Minireview

Amino acid determinants of α-synuclein aggregation: putting together pieces of the puzzle

Vladimir N. Uversky[a,b,*], Anthony L. Fink[a]

[a] *Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064, USA*
[b] *Institute for Biological Instrumentation, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia*

Received 17 April 2002; revised 14 May 2002; accepted 16 May 2002

First published online 3 June 2002

Edited by Thomas L. James

Abstract Parkinson's disease is the second most common neurodegenerative disease, and results from loss of dopaminergic neurons in the *substantia nigra*. The aggregation and fibrillation of α-synuclein in the form of intracellular proteinaceous aggregates (Lewy bodies and Lewy neurites) have been implicated as a causative factor in this disease, as well as in several other neurodegenerative disorders, including dementia with Lewy bodies, Lewy body variant of Alzheimer's disease, multiple system atrophy and Hallervorden–Spatz disease. Thus, the aggregated forms of α-synuclein play a crucial role in the pathogenesis of the synucleinopathies. However, the molecular mechanisms underlying α-synuclein aggregation into specific filamentous inclusions remained unknown until recently. Data on the aggregation and fibrillation properties of human α-, β- and γ-synucleins, mouse α-synuclein and familial Parkinson's disease mutants of human α-synuclein (A30P and A53T) are analyzed in order to shed light on the amino acid determinants of synuclein aggregation.

Key words: Parkinson's disease; Synucleinopathy; Neurodegenerative disorder; α-Synuclein; Fibrillation; Aggregation

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disease, results from loss of dopaminergic neurons in the *substantia nigra*. Some surviving nigral dopaminergic neurons contain cytosolic filamentous inclusions known as Lewy bodies (LBs) and Lewy neurites (LNs) [1,2]. Besides the *substantia nigra*, LBs and LNs also are found in other brain regions, such as the *dorsal motor nucleus* of the vagus, the *nucleus basalis* of Meynert, and the *locus coeruleus* [2]. In addition, abundant LBs and LNs in cerebral cortex are neurophathological hallmarks of dementia with LBs, a common late-life dementia that is clinically similar to Alzheimer's disease [3], in LB variant of Alzheimer's disease [4], diffuse LB disease [5], multiple system atrophy [4,6] and several other neurodegenerative disorders. The major fibrillar material of LBs and LNs was shown to be α-synuclein [7,8]. Moreover, two different missense mutations in the α-synuclein gene, corresponding to A53T and A30P substitutions in α-synuclein, have been identified in two kindreds with autosomal-dominantly inherited, early-onset PD [9,10]. The production of wild-type (WT) human α-synuclein in transgenic mice [11] or of WT, A30P and A53T human α-synuclein in transgenic flies [12] leads to motor deficits and neuronal inclusions reminiscent of PD. It has been established that all three proteins, as well as the 1–87 and 1–120 truncated forms of recombinant α-synuclein, are able to assemble readily into filaments in vitro, with morphologies and staining characteristics similar to those extracted from disease-affected brain [13–15]. Moreover, it has been shown that fibrils formed in vitro from α-synuclein and two mutant forms linked to PD are typical amyloid, i.e. structurally and morphologically they resembles fibrils formed by other amyloidogeneic proteins [14–16]. Interestingly, the peptide derived from the central hydrophobic region of α-synuclein represents a second major intrinsic constituent of Alzheimer's plaques. This 35-amino-acid peptide, known as NAC (Non-Aβ Component of Alzheimer's disease amyloid), was shown to amount to about 10% of the amyloid plaque [17]. These observations indicate that α-synuclein is a key player in the pathogenesis of several neurodegenerative disorders.

2. Peculiarities of the α-synuclein amino acid sequence

α-Synuclein was first described as a neuron-specific protein localized to the nucleus and presynaptic nerve terminals [18]. α-Synuclein from different organisms possesses a high degree of sequence conservation. For example, mouse and rat α-synucleins are identical throughout the first 93 residues, and human and canary proteins differ from them by only two residues [19]. The primary sequence of human α-synuclein is composed of 140 amino acid residues. It can be subdivided into three regions (see Fig. 1):

1. Residues 1–60 form the N-terminal region. It includes the sites of both familial PD mutations and contains four 11-amino-acid imperfect repeats with a highly conservative hexamer motif (KTKEGV). The N-terminal region is predicted to form amphipathic α-helices, typical of the lipid-binding domain of apolipoproteins ([19,20] see below).
2. The central region comprises the highly amyloidogenic NAC sequence (residues 61–95) [17,21]. It contains two additional KTKEGV repeats.

