Structural Organization of α-Synuclein Fibrils Studied by Site-directed Spin Labeling*

Received for publication, May 20, 2003
Published, JBC Papers in Press, June 18, 2003, DOI 10.1074/jbc.M305266200

Ani Der-Sarkissian‡, Christine C. Jao¶, Jeannie Chen¶¶, and Ralf Langen‡‡

From the ‡Department of Molecular Pharmacology and Toxicology, School of Pharmacy, the ¶Department of Biochemistry and Molecular Biology, the Zilkha Neurogenetic Institute and Arnold and Mabel Beckman Macular Research Center, and the **Mary D. Allen Laboratory for Vision Research, Doheny Eye Institute, Departments of Ophthalmology and Cell and Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, California 90089

Despite its importance in Parkinson's disease, a detailed understanding of the structure and mechanism of α-synuclein fibril formation remains elusive. In this study, we used site-directed spin labeling and electron paramagnetic resonance spectroscopy to study the structural features of monomeric and fibrillar α-synuclein. Our results indicate that monomeric α-synuclein, in solution, has a highly dynamic structure, in agreement with the notion that α-synuclein is a natively unfolded protein. In contrast, fibrillar aggregates of α-synuclein exhibit a distinct domain organization. Our data identify a highly ordered and specifically folded central core region of ~70 amino acids, whereas the N terminus is structurally more heterogeneous and the C terminus (~40 amino acids) is completely unfolded. Interestingly, the central core region of α-synuclein exhibits several features reminiscent of those observed in the core region of fibrillar Alzheimer's amyloid β peptide, including an in-register parallel structure. Although the lengths of the respective core regions differ, fibrils from different amyloid proteins nevertheless appear to be able to take up highly similar, and possibly conserved, structures.

The deposition of amyloid fibrils has been linked to a variety of slow-onset degenerative diseases, such as Alzheimer's disease, Parkinson's disease (PD),† type II diabetes mellitus, and spongiform encephalopathies. In the case of PD, these amyloid fibrils are formed by α-synuclein (1), which is found in intracellular inclusions of dopaminergic neurons, called Lewy bodies. Several lines of evidence suggest that α-synuclein plays a causative role in PD. Linkage studies have shown that rare familial forms of early-onset PD, inherited as an autosomal dominantly inherited trait, result from two independent missense mutations (A53T and A30P) within the α-synuclein gene (2, 3).

Moreover, experimental fly (4) and other animal (5, 6) models have suggested an important role of α-synuclein in the etiology of PD. α-Synuclein is a 140-amino-acid, thermally stable (7, 8), cytoplasmic protein, found in presynaptic terminals of neuronal cells (9, 10). Although the precise physiological role of this protein is not yet well defined, it has been suggested that α-synuclein is involved in the modulation of neurotransmitter release (11). In aqueous solution, α-synuclein has been found to be highly dynamic and, therefore, has been classified as a natively unfolded protein (8). However, upon aggregation, some not yet well-defined regions of α-synuclein undergo a conformational change and take up a cross-β-structure (12, 13), in which individual β-strands run perpendicular to the fiber axis. Originally, a 35-amino-acid fragment of α-synuclein was isolated from brain tissue of Alzheimer patients (14). This fragment (amino acids 61–95), designated NAC (non-amyloid β (Aβ) component of Alzheimer's disease amyloid), comprises the hydrophobic core of the protein and has been shown to be important in fibril formation (15). More recent analysis suggests, however, that the core domain of α-synuclein might be even shorter. In the latter study, a 12-amino-acid peptide (residues 71–82), located within the NAC region, was shown to be capable of undergoing self-aggregation (16). The importance of this hydrophobic stretch is further supported by its absence in β-synuclein, a homologue of α-synuclein (17), with strongly reduced propensity for fibril formation (12, 18). In contrast, a third line of evidence, based on protease digestion studies (19), suggests that the core region of α-synuclein is longer. In this study, a 7-kDa fragment (comprising residues 31–109) was shown to be protected from proteinase K digestion. This region contains the putative 12-residue core domain, as well as the NAC region.

