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Backbone assignment and dynamics of human α -synuclein in viscous 2 M glucose solution

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Abstract

We present $^1\text{H}^{\text{N}}$, ^{15}N , $^{13}\text{C}^{\alpha}$, $^{13}\text{C}^{\beta}$ and $^{13}\text{C}'$ assignments and ^{15}N transverse relaxation rates (R_2) of a Parkinson's disease-related intrinsically disordered protein, α -synuclein, in the presence of 2 M (360 g/liter) glucose solution.

Keywords

Synuclein; intrinsically disordered protein; glucose; NMR resonance assignments

Biological context

Human α -synuclein (α Syn) is the primary protein component of the Lewy body deposits found in Parkinson's disease (PD). Aggregation of α Syn into fibrils is thought to play an important role in PD and the mechanism of conversion from soluble α Syn to amyloid fibril is not well understood. α Syn (UnitProtKB/SwissProt entry ID: P37840) is an intrinsically disordered protein (IDP) composed of 140 amino acids with 3 defined regions: an N-terminal region (residues 1–60), a central hydrophobic NAC (non-amyloid β component) region (residues 61–95) and a highly negatively charged C-terminal region (residues 96–140). Establishing the conformation and dynamics of the intrinsically disordered protein under different solution conditions will help elucidate the mechanism of fibril formation. To date, extensive NMR studies of α Syn in water have shown transient helical conformation in the N-terminal region and weak long-range transient tertiary N- to C-terminal intramolecular interactions (Bisaglia et al. 2009) at physiological pH. Recently, we have used NMR paramagnetic relaxation enhancement to determine the existence of weak N- to C-terminal inter-chain contacts of α Syn that are driven primarily by electrostatic interactions at neutral pH (Wu and Baum 2010).

In the cell, macromolecules can reach very high concentrations (Serber et al. 2005) and viscous or macromolecular solutes have been used to model the effects of crowding. It has been shown that the intrinsically disordered protein FlgM gains helical structure in the presence of high concentrations of glucose (2.5 M) (Dedmon et al. 2002) and recent reports by White et. al indicate that the growth kinetics of fibrillar proteins in crowded environments (e.g. poly (ethylene glycol), glucose) are significantly accelerated (White et al. 2010). This implies that viscous or crowded environments may enhance or stabilize the secondary structures of IDP and may also induce self-association (Shtilerman et al. 2002; Uversky et al. 2002).

Here we describe the backbone assignments, secondary structure propensities and relaxation dynamics of α Syn in 2 M (360g/L) glucose solution in order to determine the effect of increased viscosity on these parameters and to begin to provide information that may be related to the molecular behavior of α Syn inside the cell.

Methods and experiments

Expression and purification of uniformly ^{15}N -labeled α Syn and [$^{15}\text{N}/^{13}\text{C}$]-labeled α Syn were carried out as described previously (Wu et al. 2009). 2 M glucose was dissolved in PBS buffer at pH 7.4 (Sigma Inc., MO) for NMR experiments (Wu and Baum 2010). Backbone assignments of $\sim 300\ \mu\text{M}$ doubly labeled α Syn were carried out using ^{15}N - ^{15}N connections using the 3D HNN spectrum (complex points: 1024:H (F3)/100:N (F2) /60:N (F1)) (Panchal et al. 2001) and then were verified by $^{13}\text{C}^{\alpha}$ - $^{13}\text{C}^{\alpha}$, $^{13}\text{C}^{\beta}$ - $^{13}\text{C}^{\beta}$ and $^{13}\text{C}'$ - $^{13}\text{C}'$ connections using paired triple resonance 3D experiments [HNCACB (1024:H/104:C/60:N), CBCA(CO)NH (1024:H/84:C/48:N)] and [HNCO (1024:H/104:C /72:N), HN(CA)CO (1024:H /88:C/44:N)] (Ferentz and Wagner 2000). DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) was used to directly calibrate the ^1H chemical shifts; calibrations of ^{13}C and ^{15}N chemical shifts were calculated according to the gyromagnetic ratios (Wishart et al. 2002). Interleaved transverse relaxation rate (R_2) experiments of α Syn in 2 M glucose solution were acquired using the CPMG (Carr-Purcell-Meiboom-Gill) pulse train (Farrow et al. 1994) and the relaxation delay times and recycle delays were 10, 30, 50, 70, 90, 130, 170, 210 and 250 milliseconds and 2 seconds, respectively. Dried α Syn was dissolved in 2 M glucose in the presence of 20–30 % D_2O in order to avoid crystallization of glucose when acquiring NMR data at 15°C . All NMR spectra were acquired on a Varian 800 MHz spectrometer at 15°C , processed using NMRPipe (Delaglio et al. 1995) and analyzed using Sparky (Goddard and Kneller). Secondary structural propensities (SSP) were calculated using SSP developed by the Forman-Kay group (Marsh et al. 2006).

