International Society for Neurochemistry
CAEN grant Category 1B 2012-2013
Research report Dr María Soledad Celej

Structural Biology of Parkinson´s disease: structural and functional insights into the interaction of toxic oligomeric α-synuclein with membranes

Aim

The aim of our current work is to elucidate the supramolecular arrangement of α-synuclein (AS) amyloid oligomers which have been pointed out the most toxic species in Parkinson´s disease and related disorders. Our hypothesis is that AS would exhibit different binding modes to lipid biomembranes depending on its aggregation state due to the distinctive structural signatures of monomers and oligomers, leading to both loss-in-function physiology and gain-in-function toxicity.

Specific objective and experimental strategy

In this period we focused our research on deciphering the structural organization of AS oligomers leading to the impartment of lipid biomembranes, one of the pathways of pathobiology associated to such species. It is already recognized that these prefibrillar intermediates contain β-sheet structural elements that differ from the canonical cross-β conformation of amyloid fibrils [1]. Indeed, we showed that AS oligomers adopt an antiparallel β-sheet structure, as opposed to the parallel arrangement present in fibrils [2]. Compared to the monomer, the N-terminus and the hydrophobic (NAC) region to at least position 90 are more solvent-protected in the oligomeric form[3], and therefore they are likely involved in early β-sheet interactions. We employ site-specific fluorescence of engineered single-Cys containing AS variants labeled with solvatochromic dyes in order to define the regions involved in cooperatively folded structures and to identify intermolecular contacts.

Results

We generated 8 single-Cys mutants along the AS sequence and labeled them with thiol selective fluorescent probes, such as pyrene-maleimide (PM) and acrylodan (Fig. 1). The oligomerization protocol (Fig. 1) was adapted from ref.[2] to avoid fibril formation during the purification steps. We obtained spheroidal heterogenous high molecular weight species that do not enhance Thioflavin-T fluorescence, the amyloid specific dye, suggesting that such species lack the canonical cross-β fibrillar structure (Fig. 1).

Distinctive PM emission spectra were observed depending on the labeled position (Fig 2 a,b). The ratio between bands I and III of pyrene emission is a sensitive indicator of polarity of the probe environment. This ratio is shown in Fig. 2c. As reported previously [3], the C-terminus part, at least position 107, is solvent-exposed in oligomeric AS.

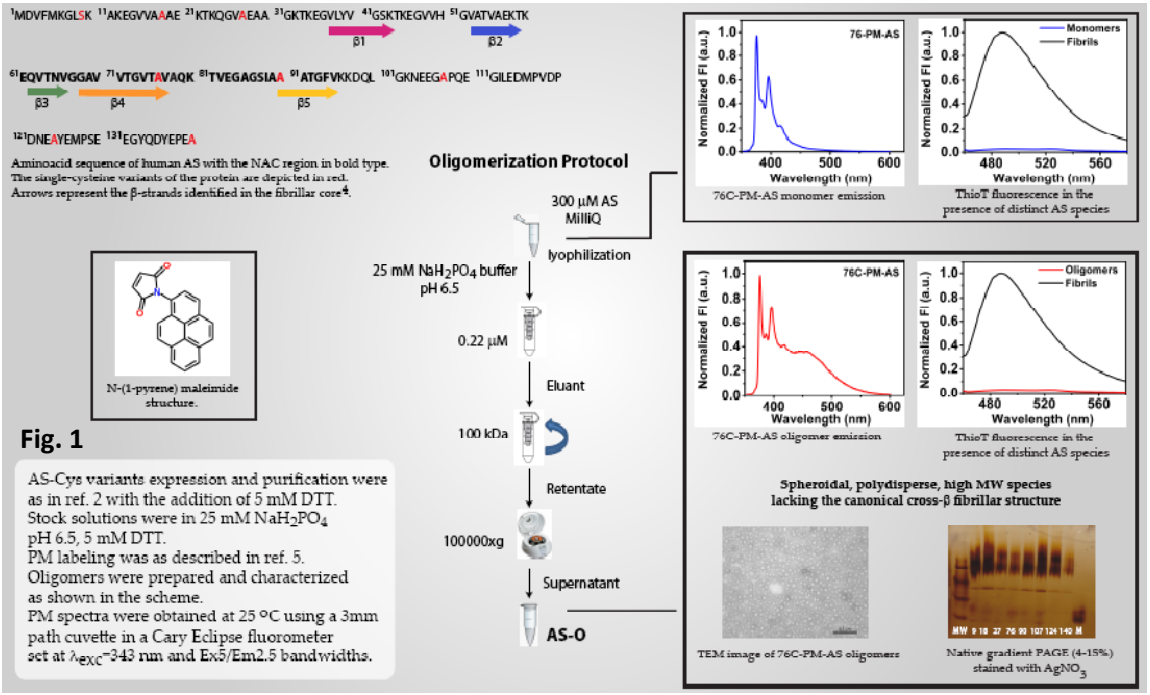

Fig. 1

AS-Cys variants expression and purification were as in ref. 2 with the addition of 5 mM DTT. Stock solutions were in 25 mM NaH_2PO_4 pH 6.5, 5 mM DTT. PM labeling was as described in ref. 5. Oligomers were prepared and characterized as shown in the scheme. PM spectra were obtained at 25 °C using a 3mm path cuvette in a Cary Eclipse fluorometer set at λ_{exc}=343 nm and Ex5/Em2.5 bandwidths.

