Spectroscopy 15 (2001) 141–150
IOS Press

Aggregation and properties of α-synuclein and related proteins

Omar M.A. El-Agnaf [a,*] and G. Brent Irvine [b]

[a] *Department of Biological Sciences, Lancaster University, Lancaster LA1 4YQ, UK*
[b] *Centre for Peptide and Protein Engineering, School of Biology and Biochemistry, Queen's University Belfast, Medical Biology Centre, Belfast BT9 7BL, UK*

Abstract. α-Synuclein has been identified as a component of intracellular fibrillar protein deposits in several neurodegenerative diseases, and two mutant forms have been associated with early onset Parkinson's disease. A fragment of α-synuclein has also been identified as the non-Aβ component of Alzheimer's disease amyloid (NAC). Ageing solutions of α-synuclein and NAC leads to formation of β-sheet, detectable by circular dichroism spectroscopy, and aggregation to form amyloid-like fibrils, detectable by electron microscopy. Differences in the rates of aggregation of the fibrils formed by α-synuclein and the two mutant proteins are presented. The toxicity of α-synuclein and related peptides towards neurons is also discussing in relation to the aetiology of neurodegenerative diseases.

Experiments on fragments of NAC have enabled the region of NAC responsible for its aggregation and toxicity to be identified.

Keywords: α-Synuclein, non-Aβ-component (NAC), Parkinson's disease, amyloid, fibrils

Abbreviations: Aβ, amyloid β-peptide; NAC, non-Aβ-component of Alzheimer's disease amyloid.

1. Introduction

Synucleins are a family of small proteins (127–140 amino acid residues for the human forms) expressed at highest levels in nervous tissue. Three members, α-, β-, and γ-synucleins, are the products of three genes present on three different chromosomes [10]. A fourth member, synoretin, is expressed most highly in retina [49]. The first indication of an involvement of α-synuclein in the pathogenesis of neurodegenerative diseases came from the isolation, from purified amyloid of Alzheimer's disease brains, of a novel peptide unrelated to Aβ. This peptide, representing about 10% of the non-SDS soluble material, was named non-Aβ-component of Alzheimer's disease amyloid (NAC). Sequencing revealed that NAC comprised at least 35 amino acids, although the N-terminal residues could not be assigned with certainty because of the specificity of the enzyme used in sequencing [53]. These 35 amino acids correspond to residues 61–95 of a 140 amino-acid precursor (NACP), later shown to be identical to α-synuclein. Interest in α-synuclein was enormously enhanced when two different mutations in the α-synuclein gene were found in inherited forms of Parkinson's disease. One mutation, α-synuclein(A53T), found in certain Italian and Greek families, results in an Ala^{53} to Thr substitution in a region predicted to adopt an α-helical structure surrounded by β-sheets [42]. The other mutation, α-synuclein(A30P), an Ala^{30} to Pro change, was detected in a family of German origin [32]. It has been suggested that these amino acid substitutions

*Corresponding author. Tel.: 44 1524 593480; Fax: 44 1524 843854; E-mail: o.el-agnaf@lancaster.ac.uk.

0712-4813/01/$8.00 © 2001 – IOS Press. All rights reserved

may disrupt local α-helical structure, extending the β-pleated sheet and so rendering mutant α-synuclein more prone to self-aggregation [32,42]. This might lead to the formation of abnormal deposits in the brain, in an analogous manner to the accumulation of protein aggregates such as amyloid β-peptide (Aβ) or tau in Alzheimer's disease, huntingtin protein in Huntington's disease, and prion protein in the transmissible spongiform encephalopathies.

Lewy bodies and Lewy neurites constitute the main pathological features in the brains of patients with Parkinson's disease and dementia with Lewy bodies. Lewy bodies and Lewy neurites contain α-synuclein in a fibrillar form [3,45,46]. Thus, these α-synuclein fibrils could be analogous to the insoluble protein aggregates found in other forms of neurodegenerative disease. Additional immunohistochemical and immunoelectron microscopy studies have shown that α-synuclein is also associated with pathological lesions in other neurodegenerative diseases involving non-neuronal cells, such as the glial cytoplasmic inclusions found in multiple system atrophy [34,47,55]. Several neurodegenerative diseases involving α-synuclein, collectively known as synucleinopathies, are discussed in a recent review [51].

Protein conformation-dependent neurotoxicity is an emerging theme in neurodegenerative disorders such as Alzheimer's disease, Huntington's disease and prion disease [33]. A similar conformation-dependent mechanism may also be relevant to the synucleinopathies. Thus there have been several studies into the aggregation of α-synuclein, its mutants and NAC, and attempts to determine what structural features govern this behaviour. We describe below studies on the aggregation of NAC and α-synuclein, wild-type and mutant forms, as well as conformational changes associated with fibril formation. Toxicity of these proteins to cultured cells is also discussed.

2. Aggregation of NAC

Since NAC was originally isolated from an amyloid preparation it was not surprising that subsequently NAC was found to aggregate *in vitro* [27,31]. These aggregates have been shown, by thioflavin-S staining, Congo Red staining and Fourier-transform infrared spectroscopy, to contain β-sheet structure, indicative of the presence of amyloid-like fibrils. Electron microscopy revealed the presence of clumps of short irregular fibrils of variable length, mainly of diameter 4–11 nm, similar in size to those found in neuritic plaques [17,18,27,31]. A representative example of fibrils formed by NAC is shown in Fig. 1.

Changes in secondary structure when solutions of proteins are aged (i.e., incubated, usually at 37°C, for varying times) can be quantified by circular dichroism spectroscopy. A transition from totally random structure to predominantly β-sheet upon ageing of NAC in solution can be seen in Fig. 2 [17,18]. The formation of amyloid can be revealed by binding to thioflavin-T, with consequent increase in fluorescence. The rate of amyloid formation by NAC using this assay is shown in Fig. 3.

