Protein misfolding and aggregation in disease

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An L-DOPA-PLP cyclic adduct exerts oxidative stress on α-Synuclein

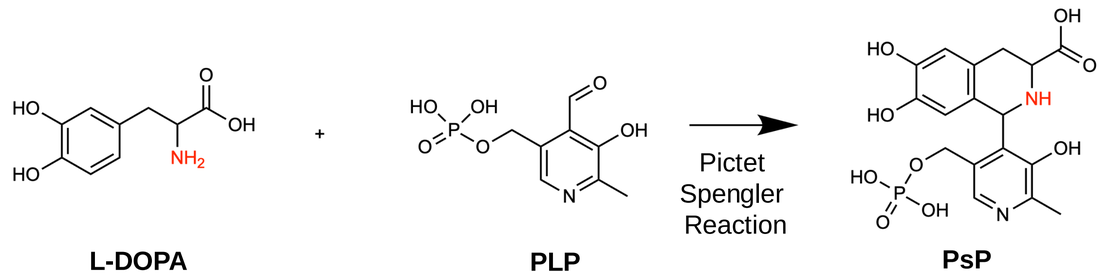
Philipp Trolese[1,2], Giovanni Bisello[2], Benedetta Fongaro[1], Laura Acquasaliente[1], Patrizia Polverino De Laureto[1] and Mariarita Bertoldi[2]

[1]*Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy*
[2]*Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Verona, Italy*

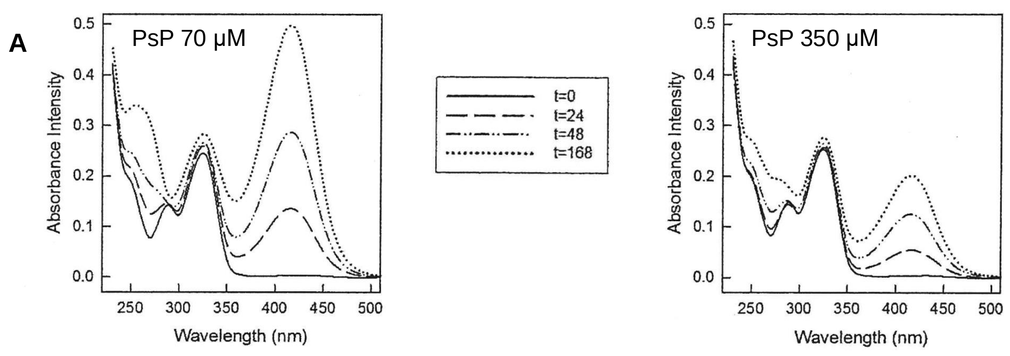
Introduction

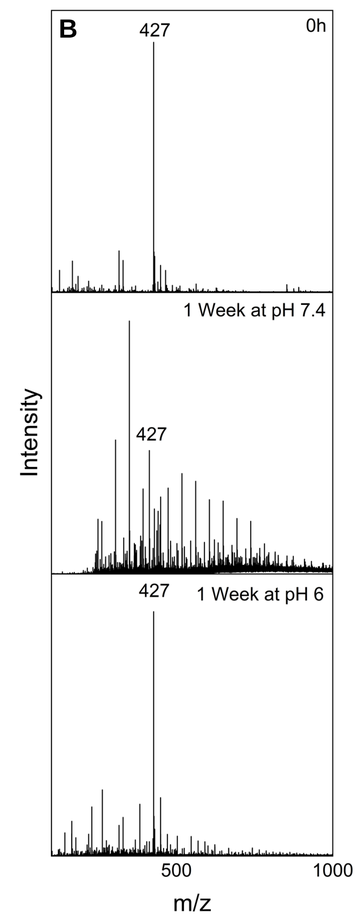
Levodopa (L-DOPA) is widely used as a therapeutic agent to alleviate motor symptoms in Parkinson disease (PD). L-DOPA is converted into dopamine by the pyridoxal 5′-phosphate (PLP)-dependent enzyme L-aromatic amino acid decarboxylase (AADC), responsible for the synthesis of this neurotransmitter. Mutations in the *DDC* gene synthesizing AADC lead to a severe inherited neurometabolic disorder: AADC deficiency. L-DOPA bound to PLP via a Schiff base covalent linkage can irreversibly cyclize into an adduct via the Pictet-Spengler condensation reaction *in vitro*, forming a Pictet-Spengler adduct (PsP), either free in solution or at the AADC active site under certain experimental conditions[1,2]. In addition, several AADC deficiency pathogenic variants undergo this nonenzymatic reaction, as a consequence of the structural alterations at their active site[3]. It is possible that unmetabolized L-DOPA may react with PLP also *in vivo*, possibly leading to PLP drop. Indeed, this depletion could impair PLP-dependent enzymes. Furthermore, PsP itself may interact with α-synuclein (Syn), contributing to neurodegenerative pathology. Here, we investigated the impact of PsP on Syn aggregation and explored the molecular mechanisms underlying this interaction.

