The role of the acidic domain of α-synuclein in amyloid fibril formation: a molecular dynamics study

SeongByeong Park, Jeseong Yoon, Soonmin Jang, Kyunghee Lee and Seokmin Shin

*Department of Chemistry, Seoul National University, Seoul 151-747, Republic of Korea; bDepartment of Chemistry, Sejong University, Seoul 143-747, Republic of Korea

Communicated by Ramaswamy H. Sarma

(Received 7 January 2015; accepted 19 March 2015)

The detailed mechanism of the pathology of α-synuclein in the Parkinson’s disease has not been clearly elucidated. Recent studies suggested a possible chaperone-like role of the acidic C-terminal region of α-synuclein in the formation of amyloid fibrils. It was also previously demonstrated that the α-synuclein amyloid fibril formation is accelerated by mutations of proline residues to alanine in the acidic region. We performed replica exchange molecular dynamics simulations of the acidic and nonamyloid component (NAC) domains of the wild type and proline-to-alanine mutants of α-synuclein under various conditions. Our results showed that structural changes induced by a change in pH or an introduction of mutations lead to a reduction in mutual contacts between the NAC and acidic regions. Our data suggest that the highly charged acidic region of α-synuclein may act as an intramolecular chaperone by protecting the hydrophobic domain from aggregation. Understanding the function of such chaperone-like parts of α-synuclein may provide novel insights into the mechanism of amyloid formation.

Keywords: amyloid formation; protein aggregation; α-synuclein; intramolecular chaperone; molecular dynamics

Introduction

Parkinson’s disease is characterized by the degeneration of dopaminergic neurons in the substantia nigra. One of the key contributing factors in Parkinson’s disease is the α-synuclein protein (Andrei, 2008, chapter 6; Spillantini, Crowther, Jakes, Hasegawa, & Goedert, 1998). In dopaminergic neurons of patients with Parkinson’s disease, the fibrillar form of α-synuclein constitutes the main protein component of the characteristic cytoplasmic aggregates called Lewy bodies (Ohama & Ikuta, 1976; Spillantini et al., 1997). It has also been found that rare, inherited forms of Parkinson’s disease may be caused by mutations in α-synuclein, which accelerate the aggregation of the protein (Conway, Harper, & Lansbury, 1998; Li, Uversky, & Fink, 2001). α-synuclein has 140 amino acids and consists of three domains: a membrane-binding domain, a nonamyloid component (NAC), and acidic domains (Davidson, Jonas, Clayton, & George, 1998; Jo, McLaurin, Yip, St. George-Hyslop, & Fraser, 2000; McLean, Kawamata, Ribich, & Hyman, 2000). α-synuclein, which is one of the intrinsically disordered proteins in solution, may aggregate into the fibrillar β-sheet structure under pathological conditions. The central NAC domain mainly consists of hydrophobic residues and confers the propensity for aggregation (Ueda et al., 1993). The polar acidic C-terminal domain comprises many carboxyl acids (Bertoncini et al., 2005; Clayton & George, 1999; Hoyer, Cherry, Subramaniam, & Jovin, 2004; Nielsen, Vorum, Lindersson, & Jensen, 2001).

The aggregation of α-synuclein into amyloid fibrils is a nucleation-dependent process with an initial lag phase, an elongation step, and a steady-state phase (Han, Weinreb, & Lansbury, 1995; Wood et al., 1999). In the lag phase, the nucleus of aggregation is formed. It has been suggested that this nucleus can be as small as a dimer of α-synuclein (Fernández et al., 2004). The nucleation process, which involves the formation of partially folded intermediates (Uversky, Li, & Fink, 2001), is usually rather slow. Once the nucleus is formed, the aggregates grow rapidly until a thermodynamic equilibrium is established between aggregates and monomers. During the aggregation process, the protein forms a secondary structure containing mainly an antiparallel β-sheet (Lin et al., 2006; Narhi et al., 1999).