Neurotoxicity has been linked with the formation of β-sheet structure and aggregation. The 25–35 region is the minimum biologically active fragment of Aβ that retains the ability to form β-sheet and aggregate, and is neurotoxic [41]. The identification of this bioactive region within Aβ has led to the design of inhibitors that are capable of blocking the aggregation and neurotoxic capabilities of this highly amyloidogenic peptide [48]. By analogy we decided to try to define the region of NAC responsible for directing its rapid aggregation. Examination of the N-terminal half (residues 1–18) of NAC revealed a degree of sequence similarity to regions crucial for aggregation and toxicity of several other amyloidogenic proteins. Secondary structure prediction analysis of NAC lent support to our hypothesis, since the N-terminal half was predicted to form β-sheet whereas the C-terminal half was predicted to form α-helix. To define the amyloidogenic region of NAC we therefore compared the biophysical properties

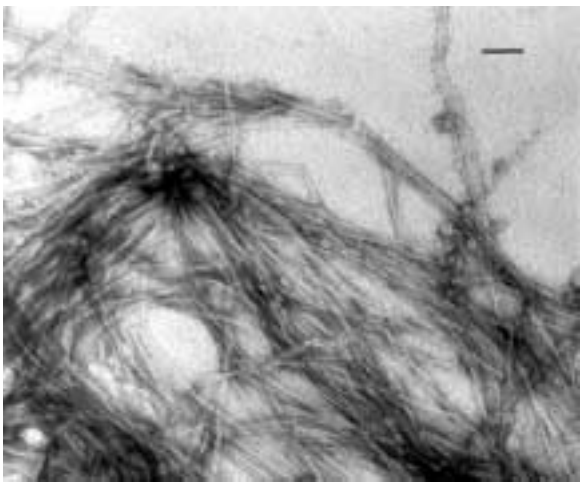


Fig. 1. Negatively stained electron micrograph (EM) of fibrils obtained from aged NAC(1–35) solution. The EM showed fibrils of different morphology of fibrils including short and long 4–12 nm wide fibrils (scale bar = 100 nm).

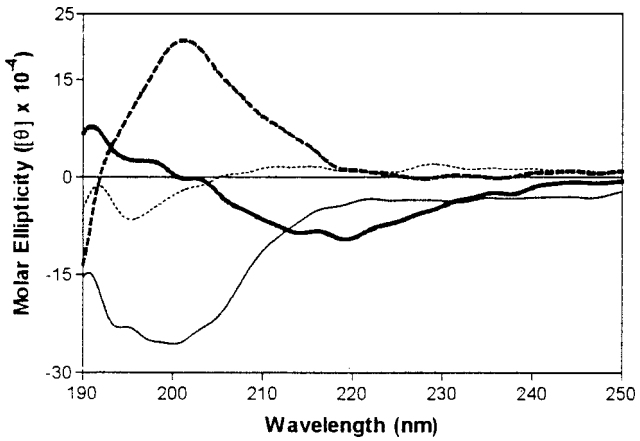


Fig. 2. Effect of ageing on the CD spectra of solutions (100 μM) NAC(1–35) and NAC(1–18) peptides in phosphate buffer saline (pH 7.4). CD spectra were obtained for freshly prepared solutions and after incubation at 37°C for 7 days: NAC(1–35) at time 0 (light solid line); NAC(1–35) at 7 days (heavy solid line); NAC(1–18) at time 0 (light dotted line); NAC(1–18) at 7 days (heavy dotted line). This figure is adapted from El-Agnaf et al. [17].

of an N-terminal fragment, NAC(1–18), a C-terminal fragment, NAC(19–35), and full length NAC [18]. Upon incubation at 37°C for 7 days the N-terminal fragment, NAC(1–18), displayed transition from random coil to β-sheet structure upon ageing (Fig. 2). In contrast, a "reverse peptide" NAC(18–1) and the C-terminal fragment, NAC(19–35), remained random coil under the same conditions (Fig. 4) [17, 18]. Aggregation was measured by percentage of peptide remaining in solution after centrifugation, using an HPLC assay. After incubation at 37°C for 3 days almost 50% of NAC(1–18) was sedimentable whereas NAC(19–35) remained in solution (Fig. 5). NAC(1–18) aggregates gave positive staining with thioflavin-S, indicative of the β-pleated sheet conformation characteristic of amyloid fibrils. Electron microscopy on the aggregated samples revealed fibrils of diameter 5–10 nm [17,18].

To pinpoint the exact region responsible for NAC aggregation we repeated similar experiments on smaller fragments of NAC. NAC(8–18) (residues 68–78 of α-synuclein) was the smallest fragment that

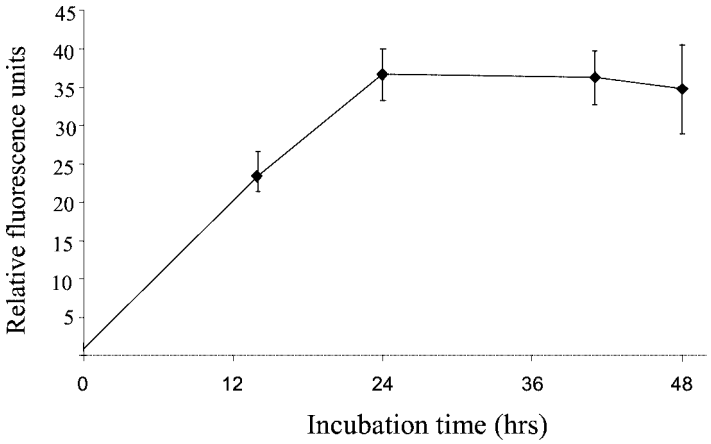


Fig. 3. NAC(1–35) aggregation as monitored by thioflavin T fluorometric assay. The increase in thioflavin T fluorescence is a measure of the concentration of aggregates with β-sheet conformation, present after incubation of a solution of peptide (1 mg/ml) at 37°C. For each data point, error bars indicate 95% confidence limits.

