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Human Cofilin-1 modified by HNO/NO on cysteine residues and their roles in aggregation

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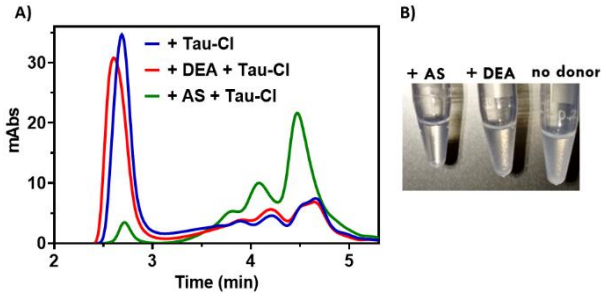
Parkinson's disease (PD) and other neurodegenerative diseases are related to the aggregation of proteins, specifically alpha-synuclein and Cofilin-1 (CFL-1). Cysteine oxidation and disulfide bond formation are related to the functions of CFL-1, as these events govern the formation of oligomers and aggregates. Many efforts have been made to understand these processes, but there is still a lack of understanding regarding the exact amyloidogenic properties of CFL-1. Furthermore, we are interested in investigating how nitric oxide and nitroxyl can influence oligomerization/aggregation by reacting with cysteines. To separate monomeric and oligomeric forms, we use analytical size exclusion chromatography (SEC), thus culminating in size distribution and concentration- and time-dependent oligomerization trends as well. Fibril formation was investigated by reaction with Thioflavin T followed by fluorescence spectroscopy. Upon oxidation, CFL-1 transitions to oligomeric forms that eventually aggregate into fibrils. This is a time/concentration-dependent process. The SEC results suggest the formation of dimers, tetramers, and octamers, in addition to large structures, which are probably fibrils, considering the increase in Thioflavin T fluorescence when the protein was treated with an oxidizing agent, while untreated protein remained quite stable, maintaining the monomeric form. After 20 hours, aggregation is complete up to a protein concentration of 40 µM, but barely occurs at 20 µM or below this concentration. Treatment with NO/HNO donors, mainly HNO, prevents the formation of fibrils and aggregation (Figure 1). Furthermore, HNO can partially disrupt fibril structures. These results highlight the importance of cysteine oxidation in CFL-1 aggregation, underlining the potential relevance of this post-translational modification in neurodegenerative disease pathways and showing a way to prevent/reduce fibril formation and protein aggregation.

Figure 1 – A) SEC of CFL-1 (40 µM) after incubation with Tau-Cl (24h, 37ºC) native form and pre-treated with NO and HNO donors. B) CFL-1 (80 µM) precipitation after incubation with Tau-Cl.



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