How do Proteins Form Amyloid? Insight from the NMR Spectroscopic Characterization of ^{13}C, ^{15}N-labeled Ribonuclease A in 40% Acetic Acid

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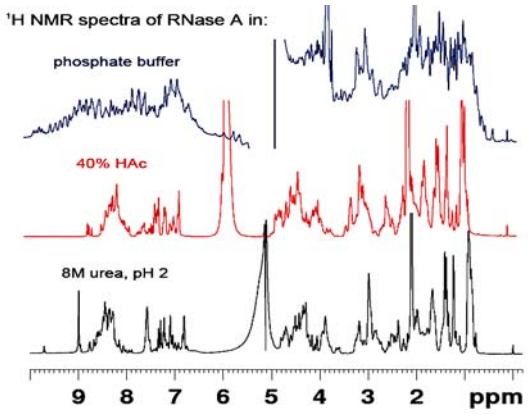
Under certain conditions, some folded proteins can adopt an alternative conformation called amyloid[1] which has been linked to over twenty mortal human diseases including diabetes, Alzheimer's and Parkinson's. Two hypothetical models have been advanced for natively folded proteins become amyloid. Dobson and coworkers proposed that proteins must fully unfold before becoming amyloid.[2] In contrast, Eisenberg and coworkers have argued that amyloid formation can occur when RNase A oligomerizes by 3D domain swapping[3,4] through a limited local unfolding event. Studies of the amyloidogenic conformations of proteins are rare and are needed to test these models. Using CD, we have observed that RNase A oligomerizes best when the protein is completely unfolded.[5] Here, we use NMR spectroscopy to characterize RNase A's structure in solution conditions that promote oligomerization; namely, 40% acetic acid, 25 ºC. ^{1}H 1D and 2D NOESY NMR spectra reveal a poor chemical shift dispersion and a loss of long-range contacts indicative of tertiary structure. The analysis of several 3D spectra using ^{13}C, ^{15}N labeled RNase A, permitted the unambiguous assignment of over 90% of the $^{13}C\beta$ and backbone ^{15}N, ^{1}HN, $^{13}C\alpha$, ^{13}CO and $^{1}H\alpha$ nuclei. The assignments were confirmed using a novel series of pulse sequences which selects the signals according to the sidechain structure. See the presentation of Prof. J. Santoro for full description of the pulse sequences. The conformational shifts of these nuclei show that the three native α-helices are present in 40% HAc, whereas the rest of the secondary structure is unfolded. A similar study of the conformation of RNase A in 8 M urea at acidic pH revealed no evidence for any preferred conformations. The conformation of RNase A in 40% HAc is not a molten globule as it lacks tertiary structure; instead it resembles the denatured state provoked by alcohols.[6] Thus, RNase A oligomerizes via a thoroughly, but not completely, unfolded state which is akin to the mechanism of amyloid formation proposed by Dobson and coworkers.[2]



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