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Conformational ensemble of human α-synuclein physiological form predicted by molecular simulations

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We perform here enhanced sampling simulations of N-terminally acetylated human α-synuclein, an intrinsically disordered protein involved in Parkinson’s disease. The calculations, consistent with experiments, suggest that the post-translational modification leads to the formation of a transient amphipathic α-helix. The latter, absent in the non-physiological form, alters protein dynamics at the N-terminal and intramolecular interactions.

Parkinson’s disease (PD) is the second most common neurodegenerative disease, affecting approximately four million people worldwide.1 Currently, there is no cure for it. The human protein α-synuclein2 represents the major component of PD’s pathologic proteinaceous deposits in the substantia nigra of human brains (‘Lewy bodies’ and ‘Lewy neuritis’).3 Although it remains unclear how α-synuclein initiates neuronal death, there is growing evidence that supports a role for α-synuclein aggregation in the pathological effects associated to PD. The aggregation pathway of α-synuclein represents then an obvious target for therapeutic intervention in PD.4, 5 The precise function of α-synuclein is unknown, as are the mechanisms underlying the structural transition from the innocuous, monomeric disordered conformations of α-synuclein to its neurotoxic forms. Recently, abundant evidence revealed that the monomeric state of α-synuclein undergoes N-terminal acetylation in vivo (AcAS, hereafter),5-7 which differs structurally in a small but subtle way from the widely studied, non-acetylated, non-physiological form (no-AcAS),5, 6 expressed in E. coli and lacking the post-translational modification. Indeed, AcAS’ hydrodynamic radius is larger by 0.5 Å than that of no-AcAS. In addition, its N-terminus has higher α-helical propensity.5, 4

Consistently, the chemical shifts of that region show the largest differences between the AcAS and no-AcAS states.9 While the structural determinants of no-AcAS’ structural ensemble in aqueous solution have been well characterized by NMR measurements,10-13 those of the physiological form have been explored only in the past few years.9, 12 Recently, some of us performed molecular dynamics (MD) simulations of no-AcAS14 based on the Amber ff99SB force fields15 with ILDN modification.16 The calculations, based on the available structural information,16 turned out to reproduce the available biophysical data, including NMR chemical shifts (CSs)17-19 and residual dipolar couplings.20 Prompted by the consistency between these simulations and experimental data, we have used here a MD enhanced sampling method based on the same force field to provide insights into the conformational ensemble of the physiological AcAS form. The predictive power of our simulations is here assessed via an extensive comparison with experimental data, that includes CSs, Circular Dichroism (CD),7 and measured hydrodynamic radii (RH).7 Comparison is also made with the reported electrostatic compaction factor, as derived by translational diffusion experiments.7

4,000 no-AcAS NMR structures10 were clustered in six representatives, covering almost three quarters of the protein’s conformations, as in ref. 21. One acetyl group was manually added to the N-terminus of each representative. Each representative was inserted in a water box using a 10 Å buffer zone of solvent around any atom of the protein. The Amber ff99SB force fields15 with ILDN modification16 and the TIP3P water model22 were used for the protein and for the solvent, respectively. The systems were neutralized by adding