In an effort to determine which of these core regions potentially correspond to the cross-β-structure and to decipher the overall structural features of α-synuclein fibrils, we used site-directed spin labeling (SDSL), together with electron paramagnetic resonance (EPR) spectroscopy. Although SDSL had originally been developed to study the structural and conformational dynamics of soluble and membrane proteins (20), this method has recently been applied successfully to structural and dynamic studies of other amyloid proteins, such as Aβ (21) and transthyretin (22). SDSL is based upon the introduction of a cysteine-specific nitroxide spin label, which serves as a reporter molecule, into selected sites of the protein to generate the side chain R1 (Fig. 1). The structural environment of the spin label can then be monitored by EPR spectroscopy. The mobility information contained in the EPR spectra can be used to distinguish between loop, surface, or buried sites (23–25). In nitroxide scanning experiments, this information

* This work was supported by the Hillblom Foundation (to R. L. and J. C.) and the Arnold and Mabel Beckman Foundation (to R. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a predoctoral fellowship from the National Institutes of Health and by NIDCR, National Institutes of Health Grant T32 DE07211.

¶ To whom correspondence should be addressed: Zilkha Neurogenetic Institute, Keck School of Medicine, Los Angeles, California 90089. Tel.: 323-442-1323; Fax: 323-442-2145; E-mail: langen@usc.edu.

† The abbreviations used are: PD, Parkinson's disease; NAC, non-Aβ component of Alzheimer's disease amyloid; SDSL, site-directed spin labeling; EPR, electron paramagnetic resonance spectroscopy; Aβ, amyloid β.

This paper is available on line at http://www.jbc.org
can furthermore be used to determine secondary structural elements (20). In addition, the distance between two R1 side chains can be estimated based on magnetic dipolar interactions (20).

Our findings show that α-synuclein, in its fibrillar state, has a distinct domain organization. We identified a core region of ~70 amino acids (residues 34–101), packed in highly ordered, parallel fashion, with the same residues from different strands in exact register. Similar parallelism was also observed in the core regions of synthetic Aβ fibrils assembled in vitro using SDS-LS (21). The N terminus of fibrillar α-synuclein is structurally more heterogeneous, displaying a less ordered structural organization. The C-terminal region of the protein remains unfolded, even within fibrillar bundles. Taken together, these findings suggest that there may be a common mechanism underlying the formation of amyloid fibrils, at least in Alzheimer’s disease and PD.

EXPERIMENTAL PROCEDURES

α-Synuclein Mutagenesis—The wild-type human α-synuclein expression construct (pRK172) was kindly provided by Dr. M. Goedert. Wild-type α-synuclein sequence contains no cysteine residues. Single cysteine mutants for spin labeling were generated using the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). The introduced mutations were verified by DNA sequencing.

α-Synuclein Expression and Purification—Escherichia coli BL21(DE3)pLys-S cells were transformed with the pRK172 construct by heat shock. Expression of α-synuclein was induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside at 25 °C overnight. Bacterial cells were harvested by centrifugation at 4,000 × g for 10 min and lysed in 500 mM NaCl, 100 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, and 1 mM phenylmethylsulfonyl fluoride. The cell lysate was then boiled for 10 min and subsequently centrifuged for 30 min at 15,000 × g. The resulting supernatant was precipitated in acid at pH 3.5 and centrifuged through Microcon YM-100 (molecular weight cut off, 10 kDa) spin filter units to ensure the absence of pre-existing aggregates. Immediately before spin labeling, di-thiothreitol was removed from the buffer using size exclusion chromatography (PD-10 columns, Amersham Biosciences). The composition of the buffer used for equilibration and spin labeling was 10 mM HEPES buffer, pH 7.4, 100 mM NaCl. Sulphydryl groups of the purified cysteine mutants were then reacted with a 3-fold molar excess of the spin label [1-oxy-2,2,5,5-tetramethyl-p-pyrrole-3-methyl]methanethiosulfonate (Toronto Research Chemicals, Toronto, Ontario, Canada) (see Fig. 1) at room temperature for 1 h. Unreacted spin label was removed using PD-10 columns. Spin-labeled proteins were concentrated using Microcon YM-3 (molecular weight cut off, 3,000) (Amicon, Bedford, MA).