The kinetics of α-synuclein aggregation can be affected by various factors. One of the interesting findings was that the lag phase was reduced in vitro in the presence of FK506 binding proteins (FKBPs) (Gerard et al., 2006, 2008). FKBPs exhibit a peptidyl-prolyl isomerase (PPlase) activity, which accelerates the cis–trans isomerization of a proline residue (Göthel & Marahiel, 1999). FK506 binds to most FKBPs and is a potent and
The role of the acidic domain of α-synuclein in amyloid fibril formation

Specific inhibitor of their PPlase activity (Gerard et al., 2006, 2008). Several immunophilin ligands such as FK506 and members of the FKBP family have been associated with Parkinson’s disease (Gerard et al., 2010). Several studies have shown that the neurotrophic activity of FK506 is mediated by the interaction with FKBP1 (Fischer, Wittmann-Liebold, Lang, Kiefhaber, & Schmid, 1989; Harding, Galat, Uehling, & Schreiber, 1989; Siekierka, Hung, Poe, Lin, & Sigal, 1989; Takahashi, Hayano, & Suzuki, 1989). Furthermore, several reports have suggested the role of the PPlase in neurodegeneration, but a conclusive evidence of this has not been presented so far (Fischer et al., 1989; Gerard et al., 2006, 2008, 2010; Göthel & Marahiel, 1999; Harding et al., 1989; Siekierka et al., 1989; Takahashi et al., 1989). Pertinent to the subject of the present study, these reports suggested that the cis–trans isomerization of a proline residue may be linked with the kinetics of the α-synuclein aggregation.

Some studies have shown that α-synuclein suppresses the aggregation of the thermally denatured alcohol dehydrogenase and chemically denatured insulin (Hoyer et al., 2004). The chaperone-like activity of α-synuclein is suppressed completely by the removal of its C-terminal residues 98–140 (Crowther, Jakes, Spillantini, & Goedert, 1998; Hoyer et al., 2004; Souza, Giasson, Lee, & Ischiropoulos, 2000). It is interesting to note that the acidic region of α-synuclein has five proline residues. The presence of proline residues reduces protein flexibility. In the absence of an enzyme-catalyzed acceleration of the proline isomerization (Lu, Finn, Lee, & Nicholson, 2007; Wedemeyer, Welker, & Scheraga, 2002), proline residues lock the structure of the peptide backbone in a rigid configuration. Recently, the aggregation kinetics of several α-synuclein mutants – in which one or more proline residues in the acidic region were mutated to alanine – have been studied to elucidate the role of proline residues in the conformation and aggregation of α-synuclein (Meuvis, Gerard, Desender, Baekelandt, & Engelderghs, 2010). It has been shown that the mutation of proline residues to alanine accelerates the aggregation kinetics of α-synuclein, while all proline mutants formed fibrils similar to those composed of the wild-type α-synuclein. It has also been demonstrated that α-synuclein that bears a quintuple mutation of all five proline residues being replaced with alanine is more structured than the wild-type α-synuclein. This observation suggested that proline residues may act as potential helix breakers in the inhibitory conformation of the C-terminus (Meuvis et al., 2010).

At low pH, α-synuclein has been shown to aggregate faster than at neutral pH (Uversky et al., 2001). It was suggested that the compaction of the protein into a partially folded conformation is responsible for the increased rate of aggregation at low pH. Structural reorganization of α-synuclein at low pH was also studied by NMR spectroscopy and molecular simulations (Wu, Weinstock, Narayanan, Levy, & Baum, 2009). It was observed that there was a significant structural reorganization within the low pH ensemble relative to that at neutral pH in terms of long-range contacts, hydrodynamic radius, and the amount of heterogeneity within the conformational ensembles.

In the present study, we performed systematic simulations of α-synuclein under various conditions in order to provide plausible scenarios for the role of the acidic region in the formation of amyloid fibrils by α-synuclein. Our main aim was to investigate structural changes of the acidic region with relation to its possible interactions with the NAC region under different conditions.

