The aggregation and membrane-binding properties of an α-synuclein peptide fragment

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Abstract
α-Synuclein is a 140 amino acid protein, which is associated with presynaptic membranes in the brain, and is the major component of protein aggregates produced during the progression of many neurodegenerative diseases. It has been shown that a central hydrophobic region of α-synuclein comprising residues 71–82 is required for aggregation of the protein into the fibrillar form found in pathogenic aggregates [Giasson, Murray, Trojanowski and Lee (2001) J. Biol. Chem. 276, 2380–2386]. In the present study, we used 2H NMR and electron microscopy to investigate the aggregation and membrane-binding properties of a synthetic peptide corresponding to this region. Results indicate that this region associates with phospholipid bilayers but also forms amyloid-like fibrils in the absence of lipid membranes.

α-Synuclein
Many neurodegenerative disorders, including Alzheimer’s and Parkinson’s diseases, are associated with misfolded protein aggregation and deposition in areas of the brain [1]. Protein aggregates can take the form of Lewy bodies and Lewy neurites. Lewy bodies have a dense core consisting of a mixture of insoluble, filamentous proteins and lipids, surrounded by a peripheral halo. The major protein component of these inclusions is the 140 amino acid protein α-synuclein [2]. At present, the function of α-synuclein is not known, although its ability to interact with presynaptic membranes suggests possible involvement in the regulation of synaptic vesicle pools [3] and dopamine release control [4]. The native protein is unfolded, but misfolds into a state with a high propensity to aggregate into amyloid fibrils [5]. The sequence of α-synuclein contains three distinct domains. The N-terminal domain (residues 1–70) contains repeat KTKEGV motifs similar to amphipathic α-helical domains of membrane-binding apolipoproteins [6]. A central hydrophobic region (residues 71–82) is believed to be responsible for aggregation based on experimental mutation of the residues and studies of synthetic peptides, which form amyloid fibrils in vitro [7]. The C-terminal domain is highly acidic and contains three tyrosine residues that are extensively nitrated in inclusions, although it is not clear whether nitration occurs before or after the formation of aggregates [8]. α-Synuclein fibrils found in brain extracts and formed in vitro are rigid, unbranched, 10-nm-wide structures, characteristic of amyloid [5]. The aim of the present study was to examine the propensity of a synthetic peptide (VTGVTA-VAQKTV) corresponding to the hydrophobic region of α-synuclein [α-syn(71–82), residues 71–82 from α-synuclein] to aggregate into fibrils and to bind to phospholipid membranes.

Aggregation
α-syn(71–82) was synthesized using solid-phase fmoc [N-(9-fluorenyl)methoxycarbonyl] L-amino acid chemistry, without acetylation or amidation. Purification was achieved using reversed-phase HPLC, and the product was confirmed by MS. Fibrils of α-syn(71–82) were formed by incubating the peptide in solution at 37°C [7]. After incubation for 6 weeks, the peptide formed a white precipitate, which reacted with the amyloid-diagnostic fluorescent agent Congo Red. Electron microscopy showed that the precipitate consisted of straight, unbranched fibrils of approx. 0.5 μm in length (Figure 1).

Membrane binding
The soluble monomeric form of α-syn(71–82) was examined for its ability to bind to multilamellar vesicles of DMPC (1,2-dimyristoyl-sn-glycerol-3-phosphocholine). DMPC was added to α-syn(71–82) solution and centrifuged to remove membrane-bound peptide. The concentration of peptide remaining in the supernatant was analysed after alkaline hydrolysis using the primary amine-reactive agent ninhydrin. Controls of DMPC added to water and centrifuged are subtracted from the data. Figure 2(A) shows that the free peptide concentration in the supernatant decreased when the lipid was added, indicating that the peptide associates with the lipid membranes. The apparent membrane partition coefficient (Kp) for the peptide was calculated using the equation

\[ K_p = \frac{(N_t - N_{aq}) \cdot V_{aq}}{N_{aq} \cdot V_m} \]

where Nt is the total number of moles of peptide, Naq the number of moles of peptide in the aqueous phase, Vaq the volume of aqueous solution andVm the membrane volume.
Electron microscopy shows fibrils formed from 0.9 mM α-syn(71–82) incubated at 37°C, in 10 mM phosphate buffer (pH 7) with continuous agitation for 6 weeks. Scale bar, 100 nm.

Experiments to examine the membrane-binding properties of α-syn(71–82)

(A) The results of a membrane-binding assay, indicating an approx. 50% reduction in α-syn(71–82) concentration, from 0.6 to 0.32 mM, on addition of DMPC to a lipid/peptide molar ratio of 20:1. Error bars were calculated from triplicate results. (B) 2H-NMR spectra of DMPC-d54 (10 mg) lipid in pure form (top trace), after the addition of α-syn(71–82) to a lipid/peptide molar ratio of 20:1 (middle trace), and after the addition of α-syn(71–82) to a lipid/peptide molar ratio of 10:1 (bottom trace). Spectra were recorded at an operating frequency of 61 MHz and are the result of accumulating 10 000 transients with a 1 s recycle delay.

A 2H-NMR spectrum for DMPC-d54 in the absence of peptide is shown in Figure 2(B) (top trace). The spectrum exhibits an outer quadrupole splitting of 25 kHz, which is typical of lipid bilayers. Addition of α-syn(71–82) to the membranes to a lipid/peptide molar ratio of 20:1 caused a flattening of the Pake doublets and a slight reduction in the outer splitting to 23.7 kHz (Figure 2B, middle trace). These changes suggest that α-syn(71–82) interacts with the bilayer surface and indirectly disrupts the bilayer centre [9]. Addition of α-syn(71–82) to a final lipid/peptide molar ratio of 10:1 did not decrease the splitting further (Figure 2B, bottom trace), suggesting that the membrane surface was saturated by this peptide at the 20:1 ratio.

Conclusions

These results suggest that the hydrophobic region α-syn(71–82) associates with the membrane surface, indirectly affecting the orientation of phospholipid molecules within the bilayer. As the hydrophobic region is involved in aggregate formation [7], membrane association may be involved in controlling aggregation of α-synuclein in vivo. Membrane interactions are under tight control within neurons, with disease states resulting when these controls are disturbed. Previous reports suggest that membrane interactions prevent fibrillization and therefore inclusion formation [10], whereas others show the presence of membrane promoting fibrillization [11]. If membrane binding prevents aggregation, disruption of the binding of α-synuclein to membranes would increase the availability of the hydrophobic region to form intermolecular inclusions, leading to aggregation and filament formation [12]. Membrane binding could also promote aggregation by increasing the local concentration of the protein, initiating seeding of protofibrillar intermediates at membrane surfaces. The effects of membrane interactions on α-synuclein structure and rates of aggregation are being investigated further in our laboratory.

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References


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