*Corresponding author. Fax: (1)-831-459 2935.
E-mail address: uversky@hydrogen.ucsc.edu (V.N. Uversky).

0014-5793/02/$22.00
PII: S0014-5793(02)02883-1

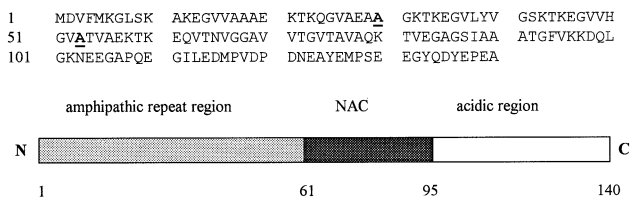

Fig. 1. α-Synuclein sequence. The two sites of early-onset PD-linked mutations (positions 30 and 53) are highlighted.

3. Finally, residues 96–149 constitute the C-terminal region. This part of α-synuclein is enriched in acidic residues and prolines, suggesting that it adopts a disordered conformation (see below). Three highly conserved tyrosine residues, which are considered as a family signature of α- and β-synucleins, are located in this region.

An important characteristic of the α-synuclein primary structure is six imperfect repeats within the first 95 residues, resulting in a variation in hydrophobicity [17,19,20–22] with a strictly conserved periodicity of 11 [20]. Such a periodicity is characteristic of the amphipathic lipid-binding α-helical domains of apolipoproteins [19,20], which have been extensively studied and assigned to subclasses according to their unique structural and functional properties [23,24]. α-Synuclein shares the defining properties of the class A2 lipid-binding helix, distinguished by clustered basic residues at the polar–apolar interface, positioned ±100° from the center of apolar face; predominance of lysines relative to arginines among these basic residues; and several glutamate residues at the polar surface [23–25]. In agreement with these structural features, α-synuclein (which belongs to the family of natively unfolded proteins, which show little or no ordered structure under physiological conditions [26–28]) binds specifically to synthetic vesicles containing acidic phospholipids [22,25]. This binding was shown to be accompanied by a dramatic increase in α-helix content [22,25].

3. Amino acid determinants of α-synuclein aggregation and fibrillation

3.1. Role of the NAC region

Analysis of literature data allows some conclusions to be made about the amino acid determinants of α-synuclein aggregation and fibrillation. First of all, the crucial role of the central hydrophobic region, known as the NAC peptide, is implicated by several lines of evidence.

1. As already mentioned, NAC is the second major component of amyloid plaques in Alzheimer's brain [17], and readily forms ordered fibrils in vitro [17,21,29]. Moreover, NAC can seed Aβ(1–40) amyloidogenesis and Aβ can also seed amyloid formation by NAC [21].
2. Although β-synuclein is highly homologous to α-synuclein, it does not fibrillate [30–32]. It has been suggested that this is because of the lack of an 11-amino-acid segment within the central part of the NAC domain (corresponding to residues 73 to 83 of α-synuclein) (see Fig. 1).
3. The rate of α-synuclein association in vitro was shown to decrease after the substitution of Ala76 with charged amino acids (either Arg or Glu) [33]. Furthermore, the deletion of amino acid residues 71–82 in human α-synuclein prevented protein aggregation [33].
4. Finally, analysis of the N-terminal sequence of NAC established the existence of some similarity to a region crucial for aggregation of three other amyloidogenic polypeptides, Aβ, prion protein, and islet amyloid polypeptide. Specifically, a four-residue motif, Gly-Ala-XX, where X is an amino acid with an aliphatic side chain, has been shown to be common to all four peptides [21,34,35].