**Computational methods**

Replica exchange molecular dynamics (REMD) simulations of wild-type and P-to-A mutated α-synuclein were performed in an attempt to understand the role of proline residues in the structure of the C-terminal region and subsequent aggregation of α-synuclein. We carried out simulations of the acidic domain (residues 101–140) and the combined sequence of the NAC and acidic regions (residues 61–140). For the mutated α-synuclein, all proline residues (P108, P117, P120, P128, and P138) were substituted by alanine residues. In order to model the acidic condition of pH 2, the negative charges on the side chains of the acidic residues are neutralized. The all-atom AMBER FF96 force field with modified hydrogen bonding radii was used in this study (Kollman, Cornell, Fox, Chipot, & Pohorille, 1997). A modified GB/SA model was used to describe solvation (Fan, Hwang, & Warshel, 1999; Onufriev, Bashford, & Case, 2000, 2004; Still, Tempczyk, Hawley, & Hendrickson, 1990). Initial configurations of the monomers prepared with the LEAP program of AMBER10™ were minimized using the steepest descent and conjugate gradient algorithms, and then monomers were heated up to 500 K using the weak-coupling algorithm (Berendsen, Postma, van Gunsteren, DiNola, & Haak, 1984). Several replicas (24 replicas between 290.0 and 621.2 K for the acidic domain only and 34 replicas between 282.8 and 750.0 K for the combined sequence of the NAC and acidic domains) were created. Their temperatures formed a geometrical series, and all the replicas were equilibrated at their respective temperatures. Next, REMD simulations with an exchange interval of 400 fs were performed using the replicas described above (Cheng, Cui, Hornak, & Simmerling, 2005; Mitsutake, Sugita, & Okamoto, 2001; Nymeyer, Gnanakaran, & García, 2004). Distributions of potential energies for the replicas were well overlapped and the swapping probability was ~0.6. The convergence of individual MD simulation was confirmed...
by examining the time evolutions of the secondary structure contents and the root mean square deviation (RMSD). The simulation times for eight different simulations ranged from 26 to 73 ns and the total simulation time for all of the replicas comprised ~13 μs. All analyses were done for the last 9 ns of the production simulation. The REMD simulations were done using the SANDER program of AMBER10™ with the SHAKE algorithm (Case et al., 2008; Ryckaert, Ciccotti, & Berendsen, 1977).

As one of the intrinsically disordered protein, it is difficult to determine a single structure representing structural properties of α-synuclein. We can define representative structures for ensemble of structures occurring during the overall trajectory in order to examine the conformational characteristics of the segments of α-synuclein (Yoon, Park, Jang, Lee, & Shin, 2008, 2009). Configurations obtained from the trajectories of simulations were clustered into four groups based on relative RMSD values. For each cluster, the representative configuration was chosen as the configuration whose RMSD value is the closest to the average RMSD of the configurations belonging to the particular cluster. The representative configuration for the cluster with the largest population can be considered as the representative structure for the simulations. Cluster analysis was also performed using analysis tools supplied in the AMBER™ software package.

**Results and discussion**

Proline residues, which have a cyclic structure, may play important roles in determining biological functions of proteins because their conformational changes are restricted by the ring strain. Using the density functional theory (B3LYP) with the basis of 6–31G(d), the activation barrier for the cis–trans isomerization reaction of an isolated proline residue was calculated to be ~12.4 kcal/mol (Becke, 1993; Lee, Yang, & Parr, 1988). Furthermore, activation barriers for the cis–trans isomerization of peptidyl-prolyl have been calculated to comprise ~30 kcal/mol (Lu et al., 2007). High activation barriers cause regions with proline residues to become rigid, and the reduction in the flexibility of the protein domain may lead to structural changes associated with a particular function.