Characterization of the adduct



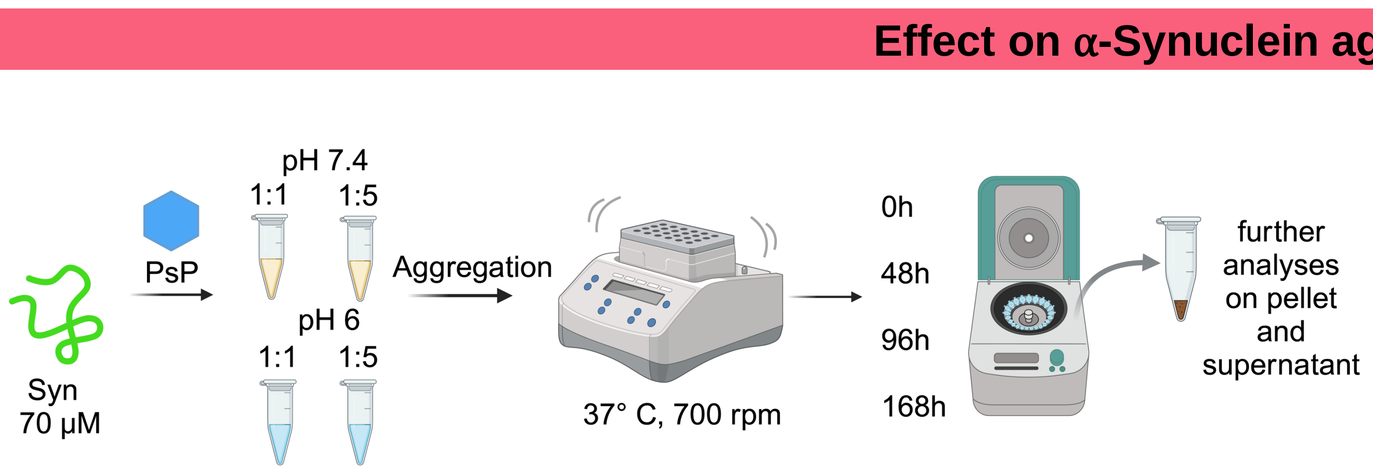
Reaction Mechanism between L-DOPA and pyridoxal 5'-phosphate (PLP) to form the cyclic Pictet Spengler Product (PsP).