These observations strongly argue in the favor of direct involvement of the NAC domain in the pathologic aggregation of α-synuclein. Several 'dissection' studies have been performed in order to understand which particular part of NAC is responsible for its tendency to form fibrils [30,34–37]. It has been shown that the amyloidogenicity is not uniformly distributed within NAC [33]. For example, the C-terminal half of the peptide (NAC residues 19–35, i.e. residues 79–95 in α-synuclein) does not fibrillate, whereas the N-terminal fragment can fibrillate [36,37]. Moreover, although some fibrils were detected for the NAC fragment 1–18 (61–78 in α-synuclein), it did not precipitate out of solution to a significant degree. The only NAC fragment that readily formed fibrils and precipitated out of solution was NAC 3–18 (or 63–78 in α-synuclein) [30]. Finally, NAC fragment 8–18 (68–78 in α-synuclein sequence) also showed propensity to fibrillate [36] (see Fig. 2). Interestingly, all the peptides considered so far contained a four-residue motif, Gly68-Ala69-Val70-Val71, which was assumed to be a signature motif of amyloidogenic proteins [34,35].

Another candidate for a major player in α-synuclein fibril formation, namely fragment 71–82, has been revealed in an independent investigation, inspired by the difference in aggregation behavior of α- and β-synucleins [33] (see Fig. 2). It has been shown that α-synuclein with residues 71–82 deleted does not fibrillate. Also, the rate of α-synuclein fibrillation was significantly reduced as a result of substitution of Ala76 with charged Arg or Glu. On the other hand, a synthetic peptide, corresponding to this 12-amino-acid stretch, fibrillated by itself and promoted fibrillation of full-length human α-synuclein in vitro [33]. Importantly, this fragment does not have a 'signature motif' GAXX (see Fig. 2), which is also lacking in fibrillation-prone γ-synuclein thus calling into question the significance of the 'signature motif' model of El-Agnaf et al. [34,35].

Interestingly, it appears that there is a limitation in length

Hum α-synuclein	^{61}EQVTNVGGAVVTGVTAVAQKTVEGAG***S***IAAATGFV^{95}
Hum β-synuclein	^{61}**ASHL****FSG----------****N******L*^{77}
Hum γ-synuclein	^{61}**ANA*SE****S*NT**T****EAE***N***VTS*F*^{95}
Mus α-synuclein	^{61}**********N********^{95}
Giasson et al. [29]	^{71}VTGVTAVAQKTV^{82}
Bodles et al. [26]	^{63}VTNVGGAVVTGVTAVA^{78}
Bodles et al. [32]	^{68}GAVVTGVTAVA^{78}

Fig. 2. Aligned sequences of the NAC regions of human α-, β- and γ-synucleins, mouse α-synuclein and six NAC fragments capable of fibrillation. *GAVV* was proposed as a signature motif of four amyloidogenic proteins, α-synuclein, Aβ, prion protein, and islet amyloid polypeptide [30]. The Ala residue, mutation of which to either an Arg or Glu decreased the rate of α-synuclein polymerization [29], is highlighted. A potential 'inhibitory' Ser87Asn substitution is shown in bold-italic.

