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Probing the Micelle-Bound Aggregation-Prone State of α-Synuclein with ^{19}F NMR Spectroscopy

Gui-Fang Wang[a], **Conggang Li**[b], and **Gary J. Pielak**[a,*]

[a]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599

[b]State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071 P.R.China

Keywords

Protein aggregation; ^{19}F NMR; Unnatural amino acid; SDS Micelle

α-Synuclein is a 140 residue protein associated with Parkinson's disease and other neurodegenerative disorders.[1,2] The protein has three distinct regions. The N-terminal region is positively charged; the hydrophobic core [also known as the non-amyloid component (NAC)] comprises residues 61–90, and the C-terminal region is negatively charged. Its filamentous inclusions are found in dementia with Lewy bodies and multiple system atrophy.[3] α-Synuclein aggregates formed *in vitro* near physiological pH are morphologically indistinguishable from those in Lewy bodies.[4] Understanding α-synuclein's fibrillation mechanism at the molecular level is key to discovering or designing drugs to slow, prevent, or even reverse its aggregation.[5]

At low sodium dodecyl sulfate (SDS) concentrations (<~2 mM), α-synuclein assembles into highly ordered amyloid fibrils.[6–9] At high SDS concentrations (>~20 mM), monomeric α-synuclein forms two stable anti-parallel curved α-helices followed by an unstructured tail.[10–17] How α-synuclein switches from an unfolded monomer in buffer to a helical conformation in SDS and why SDS promotes aggregation remain unclear. Here, we identify the conformational state through which amyloid fibril formation most likely occurs by using highly sensitive ^{19}F NMR spectroscopy and site specifically labeled a-synuclein to probe the conformational switch.

First, we used two dimensional ^{1}H-^{15}N Heteronuclear Single Quantum Correlation (HSQC) spectra to monitor conformational changes in 100 μM α-synuclein upon increasing the SDS concentration from 0.8 mM to 24 mM at 25 °C (Figure 1). No aggregation occurred during the 20 min NMR experiments. As observed previously,[9,18] resonances from residues in the C-terminal region remain unchanged, but the resonances from residues in the N-terminal region are broadened beyond detection in 0.8 and 2.4 mM SDS. The line broadening could arise from chemical exchange between the free and micelle-bound states occurring at rates approximating the difference in resonance frequencies in the two environments.[8,9] In 24 mM SDS, additional new resonances appear, but they are broad compared to the relatively sharp resonances from the C-terminal residues.

The ^{19}F chemical shift is sensitive to local environment and it is widely used to monitor conformational transitions in proteins,[8,19–27] DNAs[28] and RNAs.[29,30] We made

Fax: (+1)919-962-6714, gary_pielak@unc.edu.

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four variants of α-synuclein by substituting the unnatural amino acid L-4-trifluoromethylphenylalanine (tfmF) at phenylalanine 4, tyrosine 39, valine 70 and tyrosine 133. These positions were chosen to provide a probe in each of the three regions descibed above. Residues 4 and 39 are present in the N-terminal region, residue 70 is in the NAC-region, and residue 133 is in the C-terminal region. We chose these particular residue types because their aromatic or bulky hydrophobic side chains are similar.

One-dimensional ^{19}F NMR spectra were recorded in the absence and in the presence of 0.8, 2.4 and 24 mM SDS (Figure 2). The conditions and the molar ratio of protein to SDS were kept the same as those used to obtain the HSQC data.

Without SDS, the ^{19}F resonances from tfmF labeled proteins are sharp and show little chemical shift dispersion, as expected for a disordered protein. A sharp transition (~0.5 ppm shift) for the F4tfmF resonance takes place at 0.8 mM SDS, while the transitions for the Y39tfmF (~0.6 ppm shift) and V70tfmF (~1.2 ppm shift) resonances take place at 2.4 mM SDS. Y133tfmF does not exhibit any change from 0 to 24 mM SDS. Many studies show that α-synuclein adopts α-helical structure in SDS.8–11 Our data pinpoint the location of the changes at the residue level. Such information is unavailable from methods such as circular dichrosim (CD) and fluorescence spectroscopies. Another feature of the ^{19}F spectra is that the resonances are broad in SDS solution compared to dilute solution, except for Y133tfmF. The results from light scattering experiments show that the broadness is not due to the formation of large soluble oligomers in SDS.[7] Instead, the broadness is caused by conformational exchange in the ensemble. These resonances sharpen when the SDS concentration is increased from 0.8 to 24 mM, indicating a dynamic interaction between α-synuclein and SDS.