Fibrillar or monomeric α-synuclein was loaded into sealed glass capillaries for subsequent EPR analysis. EPR spectroscopy was performed using the Bruker EMX spectrometer (Bruker Instruments, Billerica, MA), fitted with a loop-gap resonator (26) for room temperature measurements and with an ER 4119HS resonator for measurements taken at −40 °C. X-band room temperature EPR spectra were obtained at 2 mW incident microwave power and at a field modulation of 1.5 Gauss at 100 kHz over a scan range of 150 Gauss. Spectra were accumulated over 20 scans. All first-derivative EPR spectra were normalized by double integration to represent the same number of spins. To correct for small amounts of free spin label and/or nonspecific labeling, all spectra were baseline corrected by subtracting the spectrum of spin-labeled (Cys-less) wild-type α-synuclein, which yielded a highly characteristic sharp line shape of 1.6 Gauss. This subtraction amounted to ~5% of the signal (data not shown).

Inter-spin distances can be quantified based upon magnetic dipolar interactions (20, 27–30). Here, distances between the same R1 labels within α-synuclein strands were determined using a Pake pattern-based simulation software generously provided by Drs. Hubbell and Altenbach (31), which has proven successful in measuring inter-spin distances within fibrils of Aβ (21). Using this software, distances between R1-labeled derivatives of α-synuclein were measured by comparing the EPR spectra of fully labeled protein with those co-mixed with an equal molar ratio of unlabeled wild-type protein (spin diluted), as described previously (21, 31). Any residual coupling in the spin-diluted spectra were removed by subtracting the spin-coupled spectrum using the built-in option in the program.

α-Synuclein Fibril Assembly—Purified spin labeled proteins (4 mg/ml) were incubated in 10 mM HEPES buffer, pH 7.4, 100 mM NaCl, 0.1% NaN3, at 37 °C for 7–10 days with stirring. Fibrils were isolated by centrifugation and washed twice with 10 mM HEPES buffer, pH 7.4. Purified, unlabeled wild-type α-synuclein was used for spin-dilution experiments. Spin-labeled derivatives of α-synuclein were mixed with unlabeled wild-type protein in a 1:1 molar ratio at 4 mg/ml of total protein concentrations.

RESULTS

Structural Changes upon Transition from Monomeric to Fibrillar α-Synuclein—To identify the structural organization of soluble and fibrillar α-synuclein, single spin labels were introduced at selected sites throughout the protein, and their EPR spectra were recorded in solution and in fibrils. As shown in Fig. 2, the EPR spectra for all α-synuclein derivatives in solution (monomeric; black traces) are very similar and are characterized by sharp and narrowly spaced lines. Such spectral features arise from the rapid motion of the R1 label and are typical of highly dynamic and unfolded structures, in close proximities.
agreement with the notion of α-synuclein as a “natively unfolded” protein (8).

Next, by growing fibrils from spin-labeled α-synuclein derivatives, we sought to determine how this unfolded structure is altered upon fibril formation. Fibril morphology for all sites was verified by electron microscopy (data not shown) and was found to be very similar to fibrils taken from wild-type α-synuclein (15, 32, 33). Fig. 2A shows the effect of fibril formation on the EPR spectra of α-synuclein derivatives containing spin labels at selected sites in the C terminus (103R1, 106R1, 107R1, 124R1, 127R1, 130R1, 131R1, and 136R1; green trace). As was the case in solution, these sites gave rise to very sharp and narrowly spaced lines, even after fibril formation. Thus, we found little evidence of any ordering in the C-terminal region of α-synuclein aggregates and believe it is likely that the C-terminal region remains largely unfolded, even after fibril formation (also see discussion of Fig. 3 below).