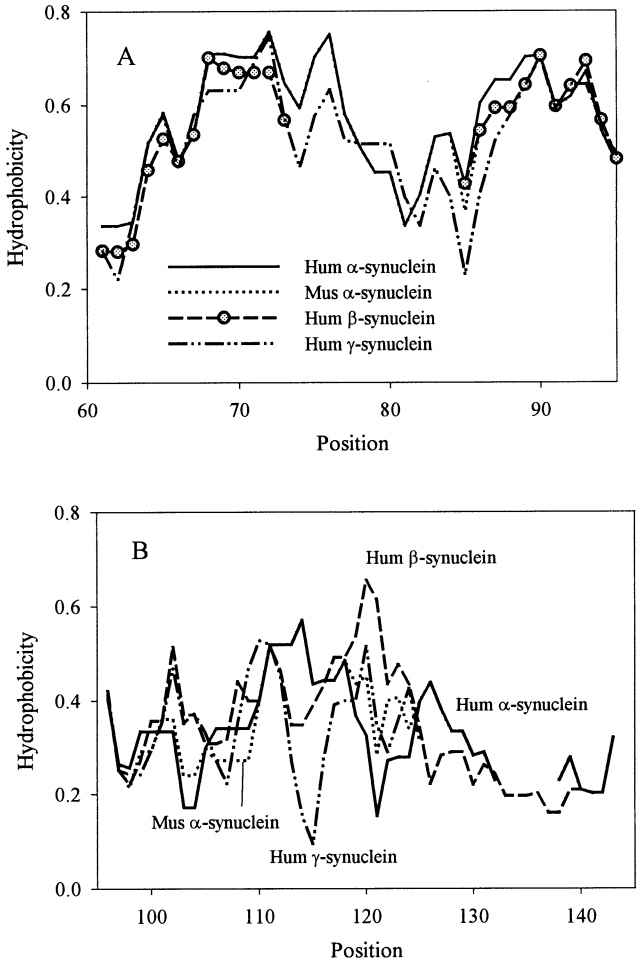

Fig. 3. Hydrophobicity distribution within NAC (A) and C-terminal (B) fragments of optimally aligned sequences of α-, β- and γ-synucleins from human and mouse α-synuclein.

for peptides derived from NAC to be amyloidogenic [36]. In fact, short NAC fragments 8–15(68–75), 8–16(68–76), 9–16(69–76) and 12–18(72–78) were shown to remain absolutely soluble over the three-day incubation, whereas NAC 8–18(68–78) showed significant aggregation, with ~70% of the peptide coming out of solution after three days [36]. Note that two non-aggregating peptides NAC 8–15 and 8–16 contained the 'signature' GAVV motif.

Stretches of hydrophobic amino acid residues, commonly found in amyloidogenic proteins, are usually considered as important prerequisites for aggregation and fibrillation. Three consecutive repeats of the GXXX motif (where X is any of Gly, Ala, Val, Ile, Leu, Phe, Tyr, Trp, Thr, Ser or Met) were implicated in the amyloidogenic behavior of several amyloidogenic proteins [38]. NAC contains three GXXX motifs, ^{68}GAVV^{71}, ^{73}GVTA^{76} and ^{84}GAGS^{87}, with two of them being located within the non-amyloidogenic C-terminal half of NAC (see Fig. 2). On the other hand, the highly amyloidogenic peptide NAC 11–22 (71–82) [33] contains only a single GXXX motif. Furthermore, two out of three such motifs are eliminated by substitutions of glycine with glutamate in fibrillation-prone γ-synuclein (Fig. 2). These observations bring into question the validity of the model of three consecutive GXXX repeats as a major amino acid determinant of fibrillation.

Some additional lessons can be taken from the comparison of the NAC regions derived from different members of the synuclein family. Fig. 2 shows that the NAC regions of human α- and γ-synucleins are rather different, as they have only 18 identical residues out of 35. On the other hand, such divergence does not significantly affect the distribution of hydrophobicity within their amino acid sequences and Fig. 3A illustrates that the NAC domains of all members of the synuclein family possess very similar profiles of hydrophobicity. Another interesting observation is that the central 11-amino-acid residue fragment, which is missing in non-amyloidogenic human β-synuclein, does not represent the most hydrophobic stretch in the NAC domain. Moreover, although between the members of synuclein family the efficiency of fibrillation decreases in the following order: mouse α-synuclein > A53T- > human α-synuclein > A30P ≥ human γ-synuclein ≫ human β-synuclein, the mean hydrophobicities of their NAC domains calculated according to [39] form another trend: human α-synuclein = A53T = A30P (0.5673) > mouse α-synuclein (0.5587) > human β-synuclein (0.5538) > human γ-synuclein (0.5209). Finally, although human and mouse α-synucleins show dramatic difference in the fibrillation rates [40], their NAC domains differ only at a single position (Ser87 in human vs. Asn87 in mouse). These observations call into question the essential role of the central hydrophobic region of α-synuclein and indicate the importance of additional interactions in α-synuclein fibrillation.

3.2. C-terminal domain

Fig. 3B shows that the hydrophobicity distributions within the C-terminal domains of different members of synuclein family vary dramatically. However, these domains possess comparable mean hydrophobicities (calculated according to [39]): human γ-synuclein (0.3457) > mouse α-synuclein (0.3449) > human β-synuclein (0.3403) > human α-synuclein = A53T = A30P (0.3348). Interestingly, the C-terminal domains also differ significantly in their charge (net charges are −4, −11, −12 and −16 for human γ-, mouse α-, human α- and human β-synucleins, respectively). In fact, the C-terminal domain of γ-synuclein, a protein which was shown to form soluble oligomers most readily among the members of synuclein family [33], has the smallest net charge (−4), whereas the carboxy-terminal part of non-amyloidogenic β-synuclein has the largest net charge (−16). This suggests that the charge and hydrophobicity distribution of the C-terminal domains may play a role in the regulation of synuclein aggregation. In accord with this hypothesis it has been shown that C-terminally truncated human α-synucleins (1–130, 1–120 and 1–110) assembled into fibrils much more readily than the full-length protein [13]. Finally, a hybrid protein, comprising the first 97 amino acids of human α-synuclein and the last 48 amino acid residues of human β-synuclein, has been designed [33]. Although this hybrid protein contained a C-terminal domain of slightly increased net charge (−16 vs. −12), it was shown to fibrillate as efficiently as WT protein and formed filaments of similar diameter and appearance [33].

