# *ATP Protects Dopamine From Fe(III)-catalyzed Oxidation*

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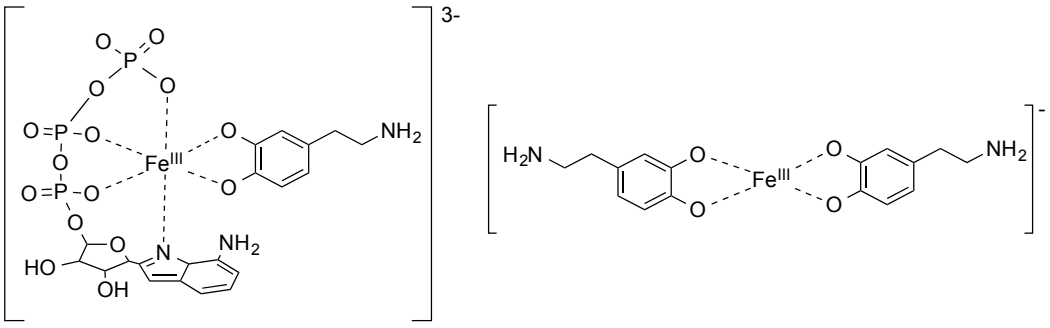
*KEYWORDS: dopamine, oxidation, Parkinson's Disease, ATP*

**ABSTRACT:** In the brain, dopamine (DA) degrades in the presence of oxygen, forming toxic byproducts. In healthy subjects, this process is tightly controlled. However, in Parkinson's Disease (PD) patients, excessive $Fe^{III}$-catalyzed DA oxidation is observed. Jiang et. al. observe that adenosine triphosphate (ATP) slows the oxidation, and hypothesize that the ATP may form a stabilizing ternary complex with DA and $Fe^{III}$. They synthesize the complex, and use UV-vis, mass spectrometry, concentration studies, and cyclic voltammograms to investigate its structure and function. The inorganic chemistry concept of ligand field theory can be used to explain the absorption bands in the UV-vis spectra.




Two $Fe^{III}$ complexes with roles in Parkinson's Disease

**Introduction:** Parkinson's Disease (PD) is a common neurodegenerative disorder which currently has no cure. The disease is characterized by degradation of dopamine (DA)-producing cells in the *substantia nigra* of the brain.[1] This degradation results in the generation of reactive oxidation species, and its cause is unknown.[7] The *substantia nigra* is involved in a number of complex processes, including motor planning, reward-seeking, and learning. Oxidative stress in this region impairs the central nervous system and causes depression, shaky and rigid movements, and dementia.[2]

Many differences have been observed between Parkinsonian brains and control brains. It is clear that Parkinsonian-*substantia nigras* are under a significant amount of oxidative stress. The diseased brains have decreased concentrations of DA, ATP, and neuromelanin (a polymer formed from DA and its oxidation products).[3,9] In contrast, concentration of iron is substantially increased.[10] Though these effects have been consistently measured, it is not clear which, if any, of these effects play a causal role in PD. Current research in neurodegenerative diseases is focused on determining possible origins for these trends.[9]

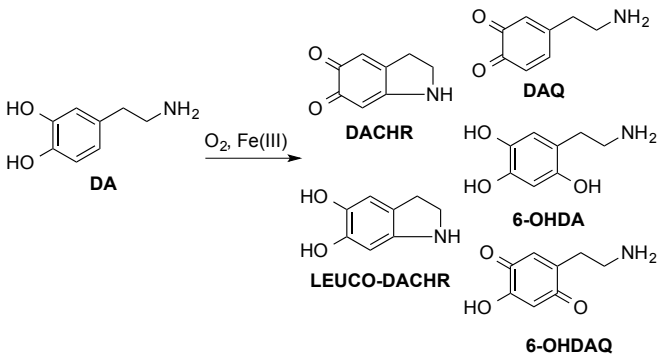



**Figure 1**. Neurotoxic intermediates in the oxidation pathway[9]

DA and the products of its oxidation (Figure 1) are known to be neurotoxic,[9,10] however, the exact mechanism of iron-catalyzed oxidation has not been determined. The authors suggest that the mechanism proceeds as shown in Figure 2.[9]

Other pathways[3,6,8] (not shown) are similar, and the authors have chosen not to discuss them for the sake of brevity. It is important to note that although only one DA molecule is shown in the mechanism, each ferric ion is actually coordinated to two DA molecules (notated DA-$Fe^{III}$-DA). Water molecules from the aqueous environment may loosely occupy the empty ligand sites, but do not seem to impede the oxygen approach shown in Figure 2.