Ahmad et al.[7] found that SDS promotes α-synuclein fibril growth at an optimal concentration range of 0.5–0.75 mM. At these SDS concentrations, the α-synuclein ensemble shows enhanced amounts of hydrophobic surface. When the SDS concentration is above 2 mM, less- or non-fibrillogenic ensembles form. These ensembles are characterized by less hydrophobic surface and maximum helix content. Our data are consistent with these conclusions and others derived from isothermal titration calorimetry (ITC), CD spectroscopy, hydrophobic dye binding, and fluorescence lifetime data.[7,31,32] Due to the site specific labeling, our ^{19}F data provide detailed conformational switch information unobtainable from other techniques.

Figure 3 describes the SDS-induced conformational switch. In dilute solution, α-synuclein is disordered. CD data indicate that α-synuclein acquires α-helical switch in SDS solution.[7,9] Changes in chemical shift from 0.8 to 2.4 mM SDS, indicate that the partial helical conformation is induced in the first several residues, including F4tfmF. But the small shift change indicates that residues after Y39tfmF remain disordered. This partial helical conformation is probably responsible for the fast aggregation of α-synuclein.

Above 2.4 mM SDS, a more folded α-synuclein with a higher helix-content forms[12] as indicated by the changes in chemical sift observed for the V70tfM protein. The C-terminal region of this species, as monitored at position 133, remains disordered. This species is known to be less aggregation prone and fully micelle bound. These conformations exchange on the ~ms time scale based on the difference in ^{19}F chemical shift between the states. The intensity increase and lack of chemical shift change with increasing SDS concentration indicates both dynamic changes and structural homogeneity.

α-Synuclein function is suggested to be related to membrane binding.[2,33–36] SDS micelles are often used to mimic the membrane environment. Recent data from NMR studies of α-synuclein-membrane binding suggest that the N-terminus (residues 3–25) binds

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membranes as a helix, while the hydrophobic non-amyloid component region remains dynamically disordered.[37,38] This bound state is prone to intermolecular interactions that progress toward disease-associated oligomers and fibrils. Our proposed aggregation prone state is consistent with the membrane bound state. Single-molecule fluorescence resonance energy transfer experiments have also been employed to investigate SDS induced species.[10,39] These studies suggests that α-synuclein adopts a metastable structure. Our ^{19}F data provide direct evidence that this metastable, aggregation prone state exists in a membrane mimetic environment.

Previously, we used ^{19}F NMR to examine the conformational change of α-synuclein by using 3 fluorotyrosine (3FY) labeling.[8] We showed that ^{19}F NMR is a good probe of conformational change at the level of individual residues.[8] tfmF labeling offers at least five advantages compared to 3FY. First, assignment is not required. Second, we obtain higher sensitivity because tfmF contains three times the ^{19}F. Third, tfmF has advantageous NMR relaxation properties. Fourth, any single ribosomally encoded amino acid can be replaced with tfmF. Fifth, ^{19}F NMR of tfmF labeling can also be applied to study large proteins or protein complexes (to 100 kDa), which is challenging for routine solution NMR spectroscopy.[40,41] It must be borne in mind, however, that the trifluromethyl group may affect protein properties. Protein conformational switching is a general mechanism in signal transduction. Here, we describe a powerful and easy ^{19}F NMR method to assess switching phenomena.

