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Targeting α-Synuclein as a possible approach to combat Parkinson's disease

B. Fongaro, L. Palazzi, M. Leri, L. Acquasaliente, M. Bucciantini, P. Polverino de Laureto
University of Padua, University of Florence

Introduction

A typical hallmark of Parkinson's disease (PD) is the presence in the dopaminergic neurons of intracellular inclusions containing aggregates of α-synuclein (Syn). The presence of mutations in Syn gene can favor the aggregation of the protein and the likelihood to develop PD. These mutations can lead to an early onset (A30P, E46K, and A53T) or late-onset (H50Q) form of the disease (Fig. 1). Among these variants, the E46K mutation causes a severe clinical phenotype and increases the toxicity of aggregated forms of Syn.

The interplay between Syn and dopamine derivatives, seems to be associated with oxidative stress-dependent neurodegeneration in early onset of PD. *In vitro*, dopamine's metabolites, such as 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylethanol (DOPET), interfere with the growth of mature amyloid fibrils of Syn.

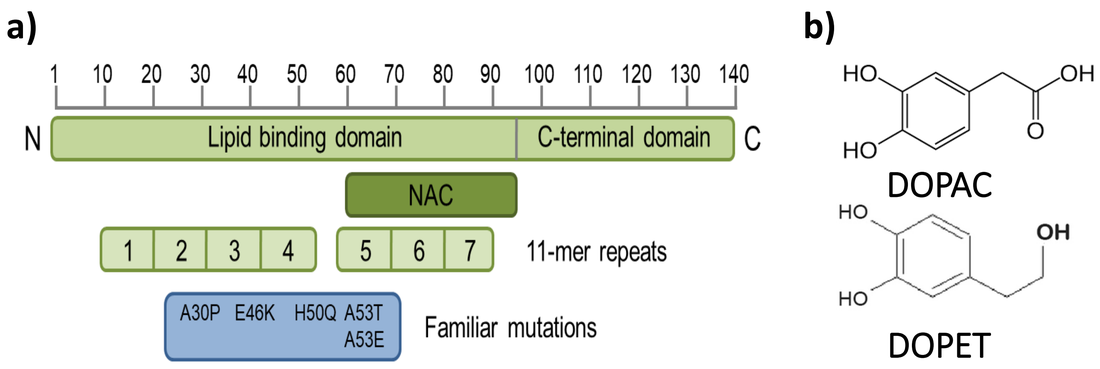


Fig.1: Sequence and structural domain organization of Syn (a). Structure of DOPAC and DOPET (b)

Aim

Here, we studied the interaction between Syn and its pathological mutant E46K with DOPAC and DOPET and the effects of this interaction on the aggregation properties of both proteins.

DOPAC and DOPET inhibit Syn WT and E46K aggregation

Thioflavin T (ThT) assay was used to monitor protein aggregation in the presence and in the absence of different protein to catechol ratios (Fig. 2). DOPAC and DOPET share the capability to inhibit the amyloid fibril formation in a dose-dependent manner for both the WT and mutated Syn. However, E46K appears to be less sensitive to catechols than Syn, and DOPAC seems to be more effective than DOPET.

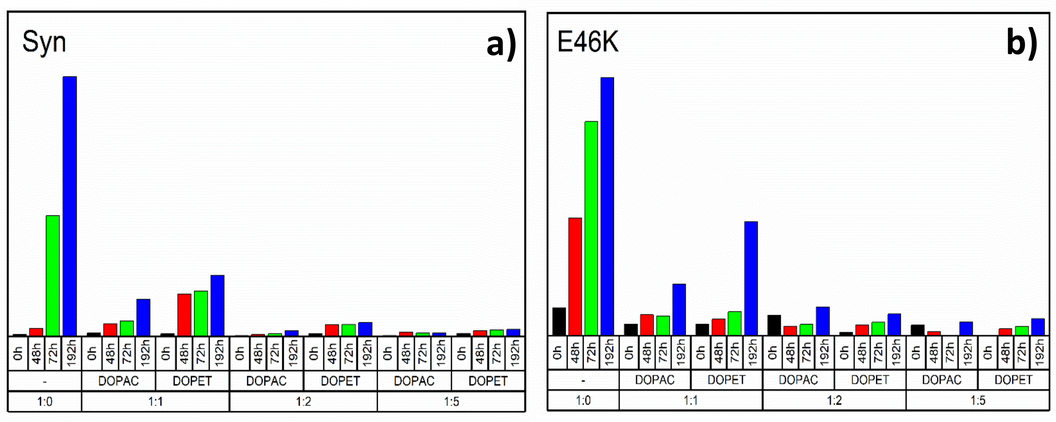


Fig.2: Fibril formation process of Syn (a) and E46K (b), in the absence (1:0) and in the presence of DOPAC and DOPET at different molar ratios (1:1, 1:2, 1:5) probed by ThT assay. Aliquots were taken at the time points 0 h (black), 48 h (green), 72 h (red) and 192 h (blue).