To test whether structural changes might occur in the N-terminal 100 amino acids of α-synuclein, we recorded the room temperature EPR spectra of fibrillar α-synuclein labeled at positions 5R1, 26R1, 68R1, and 88R1, respectively (Fig. 2B, green and red traces). Residues 5R1 and 26R1 showed some decrease in signal amplitude (Fig. 2B, green trace), whereas residues 68R1 and 88R1, located in the NAC region of the protein, showed a marked decrease in amplitude (Fig. 2B, green trace). To clearly visualize details of the line shape, spectra of fibrillar 68R1 and 88R1 were scaled at 10- and 5-fold, respectively (Fig. 2B, red trace). In addition to a strong reduction of signal intensity, we also observed a pronounced spectral broadening, characterized by the overall width of the spectrum exceeding 100 Gauss (all spectra were accumulated in a magnetic scan range of 150 Gauss). These spectral features are indicative of very strong spin-spin interactions and are reminiscent of what was observed for fibrillar Aβ peptide (21), suggesting that some regions of α-synuclein, as in Aβ, might also be arranged in parallel fashion.

α-Synuclein Fibrils Exhibit a Core Region of Parallel and In-register Structure—To further confirm the presence of spin-spin interactions and to probe the extent of a parallel, ordered structure, we recorded the EPR spectra of the R1-labeled α-synuclein fibrils in their frozen state (Fig. 3).

Two factors can significantly contribute to changes in amplitude and line shape, namely, mobility and spin-spin interactions between nearby labels because of dipolar coupling and spin exchange (20). In the frozen state, EPR spectra are no longer influenced by local structure and mobility; therefore, major changes in the EPR line shapes can be attributed solely to spin-spin interactions.

EPR spectra of sites ~34R1 through ~101R1 show clear spin-spin interactions, as reflected in the marked drop in signal intensity and a spectral line broadening beyond 100 Gauss (Fig. 3, green trace). A lesser effect was seen at the N-terminal regions of fibrillar α-synuclein, and little or no effect was observed at C-terminal sites (103R1, 106R1, 107R1, 109R1, 124R1, 127R1, 131R1, and 136R1), which exhibited relatively large amplitudes. These EPR spectra are indicative of the absence of strong spin-spin interactions, in agreement with the data shown in Fig. 2A, indicating that this region is not ordered.

To further confirm and quantify the effect of spin-spin interactions, we performed spin-dilution experiments, wherein R1-labeled and wild-type α-synuclein were co-mixed and allowed to form fibrils over time (see “Experimental Procedures”). Such experiments were conducted based upon the following rationale: if wild-type α-synuclein and its R1-labeled derivatives were to co-mix interchangeably to form fibrils, the potential of one spin label located on a given α-synuclein protein coming in close proximity to another spin label located on a neighboring protein would be expected to decrease as a function of increasing concentration of unlabeled wild-type protein. Thus, spin-spin interactions would be reduced upon co-mixing of the R1-labeled and wild-type α-synuclein. As a result, the EPR spectral line shapes would become sharper and narrower. Dilution experiments were performed, and once again, fibril formation was verified by electron microscopy (data not shown). As shown in Fig. 3, these wild-type dilutions resulted in highly similar EPR spectra for all of the sites (black traces). A comparison of the spectra from R1-labeled (green traces) and wild-type-diluted fibrils (black traces) shows that the largest spectral changes occurred in sites between ~34R1 and ~101R1. Within this region, spin dilution caused a significant loss of spin-spin interaction, as can be seen from the increase in signal intensity and the concomitant decrease in spectral breadth. In contrast, the EPR spectral changes in the N- and C-terminal regions of α-synuclein fibrils are much smaller. In addition to its importance in demonstrating spin-spin interactions, the co-mixing of wild-type and R1-labeled proteins indicates that the R1-labeled and wild-type proteins are able to adopt similar structures within the fibril. Thus, as has already been observed for Alzheimer’s Aβ (21), the introduction of R1 is tolerated remarkably well in amyloid fibrils.