Assignments, data deposition and dynamics

We have measured the translational diffusion coefficients of glucose from 1 mM to 2.5 M in our previous study to determine the changes of solvent viscosity (Wu and Baum 2010). The viscosity of 2 M glucose solution is approximately 3.5 times that of 1 mM glucose solution. The ^1H - ^{15}N HSQC spectrum of α Syn in 2 M glucose solution at 15°C (figure 1) shows the narrow chemical shift dispersion in the ^1H dimension that is typical of intrinsically disordered proteins. ^1H and ^{15}N assignments for all 135 non-proline amide protons except Met¹ are assigned in the ^1H - ^{15}N HSQC spectrum and indicated in figure 1. The 3D HNN spectrum was used primarily for sequential assignments and the ^{13}C - ^{13}C connections were used to confirm the assignments. A total of 97 % of the $^{13}\text{C}^{\alpha}$, 98% of the $^{13}\text{C}^{\beta}$, and 90% of the $^{13}\text{C}'$ chemical shifts were assigned. Comparison of the secondary structural propensities (SSP) of α Syn between the viscous solution in 2 M glucose and the dilute solution in PBS indicates that the C-terminal and NAC regions show very small differences in SSP values, as the difference is close to zero, and that the N-terminal region at residues 30 to 60 shows slightly greater differences on the order of -0.1 (Figure 2a). Overall the SSP difference spectrum shows that the secondary structure propensities are similar to one another and that the viscous glucose solution does not appear to alter the conformation relative to water.

^1H - ^{15}N NMR relaxation experiments have been performed on wild type α Syn and mutants in order to define the dynamics of the intrinsically disordered state (Wu et al. 2008). Here we investigate the effects of increased viscosity on the dynamics by performing ^1H - ^{15}N R_2 relaxation experiments of α Syn in 2 M glucose solution (Figure 2b). Comparison to published ^{15}N R_2 values of α Syn in dilute solution at 15°C (Wu et al. 2009) indicates that the R_2 profile is identical under viscous and non-viscous conditions but that the overall

magnitude of the R_2 values for each residue is increased. The average increase in R_2 is approximate 2.6 times while the viscosity elevates approximately 3.5 times in the presence of 2 M glucose. These data suggest that the tumbling time of the protein is affected by the glucose environment but that the local dynamics do not change; the five clusters of restricted motion that were identified in water (Wu et al. 2009) remain intact in glucose. The relaxation data combined with the secondary structure propensity data indicate that the conformation and dynamics of α Syn in the highly viscous 2 M glucose solvent are very similar to those in water suggesting similar structural ensembles in both conditions. Our analysis of the monomer state in 2 M glucose can be used in combination with published literature which shows accelerated aggregation kinetics of α -synuclein in viscous or crowding solution to investigate the misfolding mechanism to amyloid fibrils.

Assigned chemical shifts and transverse relaxation rates have been deposited on Biological Magnetic Resonance Bank (<http://www.bmrwisc.edu>) with accession number 16904.