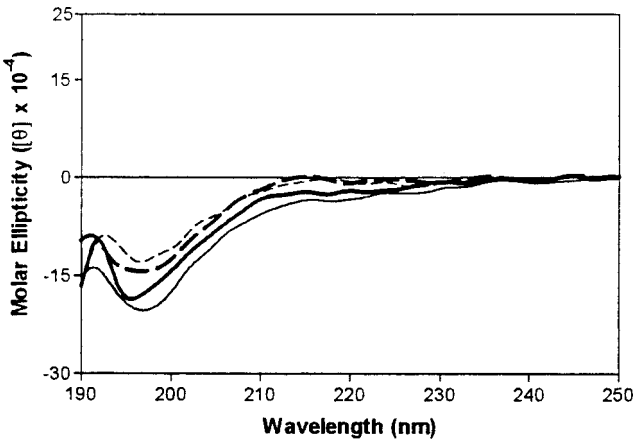


Fig. 4. Effect of ageing on the CD spectra of NAC(19–35) and NAC(18–1) solutions at 100 μM in PBS (pH 7.4). CD spectra were obtained for freshly prepared solutions and after incubation at 37°C for 7 days: NAC(19–35) at time 0 (light dashed line); NAC(19–35) at 7 days (heavy dashed line); NAC(18–1) at time 0 (light solid line); NAC(18–1) at 7 days (heavy solid line). This figure is adapted from El-Agnaf et al. [17].

aggregated, as indicated by concentration of peptide remaining in solution after 3 days, and formed fibrils, as determined by electron microscopy [8]. The importance of the NAC region in controlling aggregation of α-synuclein has also been emphasised by other workers. Consideration of the primary structure suggested residues 72–84 in α-synuclein were critical for its more rapid aggregation compared to β- and γ-synucleins [6]. A peptide comprising residues 71–82 of α-synuclein has also been shown to aggregate [26]. We believe that the fact that there is only partial overlap between the sequence defined by our work and that suggested by these other groups is a consequence of the reductionist approach we adopted. We investigated smaller and smaller fragments until we were able to define the minimum required sequence.

It should be noted that the presence of NAC in Aβ-containing plaques from Alzheimer's disease patients has been disputed by other groups who were unable to show immunostaining of plaques by

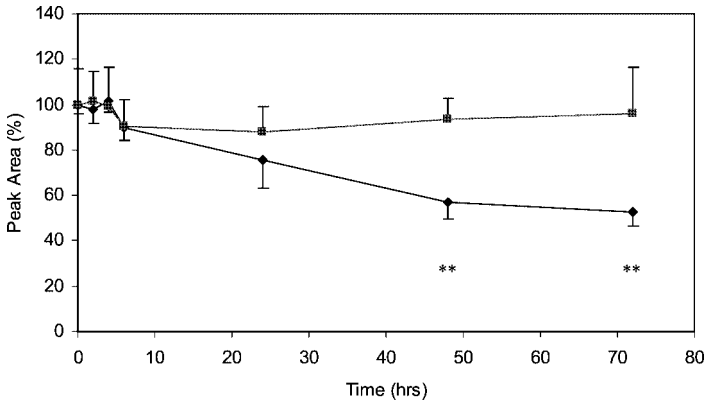


Fig. 5. Aggregation of peptides NAC(1–18) (filled diamond) and NAC(19–35) (filled square). Peptides (0.4 mM) in PBS containing 0.025% sodium azide, were incubated at 37°C and aliquots assayed at intervals over 72 h. Each data point represents the mean of at least four independent experiments. Standard deviations are shown for each point as bars above or below the mean; in some cases the spread is smaller than the symbol used and bars are not therefore visible. Statistical comparisons between the behaviour of the two peptides were performed using Student's T-test. Significant differences are indicated by ** ($p < 0.001$). This figure is reproduced from El-Agnaf et al. [18] and reprinted with permission from Blackwell Science, Oxford, UK.

antibodies to the NAC region [4,14]. It is possible that some of the confusion may arise from the diagnosis as Alzheimer's disease of cases of Lewy body variant of Alzheimer's disease. For example, α-synuclein accumulates in dystrophic neurites that decorate the core of Aβ plaques from patients with Lewy body variant, but not in comparable material from typical Alzheimer's disease patients [57].

3. Aggregation of α-synuclein

α-Synuclein comprises 140 amino acids in two domains, linked via the NAC sequence. The C-terminal domain contains many acidic amino acid residues. It can undergo post-translational modification, including phosphorylation of Ser^{87} and Ser^{129} [37] and Tyr^{125} [21]. The N-terminal domain, which is highly conserved between species, comprises 7 repeats of a degenerate 11-amino-acid motif. This feature is reminiscent of many apolipoproteins that form amphipathic α-helices [23], and indeed α-synuclein can bind to small acidic phospholipid vesicles, resulting in a marked increase in α-helicity [15]. Experimental confirmation that it is indeed the N-terminal domain of α-synuclein that binds lipid and increases its α-helicity has been obtained by NMR spectroscopy [20]. However, in its native state α-synuclein is unfolded and may function as a chaperone protein. It is also much more soluble than is NAC [56]. Indeed it was only after the link to Parkinson's disease was discovered that α-synuclein was shown to be capable of aggregating to form amyloid-like fibrils similar to those that have been isolated from Lewy bodies [28].

Although it is clear that two mutations in the α-synuclein gene can give rise to an inherited form of Parkinson's disease, the mechanism by which the disease occurs is not known. One possibility is that α-synuclein containing these mutations aggregates more rapidly, and one or both α-synuclein mutations have been reported to accelerate the aggregation process [11,16,24,35]. Aggregates formed from wild-type α-synuclein or from either mutant were thioflavine-S positive, indicative of the presence of aggregates with the β-pleated sheet conformation characteristic of amyloid fibrils [16,28]. Anti-parallel β-sheet structure in wild-type and mutant aggregates has been confirmed by Fourier transform infrared spectroscopy [13,35].

