Zinc Binding to Tau Influences Aggregation Kinetics and Oligomer Distribution

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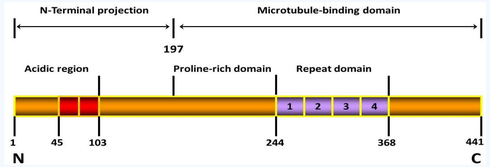
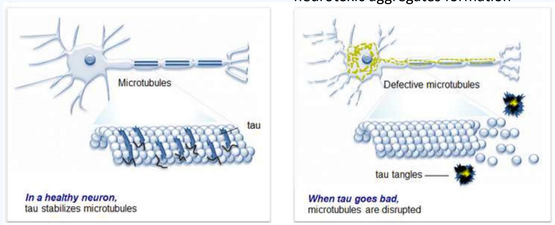
Here we report investigations using biochemical and biophysical techniques that allowed us to gain insights into effects of zinc and calcium binding to hTau441



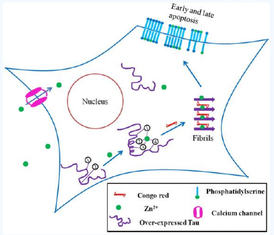
In healthy neurons, Tau stabilizes microtubules promoting axonal outgrowth and synaptic vesicle transport

Hyperphosphorylation decreases Tau stabilizing effect leading to microtubule dissociation and neurotoxic aggregates formation



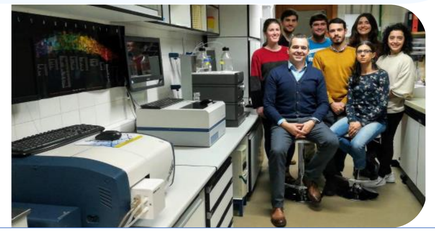
Lim, Sungsu & Haque, Md et al CSBJ (2014) 12. 10.1016/j.csbj.2014.09.011

Pathological concentrations of Zn^{2+} remarkably enhance Tau fibrillization-induced apoptosis and neurotoxicity



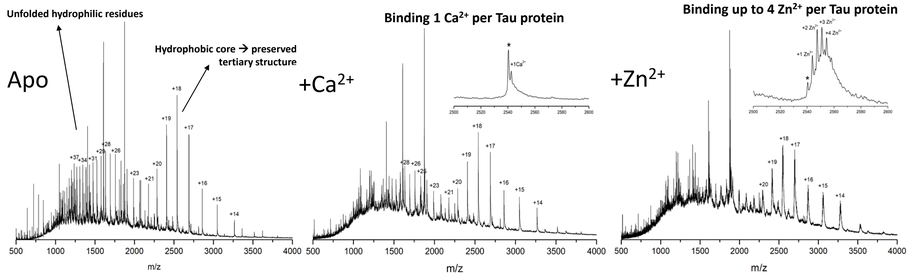
- Zn^{2+} is co-localized and highly enriched in neurofibrillary tangles with Tau
- Zinc dramatically accelerates abnormal aggregation of human Tau and significantly increases Tau toxicity in neuronal cells

Ji-Ying Hu & Yi Liang et al, Biochim Biophys Acta Mol Basis Dis. (2017) Feb;1863(2):414-427. 10.1016/j.bbadis.2016.11.022



OBJECTIVE: Metal ions are well known modulators of protein aggregation and are key players in Alzheimer's disease (AD). Also, the intraneuronal aggregation of Tau into neurofibrillary tangles (NFTs) constitutes a hallmark in AD, and there is evidences that coordination of divalent metal ions enhances Tau aggregation and neurotoxicity. These facts motivated us to study metal ion binding to full-length Tau (hTau441) and its consequences on protein structure and aggregation propensity, similarly to what takes place in AD.

Metal Ions Bind to Tau – Native-MS

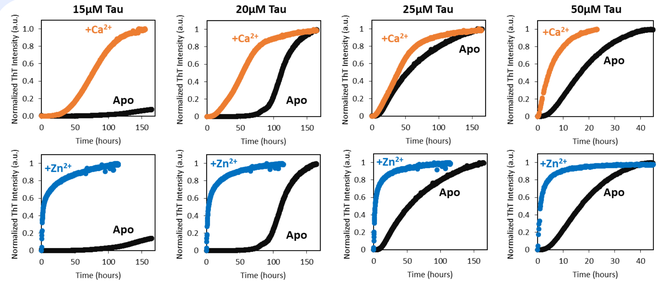


Nano-ESI mass spectrum of Tau **protein reveals a MW of 45.8kDa (45744 ± 0.8 kDa), in near native state at pH 7.4 confirming intrinsically disordered structure**

Coordination of divalent **metal ions reveals an increase in protein compactness** with consequent **loss of highly dynamic and flexible physiological structure**. This effect is more significant in the presence of zinc.