Experimental Section

The protein was labeled and expressed by using an orthogonal tRNA/tRNA synthase system.[40] Briefly, stop codons (TAG) were incorporated in the arabinose-inducible expression vector pBAD at the sites for labeling by using site-directed mutagenesis (QuickChange, Stratagene). The mutations were confirmed by DNA sequencing. A single DH10B colony from an ampicillin/tetracycline plate containing both the appropriate pBAD and pDule-tfm-Phe vectors was picked and used to inoculate 50 mL of LB media (containing 100 mg/L ampicillin and 25 mg/L tetracycline). The culture was grown overnight at 37 °C with shaking at 250 rpm. An aliquot (2.5 mL) was added to warm arabinose autoinduction medium (500 mL). tfmF was added after 30 min (1 mM final concentration) from a stock solution (100 mM) prepared by dissolution in NaOH (20 mM). This culture was grown for an additional 40 h (absorbance at 600 nm of ~5). Cultures were centrifuged at 1 200*g* for 30 min at room temperature. The cell pellets were resuspended in lysis buffer (20 mL, 10 mM Tris, pH 8.0, 1 mM EDTA, 1 mM PMSF), sonicated (FisherScientific, Sonic Dismembrator Model 500) on ice for 5 min with a duty cycle of 1 s on, 2 s off. The sonicated sample was boiled for 20 min (to precipitate globular proteins) and then centrifuged at 16 000*g* for 30 min. Nucleic acids were precipitated with streptomycin sulfate (10 mg/mL, 4 °C, 30 min) and removed by centrifugation (16 000*g*, 30 min). Proteins were precipitated with $(NH_4)_2SO_4$ (360 g/L, 4 °C, 30 min). The pellet was collected by centrifugation (16 000*g*, 30 min) and resuspended in low salt buffer (20 mL, 20 mM Tris, pH 7.7). Protein in low salt buffer was then loaded onto HiLoad 16/10 Q Sepharose High Performance column (GE Healthcare) in Tris (20 mM), pH 7.7 and eluted with in a NaCl gradient (0 – 1 M) over 160 min (α-synuclein elutes at ~300 mM). Fractions containing α-synuclein (analyzed by Coomassiestained SDS–PAGE) were concentrated with a 5K MWCO filter (Millipore, Bedford, Massachusetts), loaded onto a Supedex™ 75 column (GE Healthcare) and eluted (150 mM NaCl, 20 mM Tris, pH 8.0). Fractions containing α-synuclein were combined and lyophilized. Purity was assessed by using SDS-PAGE. The identity of the proteins was confirmed by using mass spectrometry (NanoESI-MS).

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^{19}F NMR spectra were acquired on an INOVA 600 MHz spectrometer equipped with ^{19}F(^{1}H) probes at 25 °C. The ^{19}F spectra comprised 512 transients, a 30 kHz sweep width, with a 2 s delay between transients. ^{19}F chemical shifts are referenced to trifluoroethanol at 0 ppm. All spectra were acquired under similar conditions.

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References

1. Uversky VN. J. Neurochem. 2007; 103:17. [PubMed: 17623039]
2. Lucking CB, Brice A. Cell. Mol. Life. Sci. 2000; 57:1894. [PubMed: 11215516]
3. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Nature. 1997; 388:839. [PubMed: 9278044]
4. Fink AL. Acc. Chem. Res. 2006; 39:628. [PubMed: 16981679]
5. Wu KP, Baum J. J. Am. Chem. Soc. 2010; 132:5546. [PubMed: 20359221]
6. Necula M, Chirita CN, Kuret J. J. Biol. Chem. 2003; 278:46674. [PubMed: 14506232]
7. Ahmad MF, Ramakrishna T, Raman B, Rao Ch M. J. Mol. Biol. 2006; 364:1061. [PubMed: 17054982]
8. Li C, Lutz EA, Slade KM, Ruf RA, Wang GF, Pielak GJ. Biochemistry. 2009; 48:8578. [PubMed: 19655784]
9. Rivers RC, Kumita JR, Tartaglia GG, Dedmon MM, Pawar A, Vendruscolo M, Dobson CM, Christodoulou J. Protein. Sci. 2008; 17:887. [PubMed: 18436957]
10. Ferreon AC, Gambin Y, Lemke EA, Deniz AA. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:5645. [PubMed: 19293380]
11. Bisaglia M, Trolio A, Bellanda M, Bergantino E, Bubacco L, Mammi S. Protein. Sci. 2006; 15:1408. [PubMed: 16731975]
12. Ulmer TS, Bax A, Cole NB, Nussbaum RL. J. Biol. Chem. 2005; 280:9595. [PubMed: 15615727]
13. Ulmer TS, Bax A. J. Biol. Chem. 2005; 280:43179. [PubMed: 16166095]
14. Broersen K, van den Brink D, Fraser G, Goedert M, Davletov B. Biochemistry. 2006; 45:15610. [PubMed: 17176082]
15. de Laureto PP, Tosatto L, Frare E, Marin O, Uversky VN, Fontana A. Biochemistry-us. 2006; 45:11523.
16. Bortolus M, Tombolato F, Tessari I, Bisaglia M, Mammi S, Bubacco L, Ferrarini A, Maniero AL. J. Am. Chem. Soc. 2008; 130:6690. [PubMed: 18457394]
17. Borbat P, Ramlall TF, Freed JH, Eliezer D. J. Am. Chem. Soc. 2006; 128:10004. [PubMed: 16881616]
18. Bussell R Jr, Eliezer D. J. Mol. Biol. 2003; 329:763. [PubMed: 12787676]
19. Danielson MA, Falke JJ. Annu. Rev. Biophys. Biomol. Struct. 1996; 25:163. [PubMed: 8800468]
20. Hoeltzli SD, Frieden C. Biochemistry-us. 1996; 35:16843.
21. Khan F, Kuprov I, Craggs TD, Hore PJ, Jackson SE. J. Am. Chem. Soc. 2006; 128:10729. [PubMed: 16910667]
22. Winkler GR, Harkins SB, Lee JC, Gray HB. J. Phys. Chem. B. 2006; 110:7058. [PubMed: 16571022]
23. Yu L, Hajduk PJ, Mack J, Olejniczak ET. J. Biomol. NMR. 2006; 34:221. [PubMed: 16645812]
24. Rozovsky S, Jogl G, Tong L, McDermott AE. J. Mol. Biol. 2001; 310:271. [PubMed: 11419952]
25. Song L, Teng Q, Phillips RS, Brewer JM, Summers AO. J. Mol. Biol. 2007; 371:79. [PubMed: 17560604]