The acidic domain of the wild-type α-synuclein (amino acid sequence 101–140) has five proline residues, which indicates a reduced flexibility of this domain. Changing proline residues into alanine residues is expected to make the mutant acidic domain more flexible than the same region of the wild-type α-synuclein. We calculated the radius of gyration as a function of temperature for the wild-type and P-to-A mutant of the acidic domain in neutral and acidic (pH 2) conditions (Figure 1(a)). Under the neutral condition and at low temperatures, the averaged Rg, <Rg> of the P-to-A mutant was smaller than that of the wild-type α-synuclein. As the temperature was increased, <Rg> of the P-to-A mutant showed larger changes and had larger values at high temperatures compared to the wild-type α-synuclein. Under the acidic condition, the acidic domain for both the wild-type α-synuclein and P-to-A mutant showed a much smaller <Rg> compared to the neutral condition. This is consistent with the fact that most of the negative charges in the acidic domain are neutralized at low pH, which allows the acidic domain to be more compact (See Figures S1 and S2 in Supplementary material).

We also calculated the Rg as a function of temperature for the wild-type α-synuclein and P-to-A mutant of the combined sequence of the NAC and acidic region in neutral and acidic (pH 2) conditions (Figure 1(b)). For the wild-type α-synuclein, at low temperatures <Rg> for the combined sequence of the NAC and acidic region was smaller than that of the acidic region alone. The configurations of the acidic region, in the absence of NAC domain, showed rather extended structures (See Figures S1). For the combined sequence of the NAC and acidic region, the two domains seemed to show strong interactions between each other and the resulting structure was somewhat more compact compared to the structure of the acidic region alone (See Figures S3). The value of <Rg> for the combined sequence showed large changes at approximately 350 K and became much larger than that of the acidic region alone at higher temperatures. It can be argued that the NAC and acidic domains were close to each other at lower temperatures but they became separated as the temperature was increased. As shown in Figure 1(b), when the P-to-A mutation was introduced or when pH was reduced, the value of <Rg> of the combined sequence of the NAC and acidic regions was larger than that of the wild-type α-synuclein at neutral pH. We attribute this difference mainly to the increase in the relative distance between the two regions.

The representative configurations of the combined sequence of the NAC and acidic regions for the wild-type α-synuclein and the P-to-A mutant at neutral and acidic conditions were obtained by performing a cluster analysis of trajectories of the corresponding simulations at 300 K (See Figures S3 and S4 in Supplementary material). Figure 2 shows the representative structures of the simulations under different conditions. For the wild-type α-synuclein at neutral pH, the NAC and acidic domains seem to show extensive contacts between them. After the P-to-A mutation is introduced, the interaction between the two domains appears to be reduced with the end of the flexible C-terminal region flanking away from the NAC domain. Similar conclusions can be made also for the case of the acidic pH. As the acidic region becomes more compact at lower pH, the contact region...
between the two domains is reduced. By comparing relative positions of the acidic region (red) and NAC domain (blue) in these cases, it can be argued that either the P-to-A mutation or lower pH leads to the increase in the average distance between the acidic region and the NAC domain. This observation was supported by calculations of the distribution of distances between the centers of mass for the two regions. Figure 3 clearly illustrates the fact that the relative distance between the NAC and acidic regions increased with either a reduction in the pH level or an introduction of the P-to-A mutation.

During the formation of amyloid fibrils, aggregation-prone regions of monomers, usually hydrophobic domains, such as the NAC region of α-synuclein, need to be able to move closer to each other for a fast aggregation rate. One way of examining the availability of the NAC region for the interaction with other aggregation partners is to calculate the solvent accessible surface area (SASA). We calculated the SASAs for the entire structure of NAC + acidic region and for the NAC domain alone. We compared the averaged SASA of α-synuclein at different conditions to the reference SASA calculated for the wild-type at neutral pH. For the NAC region, either the introduction of the P-to-A mutation or lowering of the pH led to an increase in the overall SASA (Table S1 in the Supplementary material). This result is consistent with the increase in the average distance between the acidic region and the NAC domain (Figure 3).