Off-pathway oligomers formation

Size exclusion chromatography (SEC) was used to monitor protein oligomerization (Fig. 3). DOPAC and DOPET induce a stable oligomerization of WT and mutated Syn. However, the major component is a specie eluting as the monomer. The protein is chemically modified by the formation of a covalent adduct on the side chains of the lysine residues. Of note, DOPAC effects seem to be stronger than DOPET effects.

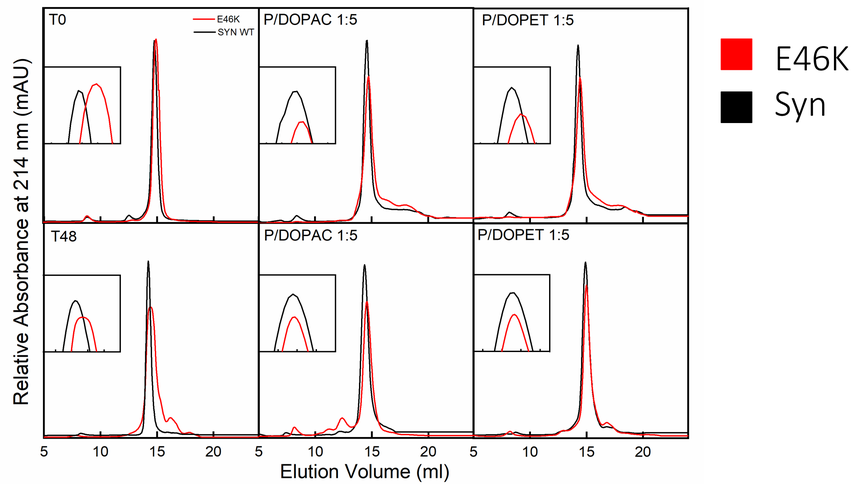


Fig.3: SEC analyses of E46K and Syn at times 0 h and 48 h of incubation in the absence and in the presence of DOPAC and DOPET at molar ratios 1:2 and 1:5.

Different monomer conformations

Native-MS shows the co-existence of three main protein conformers: (1) an extended and relaxed specie, (2) a specie with intermediate properties and (3) a compact conformer. Species 1 and 3 appear differently populated by Syn and E46K (Fig. 4). At time 0 h, the compact conformer (3) of E46K seems to be more populated at the expenses of the relaxed one (1). DOPAC and DOPET induce a marked redistribution of these species affecting their equilibrium, leading to an increase of the compact population due to the formation of oligomers after 48 h and a decrease of it when the catechol is added at time 0 h.

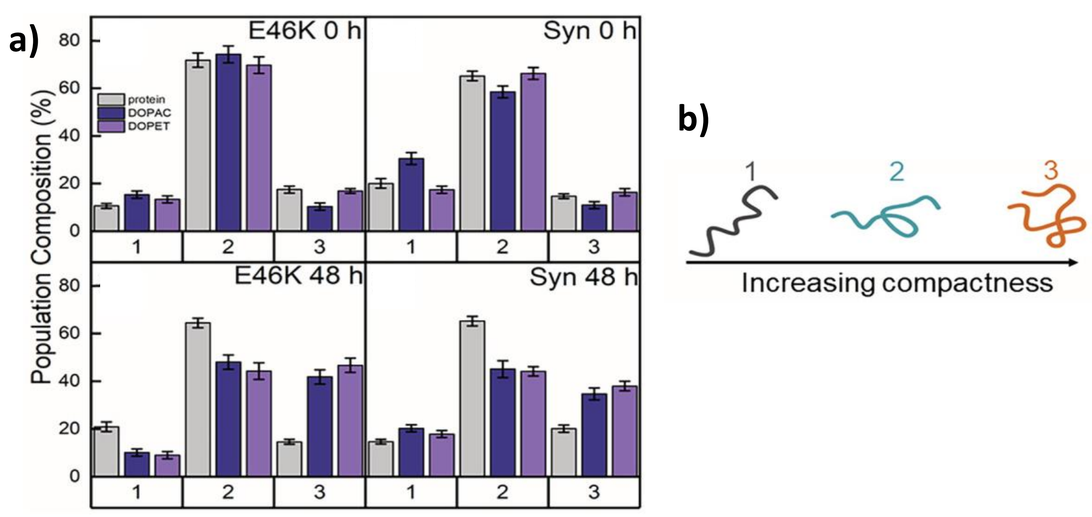


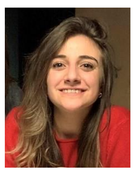
Fig.4: Histograms comparing the percentages of each population observed for the proteins in the absence and in the presence of catechol at molar ratio 1:5 at time 0 h and 48 h of incubation (a). Graphical representation of proteins conformers (b).

Conclusions

In the absence of catechols, Syn and its pathological mutant E46K undergo nucleation events followed by the formation of on-pathway toxic oligomers, that culminate in fibrils formation. In the presence of catechols, the proteins mainly bind them through non-covalent and covalent interactions giving rise to modified monomers, that then generate off-pathway harmless oligomers. The mutant seems to be less affected by catechols because of the different protein conformations at time 0 h.

References

- Fongaro et al., Journal of Molecular sciences, 2021
- Fongaro et al., Protein Science, 2022



Benedetta Fongaro
benedetta.fongaro@uliege.be