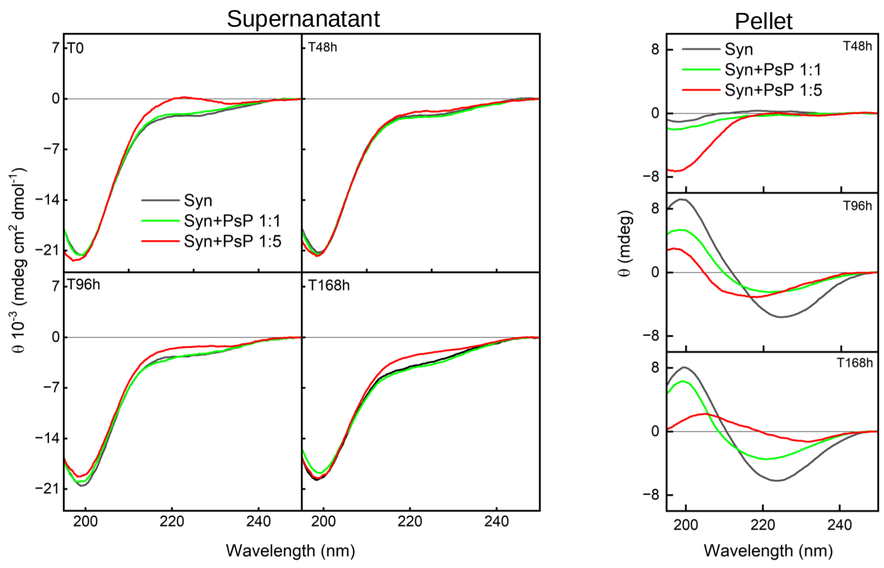


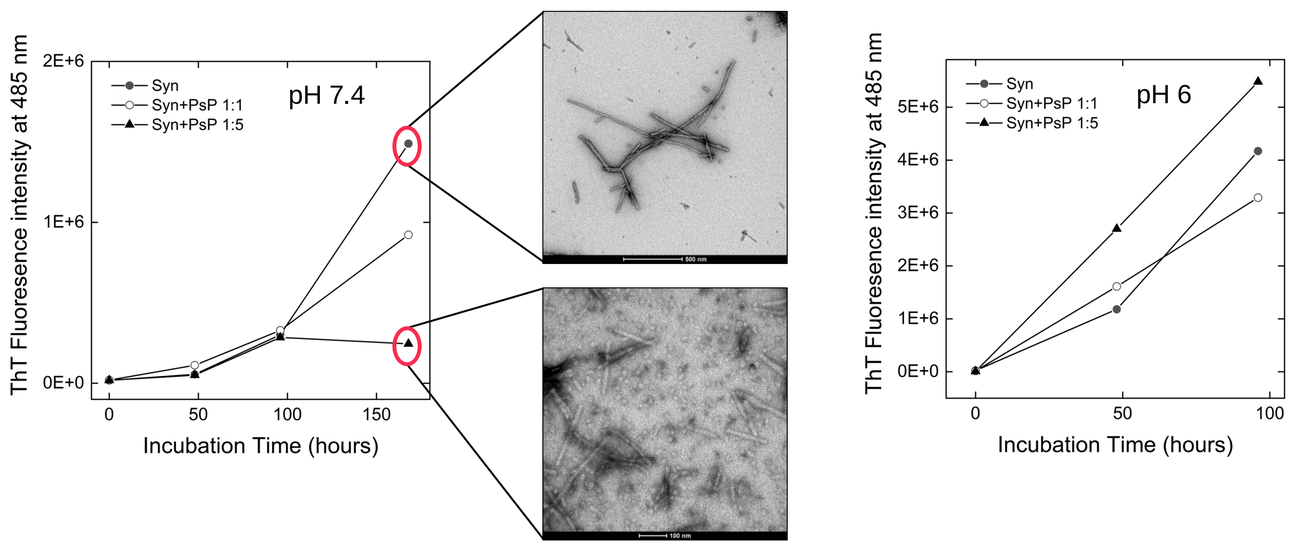


Characterization of PsP by UV spectroscopy (A) and mass spectrometry (B) over time. At pH 7.4 an additional peak absorbing at 420 nm emerges. This is accompanied by visible yellowing of the solution and a mass spectrum that suggests the presence of polymerized species. On the other hand, when incubating the compound at pH 6 the solution remains colorless, and the mass spectrum resembles the one of the freshly unfrozen sample. This suggests an intrinsic chemical instability of PsP at slighty alkaline pH values that is not present in acidic environment.