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26. Evanics F, Bezsonova I, Marsh J, Kitevski JL, Forman-Kay JD, Prosser RS. Biochemistry-us. 2006; 45:14120.
27. Cellitti SE, Jones DH, Lagpacan L, Hao X, Zhang Q, Hu H, Brittain SM, Brinker A, Caldwell J, Bursulaya B, Spraggon G, Brock A, Ryu Y, Uno T, Schultz PG, Geierstanger BH. J. Am. Chem. Soc. 2008; 130:9268. [PubMed: 18576636]
28. Puffer B, Kreutz C, Rieder U, Ebert MO, Konrat R, Micura R. Nucleic. Acids. Res. 2009; 37:7728. [PubMed: 19843610]
29. Kiviniemi A, Virta P. J. Am. Chem. Soc. 2010; 132:8560. [PubMed: 20521791]
30. Graber D, Moroder H, Micura R. J. Am. Chem. Soc. 2008; 130:17230. [PubMed: 19053191]
31. Ferreon AC, Deniz AA. Biochemistry-us. 2007; 46:4499.
32. Lee JC, Langen R, Hummel PA, Gray HB, Winkler JR. Proc. Natl. Acad. Sci. U. S. A. 2004; 101:16466. [PubMed: 15536128]
33. Clayton DF, George JM. Trends. Neurosci. 1998; 21:249. [PubMed: 9641537]
34. Davidson WS, Jonas A, Clayton DF, George JM. J. Biol. Chem. 1998; 273:9443. [PubMed: 9545270]
35. Clayton DF, George JM. J. Neurosci. Res. 1999; 58:120. [PubMed: 10491577]
36. Jo E, McLaurin J, Yip CM, St George-Hyslop P, Fraser PE. J. Biol. Chem. 2000; 275:34328. [PubMed: 10915790]
37. Bodner CR, Maltsev AS, Dobson CM, Bax A. Biochemistry-us. 2010; 49:862.
38. Bodner CR, Dobson CM, Bax A. J. Mol. Biol. 2009; 390:775. [PubMed: 19481095]
39. Veldhuis G, Segers-Nolten I, Ferlemann E, Subramaniam V. Chembiochem. 2009; 10:436. [PubMed: 19107759]
40. Jackson JC, Hammill JT, Mehl RA. J. Am. Chem. Soc. 2007; 129:1160. [PubMed: 17263397]
41. Li C, Wang GF, Wang Y, Creager-Allen R, Lutz EA, Scronce H, Slade KM, Ruf RA, Mehl RA, Pielak GJ. J. Am. Chem. Soc. 2010; 132:321. [PubMed: 20050707]