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10 Na$^+$ ions. Periodic boundary conditions were applied. Electrostatic interactions were calculated using the Particle Mesh-Ewald (PME) method.$^{23}$ All bond lengths were constrained by the LINCS algorithm$^{24}$ and an integration step of 2 fs was set for all of the MD calculations. Constant temperature and pressure conditions were achieved by coupling the system with a Nosé-Hoover thermostat$^{25,26}$ and an Andersen-Parrinello-Rahman barostat.$^{27}$ The systems were geometry-optimized and then equilibrated at 300 K and 1 atm by performing 1 ns of MD-based annealing and 1 ns of constant temperature MD. Next, each representative underwent the replica exchange with solute tempering (REST2)$^{28,29}$ enhanced sampling method at room conditions. For each REST2 simulation, 32 replicas were generated in the temperature range from 300 to 500 K (see SI). Therefore, a total of six 15 ns long independent REST2 simulations were generated. To test the convergence of our simulations we follow the same approach of Wise-Scira and co-workers$^{30,31}$ (see the SI for further details). The molecular simulations were carried out using the GROMACS 4.5.5 program package. The obtained geometries were clustered using the algorithm proposed in ref. 32 with a RMSD cut-off of 2.2 Å with the $g$ _cluster tool in GROMACS 4.5.5. The six lowest temperature replica trajectories were cumulated into a single trajectory to increase the statistic of our conformational ensemble. From the resulting cumulative trajectory, we calculated several properties: (i) CSs and CD spectra, using the SHIFTX$^{33}$ and the DichroCalc codes,$^{34}$ respectively; (ii) RH, gyration radii ($R_g$), and compaction factors ($C_F$).$^{35,37}$ $R_H$ was obtained from a linear fit between $R_g$ and RH from ref. 36; we have considered the same linear fit applies for no-AcAS and AcAS, since all the synuclein family exhibit very similar relationship between $R_g$ and RH. $R_g$ was calculated with the $g$ _gyrate tool in GROMACS 4.5.5. The $C_F$ were calculated accordingly to ref. 37. (iii) The rigid block analysis was performed as in ref. 38. The quality factor was calculated for each case as in ref. 39. (iv) Contact maps, hydrogen bonds (H Bs) and saltbridges (SBs) were calculated with the $g$ _mdmap, $g$ _hbonds, and $g$ _dist GROMACS 4.5.5 tools, respectively.

The calculated CD spectra shift mildly toward $\alpha$-helical frequencies upon acetylation, similar to what observed in experiments, (Fig. 1).$^7$ Notably, the structure featuring the best agreement with the CSs shows a clear $\alpha$-helical signal, with signature negative intensities at 208 and 222 nm. These features were also observed experimentally when AcAS samples were exposed to lipids, which facilitates the formation of helical structures.$^7$

| $R_g$ | $R_H$ |
|------|------|
| no-AcAS (best ensemble) | AcAS (best ensemble) |
| 26.4 ± 7 (24.8 Å) | 27.2 ± 8 Å (20.2) |
| 24.7 ± 5 Å | - |
| 28.8 ± 9 (28.9 Å) | 29.0 ± 10 (25.7) |
| 28.2 ± 0.3 Å | 28.7 Å |

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Contact maps for no-AcAS and AcAS, which report the smallest distance between residue pairs. The area of the map corresponding to interdomain interacting regions are highlighted with dotted lines and labeled.

The gyration ($R_g$) and hydrodynamic (RH) radii decrease upon deacetylation (Tab. 1), consistently with the experimentally observed increase in the global compaction of the protein.$^7$ The values of RH and $R_g$ compare well with experiments (RH: 28.2 and 28.7 Å for no-AcAS and AcAS, respectively), although the increase of the gyration radius due to acetylation is underestimated (0.2 Å in this work versus 0.5 Å in experiments).$^{36}$ The calculated $C_F$, that ranges between 0 (random coil) to 1 (folded structure)$^{37}$ decreases from 0.53 for no-AcAS to 0.47 for AcAS, also consistent with experimental evidence.$^{32}$ The decrease of compaction is associated with a slight decrease of intramolecular interactions: the contact maps of the two
proteins point to a modest decrease of N-terminal/C-terminal and NAC/C-terminal interactions (Fig. 2). Specifically, an overall loss of interdomain SBs (Fig. 3A-C) and a moderate loss of HBs (Fig. 3B) are observed. The Asp2-Lys6 SB interaction is however more persistent in the acetylated variant, possibly because of the increase of α-helicity at the N-terminus, which stabilizes these two residues in an apt conformation for the SB interaction. Interestingly, the intermolecular interactions involving the amino acids undergoing the three familial pathogenic mutations in Parkinson’s (A30P, E46K, and A53T) are affected by acetylation due to the looseness of SBs in such region upon acetylation.

Figure 3. (A) SBs occurrence for no-AcAS and AcAS. Interdomain SBs are in red, the intra-domain ones are in green. Only the SBs with occurrence > 2% are reported. (B) Average HB distribution for AcAS (black) and no-AcAS (red), normalized over the simulations times. (C) Details of no-AcAS structure where interdomain SB is formed.

