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PROGRESS REPORT FOR AINGRA09043

PROJECT TITLE	Studies on the structure and dynamics of misfolding proteins in neurodegenerative diseases	
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Specialist Committee	B	

SCIENTIFIC OBJECTIVES

To understand the protein misfolding pathways that convert physiologically occurring proteins into neurotoxic species. These species are the basis of the molecular pathology of Alzheimer's and Parkinson's diseases.

PROGRESS REPORT and RESEARCH OUTCOMES

Introduction

Protein misfolding to form cytotoxic species is common to all neurodegenerative diseases. The misfolding pathways may vary between diseases such as Alzheimer's, Huntington's and Parkinson's (PD) but there is a common link in that the toxic species appear to be soluble oligomers, in contrast to the prominent extra- or intra-cellular deposits that are regarded as end products of the pathological process. Understanding these pathways is important for the development of therapies that would reduce the impact of neurodegenerative disease on individuals and society.

Inclusions of aggregated alpha-synuclein (α -syn), a 140 residue natively unfolded protein, in dopaminergic neurons are a characteristic histological marker of Parkinson's disease (PD). *In vitro*, α -syn in the presence of dopamine (DA) at physiological pH forms soluble SDS-resistant oligomers lacking typical thioflavin T reacting amyloid fibril structures (Cappai et al. 2005). Previous studies have shown that oxidative intermediates of DA play a major part in this process (Leong et al 2009) and that formation of DA- α -syn adducts could explain the dopaminergic pathway of α -syn-associated neurotoxicity in PD (Conway et al. 2001) with implications for current and future PD therapeutic and diagnostic strategies. For these reasons, we have made the interaction of DA with α -syn the first target of our protein misfolding study.

We have used size exclusion chromatography, sedimentation velocity analysis, circular dichroism spectroscopy, mass spectrometry and electron spin resonance spectroscopy to characterise

DA- α -syn oligomers whose supramolecular structure was studied by small angle x-ray scattering (SAXS). Recent advances in molecular shape modelling from SAXS data, e.g the DAMMIN program (Svergun 1997, Konarev et al. 2006) make it possible to accomplish low-resolution shape and internal structure retrieval *ab initio*, not requiring a foreknowledge of the high-resolution structure of the subunits of supramolecular assemblages. We have found that the oligomers are cross linked by DA-quinone molecules that then polymerize to form neuromelanin, thus making an important contribution to our understanding of the involvement of α -syn and DA in the dopaminergic neurotoxicity in PD.

DATA

Results

Our SAXS data show that the trimers formed by the action of DA on α -syn consist of overlapping worm-like, partly unstructured monomers, lacking end-to-end associations (Figure 1). This lack of structure, confirmed by CD and frictional coefficients obtained by sedimentation velocity analysis, contrasts with the well-established, extensive β -sheet structure of the amyloid fibril form of the protein and its oligomers. We propose on the basis of these and earlier data that oxidation of the four methionine residues at the C- and N-terminal ends of α -syn molecule, stabilizes oligomers by cross linking with DA-quinone/DA-melanin formed as a result of the redox process, thus preventing formation of transient β -sheet structure needed for fibrillisation. The highly reactive DA-quinone could form Schiff bases with the amino groups of the 10 lysine residues found in the imperfect repeat series in the N-terminal half plus a single lysine at position 6 and the 4 in the terminal half of the protein, whose sequence is MDVFMKGLSKAKEGVVAAAEKTKQGVAAEAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGNIAAATGFVKKDQMGKGEEGYPQEGILEDMPVDPSSSEAYEMPSEEGYQDYEPEA.

In addition, there are 5 tyrosine residues with which the DA quinone could interact. Molecular weights obtained from the sedimentation velocity analysis of the oligomers show that 20 DA molecules are added for each additional α -syn molecule added to the dimer, suggesting that a cross linked structure similar to that shown in Figure 2 might be formed.