We found that self-oligomerisation could be detected by silver staining after SDS-PAGE of freshly dissolved or aged samples of wild-type and mutant α-synucleins [19]. Larger amounts of dimeric species and oligomers were present in aged than in fresh solutions. Furthermore, increased amounts of high molecular weight species that did not enter the separating gel were found in aged samples, suggesting the formation of larger aggregates. α-Synuclein aggregation is temperature, concentration, and time-dependent [24,28,35]. More recently, the kinetic mechanism of α-synuclein aggregation was studied and found to be rate limited by a nucleation step. Addition of preformed fibrils of α-synuclein caused rapid aggregation of a supersaturated solution, bypassing a lag phase that occurred in the absence of seeding. Aggregate growth followed first-order kinetics with respect to α-synuclein concentration, and α-synuclein(A53T) could seed the aggregation of wild-type α-synuclein. In addition, the wild-type and mutant forms of α-synuclein had similar critical concentrations, as measured by peptide remaining in solution after complete aggregation and attainment of equilibrium between peptide in solution and peptide in fibrillar form. These results led the authors to suggest that the more rapid rate of aggregation of A53T α-synuclein compared to wild-type could not be explained by decreased solubility but was due to increased nucleation rate [58]. A partially folded form of α-synuclein, stabilised by low pH and high temperatures, has been detected and may be an intermediate in the conversion between monomer and oligomeric forms [54]. A variety of non-fibrillar oligomers, including protofibril-like chains of spheres and rings that may represent circularised protofibrils, has been detected by atomic force microscopy. The disappearance of the monomeric form of the A30P mutant in the absence of fibril formation may reflect formation of such non-fibrillar forms [12]. Some differences in the morphology of fibrils formed by wild-type and mutant α-synucleins have been reported [16,24]. We used electron microscopy to examine uranyl acetate-stained fibrils preparations, made from aggregated samples of α-synucleins [16]. Fibrils of variable length and 5–25 nm in diameter from aged samples of α-synuclein proteins, similar in size to those found in Lewy bodies and Lewy neurites were detected. In general, the mutant synucleins produced fibrils of greater diameter than did wild-type.

The structures of wild-type and mutant α-synuclein proteins in solution have been studied using circular dichroism spectroscopy. α-Synuclein in freshly prepared solutions was found to exist in random conformation, a condition that has been described as "natively unfolded" [56]. However, ageing a solution of α-synuclein(A53T) led to a change in secondary structure from predominantly random to a mixture of β-sheet and random conformations [11,16]. In contrast, when a similar experiment was carried out on wild-type and mutant α-synuclein(A30P) under similar conditions, the spectrum of both proteins showed less change upon ageing [12,16]. The fact that we did not directly observe a transition to β-sheet during ageing and aggregation of wild-type α-synuclein and the mutant α-synuclein(A30P) may be due to the particular kinetics of aggregation for these proteins [16]. The use of the membrane-mimicking solvent aqueous acetonitrile, which is known to stabilise β-sheet [18,22,36,59] emphasises the structural differences between wild-type and mutant α-synucleins [16]. Freshly prepared solutions of α-synuclein mutants in acetonitrile/phosphate buffer saline mixtures (1 : 1) exhibited β-sheet conformation. The β-sheet content of mutant α-synuclein(A53T) was even higher than that of the mutant α-synuclein(A30P) as suggested by the intensity of the circular dichroism spectra. In contrast, wild-type α-synuclein under the same conditions gave a spectrum with a mixture of random and β-sheet conformations. These results would suggest that structural transition from random coil to predominantly β-sheet must occur as a prelude to aggregation for wild-type and mutant α-synucleins. Such a transition in secondary structure may well be a general prelude to the formation of toxic fibrils by amyloidogenic proteins, as has been suggested previously [33].

4. α-Synuclein toxicity

The similarity between NAC and other amyloidogenic proteins that contain neurotoxic regions, with respect to their sequence and their propensity to form fibrils consisting of β-sheet, led us to believe that NAC might also be neurotoxic [18]. Although the existence of extracellular NAC remains controversial, there have been recent reports that α-synuclein is present in CSF [9] and in extracellular Lewy bodies [50]. If α-synuclein accumulates in neurons, which eventually die, aggregates could leak out of the dead neuron and spread the disease to neighbouring cells [34]. Moreover, α-synuclein has been shown to coexist with tau in neurons in Alzheimer's disease [2] diffuse Lewy body disease [30] and multiple system atrophy [40]. The toxicity of α-synuclein and related peptides may thus have pathological relevance in several neurodegenerative diseases. On this basis we investigated the *in vitro* toxicity of wild-type and mutant α-synucleins and NAC-related peptides [17].

One indicator of toxicity is the inhibition of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction to MTT formazan, a widely used assay for measuring cell viability. Human dopaminergic neuroblastoma SH-SY5Y cells were exposed for 2 days to freshly prepared or aged solutions of α-synuclein proteins or NAC-related peptides. In cells that were treated with freshly prepared α-synucleins for 2 days at 10 μM concentration there was only about 20% loss of cell viability compared to control conditions. By contrast, there was a greater loss of about 55% of cell viability upon exposure to aged α-synucleins at the same concentration. Having previously shown that the N-terminal half of NAC is responsible for the aggregation of the full-length peptide we investigated whether toxicity too was associated with this region [7,8,17]. Toxicity of aged NAC was first detected at a concentration of 0.001 μM, whereas that of fresh NAC first arose at 0.1 μM. In contrast, NAC(1–18) was toxic at the same concentration whether prepared fresh or aged, as was the amyloidogenic control peptide Aβ(25–35). By contrast, neither fresh nor aged solutions of NAC(19–35) nor the reverse peptides Aβ(35–25) and NAC(18–1) affected cell survival compared to controls [17]. These results indicate that the toxicity of α-synuclein and NAC is sequence specific and increased by ageing in solution, meaning that it is dependent on aggregation and formation of fibrils similar to the toxicity of other amyloid peptides [33]. It is also apparent that the amyloidogenic portion of α-synuclein fragment NAC(1–18) mediated the toxic effect of α-synuclein and NAC. We therefore decided to look more closely at the N-terminal half of NAC in order to define the minimum biologically active region. NAC(3–18) was toxic, displayed β-sheet secondary structure and aggregated, forming fibrils, whereas two shorter fragments, NAC(1–13) and NAC(6–14) had none of these properties [7]. By examination of still smaller fragments we showed that NAC(8–18) and NAC(8–16) are toxic, whereas NAC(12–18), NAC(9–16) and NAC(8–15) are not. NAC(8–16) is thus the minimum toxic fragment. As mentioned above, however, NAC(8–18) was the smallest fragment that aggregated and formed fibrils. Circular dichroism spectroscopy indicated that none of the short peptides converted to β-sheet structure, but rather all remained random coil throughout 24 hours. However, in acetonitrile, an organic solvent known to induce β-sheet, fragments NAC(8–18) and NAC(8–16) both formed β-sheet structure [8].