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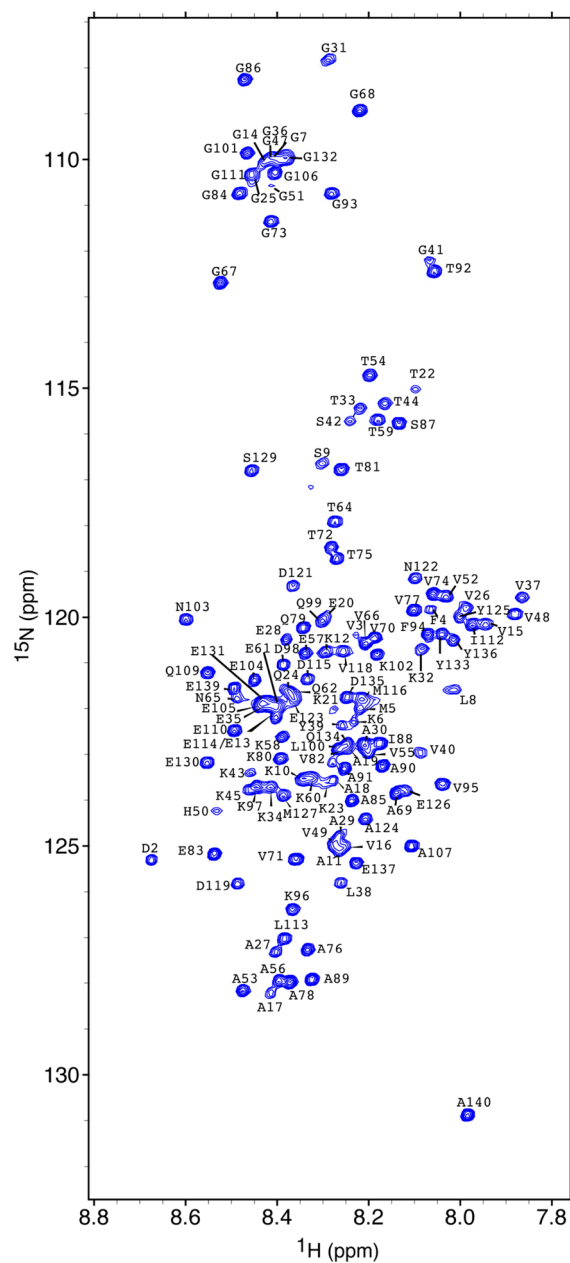


Figure 1. ^1H - ^{15}N HSQC spectrum of αSyn in 2 M (360 g/L) glucose and PBS buffer (10 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, 137 mM NaCl, and 2.7 mM KCl) at 15 °C obtained at a ^1H frequency of 800 MHz. Assigned residues are labeled with the 1-letter amino acid code.

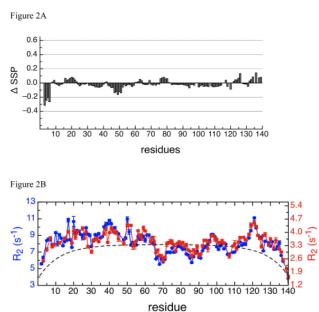


Figure 2.

Figure 2A:

The SSP (Marsh et al. 2006) difference scores of α Syn in dilute solution and in viscous solution. $^{13}\text{C}^{\alpha}$ and $^{13}\text{C}^{\beta}$ chemical shifts were used to calculate the SSP score for α Syn and reference offsets were also checked by the SSP program. ΔSSP was calculated according to the formula: $\Delta\text{SSP} = \text{SSP}(\text{glucose}) - \text{SSP}(\text{PBS})$, where SSP in phosphate buffer saline was calculated previously (Wu et al. 2009).

Figure 2B:

Relaxation dynamics of α Syn in dilute solution (10 mM MES, 100 mM NaCl, pH 6) at 15°C (Wu et al. 2009) (●) and in viscous solution at 15°C (●) show very similar trends although the magnitudes are very different. Left (colored in blue) and right (colored in red) Y-axis indicates the scales of the R_2 rates in viscous and in dilute solutions, respectively. The R_2 rates of α Syn in 2 M glucose solution are approximately 2.4-fold greater than the R_2 rates of α Syn in dilute solution. The dashed line is the theoretical R_2 values of an 140-aa random coil protein calculated as previously described (Wu et al. 2008).