Copper(II) and the pathological H50Q \( \alpha \)-synuclein mutant: Environment meets genetics

Anna Villar-Piqué\(^a\), Giulia Rossetti\(^b\)\(^c\)\(^d\), Salvador Ventura\(^e\), Paolo Carloni\(^b\), Claudio O. Fernández\(^f\)\(^g\), and Tiago Fleming Outeiro\(^a\)\(^h\)

\(^a\)Department of Neurodegeneration and Restorative Research, University Medical Centre Göttingen, Göttingen, Germany; \(^b\)Computational Biomedicine, Institute for Advanced Simulation IAS-5 and Institute of Neuroscience and Medicine INM-9, Jülich, Germany; \(^c\)Department of Oncology, Hematology, and Stem Cell Transplantation, Medical School, Rheinisch-Westfälische Technische Hochschule Aachen University, Aachen, Germany; \(^d\)Simulation Laboratory Biology - Jülich Supercomputing Centre (JSC), Jülich, Germany; \(^e\)Institut de Biotecnologia i Biomecànica and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain; \(^f\)Max Planck Laboratory for Structural Biology, Chemistry, and Molecular Biophysics of Rosario, Universidad Nacional de Rosario, Ocampo y Esmeralda, Santa Fe, Argentina; \(^g\)Instituto de Investigaciones para el Descubrimiento de Fármacos de Rosario UNR-Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina, Universidad Nacional de Rosario, Ocampo y Esmeralda, Rosario, Argentina; \(^h\)Max Planck Institute for Experimental Medicine, Göttingen, Germany.

**ABSTRACT**

Copper is one of the metals described to bind the Parkinson disease-related protein \( \alpha \)-synuclein (aSyn), and to promote its aggregation. Although histidine at position 50 in the aSyn sequence is one of the most studied copper-anchoring sites, its precise role in copper binding and aSyn aggregation is still unclear. Previous studies suggested that this residue does not significantly affect copper-mediated aSyn aggregation. However, our findings showed that the aggregation of the pathological H50Q aSyn mutant is enhanced by copper hints otherwise. Despite the existence of a model for aSyn H50Q-copper complexation, we discuss possible mechanisms by which this metal contributes to the misfolding and self-assembly of this particular aSyn mutant. Considering the genetic association of the H50Q mutation with familial forms of Parkinson disease, and the fact that copper homeostasis is deregulated in this disorder, understanding the interplay between both factors will shed light into the molecular and cellular mechanisms triggering the development and spreading of the aSyn pathology.

The abnormal accumulation of \( \alpha \)-synuclein (aSyn) is a characteristic of various neurodegenerative diseases, such as Parkinson disease (PD), that are collectively known as synucleinopathies.\(^1\) However, the pathological consequences of this process are still not clear, neither the molecular mechanisms promoting and/or causing aSyn aggregation. Factors that promote aSyn misfolding and self-assembly include point mutations, found in genetic forms of synucleinopathies, and the presence of environmental stimuli, such as pro-aggregation metals.\(^2\)\(^,\)\(^3\)

We recently exploited the unique combination of the H50Q pathological aSyn mutation and the presence of copper(II) (Cu\(^{2+}\)), a metal whose homeostasis is deregulated in many neurodegenerative conditions, and found that the neuronal damage induced by the aSyn species used in our study is not correlated with the accumulation of intracellular aSyn inclusions, supporting the dissociation between toxicity and the accumulation of large inclusion bodies.\(^4\)

Since the recent discovery of the H50Q mutation in familial forms of PD, various studies reported that it enhances the aggregation of aSyn in vitro.\(^5\)\(^\)\(^,\)\(^7\) Additionally, Cu\(^{2+}\) and other metals are known to accelerate aSyn fibrillation.\(^8\) Cu\(^{2+}\) interacts with the negatively-charged C-terminal region of the protein, but binds the N-terminal region with higher affinity.\(^9\)\(^,\)\(^10\) Two main possible complexation modes in the N-terminus are suggested: one considers the first few residues and His50 (together with neighboring residues) as 2 independent binding sites;\(^10\) the other considers the formation of a long-range bridge in the polypeptide chain through the binding of a single metal ion, in which His 50 would act as a coordination switch (Fig. 1).\(^9\) Cu\(^{2+}\)
coordination provokes compaction in aSyn.\textsuperscript{11,12} These conformational changes, even if subtle, may reduce the free energy barrier and trigger aggregation. However, these hypotheses are challenged by the recent finding that the aggregation-inducing activity of Cu\textsuperscript{2+} is stronger in the H50Q mutant than in the wild-type (WT).\textsuperscript{4} This conclusion is derived not only from cell-free aggregation experiments but also from a cellular model where a panel of pathological mutations was expressed and exposed to different metals and only the combination of Cu\textsuperscript{2+} and H50Q resulted in the formation of inclusion bodies.\textsuperscript{4} This uncovers a plethora of questions regarding the binding mode of Cu\textsuperscript{2+} in this particular mutant of aSyn, and why such event causes the rapid formation of aSyn aggregates (Table 1).

