Metal Ion Chelation and Oxidation Kinetics involving Synthetic Peptides

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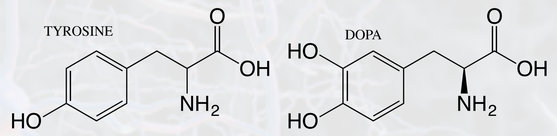
Background

The Metal Ion Hypothesis

Previous research has suggested the amyloid plaques implicated in the progression of Alzheimer's Disease (AD) contain higher levels of metal ions such as iron (Fe^{2+}) and zinc (Zn^{2+})[1]. Other literature stresses the importance of genetics in maintaining metal ion homeostasis; these genes and pathways are typically disturbed in patients with AD[1]. The metal ions thought to play a role in AD are typically strong chelators. Metal chelation occurs through coordination with amino acid residues that have hydroxyl groups, most notably tyrosine and its oxidatively damaged form dopa.



Though the actual role of the metal ions is not know, it is thought that they interact with and stabilize the oligomerized form of a peptide called amyloid-β[1]. Oligomerization reduces solubility of the peptides, thus forming the plaques indicative of Alzheimer's Disease.

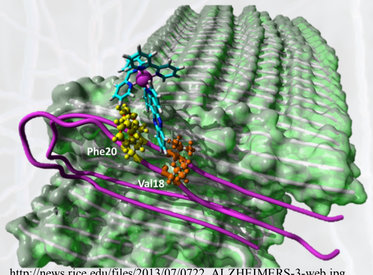


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Figure 1. A modeled oligomer of amyloid-β. The hairpin turn creates two β sheets. The meal ion causes stabilization on both Phe20 and Val18 in this figure, while stabilizing other peptides above (not pictured). The amassing of multiple oligomer systems causes a plaque, likely due to the loss of hydrophilicity and thus solubility.

Model System

All of the biological aspects of the system were eliminated, providing a bare-bones assessment of binding characteristics for specific metal ions to our synthetic peptides. Small, six amino acid peptides were synthesized to have a hairpin turn, representative of a larger protein structure, as well as a (*L*)-dopa residue, representative of an oxidatively damaged tyrosine residue. Theoretical binding would happen through chelation at the dopa residue and possibly through the coordination with other aromatic ring systems within the peptides.

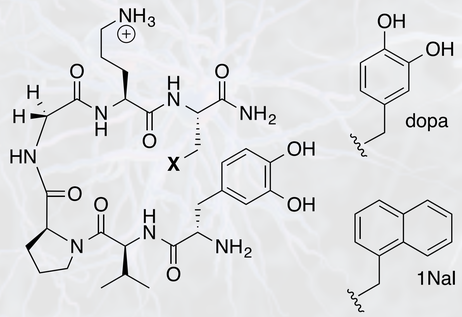

Figure 2. Synthetic generic peptide used for this research. The N-terminus is an (*L*)-dopa residue, followed by a valine, (*D*)-proline, glycine, ornithine, and a variable position; the C-terminus was amidated. The variable X position contained either a histidine, tryptophan, tyrosine, phenylalanine, 1-napthylalanine (1Nal), or 2-napthylalanine (2Nal). Both the dopa and the 1Nal are depicted.

UV-Vis

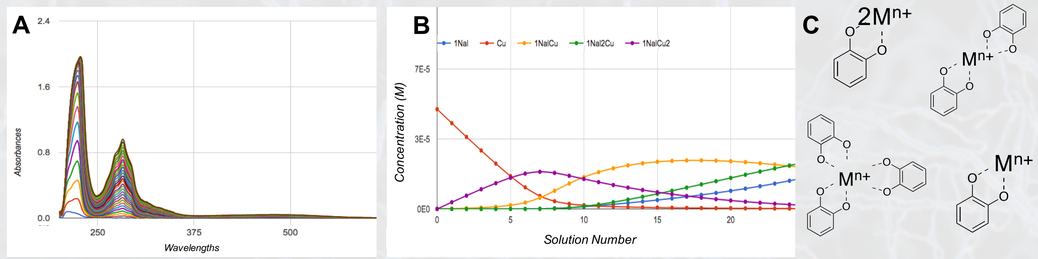

Figure 3. A. Raw ultra-violet/ visible spectral data of Cu^{2+} titrated into Dopa1Nal. The absorbance of the solution increases with the addition of Cu^{2+}, specifically at 230nm and 280nm. There is a small, broad peak around 475nm, indicative of peptide : copper binding. **B.** The solution composition and concentrations of species. As copper concentration decreases, it complexes two coppers to one Dopa1Nal, followed by one copper to one Dopa1Nal, followed by one copper bound with two Dopa1Nal peptides. **C.** Binding scheme of peptides and metal ions. The aromatic ring with two hydroxyls is representative of a (*L*)-dopa residue on the end of the synthetic peptide. One peptide can bind to one metal ion, two metal ions, two peptides to one metal ion, and three peptides to one metal ion. Each of these complexes produces a different color, rendering it possible to differentiate the prominent species found in a specific solution.

