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Dimeric structures of α-synuclein bind preferentially to lipid membranes

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Abstract

There is substantial evidence which implicates α-synuclein and its ability to aggregate and bind vesicle membranes as critical factors in the development of Parkinson's disease. In order to investigate the interaction between α-synuclein wild type (Wt) and its familial mutants, A53T and A30P with lipid membranes, we developed a novel lipid binding assay using surface enhanced laser desorption/ionisation-time of flight-mass spectrometry (SELDI-TOF MS). Wt and A53T exhibited similar lipid binding profiles; monomeric species and dimers bound with high relative affinity to the lipid surface, the latter of which exhibited preferential binding. Wt and A53T trimers and tetramers were also detected on the lipid surface. A30P exhibited a unique lipid binding profile; monomeric A30P bound with a low relative affinity, however, the dimeric species of A30P exhibited a higher binding ability. Larger order A30P oligomers were not detected on the lipid surface. Tapping mode atomic force microscopy (AFM) imaging was conducted to further examine the α-synuclein–lipid interaction. AFM analysis revealed Wt and its familial mutants can penetrate lipid membranes or disrupt the lipid and bind the hydrophobic alkyl self-assembled monolayer (SAM) used to form the lipid layer. The profile of these studied proteins revealed the presence of 'small features' consistent with the presence of monomeric and dimeric forms of the protein. These data collectively indicate that the dimeric species of Wt and its mutants can bind and cause membrane perturbations.

Keywords: Parkinson's disease; α-Synuclein; Lipid membrane; SELDI-TOF MS; AFM

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder, which is characterized by a loss of dopaminergic neurons in the substantia nigra, resulting in resting tremors, slow movement and rigidity [1]. Prevalence rates of PD are ~1% amongst individuals aged between 65–69 and increase in those aged 80 years and older to ~3% [2].

α-Synuclein is a 14.5 kDa presynaptic protein believed to play an important role in the pathogenesis of PD [3]. Evidence supporting this hypothesis includes the finding that Lewy bodies, cytostolic inclusions characteristic of PD, consist largely of fibrillar α-synuclein [4]; triplication or point mutations (A53T and A30P) in the α-synuclein gene have also been linked to PD [5–7]. Furthermore, expression of human α-synuclein in transgenic mice produces a PD phenotype, which is both age and dose-dependent [8].

The mechanisms by which α-synuclein induces toxicity in PD pathogenesis remain unclear, although there is substantial evidence which implicates lipid membrane binding and the ability of α-synuclein to aggregate as important factors. Fibril formation maybe initiated by a number of factors; (i) misfolded intermediates, which exhibit non-polar patches, binding to other

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intermediates via hydrophobic–intermolecular interactions [9], (ii) increased protein concentration arising from triplication of the α-synuclein gene [5], (iii) exposure to oxidative factors [10], or (iv) interactions with lipids [11]. Point mutations can also accelerate α-synuclein fibrilisation, supporting the premise that this event may be a critical factor in disease pathogenesis [9,12]. The process of fibril formation is often preceded by oligomeric intermediates termed protofibrils, these are consumed as more stable fibrils are formed. It is these intermediate products, rather than the fibrils, which are believed to be the toxic species responsible for the neurodegeneration associated with the disease [13].

The α-synuclein–membrane interaction is considered to be a potential cytotoxic event, resulting in the permeabilisation of the cell membrane [14]. Upon interactions with lipid membranes, α-synuclein undergoes a conformational change from a random coil to an α-helical structure [15]. The N-terminus of α-synuclein contains a series of 11 amino acid repeats with a conserved hexameric motif (KTKEGV), which is characteristic of lipid binding α-helical domains of apolipoproteins and facilitates this inducible structural change. The acidic C-terminus of the protein, however, remains essentially unstructured [15–17]. Examination of the familial point mutations revealed that A53T binds to membranes with a similar affinity to α-synuclein wild type (Wt), whilst A30P exhibits weaker binding [18–21]. Interestingly, it is the intermediate oligomers which bind to membranes with a high affinity and cause more rapid permeabilisation of cell membranes than the monomeric species [13,14,22]. Ascertaining the precise oligomeric species that associate and disrupt membranes is thus critical for further understanding of the pathogenesis of the disease process. Towards this aim, we developed a novel assay using surface enhanced laser desorption/ionisation-time of flight-mass spectrometry (SELDI-TOF MS) to measure the ability of α-synuclein Wt, A53T and A30P to bind "on-chip" to synthetic lipid membranes. The α-synuclein–lipid interaction was further examined by atomic force microscopy (AFM) imaging.

2. Materials and methods

2.1. Expression and purification of α-synuclein Wt, A30P and A53T

α-Synuclein Wt and its mutants were prepared as described by Cappai et al. [23]. Briefly, human α-synuclein Wt was cloned into pRSETB (Invitrogen, Carlsbad, CA, USA) and expressed in *Escherichia coli*, BL21 (DE3). The Quikchange mutagenesis kit (Stratagene, La Jolla, CA, USA) was used to generate A30P and A53T. Purification was performed as previously described [23].