35µM Tau in 100mM ammonium bicarbonate at pH=7.4

Metal Ions Accelerate Tau Aggregation



Ca^{2+} and Zn^{2+} enhance Tau aggregation, increasing the aggregation rate and reducing the lag time phase

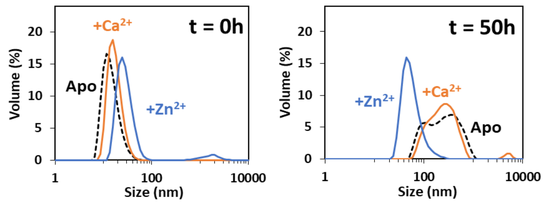
The effect of Zn^{2+} on Tau aggregation is clearly more pronounced, with no lag phase observable and a rapidly achieved fibrillar end-point

Thioflavin T monitored heparin-induced Tau aggregation kinetics were performed to assess the effects of Ca^{2+} and Zn^{2+} on hTau441 aggregation and fibrillation.

15 to 50 µM hTau441 with 0.5 mg/mL heparin, 1 mM DTT, 50 mM NaCl, 1 mM PMSF, 75 µM ThT and 1.1 mM metal ions ($CaCl_2$ and $ZnCl_2$). Aggregation at 37 °C pH 7.4, orbital agitation at 600 rpm; n=3.

Metal Ions Tau Aggregates Analysis

DLS analysis

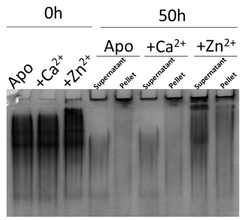


At 0h, Ca^{2+}-Tau and Zn^{2+}-Tau conformers **have dimensions that are comparable to those of apo Tau**

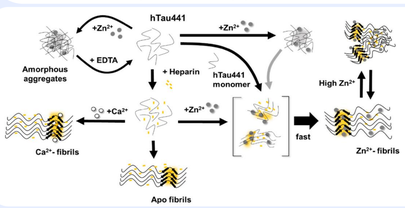
After 50h of incubation, the apo and Ca^{2+}-Tau samples both exhibited a broad and heterogeneous distribution of large oligomers, however, **zinc-bound Tau aggregates were smaller and relatively homogeneous**

50 µM Tau with 1.1 mM metal ion and 0.5 mg/mL heparin; 1 mM DTT; 50 mM NaCl; 1 mM PMSF. Incubation: 37 °C and 800 rpm; Measured in a 45-µl quartz cuvette (Hellma) at 25 °C; 17 runs each with 8 measurements

Native-PAGE



Zn^{2+}-Tau produces soluble and smaller species after 50h



Metal Ions Involvement on Tau Pathomechanisms

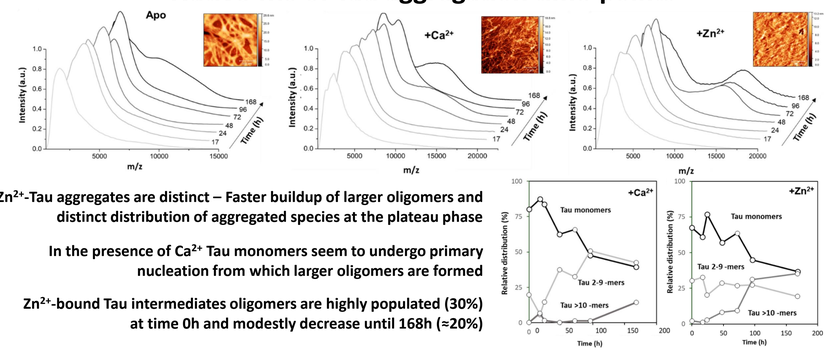
Zn^{2+} binding to the R3/R4 regions are responsible for faster ThT-reactive fibrillation

Unordered and amorphous aggregates might involve binding of Zn^{2+} to residues within the fuzzy coat regions (not observed in the presence of Ca^{2+})

Zn^{2+} binding might also interfere with ordered stacking of residues in the repeat regions that will lead to amyloid cross-beta formation

Tau Oligomers Distribution

Native-MS of Tau aggregation time-points



Zn^{2+}-Tau aggregates are distinct – Faster buildup of larger oligomers and distinct distribution of aggregated species at the plateau phase

In the presence of Ca^{2+} Tau monomers seem to undergo primary nucleation from which larger oligomers are formed

Zn^{2+}-bound Tau intermediates oligomers are highly populated (30%) at time 0h and modestly decrease until 168h (≈20%)

Mass spectrometry analysis - modified Waters QTOF Ulitima II instrument with off-line nano electrospray source; AFM: Aggregated hTau441 samples from end time points of aggregation studies $[hTau441]_{final}$ = 0,5-1 µM. The distributions were obtained from peak fitting and deconvolution of the obtained spectra at each time point according to the relative contribution of the species.

Conclusion

The binding of calcium and zinc to Tau results in substantially different effects on the protein aggregation

This is especially relevant when considering recent evidence implicating zinc binding to Tau to the generation of toxic aggregates, independently of phosphorylation

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