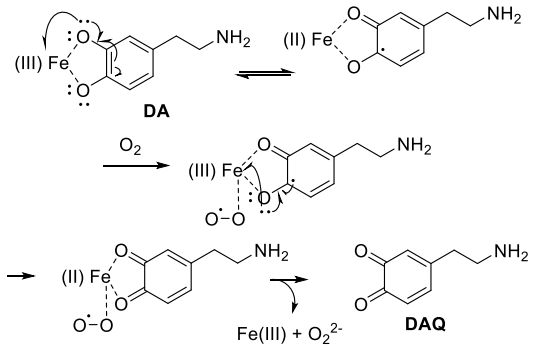



**Figure 2**: One possible mechanism of $Fe^{III}$- and $O_2$-catalyzed DA oxidation[9]

After observing that ATP slows the oxidation of DA dramatically, Jiang et al. hypothesize that ATP coordinates to the iron center of the complex as a tetradentate ligand. This coordination results in an octahedral complex in which one DA molecule and one ATP molecule are each coordinated to a ferric ion center (notated ATP-$Fe^{III}$-DA). The coordination sites of the metal are each tightly occupied, and this impedes molecular oxygen's access to the ferric ion, therefore inhibiting its ability to oxidize DA.

The Jiang Lab uses UV-vis, high resolution-MS, cyclic voltammograms, and time-dependent concentration assays to characterize the complex and to study or simulate its role in biological systems in the brain. The results of these studies and an analysis of their implications follow.

**Discussion:** As a transition metal, iron is well-known for its ability to bind to a variety of ligands.[5] Iron complexes readily undergo electronic excitations, which can be detected through UV-vis spectroscopy. The authors therefore use UV-vis to characterize the metal complexes they are examining. The spectra of these complexes are shown in Figure 3. Each complex was formed by the addition of ligands to a solution containing $Fe^{III}$ ions. Two solutions contained only DA ligands, and they each produced the same absorbance pattern. The DA:$Fe^{III}$ ratio in the first spectrum (black) was 2:1, and the ratio in the second spectrum (red) was 1:1. These two spectra have identical peak absorption wavelengths (570 nm), indicating that the complex formed in each case was the same. The lower absorbance rate in the red spectrum indicates that the concentration of the complex has decreased. An additional solution (blue line), contained the DA:ATP:$Fe^{III}$ in a 1:1:1 ratio, and displayed an absorbance peak at a slightly larger wavelength, 625 nm. (Another spectrum, not shown, of a 1:2:1 ratio, resulted in an identical absorption pattern and intensity. This indicates that there were no available coordination sites on the iron centers in the 1:1:1 ratio, since no additional ligand has bound to the complexes). This shift in peak absorption wavelength from 570 nm to 625 nm indicates that the two complexes undergo differing electronic excitations, and are, in fact, distinct complexes.

The absorption bands are the result of ligand metal charge transfers (LMCTs). Although the authors only touch briefly on this fact, a full understanding of LMCT adds clarity to the shift in the absorbance peak described in their paper. An LMCT occurs when an electron from a ligand orbital is excited into a metal orbital, formally reducing the metal. Here, one electron is transferred from DA, reducing $Fe^{III}$ to $Fe^{II}$. This electron transfer can be visualized on a molecular orbital