3.3. N-terminal domain

The N-terminal domain has been revealed as another part of the molecule that plays an important role in the modulation of α-synuclein aggregation and fibrillation, based on comparison of the fibrillation and aggregation rates of WT,

A30P and A53T human α-synucleins. It has been shown that both familial PD mutations increase the rate of α-synuclein oligomerization, whereas the rate of mature fibril formation was increased by one (A53T) and decreased by the other (A30P) [40–44]. High-resolution NMR analysis of these three proteins established that the A30P mutation abolished a significant helical propensity found in an N-terminal stretch of the WT protein [45]. From analysis of the Cα chemical shifts of this region it has been suggested that it preferentially adopted a helical conformation (~10% of the time) in the WT and A53T protein, but not in the A30P mutant [45]. As for A53T, the structural alterations resulting from this mutation were shown to be quite minor compared with those induced by A30P, leading to the creation of a short contiguous region with a local preference for extended backbone conformation [45]. These observations are in a good agreement with the results of the WT, A30P and A53T amino acid sequence analyses in terms of hydrophobicity and propensity to form α-helical or β-sheet structure [43]. The hydrophobicity of both mutants is slightly reduced in the vicinity of substitutions [43]. This is an interesting observation, as it has been established that both mutations accelerate aggregation and fibrillation of α-synuclein in vitro [40–44]. On the other hand, the propensity to form α-helical structure is somewhat diminished in the N-terminal regions of both the mutants. However, it has been shown that A30P and A53T α-synucleins might be more prone to form β-structure than WT α-synuclein [43,44]. It is known that aggregated species of many proteins are enriched in β-structure. Moreover, it has been established that transformation of α-helical (or disordered) structure to β-sheets (including intermolecular ones) is a hallmark of aggregation and fibrillation processes [46–55]. Taking into account these observations it has been assumed that the increased propensity for β-structure of A30P and A53T may not be enough to alter the structure of the monomeric proteins, but may affect their aggregation behavior through specific stabilization of an intermolecular β-structure.

3.4. 'Inhibitory' substitutions

It has been shown that β- and γ-synucleins may inhibit the fibrillation of α-synuclein [31,32]. Similarly, inhibition of fibril formation has been reported for a mixture of human and mouse α-synucleins [40]. Comparison of the sequences of mouse α- and human α-, β- and γ-synucleins leads to an interesting hypothesis concerning the existence of a unique 'inhibitory' substitution. There is only a single common residue, Asn87 (located in the C-terminal part of NAC domains, see Fig. 2) in the NAC domain in mouse α-, human β- and γ-synucleins that is different from human α-synuclein, which has serine at this position. Thus, we propose that the soluble heterooligomers of human α- with mouse α-, or human β-, or human γ-synucleins, which lead to the effective suppression of human α-synuclein fibrillation, may be stabilized via specific contacts involving Ser87-Asn87.

4. Concluding remarks

The following conclusions can be made about the sequence determinants of α-synuclein aggregation and fibrillation:

1. The NAC region is *absolutely necessary* for the aggregation and fibrillation of synucleins.

The C-terminal part of NAC (79–95 fragment) is not amyloidogenic, whereas the amino-terminal half of NAC, as well as stretches of nine and more amino acid residues derived from this part, is able to fibrillate and to seed fibrillation of full-length α-synuclein.

GXXX motifs of the NAC domain are not involved in regulation of synuclein fibrillation.

The C-terminal and N-terminal domains of α-synuclein are important for the modulation of its aggregation and/or fibrillation.

The amino acid residue at the position 87 may play an important role in the inhibition of human α-synuclein fibrillation by mouse α- or human β- or γ-synucleins.