We combined the polarity-sensitivity of the probe with chemical denaturation to define cooperatively folded units. The rational behinds our approach is that regions involved in regular structures will display a somehow cooperative denaturation profile whereas collapsed regions will show a progressive change as a function of the denaturant. On the other hand, those regions that are solvent-exposed will remain constant along the denaturation curve.

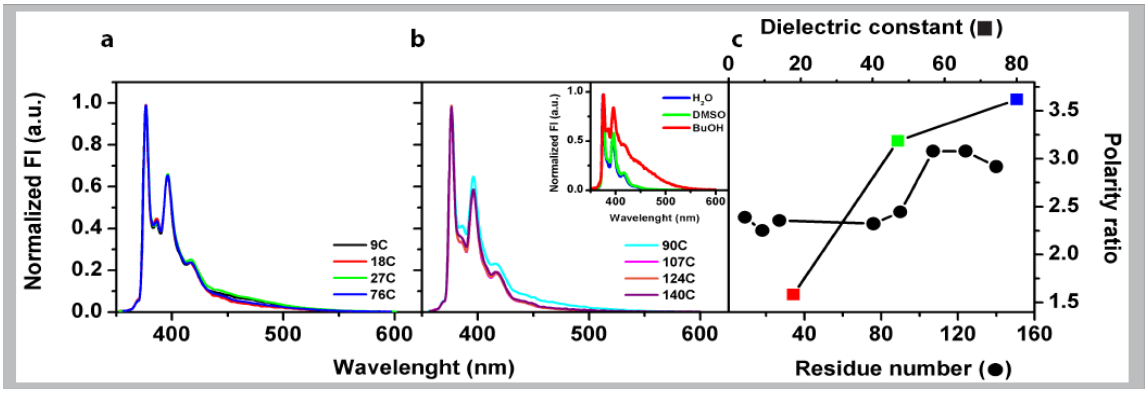

Fig.2 a-b | Normalized emission spectra of AS oligomers at 5% PM-AS. Inset: normalized emission spectra of PM-labeled glutathione (PM-GSH) in solvents with different dielectric constants. **c |** Polarity ratio (FI376nm / FI386nm) along the AS sequence (•) and PM-GSH (■) as a function of the dielectric constant.

Distinctive emission spectral changes were observed for the several positional pyrene labeled oligomeric AS as a function of Guanidinium.HCl. Fig 3 shows the emission spectra of 27C and 107C PM labeled AS oligomers as representative cases for solvent-protected and solvent-exposed variants, respectively. The polarity ratio for PM labeled glutathione (GSH) was high and invariant with the increase in denaturant concentration demonstrating that the observed changes in the PM labeled AS oligomers were due to dissociation/unfolding events.

According to the denaturation profiles, position 9C, 27C and 90C would be located in structured regions (Fig. 3d).

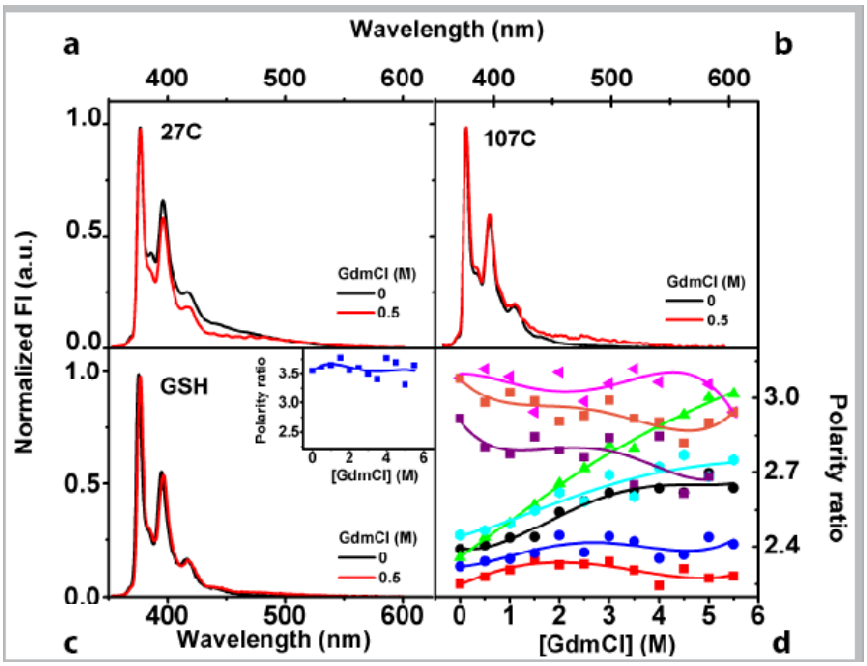

Fig. 3 Emission spectra of **a-b|** representative oligomeric PM-AS variants and **c |** PM-GSH at 0 and 5.5 M GdmCl. Inset in **c |** shows the polarity ratio of PM-GSH at different concentration of GdmCl. **d |** GdmCl denaturation profiles of oligomeric PM-AS variants ●9C ●18C ●27C ●76C ●90C ●107C ●124C ●140C.