We also investigated the mechanism involved in the toxicity induced by α-synucleins and NAC peptides. Morphological examination of cell nuclei, stained with the DNA-binding fluorochrome Hoechst 33258, showed that several cells exposed to α-synucleins or α-synuclein fragments NAC and NAC(1–18) presented a typical apoptotic morphology, including condensation of chromatin and nuclear fragmentation [17].

There have been several reports that implicate oxidative damage to the aetiology of neurodegenerative diseases. Incubation of α-synuclein in an oxidative system derived from cytochrome *c* and hydrogen

peroxide increased the rate of aggregation of the former protein. Moreover, cytochrome c and α-synuclein were shown, by double labelling, to be co-localised in Lewy bodies from brains of Parkinson's disease patients [29]. Oxidative damage, in the form of nitrated α-synuclein in Lewy bodies, has been directly demonstrated using an antibody that recognised the nitrated protein [25]. Overexpression of α-synuclein is toxic to neurons [38,43]. Iron and free radical generators stimulate the production of intracellular aggregates of α-synuclein [39]. NAC and α-synuclein, but not β- or γ-synucleins, liberate hydroxyl radicals when incubated with iron(II) [52].

Inclusions containing ubiquitinated proteins have been detected in several neurological disorders and ubiquitin has been used as a marker for Lewy bodies. Ubiquitin tags proteins earmarked for degradation by the proteasome. It has been suggested that failure to degrade ubiquitinated protein aggregates may be part of the pathology of neurodegenerative diseases [1] and indeed it has been shown that inhibition of proteasome activity results in decreased α-synuclein turnover [5]. In a recent significant development, a link has been discovered between two proteins, parkin and α-synuclein, that have mutant forms known to be implicated in Parkinson's disease. Normal parkin is an E3 ubiquitin ligase, and a mutation in this protein is associated with autosomal recessive Parkinson's disease. Normal parkin, but not the mutant type, forms a complex involving a glycosylated form of α-synuclein. Defective parkin thus resulted in accumulation of non-ubiquinated synuclein [44].

Acknowledgements

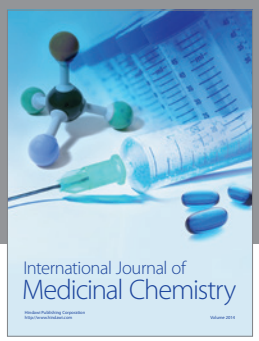
Parts of studies summarised here were supported by grant from The Parkinson's Disease Society UK to Dr. Omar El-Agnaf. We also thank Dr. Yvonne Davies (Lancaster University, UK) for the EM.

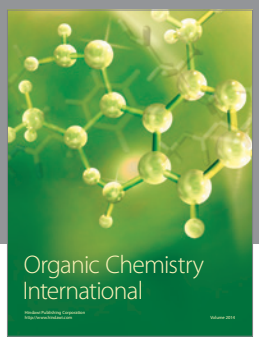
References

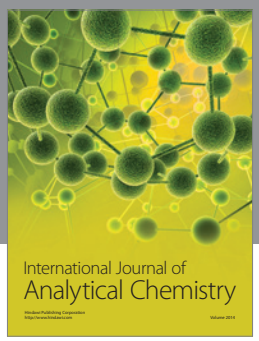
[1] A. Alves-Rodrigues, L. Gregori and F. Figueiredo-Pereira, Ubiquitin, cellular inclusions and their role in neurodegeneration, *Trends Neurosci.* **21** (1998), 516–520.
[2] Y. Arai, M. Yamazaki, O. Mori, H. Muramatsu, G. Asano and Y. Katayama, α-Synuclein-positive structures in cases with sporadic Alzheimer's disease: morphology and its relationship to tau aggregation, *Brain Res.* **888** (2001), 287–296.
[3] M. Baba, S. Nakajo, P.-H. Tu, T. Tomita, K. Nakaya, V.M.-Y. Lee, J.Q. Trojanowski and T. Iwatsubo, Aggregation of α-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies, *Am. J. Pathol.* **152** (1998), 879–884.
[4] T.A. Bayer, P. Jäkälä, T. Hartmann, L. Havas, C.A. McLean, J.G. Culvenor, Q.-X. Li, C.L. Masters, P. Falkai and K. Beyreuther, α-Synuclein accumulates in Lewy bodies in Parkinson's disease and dementia with Lewy bodies but not in Alzheimer's disease β-amyloid plaque cores, *Neurosci. Letts.* **266** (1999), 213–216.
[5] M.C. Bennett, J.F. Bishop, Y. Leng, P.B. Chock, T.N. Chase and M.M. Mouradian, Degradation of α-synuclein by proteasome, *J. Biol. Chem.* **274** (1999), 33 855–33 858.
[6] A.L. Biere, S.J. Wood, J. Wypych, S. Steavenson, Y. Jiang, D. Anafi, F.W. Jacobsen, M.A. Jarosinski, G.-M. Wu, J.-C. Louis, F. Martin, L.O. Narhi and M. Citron, Parkinson's disease-associated α-synuclein is more fibrillogenic than β- and γ-synuclein and cannot cross-seed its homologs, *J. Biol. Chem.* **275** (2000), 34 574–34 579.
[7] A.M. Bodles, D.J.S. Guthrie, P. Harriott, P. Campbell and G.B. Irvine, Toxicity of non-Aβ component of Alzheimer's disease amyloid, and N-terminal fragments thereof, correlates to formation of β-sheet structure and fibrils, *Eur. J. Biochem.* **267** (2000), 2186–2194.
[8] A.M. Bodles, D.J.S. Guthrie, B. Greer and G.B. Irvine, Identification of the region of non-Aβ component of Alzheimer's disease amyloid responsible for its aggregation and toxicity, *J. Neurochem.* **78** (2001), 384–395.
[9] R. Borghi, R. Marchese, A. Negro, L. Marinelli, G. Forloni, D. Zaccheo, G. Abbruzzese and M. Tabaton, Full length α-synuclein is present in cerebrospinal fluid from Parkinson's disease and normal subjects, *Neuroscience Letts.* **287** (2000), 65–67.
[10] D.F. Clayton and J.M. George, Synucleins in synaptic plasticity and neurodegenerative disorders, *J. Neurosci. Res.* **58** (1999), 120–129.