For a more quantitative evaluation of the spin-spin interactions, we determined the distances between spin labels, as described under “Experimental Procedures.” Distances obtained by SDSL are very accurate, as reported previously in a comparison of distances obtained by x-ray crystallographic methods with those obtained by SDSL in T4 lysozyme (28). It is
important to note, however, that SDSL measures distances between the nitroxide side chains of the spin labels rather than between the protein backbone atoms. A summary of distance distributions of filamentous α-synuclein using SDSL is shown in Fig. 4A, wherein the percentage of distance distributions is plotted against the corresponding residue number. Red columns represent distance distributions beyond 20 Å, yellow columns indicate distance distributions between 15 and 20 Å, and black columns indicate distance distributions of <15 Å. As depicted in this figure, the distance contributions from residues 34 to 101 are <15 Å (typically 8–11 Å) (black columns), with negligible amounts of distance contributions from longer distances (red columns), which is indicative of a highly specific, in-register arrangement of α-synuclein amyloid fibrils. Interestingly, the degree of parallelism includes but extends beyond residues 71–82 and the amyloidogenic NAC region. The C-terminal residues of the protein primarily exhibit distance distributions of >20 Å (red columns), which is consistent with the data presented in Figs. 2 and 3, showing that the C-terminal region of α-synuclein is unstructured. A much more heterogeneous distance distribution is observed in the N-terminal region of α-synuclein. Structural heterogeneity may arise from the existence of multiple conformations as observed in other amyloid fibrils such as Aβ (21) studied by EPR and by protease digestion studies of the Aβ peptide (34).

**DISCUSSION**

The principal objective of this study was to identify the overall structural organization of α-synuclein fibrils. Using SDSL and EPR analysis, we observed a distinctive domain organization in α-synuclein fibrils. We found a core region of ~70 residues that is flanked by shorter N- and C-terminal domains of ~30 and 40 amino acids, respectively (Fig. 4B). Interestingly, the core region is characterized by strong spin-spin interactions arising from the close proximity of R1 side chains (mostly, 8–11 Å distances between nitroxide groups) of the same residues from different α-synuclein molecules, thus indicating the formation of highly ordered packing interactions that result in a homogeneous, parallel, and in-register structure. The core region of α-synuclein, identified by SDSL and EPR, contains the NAC domain, as well as the hydrophobic 12-residue peptide VTGVTAVAQKTV, which was found to be necessary and sufficient for fibril formation (16). However, our data demonstrate that the core region of α-synuclein fibrils extends beyond these regions to a significant extent, a finding that is further supported by a proteinase K-resistant fragment (residues 31–109) identified recently by Miake et al. (19).

It is important to note that a parallel arrangement of amyloid fibrils is not limited to α-synuclein alone. In fact, a number of studies on Alzheimer’s Aβ peptide by SDSL (21) and solid-state NMR (35) have revealed that this peptide is also packed in a parallel and highly specific manner. Studies involving many different amyloid fibril-forming proteins have revealed an overwhelming number of similarities in the mechanism(s) of fibril formation. For example, amyloid fibrillogenesis consistently appears to be a nucleation-dependent process (36) that ultimately results in a cross-β structure, where individual strands run perpendicular to the fiber axis (37). Moreover, all amyloid fibrils exhibit specific tinctorial properties, i.e. they bind Congo red and Thioflavin S (38). Common structural features of amyloids are furthermore supported by conformational antibodies that appear to recognize a common amyloid fibril fold that seems to be shared by a number of different amyloid fibrils (39). This overall structural similarity might even extend to prefibrillar structures, considering that another conformationally specific antibody recognizes protofibrillar aggregates from various amyloidogenic proteins, including α-synuclein (40). Thus, in light of these similarities, it is possible that parallel arrangement is a general feature of the core regions of amyloid fibrils.