2.2. Preparation of α-synuclein Wt, A30P and A53T for functional studies

α-Synuclein Wt, A30P and A53T were initially filtered (0.2 μm Minisart RC4 filters, Sartorius, Goettingen, DEU) to remove pre-formed aggregates and prepared in 10 mM phosphate buffer, pH 7.4 (PB) at a final concentration of 200 μM.

2.3. Preparation of vesicles

Equal amounts of *1*-palmitoyl-2-oeoyl-*sn*-glycero-3 [phospho-L-serine] (POPS) and *1*-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Avanti Polar Lipids, Inc., Alabaster, AL, USA) (16 mg) were dissolved in 2 ml of ethanol-free chloroform. The solvent was evaporated and lipids were re-suspended in 1 ml of PB by mixing (200 rpm) with glass beads for 1 h at 37 °C. The lipids were sonicated for 15 min and subjected to several freeze thaw cycles in liquid nitrogen. The lipids were finally passed through a 0.05 μM filter (Millipore, Bedford, MA, USA) in an Avanti "mini extruder" apparatus until the solution was translucent.

2.4. Formation of a lipid monolayer on H50 ProteinChip arrays

Hydrophobic H50 ProteinChip arrays (Bio-Rad Laboratories, CA, USA) with functional C8 groups were placed in a humidity chamber. Each spot was loaded with 5 μl of CHAPS (30 mg/ml), which was immediately wicked off, followed by three 2 min washes with 5 μl of PB on a shaking platform. The POPC/POPS mixture or PB (5 μl) was applied to each spot and incubated for 2 h at 37 °C. Arrays were washed twice with PB to remove unbound lipid.

2.5. Protein binding to the lipid coated H50 ProteinChip arrays

BSA (Sigma Aldrich, Munich, DEU), melitin (Sigma Aldrich), α-synuclein Wt, A30P or A53T (5 μl, 50 μM) were loaded onto spots coated with either the POPC/POPS lipid mixture or PB and incubated for 5 min with agitation. Arrays were washed twice with PB for 2 min, followed by two 1 min washes with 1 mM HEPES, pH 7.2. The arrays were air dried and l μl of 50% saturated sinapinic acid in 50% (v/v) acetonitrile, 0.5% trifluoroacetic acid was applied onto each spot twice, arrays were air dried between each application. Sinapinic acid was chosen since high mass proteins were to be assessed. Arrays were finally analysed by SELDI-TOF MS (PBSIIc) and subsequent spectra was examined using ProteinChip software® (Bio-Rad Laboratories). Every 5th position on the spot was scanned, 5 transients per position (65 total) were collected.

2.6. SELDI-TOF MS analysis of α-synuclein Wt, A53T and A30P on NP20 ProteinChip arrays

1 μl of α-synuclein Wt, A30P and A53T was loaded onto spots of a NP20 ProteinChip array (Bio-Rad Laboratories) and air dried. Matrix was prepared and applied as described above. Arrays were analysed by SELDI-TOF MS and spectra was examined using ProteinChip software®.

2.7. Preparation of samples for AFM Imaging

The surfaces used were prepared via a 3 step procedure. Glass slides were cleaned with piranha solution (sulphuric acid: H_2O_2 (3:1, v:v) for 1 h and then rinsed with MilliQ water. A mask was applied to the surface in order to generate a spherical surface on the glass substrate allowing improved localization of the sample. 5 nm of chromium (Proscitech, Thuringowa, QLD, AU) and 50 nm of gold (Proscitech) were sequentially sputter coated on the substrates with an EMITECH K575 sputter-coater apparatus (Emitech Ltd., Ashford Kent, UK). Finally, a SAM of octadecanethiol was organized on the gold spherical surface of the substrate by leaving the substrate in a 1 mM solution overnight. The samples were then ready for lipid adsorption. The contact angle for the SAM was measured at 108°±4° with a Dataphysics Contact Angle System OCA 15 plus, at ambient temperature with a CCD camera (Teli) and the computer software 'SCA 20'.

2.8. Formation of a lipid monolayer

The AFM imaging samples were placed in a humidity chamber. Each slide was loaded with 10 μl of CHAPS (30 mg/ml), which was immediately wicked off, followed by five 2 min washes with 10 μl of PB. The POPC/POPS mixture or PB (10 μl) was applied to each spot and incubated for 2 h at 37 °C. Slides were washed with PB three times to remove unbound lipid.

2.9. Protein binding to the lipid coated surfaces

BSA, melitin, α-synuclein Wt, A30P or A53T (10 μl, 50 μM) were loaded onto slides coated with the POPC/POPS lipid mixture or PB and incubated for