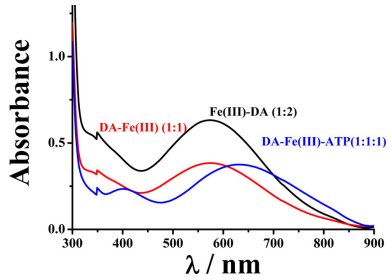



**Figure 3**: Absorbance spectra of ATP-$Fe^{III}$-DA and DA-$Fe^{III}$-DA complexes (Figure taken from paper)[9]

diagram constructed using ligand field theory. Ligand field theory is an inorganic chemistry model which considers the effects of ligands on octahedral metal complexes. These ligands either stabilize or destabilize the metal by accepting or donating electron density to the iron center. Although the DA-$Fe^{III}$-DA complex appears to be square planar, there are water molecules coordinated to the axial sites of the metal which participate in bonding with the complex. Therefore the DA-$Fe^{III}$-DA and the DA-$Fe^{III}$-ATP complexes each have an octahedral geometry, and can be examined using ligand field theory. The electron transfers associated with the absorption band of each complex are shown in Figure 4. In DA-$Fe^{III}$-DA, shown on the left of the figure, the electron is excited from a ligand orbital to a metal orbital. On the right, an electron in the ATP-$Fe^{III}$-DA complex undergoes the same process. However, in this second complex, the nitrogen –bearing ATP ring, a pi acceptor ligand stabilizes the metal orbitals by accepting electron density from the metal center (as compared to the water molecules which destabilize the corresponding DA complex by donating electron density to the metal center). The excited electron in the ATP-$Fe^{III}$-DA complex therefore absorbs a smaller frequency of energy in its excitation process. Because of this, the energy transition associated with the ATP-$Fe^{III}$-DA complex is also smaller, resulting in a larger peak absorption wavelength.

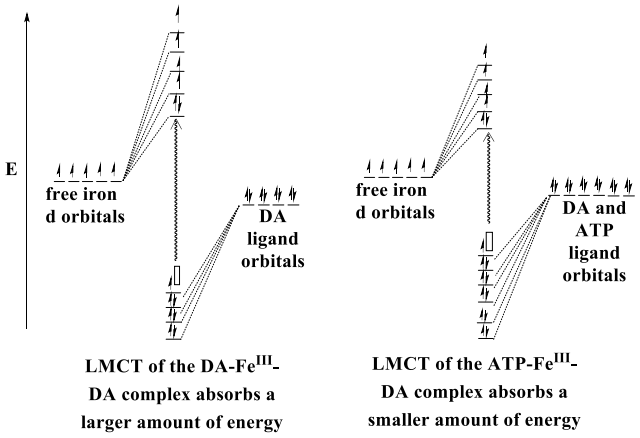



**Figure 4:** a simplified depiction of electron excitation in ATP-$Fe^{III}$-DA and DA-$Fe^{III}$-DA metal complexes. The elec-

tron is excited from the ligand orbital (the empty rectangle) to the higher level (with two electrons)

In their second UV-vis experiment (Figure 5), the authors attempt to simulate "real-life" formation of the ATP-$Fe^{III}$-DA. In these solutions, iron was introduced to the system in the form of ferritin, an iron storage protein, instead of in its free form. Jiang et. al hypothesized that ferric ions would dissociate from ferritin to form ATP-$Fe^{III}$-DA, because of the stability inherent in the ternary complex. In this experiment, a spectrum was taken of a ferritin, DA, and ATP solution at 0 hours (blue), and again after 21 hours (green). The reaction was then separated from remaining ferritin, and another spectrum (red) was taken of the purified solution. Each of these spectra are compared to a reference of DA-$Fe^{III}$-ATP (black). As seen in Figure 5, each 21-hour curve shows evidence of a peak at 625 nm, indicating formation of the DA-$Fe^{III}$-ATP complex. The formation of this complex indicates the relative stability of the complex as compared to ferritin.

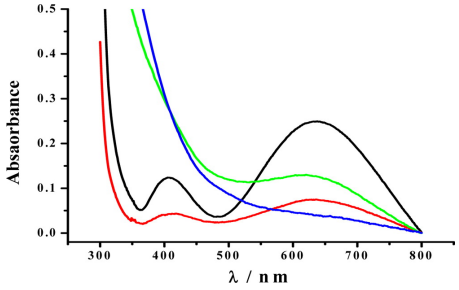



**Figure 5:** Absorbance spectra demonstrating the formation of the ATP-$Fe^{III}$-DA complex (figure taken from paper)[9]

Though these UV-vis experiments demonstrate that the formation of DA-$Fe^{III}$-ATP is possible, they fail to prove that the complex occurs *in vivo*. To take this next step, the authors examined tissue extracts from various regions of rat brains. An electrospray high-resolution mass spectrum of extract from the *substantia nigra* of a rat brain sample indicated a peak which the authors attribute to ATP-$Fe^{III}$-DA. They offer several rationalizations for their assignment. (1) The masses of the three common isotopes of the complex are accurate to within 5 ppm. (2)When pure ATP-$Fe^{III}$-DA was added to the extract, the size of the assigned peak increased. (3) When EDTA (a strong iron chelator) was added to the tissue, the peak disappeared, presumably because the ferric ions bound to EDTA instead of DA and ATP. (4) Extracts from other areas of the rat brain were also examined, and did not contain this peak. Although none of these facts would be sufficient proof on their own, together they offer a convincing argument.