PM is a probe of the proximity between pairs of labeled residues because of its ability to form excited-state dimers (excimers), when two PM moieties are <10 Å apart. Excimer formation gives rise to an emission band in the 430–470 nm region; the presence or absence of this band therefore allows us to determine whether a labeled position is close (≤10 Å) or distant (>10 Å), respectively, to the same labeled position in an adjacent protein molecule in the oligomer.

The fluorescence emission spectra of samples containing AS labeled with PM at residues 107 and 124 did not feature an excimer component whereas an excimer peak centered at about 460nm was detected for positions 9C, 27C and 76C (Fig. 4). The excimer ratio (*FI*465nm/*FI*376nm) for each labeled residue revealed that at least positions 9 and 76 would be involved in adjacent intermolecular contacts (Fig. 4c).

The high excimer ratio of position 9C (Fig. 4) was quite surprising taking into account that this region has a low propensity to aggregate and is excluded from the amyloid fibrillar core [4]. This result led us to postulate that the N-terminus may be arranged as a coiled-coil. Helical intermediates might promote amyloid formation by increasing the local concentration of amyloidogenic sequences. This finding is the basis of a recently established international collaboration (see below).

Currently we are performing additional experiments with mixed PM-AS variants, additional AS-Cys variants and the polar-sensitivity dye acrylodan to depict the AS oligomer supramolecular organization.

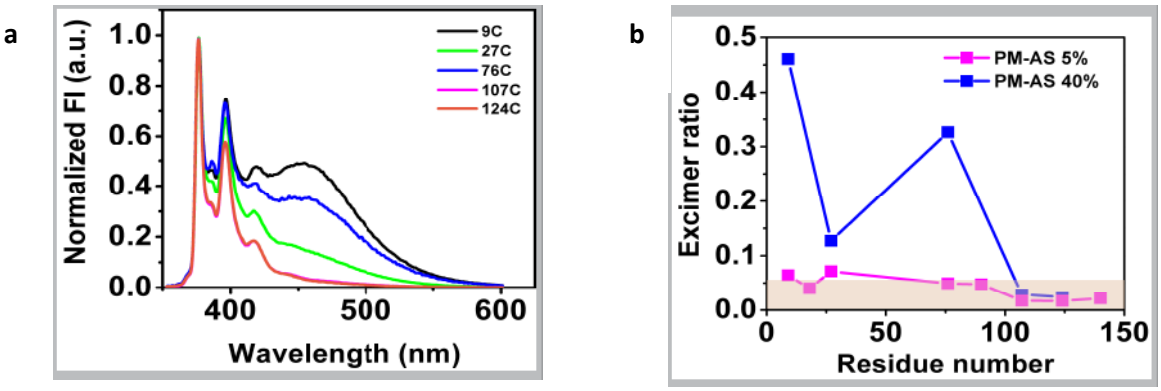

Fig. 4 a | Emission spectra of oligomeric PM-AS variants at 40% PM-AS. **b |** Excimer ratio along the AS sequence. The rectangle indicates the range found for the monomers

Results communications

The results summarized above were presented at the XLI Annual meeting Argentinean Biophysical Society, San Javier, Tucumán, Argentina 12/12 (*Insights into the supramolecular architecture of α-synuclein amyloid oligomers.* JI Gallea, Celej MS.)

The ongoing work will be presented at:

-13th Conference on Methods and applications of fluorescence, Genoa, Italy, 8-11/06/2013 (*Sculpting the internal architecture of α-synuclein amyloid oligomers with residue-specific fluorescence resolution*, Celej MS ,JI Gallea)

-2nd Workshop on Photoinduced processes in proteins, Invited speaker, Córdoba, Argentina, October 22nd, 2013 (*Lightening the architecture of α-synuclein amyloid oligomers*)

We expect to conclude this part of our work and send a written communication for peer-review by the end of 2013.

Additional benefits from the grant

The financial support from the ISN helped me to develop my own line of research and gave me the possibility to incorporate my first PhD student under my supervision. In addition, the research we have been developing during this last year allowed us to establish diverse international collaborations upgrading our group to the international level:

1-Dr. Bongarzone's group (UIC, Chicago, USA). We are investigating the role of glycolipids in Lewy body formation in idiopathic Parkinson's disease.

2-Dr. Goormightight's group (ULB, Brussels, Belgium): We are investigating the link between AS and neuroinflammation underlying Parkinson's disease pathobiology.

3-Dr. Bertoncini´s group (IRB, Barcelona, Spain): We will investigate the potential structural arrangement of the N-terminus in oligomeric AS as a coiled-coil.

References

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