We constructed contact maps for the combined sequence of the NAC and acidic regions in different conditions (See Figure S5 in the Supplementary material). In representative structures of the wild-type α-synuclein at neutral pH, contact maps showed strong contacts between the middle parts of the NAC and acidic regions. This is consistent with the earlier suggestion that...
the combined sequence of the NAC and acidic region shows rather compact structure with extensive contacts between the two domains. The strength of these contacts was reduced when the P-to-A mutation was introduced or when pH became acidic. Figure 4 shows the number of contacts for the combined sequence of the NAC and acidic regions at different conditions. It also clearly demonstrates that the interaction between the two regions, as determined by the number of close contacts, is significantly reduced by the P-to-A mutation or a pH change. These results support the idea that the increase in the exposure of the aggregation-prone NAC region results from the reduction in close contacts between the acidic region and the NAC domain due to the P-to-A mutation or acidic pH.

In contrast to the Aβ peptide, α-synuclein, as an amyloid forming protein, has a rather long sequence consisting of three different domains. These three domains exhibit different properties: the membrane-binding N-terminal region is amphipathic, the NAC domain is hydrophobic, and the polar C-terminal tail is acidic. The middle part of the hydrophobic NAC domain is usually considered to be important for the aggregation of α-synuclein. While the N-terminal region is known to form membrane-bound α-helices, the acidic C-terminal region is unstructured. The possible role of the acidic region during the formation of amyloid fibrils by α-synuclein is not known at present. Recent experiments have provided interesting insights suggesting that structural changes in the acidic region may affect the aggregation processes (Crowther et al., 1998; Souza et al., 2000). Most notably, the mutation of proline residues to alanine residues in the acidic region accelerates the aggregation kinetics (Meuvis et al., 2010).

In the present study, we performed systematic simulations of α-synuclein conformations under various conditions in order to elucidate structural characteristics of the acidic region responsible for changes in the aggregation behavior. The results of the present study provide plausible scenarios for the role of the acidic region in prevention of amyloid formation by α-synuclein. We observed that under neutral pH, the acidic C-terminal domain of the wild-type α-synuclein was fairly stretched and rigid owing to multiple negative charges and five proline residues. The results of our simulations showed that the distance between the acidic region and the aggregation-prone NAC domain was relatively small. We also found that there exist close contacts

Figure 3. Distribution of the relative distances between the NAC domain and the acidic region for the wild-type and P-to-A mutant of α-synuclein under the neutral condition and the acidic condition.

Figure 4. (a) Time evolution and (b) distribution of number of contacts for the combined sequence of the NAC and acidic region of the wild-type and P-to-A mutant under the neutral condition and the acidic condition. The contact is defined when distances between any two atoms are equal to or less than 4.5 Å.
between the middle parts of the NAC domain and the acidic region. One may argue that the acidic region is locked in a position where it covers main parts of the NAC domain and blocks the approach of other hydrophobic chains such as the NAC domain of the other α-synuclein. Therefore, the natural role of the acidic region is to prevent the aggregation of the hydrophobic NAC domain and maintain the functional form of α-synuclein. This argument is supported by experimental findings that revealed an acceleration of the aggregation following the removal of the acidic region (Crowther et al., 1998; Souza et al., 2000). The acidic C-terminal region, as described above, may be therefore viewed as an “intramolecular chaperone.”

The chaperone-like role of the acidic region can be affected by environmental changes or mutations. The present study has shown the structural basis for such effects. Lowering the pH to an acidic condition neutralizes negative charges on the C-terminal domain. As a result, the acidic region may form a more compact structure, greatly limiting the interaction with the NAC domain (Wu et al., 2009). Mutations of the five proline residues to alanine make the acidic region much more flexible. The increased flexibility prevents the C-terminal domain from maintaining a close contact with the NAC domain. In either case, i.e. whether the structure is flexible or compact, the acidic C-terminal region can no longer protect the NAC domain from interacting with other chains for aggregation.