Acknowledgements: This work was supported in part by a grant from NIH (NS39985).

References

[1] Lewy, F.H. (1912) in: Handbuch der Neurologie (Lewandowski, M., Ed.), Springer, Berlin.
[2] Forno, L.S. (1996) J. Neuropathol. Exp. Neurol. 55, 259–272.
[3] Okazaki, H., Lipkin, L.E. and Aronson, S.M. (1961) J. Neuropathol. Exp. Neurol. 21, 422–449.
[4] Trojanowski, J.Q., Goedert, M., Iwatsubo, T. and Lee, V.M. (1998) Cell Death Differ. 5, 832–837.
[5] Takeda, A., Mallory, M., Sundsmo, M., Honer, W., Hansen, L. and Masliah, E. (1998) Am. J. Pathol. 152, 367–372.
[6] Lucking, C.B. and Brice, A. (2000) Cell. Mol. Life Sci. 57, 1894–1908.
[7] Spillantini, M.G., Schmidt, M.L., Lee, V.M.Y., Trojanowski, J.Q., Jakes, R. and Goedert, M. (1997) Nature 388, 839–840.
[8] Spillantini, M.G., Crowther, R.A., Jakes, R., Hasegawa, M. and Goedert, M. (1998) Proc. Natl. Acad. Sci. USA 95, 6469–6473.
[9] Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R.C., Di Iorio, G., Golbe, L.I. and Nussbaum, R.L. (1997) Science 276, 2045–2047.
[10] Krüger, R., Kuhn, W., Müller, T., Woitalla, D., Graeber, M., Kösel, S., Przuntek, H., Epplen, J.T., Schöls, L. and Riess, O. (1998) Nat. Genet. 18, 106–108.
[11] Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagara, Y., Sisk, A. and Mucke, L. (2000) Science 287, 1265–1269.
[12] Feany, M.B. and Bender, W.W. (2000) Nature 404, 394–398.
[13] Crowther, R.A., Jakes, R., Spillantini, M.G. and Goedert, M. (1998) FEBS Lett. 436, 309–312.
[14] Wood, S.J., Wypych, J., Steavenson, S., Louis, J.C., Citron, M. and Biere, A.L. (1999) J. Biol. Chem. 274, 19509–19512.
[15] Serpell, L.C., Berriman, J., Jakes, R., Goedert, M. and Crowther, R.A. (2000) Proc. Natl. Acad. Sci. USA 97, 4897–4902.
[16] Conway, K.A., Harper, J.D. and Lansbury Jr., P.T. (2000) Biochemistry 39, 2552–2563.
[17] Ueda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D.A.C., Kondo, J., Ihara, Y. and Saitoh, T. (1993) Proc. Natl. Acad. Sci. USA 90, 11282–11286.
[18] Maroteaux, L., Campanelli, J.T. and Scheller, R.H. (1988) J. Neurosci. 8, 2804–2815.
[19] Clayton, D.F. and George, J.M. (1998) Trends Neurosci. 21, 249–254.
[20] George, J.M., Jin, H., Woods, W.S. and Clayton, D.F. (1995) Neuron 15, 361–372.
[21] Han, H., Weinreb, P.H. and Lansbury Jr., P.T. (1995) Chem. Biol. 2, 163–169.
[22] Davidson, W.S., Jinas, A., Clayton, D.F. and George, J.M. (1998) J. Biol. Chem. 273, 9443–9449.
[23] Segrest, J., De Loof, H., Dohlman, J., Brouilette, C. and Anantharamaiah, G. (1990) Proteins Struct. Funct. Genet. 8, 103–117.