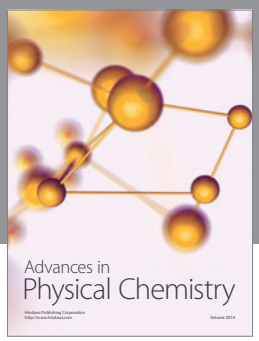
[11] K.A. Conway, J.D. Harper and P.T. Lansbury, Jr., Accelerated *in vitro* fibril formation by a mutant α-synuclein linked to early-onset Parkinson disease, *Nat. Med.* **4** (1998), 1318–1320.
[12] K.A. Conway, S.J. Lee, J.-C. Rochet, T.T. Ding, R.E. Williamson and P.T. Lansbury, Jr., Acceleration of oligomerization, not fibrillization, is a shared property of both α-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy, *Proc. Natl. Acad. Sci. USA* **97** (2000), 571–576.
[13] K.A. Conway, J.D. Harper and P.T. Lansbury, Jr., Fibrils formed in vitro from α-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid, *Biochemistry* **39** (2000), 2552–2563.
[14] J.G. Culvenor, C.A. McLean, S. Cutt, B.C.V. Campbell, F. Maher, P. Jäkälä, T. Hartmann, K. Beyreuther, C.L. Masters and Q.-X. Li, Non-Aβ component of Alzheimer's disease amyloid (NAC) revisited, *Am. J. Pathol.* **155** (1999), 1173–1181.
[15] W.S. Davidson, A. Jonas, D.F. Clayton and J.M. George, Stabilization of α-synuclein secondary structure upon binding to synthetic membranes, *J. Biol. Chem.* **273** (1998), 9443–9449.
[16] O.M.A. El-Agnaf, R. Jakes, M.D. Curran and A. Wallace, Effects of the mutations Ala^{30} to Pro and Ala^{53} to Thr on the physical and morphological properties of α-synuclein protein implicated in Parkinson's disease, *FEBS Letts.* **440** (1998), 67–70.
[17] O.M.A. El-Agnaf, R. Jakes, M.D. Curran, D. Middleton, R. Ingenito, E. Bianchi, A. Pessi, D. Neill and A. Wallace, Aggregates from mutant and wild-type α-synuclein proteins and NAC peptide induce apoptotic cell death in human neuroblastoma cells by formation of β-sheet and amyloid-like filaments, *FEBS Letts.* **440** (1998), 71–75.
[18] O.M.A. El-Agnaf, A.M. Bodles, D.J.S. Guthrie, P. Harriott and G.B. Irvine, The N-terminal region of non-Aβ component of Alzheimer's disease amyloid is responsible for its tendency to assume β-sheet and aggregate to form fibrils, *Eur. J. Biochem.* **258** (1998), 157–163.
[19] O.M.A. El-Agnaf and G.B. Irvine, Review: Formation and properties of amyloid-like fibrils derived from α-synuclein and related proteins, *J. Struct. Biol.* **130** (2000), 300–309.
[20] D. Eliezer, E. Kutluay, R. Bussell, Jr. and G. Brown, Conformational properties of α-synuclein in its free and lipid-associated states, *J. Mol. Biol.* **307** (2001), 1061–1073.
[21] C.E. Ellis, P.L. Schwartzberg, T.L. Grider, D.W. Fink and R.L. Nussbaum, α-Synuclein is phosphorylated by members of the Src family of protein-tyrosine kinases, *J. Biol. Chem.* **276** (2001), 3879–3884.
[22] H. Fabian, G.I. Szendrei, H.H. Mantsch and L. Otvos, Comparative analysis of human and Dutch-type Alzheimer β-amyloid peptides by infrared spectroscopy and circular dichroism, *Biochem. Biophys. Res. Comm.* **191** (1993), 232–239.
[23] J.M. George, H. Jin, W.S. Woods and D.F. Clayton, Characterization of a novel protein regulated during the critical period for song learning in the Zebra finch, *Neuron* **15** (1995), 361–372.
[24] B.I. Giasson, K. Uryu, J.Q. Trojanowski and V.M.-Y. Lee, Mutant and wild type human α-synucleins assemble into elongated filaments with distinct morphologies *in vitro*, *J. Biol. Chem.* **274** (1999), 7619–7622.
[25] B.I. Giasson, J.E. Duda, I.V.J. Murray, Q. Chen, J.M. Souza, H.I. Hurtig, H. Ischiropoulos, J.Q. Trojanowski and V.M.-Y. Lee, Oxidative damage linked to neurodegeneration by selective α-synuclein nitration in synucleinopathy lesions, *Science* **290** (2000), 985–989.
[26] B.I. Giasson, I.V.J. Murray, J.Q. Trojanowski and V.M.-Y. Lee, A hydrophobic stretch of 12 amino acid residues in the middle of α-synuclein is essential for filament assembly, *J. Biol. Chem.* **276** (2001), 2380–2386.
[27] H. Han, P.H. Weinreb and P.T. Lansbury, Jr., The core Alzheimer's peptide NAC forms amyloid fibrils which seed and are seeded by β-amyloid: is NAC a common trigger or target in neurodegenerative disease?, *Chemistry & Biology* **2** (1995), 163–169.
[28] M. Hashimoto, L.J. Hsu, A. Sisk, Y. Xia, A. Takeda, M. Sundsmo and E. Masliah, Human recombinant NACP/α-synuclein is aggregation and fibrillated in vitro: Relevance for Lewy body disease, *Brain Res.* **799** (1998), 301–306.
[29] M. Hashimoto, A. Takeda, L.J. Hsu, T. Takenouchi and E. Masliah, Role of cytochrome c as a stimulator of α-synuclein aggregation in Lewy body disease, *J. Biol. Chem.* **274** (1999), 28 849–28 852.
[30] E. Iseki, W. Marui, H. Akiyama, K. Uéda and K. Kosaka, Degeneration process of Lewy bodies in the brains of patients with dementia with Lewy bodies using α-synuclein-immunohistochemistry, *Neurosci. Letts.* **286** (2000), 69–73.
[31] A. Iwai, M. Yoshimoto, E. Masliah and T. Saitoh, Non-Aβ component of Alzheimer's disease amyloid (NAC) is amyloidogenic, *Biochemistry* **34** (1995), 10 139–10 145.
[32] R. Kruger, W. Kuhn, T. Muller, D. Woitalla, M. Graeber, S. Kosel, H. Przuntek, J.T. Epplen, L. Schols and O. Riess, Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease, *Nature Gen.* **18** (1998), 106–108.
[33] P.T. Lansbury, Jr., Evolution of amyloid: what normal protein folding may tell us about fibrillogenesis and disease, *Proc. Natl. Acad. Sci. USA* **96** (1999), 3342–3344.
[34] E. Mezey, A. Dehejia, G. Harta, M.I. Papp, M.H. Polymeropoulos and M.J. Brownstein, Alpha synuclein in neurodegenerative disorders: murderer or accomplice?, *Nature Med.* **4** (1998), 755–757.
[35] L. Narhi, S.J. Wood, S. Steavenson, Y. Jiang, G.M. Wu, D. Anafi, S.A. Kaufman, F. Martin, K. Sitney, P. Denis, J.-C. Louis, J. Wypych, A.L. Biere and M. Citron, Both familial Parkinson's disease mutations accelerate α-synuclein aggregation, *J. Biol. Chem.* **274** (1999), 9843–9846.