Having identified the ternary complex *in vitro* and *in vivo*, Jiang et al. next examine its effect on the oxidation of the DA. Once again, the authors considered three solutions; one contained only DA, a second contained DA and $Fe^{III}$, and a third contained DA, ATP, and $Fe^{III}$. Cyclic voltammograms of each solution were obtained and the results were analyzed. The voltammograms were conducted over specific voltage ranges, capable of oxidizing DA in its various complexes. In their analysis, the authors choose to focus on the oxidation potential of DA, and these results are summarized below in Table 1. Although data concerning the oxidation potential of the iron centers has been omitted from this table, it strengthens the trend shown by the DA potentials.

**Table 1:** Oxidation Potentials of DA

| Form of DA | Oxidation potential (V) |
|---|---|
| DA (free) | 0.25 V |
| DA-$Fe^{III}$-DA | 0.37 V |
| ATP-$Fe^{III}$-DA | 0.44 V |

A second oxidation study was also performed. In this experiment, the authors prepared the same three solutions and monitored concentration of DA over time. The results, shown in Figure 6 below, indicate that ATP slows $Fe^{III}$-catalyzed oxidation of DA to near-$Fe^{III}$-free conditions, while the DA in the $Fe^{III}$ solution is quickly oxidized.

At first, these results may seem to contradict each other. The voltammogram suggests that DA is more difficult to oxidize with $Fe^{III}$ present than without, yet in the assays, the free DA was oxidized much less than the DA in the presence of $Fe^{III}$. However, it is important to note that the voltammogram measurements were performed in an air-free environment, and therefore do not consider the effect of oxygen gas on DA oxidation. As has been discussed, oxygen rapidly oxidizes DA through an iron-mediated mechanism. If the voltammograms were performed in the presence of oxygen, the DA-$Fe^{III}$-DA would be the most easily oxidized. Since this cannot occur in the voltammogram studies, the two experiments are not comparable.

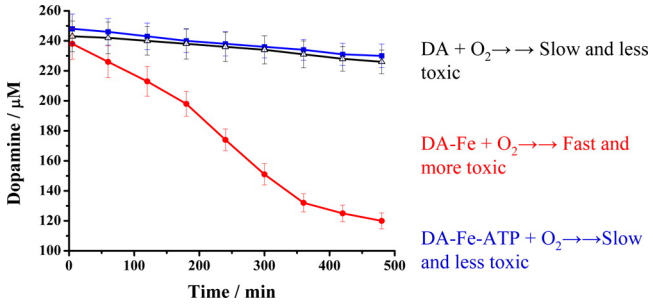



**Figure 6:** Degradation of DA in various chemical environments over time (figure taken from paper)[9]

**Conclusion:** Jiang et. al. provide a convincing argument for the existence of the ATP-$Fe^{III}$-DA complex, both *in vitro* and *in vivo*. Through high-resolution MS and absorbance spectra, they have provided definitive identification parameters of the complex. Additional absorbance spectra demonstrate the stability of the complex. Time-dependent concentration assays and oxidation potential measurements reveal the protective quality of the ATP ligand. In each of the measurements performed, the authors are comprehensive in their presentation and rationalization of the data. They thoroughly explore all possible explanations of their results, and defend their interpretation soundly.

Although Jiang et al. have successfully synthesized and characterized the ATP-$Fe^{III}$-DA complex, this is only the first of many steps which will be required before their conclusions can have an impact on current PD understanding or treatment. At best, their results indicate one method by which the healthy *substantia nigra* protects DA from oxidation. It is possible that decreased levels of ATP in this region accelerated the oxidation of DA in PD patients. However, it seems more likely that this oxidation and the subsequent death of dopaminergic neurons seen in PD brains are caused by a combination of several factors, rather than one alone.

If the authors wish to further develop this area of their research, there are multiple avenues that they could pursue. Other biological small molecules could be assessed for their ability to bind in the same manner as ATP. Alternatively, the lab could attempt to develop more biologically stable compounds that provide the same tetradentate ligand protection as ATP. If these compounds can be transported across the blood-brain barrier, they could be used as medications in the treatment and prevention of PD.

The cause and treatment of PD is currently a field with many unknowns. Jiang et. al. have brought clarity to a small area of this field, but future research will be required to implement their findings, and to successfully understand and treat the disease.

## AUTHOR INFORMATION

371-2201B5 is a chemistry major in the class of 2014 at Amherst College.

## ABBREVIATIONS

ATP, adenosine triphosphate; DA, dopamine; EDTA, ethylenediaminetetraacetic acid; PD, Parkinson's Disease

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