It is now generally believed that most of the small peptides or large proteins can form fibrils if they contain a sequence of residues with a strong propensity for aggregation. Under normal conditions, functional proteins avoid being involved in amyloid formation by preventing aggregation. The simplest way to block aggregation is to prevent the aggregation-prone region from interacting with its counterparts from neighboring chains. The results of the present study suggest that the highly charged acidic C-terminal region of α-synuclein may act as an intramolecular chaperone that protects the NAC domain from aggregation. Understanding the function of such chaperone-like parts of fibril-forming proteins should provide novel insights into the mechanism of amyloid formation and suggest new avenues for treatment of diseases caused by protein aggregation.

**Funding**

This work was supported by National Research Foundation of Korea (NRF) [grant number 2007-0056095 (CMD)], [grant number 2012M3C1A6035358 (EDISON)], [grant number 2012M3A9D1054622 (BIT)]; Supercomputing Center (KISTI).

**References**

Andrei, S. (2008). Molecular and cellular biology of synucleins. In W. J. Kwang (Ed.), *International review of cell and molecular biology* (Vol. 270, pp. 225–317). San Diego, CA: Academic Press.

Becke, A. D. (1993). Density-functional thermochemistry. III. The role of exact exchange. *The Journal of Chemical Physics*, 98, 5648–5652.

Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., DiNola, A., & Haak, J. R. (1984). Molecular dynamics with coupling to an external bath. *The Journal of Chemical Physics*, 81, 3684–3690.

Bertoncini, C. W., Jung, Y.-S., Fernandez, C. O., Hoyer, W., Griesinger, C., Jovin, T. M., & Zweckstetter, M. (2005). Release of long-range tertiary interactions potentiates aggregation of natively unstructured α-synuclein. *Proceedings of the National Academy of Sciences*, 102, 1430–1435.

Case, D. A., Darden, T. A., Chea, L., Wang, J., Duke, R. E., & Luo, R. (2008). *AMBER10*. San Francisco: University of California San Francisco Press.

Cheung, X., Cai, G., Hornak, V., & Simmerling, C. (2005). Modified replica exchange simulation methods for local structure refinement. *The Journal of Physical Chemistry B*, 109, 8220–8230.

Clayton, D. F., & George, J. M. (1999). Synucleins in synaptic plasticity and neurodegenerative disorders. *Journal of Neuroscience Research*, 58, 120–129.

Conway, K. A., Harper, J. D., & Lansbury, P. T. (1998). Accelerated in vitro fibril formation by a mutant α-synuclein linked to early-onset Parkinson disease. *Nature Medicine*, 4, 1318–1320.

Crowther, R. A., Jakse, R., Spillantini, M. G., & Goedert, M. (1998). Synthetic filaments assembled from C-terminally truncated α-synuclein. *FEBS Letters*, 436, 309–312.

Davidson, W. S., Jonas, A., Clayton, D. F., & George, J. M. (1998). Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *Journal of Biological Chemistry*, 273, 9443–9449.

Fan, Z. Z., Hwang, J. K., & Warshel, A. (1999). Using simplified protein representation as a reference potential for all-atom calculations of folding free energy. *Theoretical Chemistry Accounts: Theory, Computation, and Modeling (Theoretica Chimica Acta)*, 103, 77–80.

Fernández, C. O., Hoyer, W., Zweckstetter, M., Jares-Erijman, E. A., Subramaniam, V., Griesinger, C., & Jovin, T. M. (2004). NMR of α-synuclein-polyamine complexes elucidates the mechanism and kinetics of induced aggregation. *The EMBO Journal*, 23, 2039–2046.

Fischer, G., Wittmann-Liebold, B., Lang, K., Kielhaber, T., & Schmid, F. X. (1989). Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature*, 337, 476–478.

Gerard, M., Debyser, Z., Desender, L., Baert, J., Brandt, I., Baekelandt, V., & Engelborghs, Y. (2008). FK506 binding protein 12 differentially accelerates fibril formation of wild

**Supplementary material**

The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2015.1033016.

**Disclosure statement**

No potential conflict of interest was reported by the authors.
type-alpha-synuclein and its clinical mutants A30P or A53T. Journal of Neuroscience, 106, 121–133.