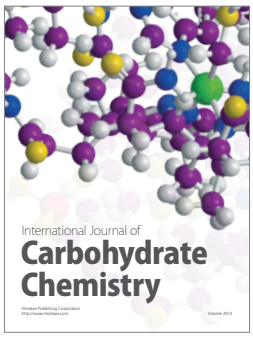
[36] J. Nguyen, M.A. Baldwin, F.E. Cohen and S.B Prusiner, Prion protein-peptides induce alpha-helix to beta-sheet conformational transitions, *Biochemistry* **34** (1995), 4186–4192.
[37] M. Okochi, J. Walter, A. Koyama, S. Nakajo, M. Baba, T. Iwatsubo, L. Meijer, P.J. Kahle and C. Haass, Constitutive phosphorylation of the Parkinson's disease associated-synuclein, *J. Biol. Chem.* **275** (2000), 390–397.
[38] N. Ostrerova, L. Petrucelli, M. Farrer, N. Mehta, P. Choi, J. Hardy and B. Wolozin, α-Synuclein shares physical and functional homology with 14-3-3 proteins, *J. Neurosci.* **19** (1999), 5782–5791.
[39] N. Ostrerova-Golts, L. Petrucelli, J. Hardy, J.M. Lee, M. Farer and B. Wolozin, The A53T α-synuclein mutation increases iron-dependent aggregation and toxicity, *J. Neurosci.* **20** (2000), 6048–6054.
[40] Y.-S. Piao, S. Hayashi, M. Hasegawa, K. Wakabayashi, M. Yamada, M. Yoshimoto, A. Ishikawa, T. Iwatsubo and H. Takahashi, Co-localization of α-synuclein and phosphorylated tau in neuronal and glial cytoplasmic inclusions in a patient with multiple system atrophy of long duration, *Acta Neuropathol.* **101** (2001), 285–293.
[41] C.J. Pike, A.J. Walencewicz-Wasserman, J. Kosmoski, D.H. Cribbs, C.G. Glabe and C.W. Cotman, Structure-activity analyses of β-amyloid peptides: contributions of the β25–35 region to aggregation and neurotoxicity, *J. Neurochem.* **64** (1995), 253–265.
[42] M.H. Polymeropoulos, C. Lavedan, E. Leroy, S.E. Ide, A. Dehejia, A. Dutra, B. Pike, H. Root, J. Rubenstein, R. Boyer, E.S. Stenroos, S. Chandrasekharappa, A. Athanassiadou, T. Papapetropoulos, W.G. Johnson, A.M. Lazzarini, R.C. Duvoisin, G. Di Iorio, L.I. Golbe and R.L. Nussbaum, Mutation in the α-synuclein gene identified in families with Parkinson's disease, *Science* **276** (1997), 2045–2047.
[43] A.R. Saha, N.N. Ninkina, D.P. Hanger, B.H. Anderton, A.M. Davies and V.L. Buchman, Induction of neuronal death by α-synuclein, *Eur. J. Neurosci.* **12** (2000), 3073–3077.
[44] H. Shimura, M.G. Schlossmacher, N. Hattori, M.P. Frosch, A. Trockenbacher, R. Schneider, Y. Mizuno, K.S. Kosik and D.J. Selkoe, Ubiquitination of a new form of α-synuclein by parkin from human brain: implications for Parkinson's disease, *Science* **293** (2001), 263–269.
[45] M.G. Spillantini, M.N. Schmidt, V.M.Y. Lee, J.Q. Trojanowski, R. Jakes and M. Goedert, Alpha-synuclein in Lewy bodies, *Nature* **388** (1997), 839–840.
[46] M.G. Spillantini, R.A. Crowther, R. Jakes, M. Hasegawa and M. Goedert, Alpha-synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies, *Proc. Natl. Acad. Sci. USA* **95** (1998), 6469–6473.
[47] M.G. Spillantini, R.A. Crowther, R. Jakes, M. Hasegawa, N.J. Cairns, P.L. Lantos and M. Goedert, Filamentous α-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies, *Neurosci. Lett.* **251** (1998), 205–208.
[48] C. Soto, E.M. Sigurdsson, L. Morelli, R.A. Kumar, E.M. Castano and B. Fragione, Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer's therapy, *Nature Med.* **4** (1998), 822–826.
[49] A. Surguchov, I. Surgucheva, E. Solessio and W. Baehr, Synoretin – a new protein belonging to the synuclein family, *Mol. Cell. Neurosci.* **13** (1999), 95–103.
[50] T. Togo, E. Iseki, W. Marui, H. Akiyama, K. Uéda and K. Kosaka, Glial involvement in the degeneration process of Lewy body-bearing neurons and the degradation process of Lewy bodies in brains of dementia with Lewy bodies, *J. Neurol. Sci.* **184** (2001), 71–75.
[51] J.Q. Trojanowski and V.M.-Y. Lee, Parkinson's disease and related neurodegenerative synucleinopathies linked to progressive accumulations of synuclein aggregates in brain, *Parkinsonism & Related Disorders* **7** (2001), 247–251.
[52] S. Turnbull, B.J. Tabner, O.M.A. El-Agnaf, S. Moore, Y. Davies and D. Allsop, α-Synuclein implicated in Parkinson's disease catalyses the formation of hydrogen peroxide *in vitro*, *Free Radical Biology and Medicine* **30** (2001), 1163–1170.
[53] K. Ueda, H. Fukushima, E. Masliah, Y. Xia, A. Iwai, D. Otero, J. Kondo, Y. Ihara and T. Saitoh, Molecular cloning of cDNA encoding an unrecognised component of amyloid in Alzheimer disease, *Proc. Natl. Acad. Sci. USA* **90** (1993), 11 282–11 286.
[54] V.N. Uversky, J. Li and A.L. Fink, Evidence for a partially folded intermediate in α-synuclein fibril formation, *J. Biol. Chem.* **276** (2001), 10 737–10 744.
[55] K. Wakabayashi, S. Hayashi, A. Kakita, M. Yamada, Y. Toyoshima, M. Yoshimoto and H. Takahashi, Accumulation of α-synuclein/NACP is a cytopathological feature common to Lewy body disease and multiple system atrophy, *Acta Neuropathol.* **96** (1998), 445–452.
[56] P.H. Weinreb, W. Zhen, A.W. Poon, K.A. Conway and P.T. Lansbury, Jr., NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded, *Biochemistry* **35** (1996), 13 709–13 715.
[57] O. Wirths, S. Weickert, K. Majtenyi, L. Havas, P.J. Kahle, M. Okochi, C. Haass, G. Multhaup, K. Beyreuther and T.A. Bayer, Lewy body variant of Alzheimer's disease: α-synuclein in dystrophic neuritis of Aβ plaques, *Clin. Neurosci. Neuropathol.* **11** (2000), 3737–3741.
[58] S.J. Wood, J. Wypych, S. Steavenson, J.-C. Louis, M. Citron and A.L. Biere, α-Synuclein fibrillogenesis is nucleation-dependent, *J. Biol. Chem.* **274** (1999), 19 509–19 512.
[59] H. Zhang, K. Kaneko, J.T. Nguyen, T.L. Livshits, M.A. Baldwin, F.E. Cohen, T.L. James and S.B. Prusiner, Conformational transitions in peptides containing 2 putative alpha-helices of the prion protein, *J. Mol. Biol.* **250** (1995), 514–526.