Existing data indicate that the binding profile of aSyn-Cu\textsuperscript{2+} is not significantly disturbed in the H50Q mutant, despite the absence of the His50 site, suggesting the absence of coordination of Cu\textsuperscript{2+} with Glu50.\textsuperscript{5} However, the faster aggregation of H50Q in the presence of Cu\textsuperscript{2+}, when compared with WT aSyn, indicates that position 50 likely plays a role in modulating the effect of Cu\textsuperscript{2+}. One possibility is that His50 lies on a “strategical” position in the sequence, in such a way that the binding of Cu\textsuperscript{2+} to this residue may induce a local turn-like structure, thereby favoring $\beta$-sheet nucleation.\textsuperscript{13} The lack of this process in the H50Q variant, due to the lack of Cu\textsuperscript{2+} binding at position 50, would trigger other misfolding mechanisms, resulting in different aggregation characteristics. Another possibility is that, indeed, the differences between Cu\textsuperscript{2+}–promoted aggregation of WT and H50Q do not depend on conformational changes in the beginning of the aggregation process, but rather on the subsequent elongation stages. However, previous studies reported that, at least with WT aSyn, Cu\textsuperscript{2+} mainly influences the lag phase of aggregation, promoting accelerated nucleation.\textsuperscript{14} Similarly, although the Cu\textsuperscript{2+} effect on WT aSyn seems restricted to intramolecular phenomena,\textsuperscript{11} promotion of intermolecular aberrant interactions could occur in the H50Q. Additionally, it is also possible that the increased flexibility of the C-terminal region in the H50Q mutant\textsuperscript{15} could change the Cu\textsuperscript{2+}-binding features in this domain and, thereby affect the overall aggregation process. Although the absence of the imidazole ring of

**Table 1.** Effects of Cu\textsuperscript{2+} on H50Q vs. WT aSyn.

| Metal binding profile | No major alterations detected besides the loss of the His50 binding site (measured by electrospray ionization—ion mobility spectrometry—mass spectrometry,\textsuperscript{18} electron paramagnetic resonance\textsuperscript{19} and nuclear magnetic resonance\textsuperscript{5}) |
|-----------------------|-------------------------------------------------------------------------------------------------------------------------------|
| In vitro aggregation  | FASTER AGGREGATION RATE\textsuperscript{4,18} Fibrillar morphology is replaced by amorphous aggregates\textsuperscript{6} |
| Aggregate morphology  | Mature aggregates display very low homoseedding capacity\textsuperscript{6} Reduced thioflavin-T binding and $\beta$-sheet structure\textsuperscript{6} |
| Seeding activity      | Stronger inclusion formation capacity of H50Q aSyn in the presence of Cu\textsuperscript{2+}\textsuperscript{4} |
| Amyloid structure     | H50Q aggregates formed with Cu\textsuperscript{2+} are stronger inducers of intracellular aSyn aggregation, but display reduced neurotoxicity\textsuperscript{4} |
| Cellular aggregation  | H50Q aggregates form with Cu\textsuperscript{2+} are stronger inducers of intracellular aSyn aggregation, but display reduced neurotoxicity\textsuperscript{4} |
| Exposure of neurons to exogenous aSyn aggregates | |

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Figure 1. The H50 residue is key for anchoring Cu\textsuperscript{2+} binding to aSyn. Quantum Mechanics / Molecular Mechanics (QM/MM) simulations\textsuperscript{21} were performed to test the stability of all the possible coordination geometries of Cu\textsuperscript{2+} at H50 site, fitting available experimental evidences.\textsuperscript{14,17,20} Models A and B results to be the most plausible. The Cu\textsuperscript{2+} ion binds to H50 side chain in both of them. The 3 additional ligands are the H50 amide group, a water molecule, and either V48 carbonyl O (model A) or V49 deprotonated amide (model B). All of the coordination bonds exhibited relatively small fluctuations around their average values, except for the Cu\textsuperscript{2+}-V48 carbonyl and Cu\textsuperscript{2+}-V49 amide. This suggests an interconversion between the 2 forms. Residues and water molecules are shown in ball-and-stick representation: red, blue, gray and white for oxygen, nitrogen, carbon and hydrogen, respectively. Cu\textsuperscript{2+} is shown as a golden ball.

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His50 may disturb Cu\(^{2+}\)-triggered aSyn aggregation. Gln50 may also promote a toxic gain-of-function role. The aggregation of different aSyn point-mutants at position 50 revealed that, while H50Q was the more aggregation-prone among the mutants tested, a positive charge at this site (WT or H50R) probably protects from fibrilization.\(^{15}\) A similar experiment performed in the presence of Cu\(^{2+}\) would contribute to defining the effect of Gln50 in Cu\(^{2+}\) binding. Even if the Gln50 is not directly involved in metal coordination, it could play a role in stabilizing protein-metal complexation.\(^{16}\)

Electron microscopy confirmed that fibrils formed by the H50Q mutant resemble those formed by WT aSyn.\(^{4-6}\) Cu\(^{2+}\) also does not alter the morphology of fibers formed by WT aSyn.\(^{14}\) However, the combination of the H50Q mutation and Cu\(^{2+}\) results in the formation of amorphous aggregates.\(^{4}\) Although this could be the mere consequence of the very rapid self-assembly rate, it will be worthwhile exploring this issue further to understand when and where those morphological changes arise.

Finally, certain physiologic circumstances may also cause the loss of Cu\(^{2+}\)-binding, such as N-terminal acetylation of aSyn or a reduction in the micro-environmental pH.\(^{17,18}\) The latter would result in protonation of the His50 imidazole ring, impairing metal binding. In this case, the H50Q substitution could create a similar scenario, forcing Cu\(^{2+}\) to coordinate only to the N-terminal cluster. The lack of a potential intramolecular bridge supported by a Cu\(^{2+}\) ion might alter the entire conformation, probably inducing more open Cu\(^{2+}\)-aSyn complexation modes, affecting the aggregation process and eventually the toxicity of the resultant species.

In the future, when further addressing the role of Cu\(^{2+}\) as an enhancer of the H50Q aggregation, physiologic micro-environments and posttranslational modifications will have to be considered. A molecular understanding of the interplay between genetic factors, such as aSyn mutations and environmental factors, such as the presence of Cu\(^{2+}\), might greatly contribute for our understanding of the pathological events causing PD.

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No potential conflicts of interest were disclosed.

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