Oxidation

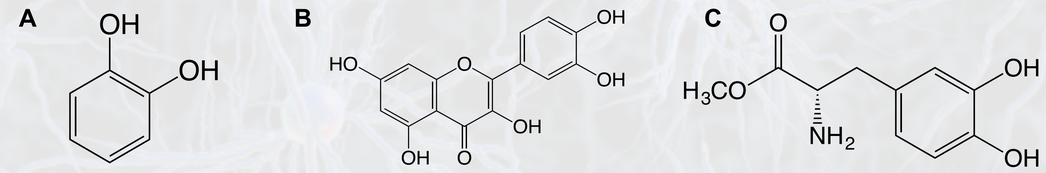

Figure 4. A. A catechol moiety. Catechols are exceptionally good oxidizers; the basic structure of a catechol is contained within a (*L*)-dopa reside. **B.** The flavonoid quercetin, found in antioxidant rich foods. Flavonoids and flavonols, among other antioxidants, are responsible for scavenging free radicals within the human body, making them extremely important in maintaining normal bodily function. They often contain catechol moieties. **C.** 3,4-dihydroxyphenyl methyl ester (D1507), used as a surrogate for (*L*)-dopa. This molecule was used in these oxidation experiments.

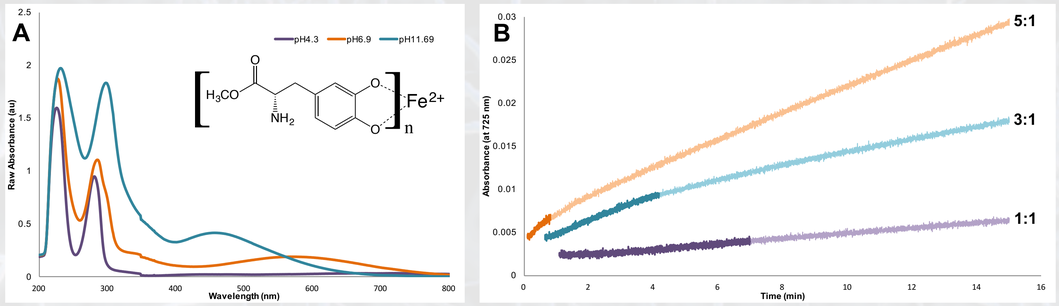

Figure 5. A. UV/Vis spectra of a pH titration of iron and D1507. At high pH (11.69), complexation is most evident in a 3:1 species of D1507 to iron, observed at 298 nm. At neutral pH (6.9), complexation is most evident in a 2:1 species, observed at 575 nm. At low pH (4.3), complexation happens most readily in a 1:1 species, observed at 725 nm. **B.** Kinetics spectra of complexation of D1507 and iron, assessed at pH 4.5 and a wavelength of 725 ±3 nm. Concentration of D1507 was increased in comparison to iron, starting at 1:1 equivalents of D1507 to iron, increasing to 3:1 equivalents and 5:1 equivalents. As the concentration of D1507 increased, so did the initial rate of product formation (shown in a darker shade).