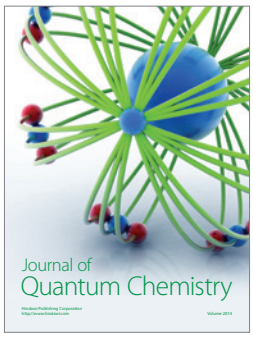


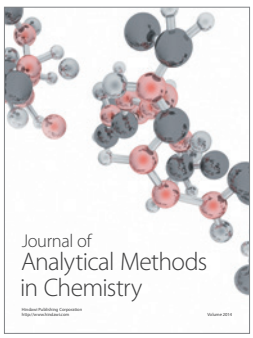


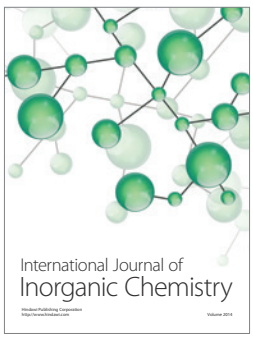


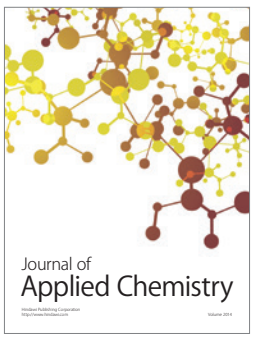


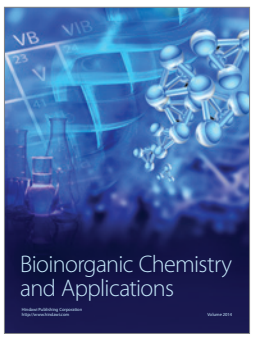


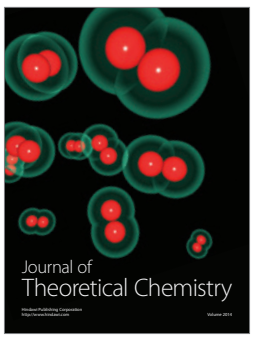


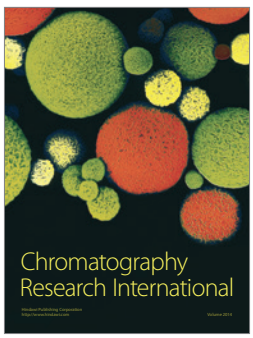












Hindawi

Submit your manuscripts at
http://www.hindawi.com