Ligand and Metal Binding to Wild Type and Mutant α-Synuclein

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Abstract

Parkinson's disease (PD) is a neurodegenerative disorder caused by aggregation of the protein α-synuclein (α-syn) into Lewy Bodies which affects communication between neurons and contributes to cell death. Previous native mass spectrometry (MS) research has shown that metal ions and small anti-aggregation compounds such as CLR01 bind to α-syn. However, no work has been done characterizing CLR01 binding to α-syn mutants, specifically mutant A30P, and α-syn/metal complexes. MS research indicates that multiple CLR01 molecules and multiple metal ions can simultaneously bind α-syn. Ion mobility-mass spectrometry (IM-MS) shows that CLR01 compacts the structure of α-syn, A30P α-syn, and α-syn /metal complexes. Using Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR) with electron capture dissociation (ECD), it is found that CLR01 and copper bind to the n-terminus of α-syn, and manganese binds toward the c-terminus of α-synuclein. This data can be utilized to learn more about aggregate formation of α-syn and a possible way to inhibit the formation of those aggregates.

Background

α-Synuclein is an amyloid protein that has been shown to aggregate into Lewy Bodies in Parkinson's Disease patients. A mutant form of the protein, A30P α-syn, aggregates more readily than wild type.[1] In addition, metals have also been shown to increase aggregation of amyloid proteins.[2,3] A small aromatic compound known as CLR01 has been shown to bind the-n-terminus of wild type α-synuclein and inhibit fibril formation.[4] Although, the interaction between A30P α-synuclein and CLR01 and α-synuclein/metal and CLR01 is not known. In this study we explore the interaction of CLR01 with mutant and metal bound α-Synuclein to better understand the interaction of CLR01 with amyloid proteins.

Materials and Methods

Sample preparation: Proteins were made in 20mM Ammonium Acetate. All α-Syn solutions had a concentration of 10uM. The concentrations of copper, manganese, and CLR01 varied from 10uM to 50uM.

Ion Mobility Mass Spectrometry: Either Thermo tips or pulled glass tip capillaries coated in platinum or gold were used to spray the protein. Ion mobility experiments were performed on a Waters Synapt G2-Si system with a capillary voltage of 1kV and a cone voltage of 20V. Deconvolution was performed using UniDec software.[5]

FT-ICR Mass Spectrometry: FT-ICR experiments were performed using a 15-T Bruker SolariX FT-ICR MS with an infinity cell. ECD-MS was performed with a pulse length of 10ms to 40ms and a bias of 1eV.

IM-MS of α-Syn/CLR01 Complexes

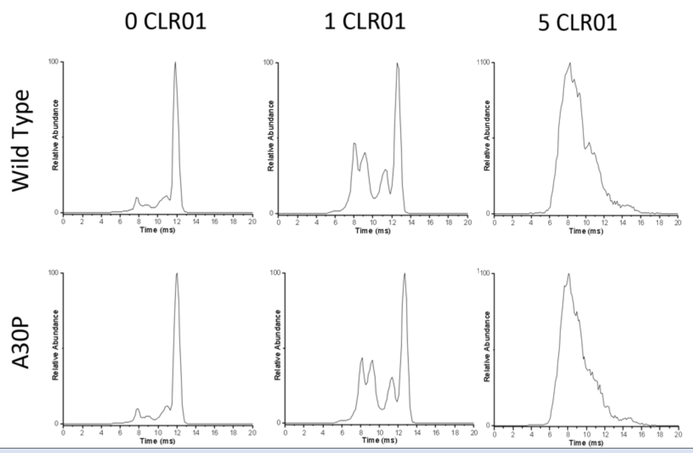
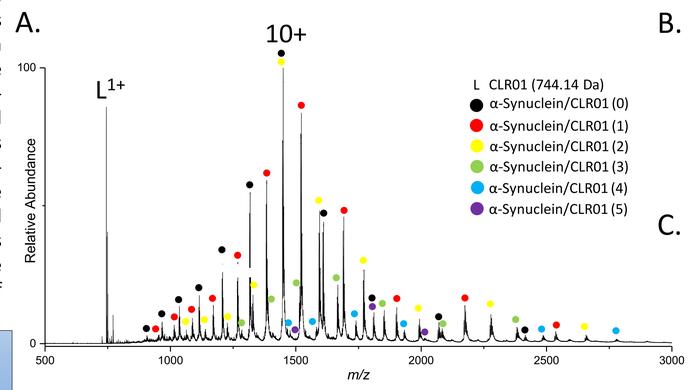


Figure 1: IM-MS data showing that CLR01 compacts the structure of WT and A30P α-Syn. Binding of additional CLR01 molecules show greater compaction. The structure of WT α-syn and A30P α-syn does not seem to vary significantly from one another.

