{rigid}}$ as described by the scaling of effective dipolar interactions (δ_{exp}) below its rigid value (δ_{rigid}) to be $S \approx 0$ (Schanda and Ernst 2016). Assuming a rotational diffusion on a cone (with angle θ) model $S^2 = (1/2 \cos \theta)(1 + \cos \theta)^2$, a vanishing order parameter describes a motion that is essentially isotropic. We estimated the correlation time of the motion via the transverse relaxation of the backbone ^{15}N because it can be sufficiently approximated by the H-N dipolar coupling and its chemical shift anisotropy (CSA). Using the expression for the transverse relaxation rate under MAS by Schanda & Ernst and approximating the ^{15}N transverse relaxation rate R_2 via the ^{15}N linewidth of ~ 40 Hz, we estimate the correlation time of the dynamic regions to be $\tau_C \approx 95$ ns (see Supplementary Material). Under these assumptions, the motional model would be the same as in solution and the correlation time corresponds to the rotational correlation time of a globular protein with a mass of about 300

kDa. This correlation time is only an estimate because factors such as dipolar dephasing, sample and field heterogeneity also contribute to the linewidth and the order parameter could be larger than $S^2 = 0$. Overestimating the relaxation rate from the linewidth leads to larger correlation times and underestimating the order parameter leads to smaller correlation times. Assuming non R_2 contributions to the linewidth of up to 20 Hz and order parameters of up to $S^2 = 0.2$, the correlation time is in the range of 47–119 ns.

Our results demonstrate that ^1H detected J -coupling based 3D pulse sequences can be used to assign the most dynamic domains of non-soluble proteins in the absence of deuteration. We think that this approach will be generally applicable to amyloid systems that have considerable disordered domains outside the fibril core (Siemer et al. 2006; Isas et al. 2015; Raveendra et al. 2013; Heise et al. 2005; Helmus et al. 2010; Loquet et al. 2009) and potentially other insoluble protein complexes that retain intrinsically disordered domains. Many intrinsically disordered domains adopt well defined 3D structures when interacting with binding partners (Uversky 2013). Using this approach allows site-specific monitoring of conformational changes in intrinsically disordered domains that are present in amyloid fibrils or larger protein complexes.

Additional ^1H detected, J -coupling based experiments might be possible depending on the degree of dynamics and amount of sample. Only the most dynamic domains of the protein were detected using this approach and less dynamic domains that give signals in straight-through ^{13}C detected experiments such as the adiabatic TOBSY could not be assigned sequentially. In addition, there is a regime of protein dynamics in which neither dipolar based cross polarization spectra nor J -coupling based spectroscopy is applicable and only direct excitation can be used to detect these domains. Sometimes even this is not possible if intermediate exchange is broadening the lines below the detection limit. A possible approach to extend this method to less dynamic regions is to combine it with partial deuteration, similar to approaches used to reduce linewidth for large proteins in solution (Yamazaki et al. 1994) or to allow ^1H based spectroscopy on proteins with little dynamics in the solid-state (Reif 2012). Furthermore, TROSY based methods might allow the extension of our method to less dynamic regions as demonstrated by Linser et al. (2010). Where full perdeuteration combined with ^1H back exchange allows the use of ^1H based spectroscopy on relatively static protein samples, partial deuteration and TROSY might allow our experiments to be performed on protein domains that are less dynamic than those identified here. The current approach, however, has the advantage of being selective to the most dynamic domains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

The most dynamic domains of amyloid fibrils have a linewidth narrow enough to allow HSQC and HNCO spectra in the absence of perdeuteration. **a** ^1H - ^{15}N HSQC spectrum of fibrils formed by Orb2A. 1D slices and linewidths for one residue are shown to illustrate spectral quality. Site-specific assignments are indicated. **b** Strip plot of a HNCO spectrum from the same cross peak as in **a**). The 1D slice through the ^{13}C dimension is shown to illustrate spectral quality

Fig. 2.

The combination of HNcoCA and HNCA experiments allows the site-specific backbone assignment of dynamic amyloid fibril domains. Strip plot of HNcoCA (*blue*) and HNCA (*red*) experiments showing residues G159-G162. The HNCA experiment shows both the $H_i^N - N_i^H - C_i^\alpha$ and $H_i^N - N_i^H - C_{i-1}^\alpha$ correlations whereas the HNcoCA shows only the $H_i^N - N_i^H - C_{i-1}^\alpha$, allowing assignment of both the C_i^α and C_{i-1}^α shift. The large peak associated with G159 is likely because G155-G159 are all glycine

Fig. 3.

The most dynamic domain of the Orb2A amyloid fibril is located in the Glycine and Serine rich region and is intrinsically disordered. **a** Domain structure of Orb2A. The N-terminus, Glnrich domain (Q), Glycine and Serine rich domain (G/S), RNA recognition motifs (RRMs), and zinc finger (Zn) are highlighted. The residues that we assigned sequentially are highlighted in red in the sequence excerpt below. **b** Secondary C^α shifts of sequentially assigned residues. The relatively small values indicate the absence of secondary structure for this domain