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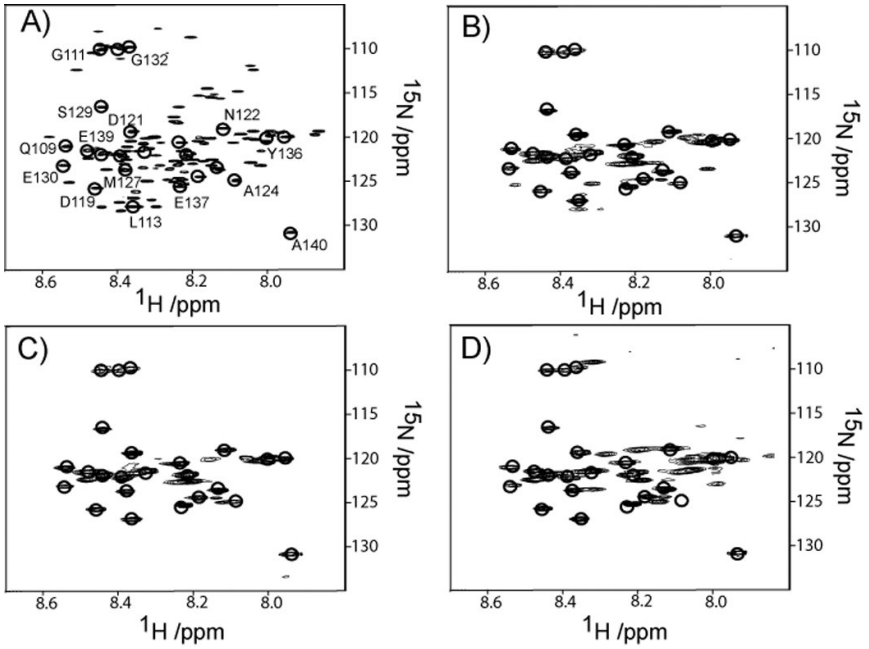

Figure 1.

HSQC spectra of 100 μM uniformly ^{15}N enriched α-synuclein in A, 0 mM; B, 0.8 mM, C, 2.4 mM, and D, 24 mM SDS. Samples are prepared in 50 mM sodium phosphate buffer (pH 7.4). Spectra were acquired on an INOVA 600 MHz spectrometer equipped with a HCN triple resonance probe at 25 °C. Several Resonances from C-terminal residues [9.18] are circled and labelled individually.

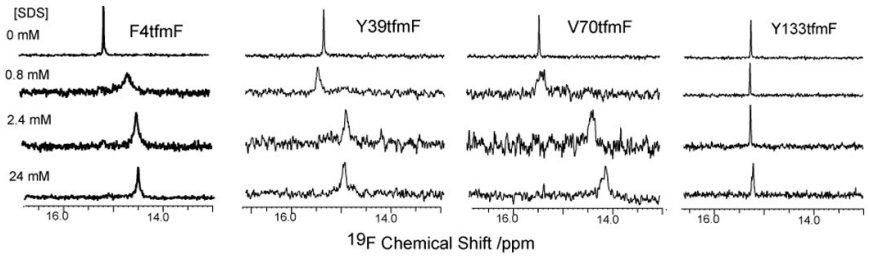

Figure 2.

^{19}F NMR spectra of labeled 100 μM α-synucleins with tfmF residue at position 4, 39, 70 and 133 in 0, 0.8, 2.4 and 24 mM SDS. The residues mutated to tfmF are indicated at the top of each spectrum. Samples are prepared in 50 mM sodium phosphate buffer (pH 7.4). The apparent lower signal-to-noise ratio of some spectra is the result of expanding the y-axis to facilitate observation of broad, low-sensitivity resonances.

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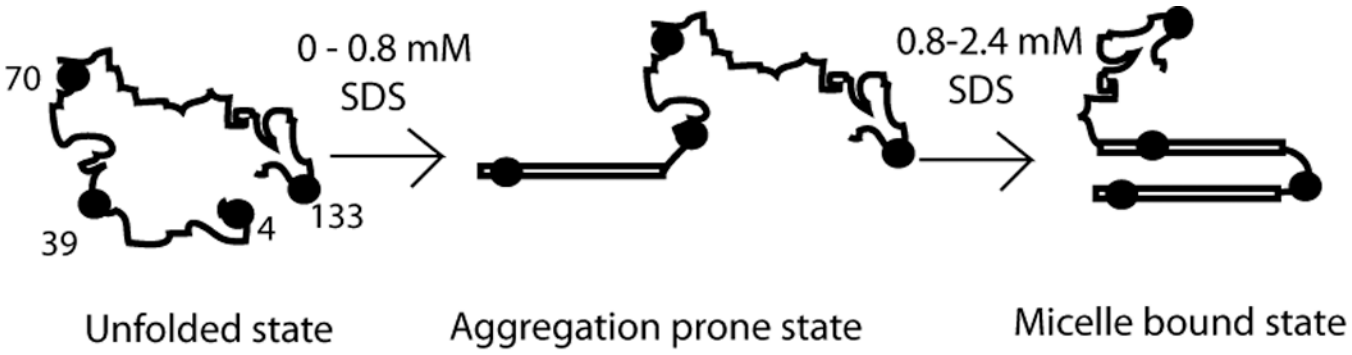

Figure 3.
Illustration of the conformational switch induced by SDS. Dots represent the tfmF sites. The thick lines represent α-helices.

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