Novel information is obtained on the conformation of the amino acids in the N-terminal region of AcAS. The Pearson’s correlation coefficient (CC) between calculated and experimental CSs is 0.86 (Fig. 4A). However, it is as high as 0.94 (Fig. 4A) for residues at the N-term (residues 1 to 12). Residues 2-8 form the α-helix (Fig. 4B) with charged residues (Asp2, Lys6, and Lys10) on one side of the helix and hydrophobic residues (Met1, Val3, Phe4, Met5, Gly7, and Leu8) on the other side (Fig. 4B). The helix is thus amphipathic. Similar conclusions are obtained by NMR studies, which localize the helix between residues 1-9 or 1-12 of AcAS. The helix may be formed because of favourable electrostatic interactions between the α-helix dipole moment and the acetylated moiety and the consequent formation of backbone hydrogen bonding interactions. Consistently with the NMR studies, 15% of AcAS conformations exhibit a α-helix in the first twelve residues of AcAS (see Fig. 4B as an example). These features are not observed in the no-AcAS form of the protein.
In conclusion, our molecular modeling study - as demonstrated by passing from AcAS to no-AcAS (29% and 25%, respectively). random coil and consistent with a decrease of secondary structure in a less correlated way than AcAS ones, closer to the behavior of a similar to a random coil than AcAS. Thus, residues in no-AcAS move the protein. suggested to depend on the stabilization of the N-terminal region of AcAS fibrillation rate observed in experiments upon acetylation was helix conformation at the N-terminal. On one hand this confers that, upon acetylation, α-synuclein adopt a broken amphipathic 4 domains that move in a concerted motion, preserving the shape, and, expectedly, they are compatible with those of a helix (Fig. 6). Ramachandran angles of residues 1-12 decreases upon acetylation acetyl group is restricted to the correlation of motion to residues 1-12 of AcAS behaves as a dynamically independent domain, possibly because of the presence of the helix (Fig. 5A). This is not the case for no-AcAS (Fig. 5B), where the removal of the acetyl group is restricted to the correlation of motion to residues 1-6 only. Consistently, the calculated spread of the Φ and Ψ Ramachandran angles of residues 1-12 decreases upon acetylation and, expectedly, they are compatible with those of a helix (Fig. 6).

![Figure 6. Ramachandran plots of the first 12 residues for AcAS (left) and no-AcAS (right).](image)

The rest of the protein moves in a uncorrelated way in both proteins, consistently with the same NMR properties of residues 13-140, in detail, the domain decomposition in no-AcAS is more similar to a random coil than AcAS. Thus, residues in no-AcAS move in a less correlated way than AcAS ones, closer to the behavior of a random coil and consistent with a decrease of secondary structure on passing from AcAS to no-AcAS (29% and 25%, respectively). In conclusion, our molecular modeling study - as demonstrated by NMR, CD, and translational diffusion experiments - suggests that, upon acetylation, α-synuclein adopt a broken amphipathic helix conformation at the N-terminal. On one hand this confers more rigidity to the N-terminal domain; Consistently, the change in AcAS fibrillation rate observed in experiments upon acetylation was suggested to depend on the stabilization of the N-terminal region of the protein. On the other hand, it causes a loss of HBs and SBs interactions, decreasing the compactness of the protein and affecting the intermolecular interaction of relevant regions for aggregation. Using NMR chemical shifts and fluorescence experiments, Kang and co-workers indeed showed that secondary structural propensity in residues 5–8, 14–31, and 50–57 are highly correlated to fibril growth rate. 5 14–31, and 50–57 are the regions in which we observe loss of SBs. Therefore, our calculations support Kang and co-workers’ idea that N-terminal acetylation inhibits the formation of the “fibrillation promoting” transient helix at residues 14–31 and 50–57, while stabilizing the formation of the “fibrillation inhibiting” transient helix in residues 1–12, thereby resulting in slower fibrillation rates relative to the no-AcAS:“

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**Notes and references**

Note 1. The reported error on Rg is the standard deviation of the gyration radius measurements along the entire trajectory. It might be rather large because of the large conformational diversity of an IDP such as α-synuclein. The theoretical RH error was obtained by propagation from Rg error. For experiments, RH errors come from the measurement of diffusion coefficients, using the Stokes-Einstein-Sutherland equation. The error reported in those cases is the error in the experimental measurement, which has nothing to do with the error associated with conformational diversity of the protein.

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