MS of α-Syn/A30P/CLR01 Complexes

Mass Spectrum



UniDec

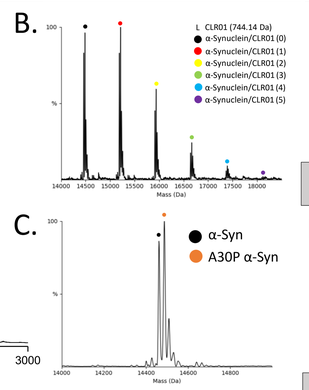


Figure 2: A.) The mass spectrum of a solution with 1:1:10 molar ratio of WT α-Syn/A30P α-Syn/CLR01 showing that CLR01 binds to both WT α-Syn and A30P α-Syn. B.) Unidec deconvolution indicates up to 5 CLR01 molecules are bound to both proteoforms. C.) A UniDec spectrum indicating proteoforms of α-Syn can be resolved.

Metal Binding on α-Syn

α-Syn/Copper Complexes

α-Syn/Manganese Complexes

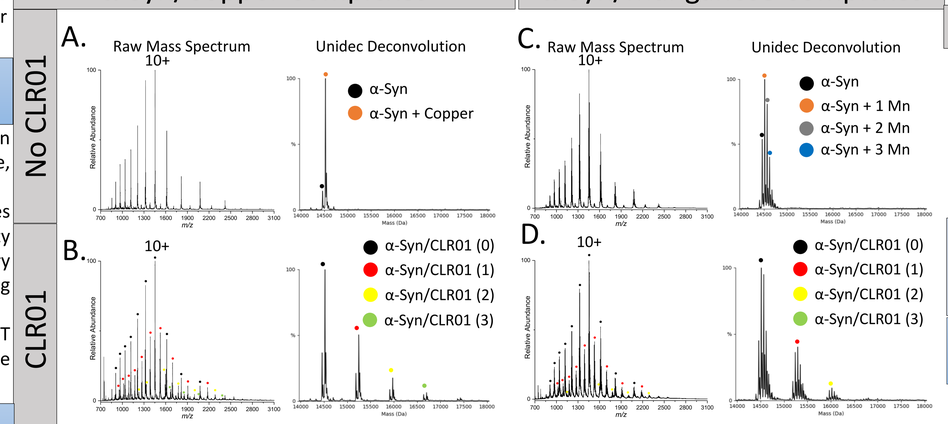
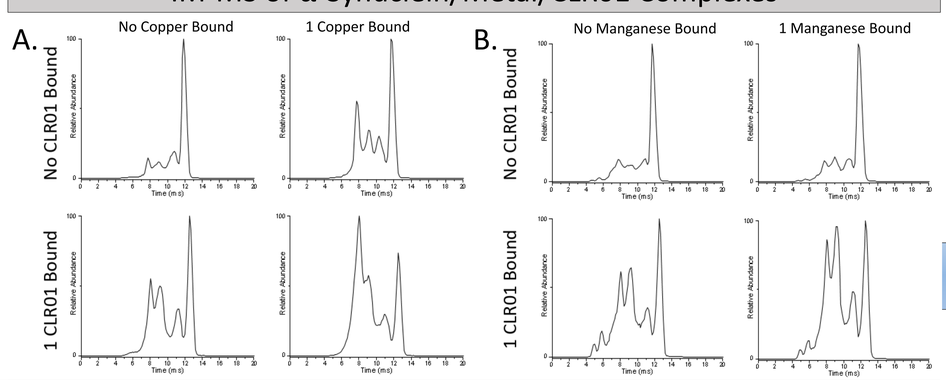


Figure 3: MS1 spectrum of A.) α-syn/copper, B.) α-syn/copper/CLR01, C.) α-syn/manganese and D.) α-syn/manganese/CLR01 solutions with the corresponding Unidec spectrum. The data indicates that CLR01 competes with copper for binding yet enhances the binding of manganese.