In contrast to this possibly conserved and highly organized structural arrangement in the core region of α-synuclein fibrils, we find little evidence of any ordered structure in the C terminus of α-synuclein fibrils. It is therefore unlikely that the C terminus contributes to the stability of fibrils. In fact, it is conceivable that the high negative charge density of the C terminus could cause significant electrostatic repulsions, a feature that may serve to prevent a further extension of the parallel-arranged core region beyond residue 101. A fibril-de-
stabilizing role of the C terminus is further supported by kinetic analysis that has shown a significant enhancement in fibril formation upon removal of the C-terminal regions (12, 41). Although it is unclear if removal of this destabilizing region contributes to enhanced fibril formation in vivo, it is interesting to note that partially truncated α-synuclein at the C terminus has been isolated from Lewy bodies of patients suffering from dementia with Lewy bodies (42).

Identification of a distinct domain organization in α-synuclein fibrils, together with the finding of strong parallelism in the core region, constitutes a first step toward understanding the mechanism of fibrillogenesis and places constraints on possible structural models. Additional structural constraints can be obtained from simple geometrical considerations. For example, we can eliminate the possibility of parallel, fully extended strands containing 70 amino acids. Given the 3.5-Å axial distance between adjacent amino acids in an extended β-strand, the parallel polypeptide core region of α-synuclein would be ~250 Å (25 nm) in length; however, this length exceeds the narrowest fiber dimension (6 nm) reported for α-synuclein fibrils (12). Thus, several turn and bend regions must exist to account for such geometrical dimensions (21, 43).

The existence of hairpins consisting of anti-parallel β-strands has been proposed for α-synuclein (13, 44) and Aβ fibrils (45, 46), based upon Fourier Transform Infrared spectroscopy analysis of amyloid fibrils and those of soluble proteins containing such antiparallel strands. A general model for a two-stranded hairpin with anti-parallel β-strands is shown in Fig. 5A, where the interstrand distance is ~5 Å, the intersheet distance is estimated at ~10 Å, and the β-strands run perpendicular to the fiber axis (37). To observe parallelism, one might envision a parallel arrangement of individual hairpins in which the same residues will be at ~10 Å distance from each other (Fig. 5A, red marks). Such a structure could be taken up entirely by shorter peptides, such as NAC or the Aβ peptide. However, for the much longer core region of α-synuclein, this arrangement would not be likely because additional strands (probably at least four) and bend regions are required to accommodate ~70 amino acids under the constraints of the fiber dimensions (see Fig. 5B). The resulting distances between the same residues in different molecules, repeated along the fibril axis (~25 Å), would be outside the detectable range using the current approach and clearly too long to account for the strong spin-spin interaction observed from the EPR data presented herein. Nevertheless, it is still possible to account for parallelism if the sheets are arranged in parallel fashion, as depicted in Fig. 5C. Although we cannot exclude such a model based on the EPR data alone, this model could not readily explain the growth and propagation properties of amyloid fibrils.

example, the contact surface for growth in the fibril direction would be limited to only a single β-strand region, as schematically illustrated in Fig. 5D. Furthermore, if the parallel sheet packing is stabilizing, one might expect greater variations in the number of stacked sheets; in turn, this could result in highly irregular fibril thickness, a feature not generally observed in fibrillar assemblies.

Problems of this nature would not be encountered with the model shown in Fig. 5E. This model is based on the existence of parallel strands that are interrupted by a number of regions that cause bending of the entire sheet. The model shown in Fig. 5E represents one of several possible organizations to which such bent regions could be achieved. One positive feature of this model is that the fibril propagation can be easily explained, i.e. both fibril ends would expose an extensive surface of hydrogen bond donors and acceptors that could act as templates for incoming molecules (see Fig. 5F). Such “template-guided folding” would further ensure that precise conformation and fibril type would be maintained. Additional support for such a model is derived from a recent solid-state NMR analysis of Aβ1–40 peptide (47), which showed that individual strands, rather than sheets of hairpins, are arranged in parallel.