Effect on α-Synuclein aggregation



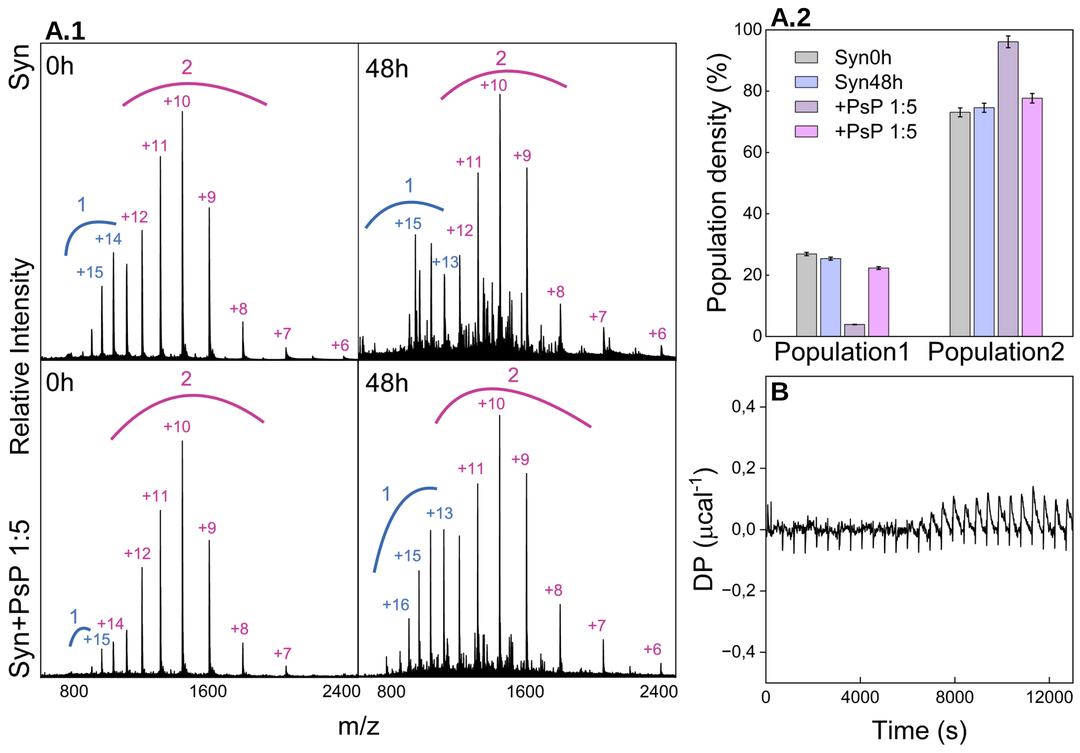




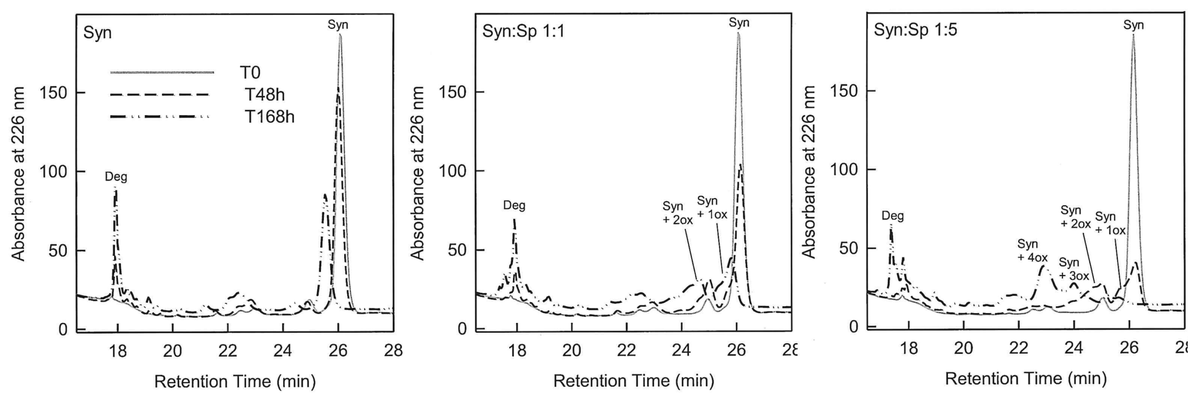
Far-UV CD spectra (up) of supernatants and resuspended pellets at each time point. The spectra of the supernatants exhibit characteristics of a disordered protein, indicating that PsP does not induce secondary structure formation in monomeric Syn over time. In contrast, the pellet spectra show a progressive increase in β-sheet formation, consistent with Syn aggregation. Notably, the Syn+PsP (1:5) sample exhibits the least secondary structure formation. Interestingly, this sample also displays a shift in the typical β-sheet maxima and minima, suggesting a slightly altered conformation.

ThT fluorescence measurements (left) of Syn alone and in the presence of PsP (1:1 and 1:5) at pH 7.4 and pH 6. At pH 7.4, where PsP is chemically unstable, fibrillation appears to be inhibited in a dose-dependent manner. TEM images at 168 h show fibrils in Syn alone, while Syn+PsP (1:5) primarily contains smaller oligomers. In contrast, at pH 6, where PsP remains chemically stable, no inhibition is observed, with ThT fluorescence intensities more than double of those at pH 7.4. These results suggest that PsP's ability to inhibit Syn fibrillation is closely linked to its chemical instability at physiological pH.

Interaction with α-Synuclein



Interaction between Syn and PsP analyzed by Native Mass Spectrometry (MS) (A1, A2) and Isothermal Titration Calorimetry (ITC) (B). Native MS provides insights into Syn conformations by analyzing mass-to-charge (m/z) ratios[4], where lower m/z values indicate a more extended state due to increased solvent exposure of protonable groups. At 0 h and 48 h, Syn alone exhibits two distinct populations: a more extended conformation (~25%) and a more compact one (~75%). Upon adding PsP at 0 h, the extended population drastically decreases to ~5%, favoring the compact conformation. However, after 48 h, this compaction is lost, with the complexed protein behaving similarly to Syn alone. This initial compaction suggests a potential binding event between Syn and PsP, leading to conformational changes. ITC analysis further explored this interaction, yielding a thermogram with minimal enthalpic contribution. This suggests that the compaction observed in Native MS may be non-specific and/or entropically driven, supporting the hypothesis that PsP interacts hydrophobically with the NAC region of Syn.



RP-HPLC chromatograms of Syn incubated with PsP over time at 1:1 and 1:5 ratios (pH 7.4). Only the chromatograms from pH 7.4 are shown. At a 1:1 ratio, additional peaks appear over time, eluting before Syn, and were identified as oxidized protein forms. This effect is more pronounced at a 1:5 ratio, where even Syn with up to four oxidations was detected. In contrast, at pH 6, only wild-type (WT) Syn is observed in the eluting peaks. These findings suggest that PsP's chemical instability—previously linked to fibrillation inhibition—also promotes Syn oxidation over time, potentially leading to the formation of smaller oligomers.

Conclusion

PsP, a cyclic product formed by PLP and L-DOPA via a Picter Spengler reaction, may form in vivo under certain conditions of AADC deficiency. Here, we studied how it may interact with Syn contributing to the pathogenesis of PD. While PsP seems to exhibit a dose dependent inhibition of fibrillation, this does not seem to be linked by a specific interaction between the compound and the protein, but rather to the chemical instability of PsP that in turn cuases Syn oxidation.

References

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