Detection and quantification of alpha- synuclein using Fast Field- Cycling magnetic resonance techniques

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Introduction: Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of the dopaminergic neurons in the substantia nigra pars compacta (SNpc). One of the pathological hallmarks of the disease is the formation of cellular inclusions, called Lewy-bodies (LB). Aggregated α- synuclein (α- syn) is the main constituent of these intraneuronal inclusions in the SNpc (Figure 1) [1]. Point mutations in α-synuclein as well as triplication, are known genetic causes of PD [2]. Wild type and mutant alpha-synuclein can form insoluble fibrillar aggregates with antiparallel beta-sheet structure upon incubation at physiological temperature in vitro which is accelerated by PD-linked mutations. It remains unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates (Lewy body). The aim of this research is to investigate the feasibility of detecting and quantifying aggregated proteins using fast field-cycling (FFC) nuclear magnetic resonance relaxometry in conjunction with laboratory protein measurement techniques. This technique is applied to the detection of the $^{14}N^{1}H$ quadrupolar relaxation process [3], which can be seen in various biological samples as characteristic peaks in the R_1 spectrum of water ^{1}H, called quadrupolar peaks. α- syn, like proteins in general, is rich in ^{14}N and when mobility is reduced due to the filamentous web-like structure formed when aggregated; it is a potential source of ^{14}N quadrupole dips in a ^{1}H T_1 dispersion plot [4].

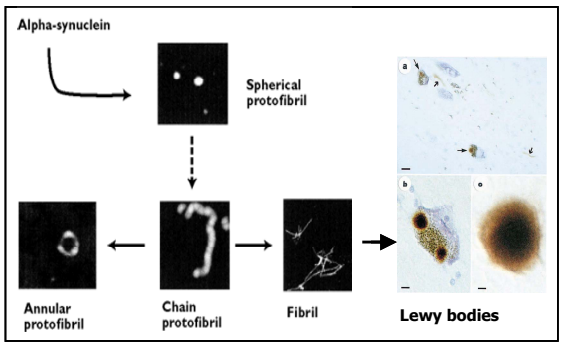

Figure 2: *The change from unfolded soluble α-syn to dense aggregated insoluble α-synuclein (densely stained core) deposits in LB [1] [7]*

Methods: The bacterial expression vector pTrc99A (contains a C-terminal His6 tag) with an inserted human WT α- syn cDNA into the Nco1/ Xho1 site were expressed in *Escherichia coli* BL21 (DE3). The bacterial cell pellets were lysed and the filtered sample was passed over a nickel- agarose affinity column, washed and eluted with imidizole to purify α- syn. A western blott was used to confirm α-syn purification. Aggregation was induced by incubation at 37˚ C with 100 mM sodium acetate pH 6.9 with agitation. 0.2 % agarose was added to ensure homogeneity. Samples were analysed by NMR relaxometry using a STELAR SMARtracer FFC relaxometer. This provided a measure of the T_1 dispersion curve between 1.5 and 3.5 MHz, which included the region of the two main quadrupole dips of ^{14}N (at 49 mT and 65 mT – i.e. 2.1 MHz and 2.8 MHz), using an inversion recovery pulse sequence.

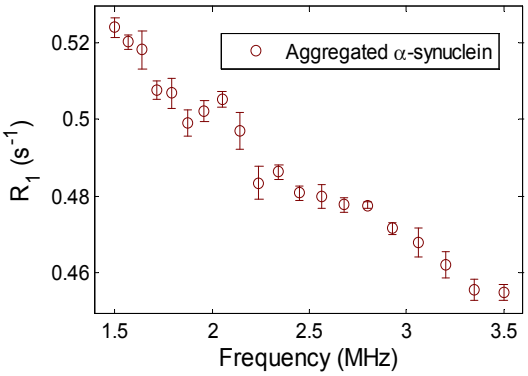

Figure 1: *Illustrated is the R_1 measurement (circle) after aggregation in the region of the quadrupole peaks.*

Results: Preliminary results (Figure 2) indicate that a quadrupolar signal may be achieved from aggregated α-syn. Further work is being done to improve the protocol and determine whether there is a linear relationship [5, 6] between the amplitude of the ^{14}N quadrupolar signal and its concentration. The results from this study may provide more information about this disease model. This could potentially lead to a novel method using FFC MRI to estimate the concentration of in vivo α-syn, which has clinical relevance for LB detection and PD treatment.

References:

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