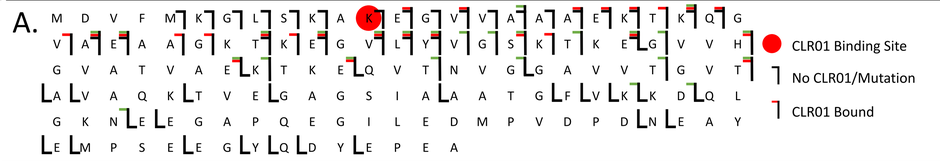
IM-MS of α-Synuclein/Metal/CLR01 Complexes



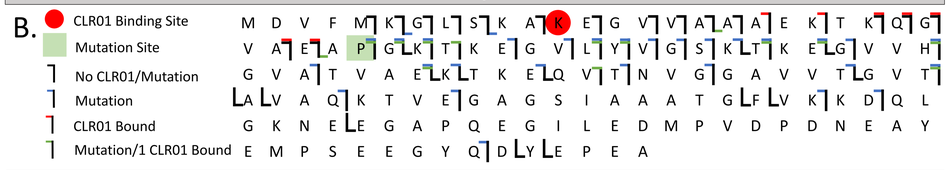
Figures 4: IM-MS data of the 9+ charge state for the A.) α-syn/copper/CLR01 solution and the B.) α-syn/manganese/CLR01 solution. Copper seems to compact the structure of α-syn while manganese only has a slight effect. CLR01 compacts the structure of both copper bound and manganese bound α-Syn.

ECD of α-Syn/CLR01 Complexes

Wild Type α-Synuclein



A30P α-Synuclein



α-Synuclein/Copper/CLR01

α-Synuclein/Manganese/CLR01

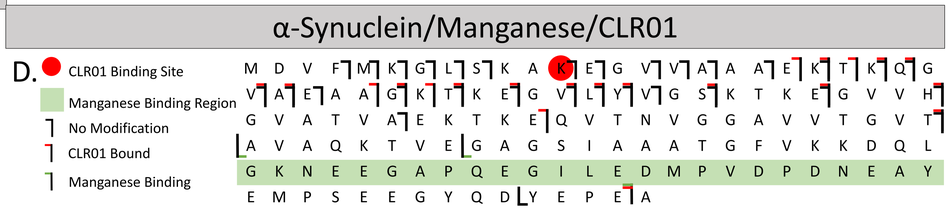


Figure 6: ECD fragmentation of A.) WT α-Syn, B.) A30P α-Syn, C.) α-Syn/copper complex, and D.) α-Syn/manganese complex with 1 CLR01 molecule bound. The approximate binding location of CLR01 is indicated with a red circle and the binding region of the metal ions is indicated by a green box. CLR01 is found to bind to the n-terminus of all complexes. ECD of α-Syn/metal complexes indicates that copper binds to the n-terminus while manganese binds to the c-terminus.

Conclusions

These data indicate the relationship WT α-Syn and A30P α-Syn have with various ligands. Up to 5 CLR01 binding events can be observed on both proteoforms of α-Syn when CLR01 is added at a 5x molar concentration. IM-MS data showed that CLR01 binding events lead to compaction of WT and A30P α-syn. Additional binding of CLR01 molecules promotes greater compaction of α-syn structure. ECD fragmentation of WT α-syn/CLR01 and A30P α-syn/CLR01 complexes indicates CLR01 binds to the n-terminus of both proteoforms. MS analysis indicates that CLR01 binds to α-syn/copper and α-syn/manganese complexes. CLR01 seems to compete with copper for binding but enhances manganese binding on α-Syn. Furthermore, IM-MS data indicates that CLR01 compacts α-syn/metal complexes. ECD of the α-Syn/copper/CLR01 complex indicates copper and CLR01 bind to the n-terminus. The proximity of their binding events may be the reason that copper competes for binding with CLR01. ECD fragmentation of the α-Syn/manganese/CLR01 complex indicates that CLR01 binding remains on the n-terminus while manganese binds toward the c-terminus. It is possible a structural change caused by CLR01 binding may enhance manganese binding. Structural changes due to CLR01 binding could indicate a mechanism for aggregation inhibition of α-syn. In the future, we hope to perform CLR01 binding experiments on oligomers of amyloid proteins to bring to light how CLR01 interacts with protein aggregates. This research could indicate how aggregation inhibiting compounds like CLR01 prevent the formation of Lewy Bodies in the brains of neurodegenerative disease patients.

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