Gerard, M., Debyser, Z., Desender, L., Kabriel, P. J., Baert, J., Baekelandt, V., & Engelborghs, V. (2006). The aggregation of alpha-synuclein is stimulated by FK506 binding proteins as shown by fluorescence correlation spectroscopy. FASEB Journal, 20, 524–526.

Gerard, M., Deleersnijder, A., Daniels, V., Schreurs, S., Munck, S., Reumers, V., & Pottel, H. (2010). Inhibition of FK506 binding proteins reduces alpha-synuclein aggregation and Parkinson’s disease-like pathology. Journal of Neuroscience, 30, 2454–2463.

Göttel, S. F., & Marahiel, M. A. (1999). Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. Cellular and Molecular Life Sciences (CMLS), 53, 423–436.

Han, H., Weinreb, P. H., & Lansbury, P. T. (1995). The core Alzheimer’s peptide NAC forms amyloid fibrils which seed and are seeded by beta-amyloid: Is NAC a common trigger or target in neurodegenerative disease? Chemistry & Biology, 2, 163–169.

Harding, M. W., Galat, A., Uehling, D. E., & Schreiber, S. L. (1989). A receptor for the immuno-suppressant FK506 is a cis-trans peptidyl-prolyl isomerase. Nature, 341, 758–760.

Hoyer, W., Cherny, D., Subramaniam, V., & Jovin, T. M. (2004). Impact of the Acidic C-Terminal Region Comprising Amino Acids 109–140 on a-Synuclein Aggregation in Vitro. Biochemistry, 43, 16233–16242.

Jo, E., McLaurin, J., Yip, C. M., St. George-Hyslop, P., & Harding, M. W., Galat, A., Uehling, D. E., & Schreiber, S. L. (1999). Peptidyl-prolyl cis-trans isomerase as shown by Baekelandt, V., & Engelborghs, Y. (2006). The aggregation and the conformation and the aggregation of alpha-synuclein as revealed by g-factor of solid-state circular dichroism. Biopolymers, 83, 226–232.

Kollman, P. A. D., Cornell, W., Fox, T., Chipot, C., & Pohorille, A. (1997). The development/application of a ‘minimalist’ organic/biochemical molecular mechanic force field using a combination of ab initio calculations and experimental data. In A. Wilkinson, P. Weiner, & W. F. van Gunsteren (Eds.), Computer simulation of biomolecular systems (Vol. 3, pp. 83–96). Dordrecht: Kluwer Academic.

Lee, C., Yang, W., & Parr, R. G. (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Physical Review B, 37, 785–789.

Li, J., Uversky, V. N., & Fink, A. L. (2001). Effect of familial Parkinson’s disease point mutations A30P and A53T on the structural properties, aggregation, and fibrillation of human α-synuclein. Biochemistry, 40, 11604–11613.

Lin, X.-J., Zhang, F., Xie, Y.-Y., Bao, W.-J., He, J.-H., & Hu, H.-Y. (2006). Secondary structural formation of α-synuclein amyloids as revealed by g-factor of solid-state circular dichroism. Biopolymers, 83, 226–232.

Lu, K. P., Finn, G., Lee, T. H., & Nicholson, L. K. (2007). Prolyl cis-trans isomerization as a molecular timer. Nature Chemical Biology, 3, 619–629.

McLean, P. J., Kawamata, H., Ribich, S., & Hyman, B. T. (2000). Membrane Association and Protein Conformation of α-Synuclein in Intact Neurons. Journal of Biological Chemistry, 275, 8812–8816.

Meuvis, J., Gerard, M., Desender, L., Baekelandt, V., & Engelborghs, Y. (2010). The conformation and the aggregation kinetics of α-synuclein depend on the proline residues in its C-terminal region. Biochemistry, 49, 9345–9352.

Mitsutake, A., Sugita, Y., & Okamoto, Y. (2001). Generalized ensemble algorithms for molecular simulations of biopolymers. Biopolymers, 60, 96–123.