Through future studies using SDS and EPR, we hope to prove the validity of the model in Fig. 5E (and variations thereof, possibly including some β-helical structures) and to identify the location(s) of the bend or turn regions. Similarly, this approach should also enable a more detailed structural analysis of prefibrillar intermediates and their membrane-bound forms.

Acknowledgments—We thank Dr. M. Goedert for the provision of the α-synuclein DNA construct and are grateful to Lena Te and Winnie Tse for their outstanding technical support.

REFERENCES
1. Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., and Goedert, M. (1997) Nature 388, 839–840
2. Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenness, E. S., Chandrasekharappa, S., Athanassiadou, A., Papaetropoulos, T., Johnann, W. G., Lazzarini, A. M., Buvuisin, R. C., Di Iorio, G., Golbe, L. I., and Nussbaum, R. L. (1997) Science 276, 2045–2047
3. Kruger, R., Kuhn, W., Muller, T., Wittalla, D., Graeber, M., Koel, S., Przuntek, H., Epplen, J. T., Schols, L., and Riez, O. (1998) Nat. Genet. 18, 106–108
4. Peany, M. B., and Bender, W. W. (2000) Nature 404, 394–398
5. Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagara, Y., Siak, A., and Mucke, L. (2000) Science 287, 1265–1269
6. van der Putten, H., Wiederhold, K. H., Probst, A., Barbieri, S., Miotl, C., Danner, S., Kauffmann, S., Hofele, K., Spooren, W. P., Roegge, M. A., Lin, S., Carnoni, P., Sommer, B., Tolnay, M., and Bihle, G. (2000) J. Neurosci. 20, 6021–6029
7. Kim, T. D., Ryu, H. J., Cho, H. I., Yang, C. H., and Kim, J. (2000) Biochemistry 39, 14839–14846
8. Wenzel, P. H., Zhen, W., Poon, A. W., Conway, K. A., and Lansbury, P. T., Jr.
Organization of α-Synuclein in Amyloid Fibrils