Figure 1 Models of (a), α -sn monomer and (b), α -sn trimer produced using the DAMMIN software. Each model is the result of a different modelling session. (c) possible different modes of assembly of the α -sn trimers, each model corresponds to the one in b

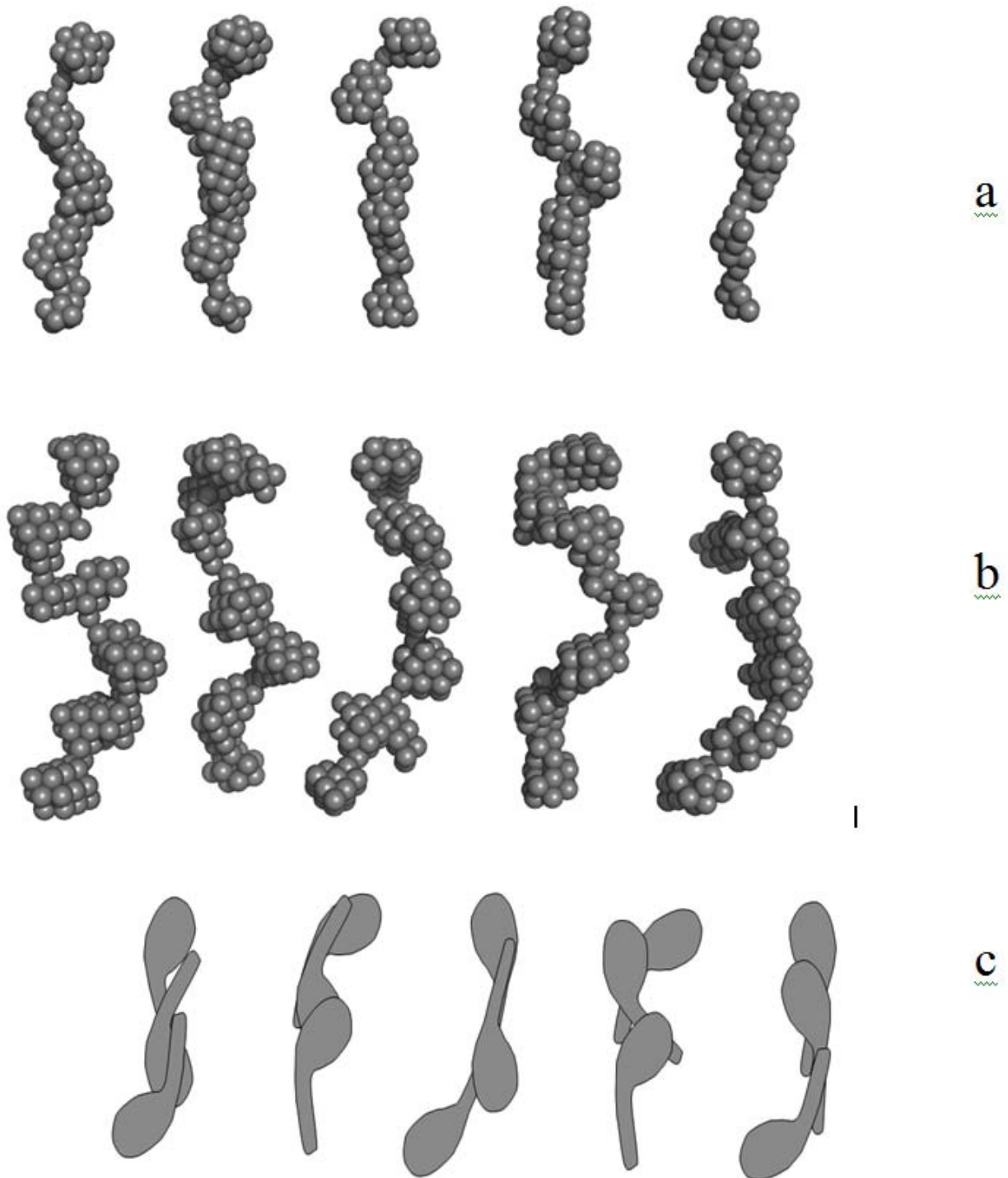
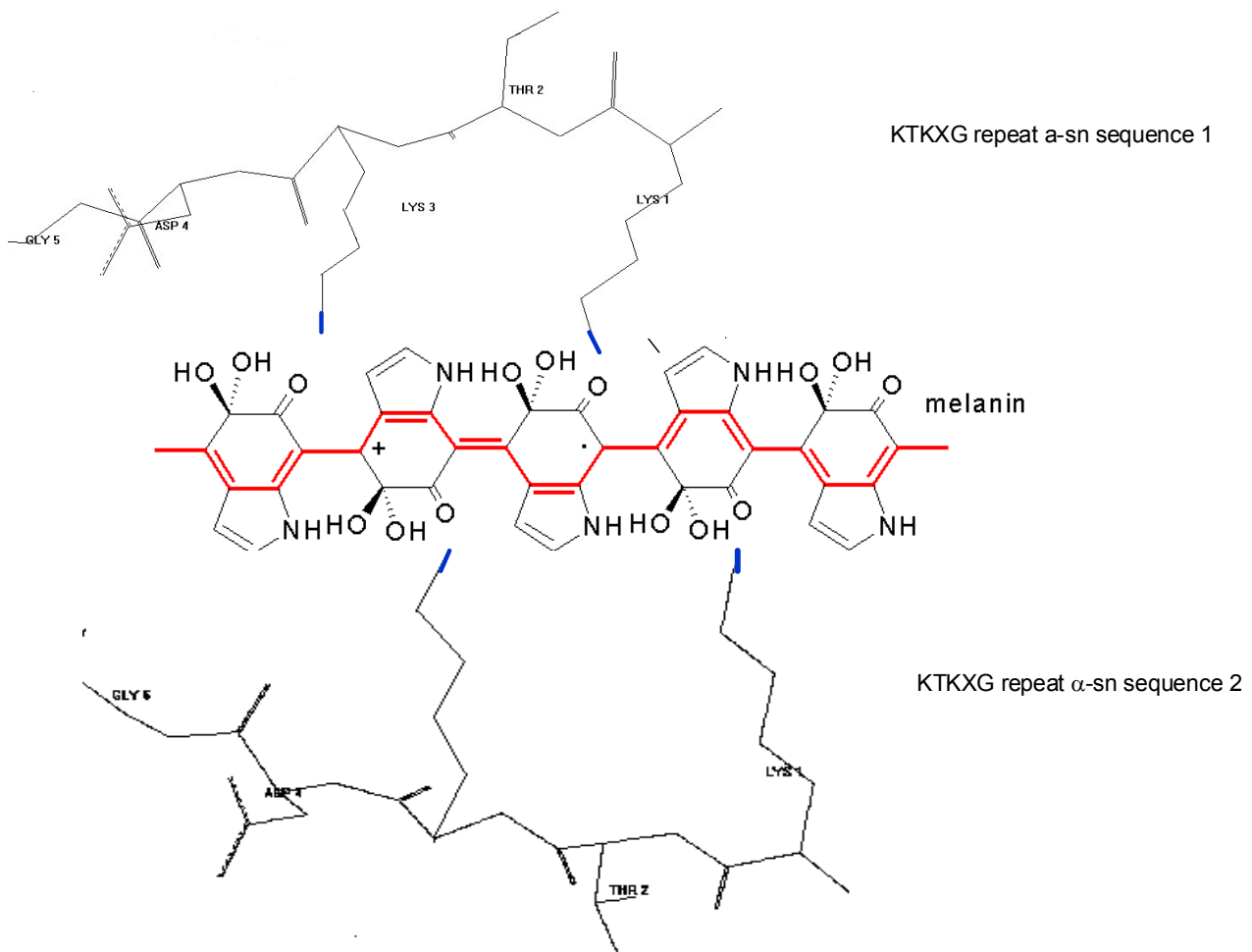


Figure 2 How melanin could cross-link α -syn molecules



As can be seen from Figure 1c the ambiguity of the *ab initio* shape reconstruction permits the arrangement of the monomers in several ways, all compatible with the SAXS data. However, partially overlapping arrangement of monomer units within a trimer is consistent with our model of multiple-site crosslinking of α -syn molecules, which would produce large contact areas which can be potentially mapped using SANS measurements of trimers following deuterium-exchange in solution. Reconstruction of the trimers from SANS data could tell us whether there is a fixed mode of monomer association.

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