Narhi, L., Wood, S. J., Stevenson, S., Jiang, Y., Wu, G. M., Anafi, D., & Kaufman, S. A. (1999). Both familial Parkinson’s disease mutations accelerate α-synuclein aggregation. Journal of Biological Chemistry, 274, 9843–9846.

Nielsen, M. S., Vorum, H., Lindersson, E., & Jensen, P. H. (2001). Ca2+ binding to α-synuclein regulates ligand binding and oligomerization. Journal of Biological Chemistry, 276, 22680–22684.

Nymeyer, H., Gnanakaran, S., & Garcia, A. E. (2004). Atomic simulations of protein folding, using the replica exchange algorithm. Methods in Enzymology, 383, 119–149.

Ohama, E., & Ikuta, F. (1976). Parkinson’s disease: Distribution of Lewy bodies and monoamine neuron system. Acta Neuropathologica, 34, 311–319.

Onufriev, A., Bashford, D., & Case, D. A. (2000). Modification of the generalized born model suitable for macromolecules. The Journal of Physical Chemistry B, 104, 3712–3720.

Onufriev, A., Bashford, D., & Case, D. A. (2004). Exploring protein native states and large-scale conformational changes with a modified generalized born model. Proteins: Structure, Function, and Bioinformatics, 53, 383–394.

Ryckaert, J.-P., Ciccotti, G., & Berendsen, H. J. C. (1977). Numerical integration of the cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. Journal of Computational Physics, 23, 327–341.

Sieckiera, J. J., Hung, S. H. Y., Poe, M., Lin, C. S., & Sigal, N. H. (1989). A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature, 341, 755–757.

Souza, J. M., Giasson, B. I., Lee, V. M. Y., & Ischiropoulos, H. (2000). Chaperone-like activity of synucleins. FEBS Letters, 474, 116–119.

Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M., & Goedert, M. (1998). α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson’s disease and dementia with Lewy bodies. Proceedings of the National Academy of Sciences, 95, 6469–6473.

Spillantini, M. G., Schmidt, M. L., Lee, V. M. Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). α-synuclein in Lewy bodies. Nature, 389, 839–840.

Still, W. C., Tempczyk, A., Hawley, R. C., & Hendrickson, T. (1990). Semianalytical treatment of solvation for molecular mechanics and dynamics. Journal of the American Chemical Society, 112, 6127–6129.

Takahashi, N., Hayano, T., & Suzuki, M. (1989). Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. Nature, 337, 473–475.

Ueda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D. A. C., Kondo, J., Ihara, Y., & Saitho, T. (1993). Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proceedings of the National Academy of Sciences, 90, 11282–11286.

Uversky, V. N., Li, J., & Fink, A. L. (2001). Evidence for a partially folded intermediate in alpha-synuclein fibril formation. Journal of Biological Chemistry, 276, 10737–10744.

Wedemeyer, W. J., Welker, E., & Scheraga, H. A. (2002). Proline cis–trans isomerization and protein folding. Biochemistry, 41, 14637–14644.

Wood, S. J., Wypych, J., Stevenson, S., Louis, J.-C., Citron, M., & Biere, A. L. (1999). α-Synuclein Fibrillogenesis Is Nucleation-dependent. Journal of Biological Chemistry, 274, 19509–19512.
Wu, K.-P., Weinstock, D. S., Narayanan, C., Levy, R. M., & Baum, J. (2009). Structural reorganization of α-synuclein at low pH observed by NMR and REMD simulations. *Journal of Molecular Biology, 391*, 784–796.

Yoon, J., Park, J., Jang, S., Lee, K., & Shin, S. (2008). Conformational characteristics of unstructured peptides: α-Synuclein. *Journal of Biomolecular Structure & Dynamics, 25*, 505–515.

Yoon, J., Park, J., Jang, S., Lee, K., & Shin, S. (2009). Structural properties of fibril-forming segments of α-synuclein. *Bulletin of Korean Chemical Society, 30*, 654–660.