1996 Biochemistry 35, 13709–13715
9. Iwai, A., Masliah, E., Yoshimoto, M., Ge, N., Flanagan, L., de Silva, H. A., Kittel, A., and Saitoh, T. (1995) Neuron 14, 467–475
10. Marsteaux, L., and Scheller, R. H. (1991) Brain Res. Mol. Brain Res. 11, 335–343
11. Murray, I. V., Lee, V. M.-Y., and Trojanowski, J. Q. (2001) Neurology 57, 1655–1661
12. Serag, A. A., Altenbach, C., Gingery, M., Hubbell, W. L., and Yeates, T. O. (2000) Biochemistry 39, 2552–2563
13. Conway, K. A., Harper, J. D., and Lansbury, P. T., Jr. (2000) J. Biol. Chem. 275, 735–739
14. Ueda, K., Fukushina, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D. A., Kondo, J., Ibara, Y., and Saitoh, T. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11283–11286
15. Iwai, A., Yoshimoto, M., Masliah, E., and Saitoh, T. (1995) Biochemistry 34, 10139–10145
16. Giasson, B. I., Murray, I. V., Trojanowski, J. Q., and Lee, V. M. (2001) J. Biol. Chem. 276, 2380–2386
17. Kerjaschki, D., Heizmann, C. W., and Glabe, C. G. (2003) FEBS Lett. 539, 19213–19219
18. Biere, A. L., Wood, S. J., Wypych, J., Steavenson, S., Jiang, Y., Anafi, D., Jacobsen, F. W., Jarosinski, M. A., Wu, G. M., Louis, J. C., Martin, F., Narhi, L. O., and Citron, M. (2000) J. Biol. Chem. 275, 34574–34579
19. Make, H., Mizusawa, H., Iwatsubo, T., and Hasegawa, M. (2002) J. Biol. Chem. 277, 19215–19219
20. Hubbell, W. L., Cafiso, D. S., and Altenbach, C. (2000) Nat. Struct. Mol. Biol. 7, 375–379
21. To¨ro¨k, M., Milton, S., Kayed, R., Wu, P., McIntire, T., Glabe, C. G., and Langen, R. (2002) J. Biol. Chem. 277, 40810–40815
22. Serag, A. A., Altenbach, C., Gingery, M., Hubbell, W. L., and Yeates, T. O. (2001) Biochemistry 40, 9689–9696
23. Margittai, M., Faasbauer, D., Pabet, S., Jahn, R., and Langen, R. (2001) J. Biol. Chem. 276, 13169–13177
24. Issa, J. M., Langen, R., Haigler, H. T., and Hubbell, W. L. (2002) Biochemistry 41, 1464–1473
25. Mchaourab, H. S., Lietzow, M. A., Hideg, K., and Hubbell, W. L. (1996) Biochemistry 35, 7692–7704
26. Hubbell, W. L., Francioli, W., and Hyde, J. S. (1987) Rev. Sci. Instrum. 58, 1879–1886
27. Hustede, E. J., Smirnov, A. L., Laub, C. F., Cobb, C. E., and Beth, A. H. (1997) Biophys. J. 72, 1861–1877
28. Mchaourab, H. S., Oh, K. J., Fang, C. J., and Hubbell, W. L. (1997) Biochemistry 36, 307–316
29. Rabenstein, M. D., and Shin, Y. K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8239–8243
30. Steinhoff, H. J., Radzwill, N., Thevis, W., Lenz, V., Brandenburg, D., Antson, A., Dobson, G., and Wellner, A. (1997) Biophys. J. 74, 3287–3288
31. Altenbach, C., Oh, K. J., Trabarnano, R. J., Hideg, K., and Hubbell, W. L. (2001) Biochemistry 40, 15471–15482
32. El-Agnaf, O. M., Jakes, R., Curran, M. D., Middleton, D., Ingenito, R., Bianchi, E., Pessi, A., Neill, D., and Wallace, A. (1998) FEBS Lett. 440, 71–75
33. Han, H., Weinreb, P. H., and Lansbury, P. T., Jr. (1995) Chem. Biol. 2, 163–169
34. Ketherpal, I., Williams, A., Murphy, C., Bledsoe, B., and Wetzel, R. (2001) Biochemistry 40, 11757–11767
35. Tycko, R. (2003) Biochemistry 42, 3151–3159
36. Lansbury, P. T., Jr. (1997) Neuron 19, 1151–1154
37. Makin, O. S., and Serpell, L. C. (2002) Biochem. Soc. Trans. 30, 521–525
38. Soto, C. (2003) Nat. Rev. Neurosci. 4, 49–60
39. O’Nuallain, B., and Wetzel, R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 1485–1490
40. Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W., and Glabe, C. G. (2003) Science 300, 486–489
41. Crowther, R. A., Jakes, R., Spallantini, M. G., and Goedert, M. (1998) FEBS Lett. 436, 309–312
42. Baba, M., Nakajo, S., Su, P. H., Tomita, T., Nakaya, K., Lee, V. M., Trojanowski, J. Q., and Iwatsubo, T. (1996) J. Biol. Chem. 271, 3301–3305
43. Uversky, V. N., Li, J., and Fink, A. L. (2001) J. Biol. Chem. 276, 10737–10744
44. Narhi, L., Wood, S. J., Steavenson, S., Jiang, Y., Wu, G. M., Anafi, D., Kaufman, S. A., Martin, F., Sitney, K., Denis, P., Louis, J. C., Wypych, J., Biere, A. L., and Citron, M. (1999) J. Biol. Chem. 274, 8843–8846
45. Fraser, P. E., Nguyen, J. T., Inouye, H., Surewicz, W. K., Selkoe, D. J., Podlisny, M. B., and Kirschner, D. A. (1992) Biochemistry 31, 10716–10723
46. Hilbich, C., Kisters-Woike, B., Reed, J., Masters, C. L., and Beyreuther, K. (1991) J. Mol. Biol. 218, 149–163
47. Petkova, A. T., Ishii, Y., Balbach, J. J., Anzur, O. N., Leipman, R. D., Delaglio, F., and Tycko, R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 16742–16747