MALDI Mass Spectrometry

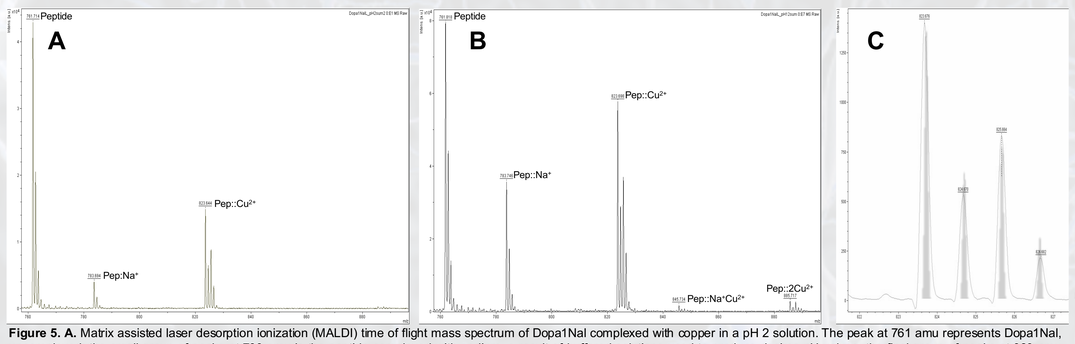

Figure 5. A. Matrix assisted laser desorption ionization (MALDI) time of flight mass spectrum of Dopa1Nal complexed with copper in a pH 2 solution. The peak at 761 amu represents Dopa1Nal, uncomplexed; the small group of peaks at 783 amu is the peptide complexed with sodium, a result of buffered solutions used to produce desires pH values; the final group of peaks at 823 amu represent the peptide complexed with copper. **B.** MALDI mass spectrum of Dopa1Nal complexed with copper in a pH 12 solution. The first three sets of peaks are the same as presented in the pH 2 spectrum: bare peptide, peptide with sodium, and peptide with one copper ion. The spectrum also indicated the presence of peptide bound to both sodium and copper at 845 amu. A two copper and one peptide complex is also evident, presented on the spectrum at 885 amu. **C.** Isotopic distribution of copper found to complex with peptide. This peak distribution is characteristic of all complexes with copper and can be seen in both the one peptide one ion complex as well as the one peptide to two ions complex, shown in theoretical form in light grey, superimposed on the image.

NMR Spectroscopy

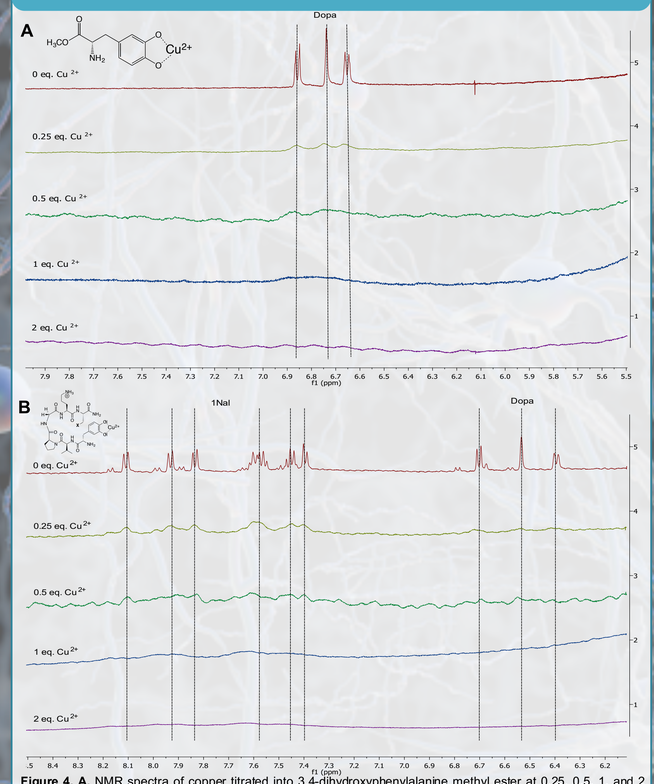

Figure 4. A. NMR spectra of copper titrated into 3,4-dihydroxyphenylalanine methyl ester at 0.25, 0.5, 1, and 2 equivalents of copper to methyl ester. As cmore opper is titrated into the system, the hydroxyl peaks broaden and eventually flat line. **B.** NMR spectra of copper titrated into Dopa1Nal, at same equivalents. The dopa peaks on the right side of the spectra broaden and disappear by 0.5 equivalents of copper, whereas the 1Nal peaks, on the left side of the spectra, remain into 1 equivalent of copper added.

Conclusions

It can be reasonably concluded that metal ions, specifically copper, interact strongly with oxidatively damaged amino acid residues. Characterization of solutions using mass spectrometry support complexation of both a 1:1 metal to peptide complex and a 2:1 metal peptide complex. In the solution phase, not only can one peptide coordinate one metal ion, but species containing multiple of each can also be readily formed, which seems somewhat dependent on pH value. These peptides may also play a role in the oxidation of metal ions, as evidenced by the increase in rate of oxidation of iron in higher concentrations of D1507. These findings may lend further credence to the metal ion hypothesis of Alzheimer's Disease.

Acknowledgments and References

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[1]Kepp, Kasper P. "Bioinorganic chemistry of Alzheimer's disease." *Chemical reviews* 112.10 (2012): 5193-5239.

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