[24] Segrest, J., Jones, M., De Loof, H., Brouilette, C., Venkatachalapathi, Y. and Anantharamaiah, G. (1992) J. Lipid Res. 33, 141–166.
[25] Perrin, R.J., Woods, W.S., Clayton, D.F. and George, J.M. (2000) J. Biol. Chem. 275, 34393–34398.
[26] Weinreb, P.H., Zhen, W.G., Poon, A.W., Conway, K.A. and Lansbury Jr., P.T. (1996) Biochemistry 35, 13709–13715.
[27] Uversky, V.N., Li, J. and Fink, A.L. (2001) J. Biol. Chem. 276, 10733–10744.
[28] Eliezer, D., Kutluay, E., Bussell Jr., R. and Browne, G. (2001) J. Mol. Biol. 307, 1061–1073.
[29] Iwai, A., Yoshimoto, M., Masliah, E. and Saitoh, T. (1995) Biochemistry 34, 10139–10145.
[30] Bodles, A.M., Guthrie, D.J.S., Harriott, P., Campbell, P. and Irvine, G.B. (2000) Eur. J. Biochem. 267, 2186–2194.
[31] Uversky, V.N., Li, J., Souillac, P.O., Jakes, R., Millett, I.S., Doniach, S., Goedert, M. and Fink, A.L. (2002) J. Biol. Chem. 277, 11970–11978.
[32] Hashimoto, M., Rockenstein, E., Mante, M., Mallory, M. and Masliah, E. (2001) Neuron 32, 213–223.
[33] Giasson, B.I., Murray, I.V.J., Trojanowski, J.Q. and Lee, V.M.-Y. (2001) J. Biol. Chem. 276, 2380–2386.
[34] El-Agnaf, O.M.A. and Irvine, G.B. (2000) J. Struct. Biol. 130, 300–309.
[35] El-Agnaf, O.M.A., Bodles, A.M., Guthrie, D.J.S., Harriot, P. and Irvine, G.B. (1998) Eur. J. Biochem. 258, 157–163.
[36] Bodles, A.M., Guthrie, D.J.S., Greeg, B. and Irvine, G.B. (2001) J. Neurochem. 78, 334–395.
[37] El-Agnaf, O.M.A., Jakes, R., Currar, M.D., Middleton, D., Ingenito, R., Bianchi, E., Pesse, A., Neill, D. and Wallace, A. (1998) FEBS Lett. 440, 71–75.
[38] Jarrett, J.T. and Lansbury Jr., P.T. (1992) Biochemistry 31, 12345–12352.
[39] Kyte, J. and Doolittle, B.F. (1982) J. Mol. Biol. 157, 105–132.
[40] Rochet, J-C., Conway, K.A. and Lansbury Jr., P.T. (2000) Biochemistry 39, 10619–10626.
[41] Conway, K.A., Harper, J.D. and Lansbury, P.T. (1998) Nat. Med. 4, 1318–1320.
[42] 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, 9843–9846.
[43] Li, J., Uversky, V.N. and Fink, A.L. (2001) Biochemistry 40, 11604–11613.
[44] Li, J., Uversky, V.N. and Fink, A.L. (2002) NeuroToxicology, in press.
[45] Bussell Jr., R. and Eleizer, D. (2001) J. Biol. Chem. 276, 45996–46003.
[46] Oberg, K., Chrunyk, B.A., Wetzel, R. and Fink, A.L. (1994) Biochemistry 33, 2628–2634.
[47] Uversky, V.N., Khurana, R., Karnoup, A.S., Segel, D., Doniach, S. and Fink, A.L. (1999) Protein Sci. 8, 161–173.
[48] Ismail, A.A., Mantsch, H.H. and Wong, P.T.T. (1992) Biochim. Biophys. Acta 1121, 183–188.
[49] Przybycien, T.M., Dunn, J.P., Valax, P. and Georgiou, G. (1994) Protein Eng. 7, 131–136.
[50] Kendrick, B.S., Cleland, J.L., Lam, X., Nguyen, T., Randolph, T.W., Manning, M.C. and Carpenter, J.F. (1998) J. Pharm. Sci. 87, 1069–1076.
[51] Clark, A.H., Saunderson, D.H. and Suggett, A. (1981) Int. J. Pept. Protein Res. 17, 353–364.
[52] Fink, A.L. (1998) Fold. Des. 3, 9–15.
[53] Nielsen, L., Frokjaer, S., Carpenter, J.F. and Brange, J. (2001) J. Pharm. Sci. 90, 29–37.
[54] Souillac, P.O., Uversky, V.N., Millett, I.S., Khurana, R., Doniach, S. and Fink, A.L. (2002) J. Biol. Chem. 277, 12657–12665.
[55] Souillac, P.O., Uversky, V.N., Millett, I.S., Khurana, R., Doniach, S. and Fink, A.L. (2002) J. Biol. Chem. 277, 12666–12679.