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Cisplatin Inhibits the Formation of a Reactive Intermediate during Copper-Catalyzed Oxidation of Amyloid β Peptide

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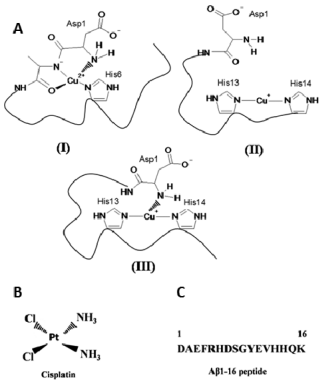
Supporting Information

ABSTRACT: Cisplatin was studied for its effect on the copper-catalyzed oxidation of amyloid β (Aβ) peptide. The interaction of cisplatin with Aβ1-16 in the presence of Cu^{II} was investigated using cyclic voltammetry and mass spectrometry. The positive shift in the $E_{1/2}$ value of Aβ1-16-Cu^{II} suggests that the interaction of cisplatin alters the copper-binding properties of Aβ1-16. The mass spectrometry data show complete inhibition of copper-catalyzed decarboxylation/deamination of the Asp1 residue of Aβ1-16, while there is a significant decrease in copper-catalyzed oxidation of Aβ1-16 in the presence of cisplatin. Overall, our results provide a novel mode by which cisplatin inhibits copper-catalyzed oxidation of Aβ. These findings may lead to the design of better platinum complexes to treat oxidative stress in Alzheimer's disease and other related neurological disorders.

Alzheimer's disease (AD) is an irreversible and progressive neurodegenerative disorder.[1] The formation of aggregates of amyloid β (Aβ) peptide in the brain is a hallmark of AD.[2] The formation of reactive oxygen species (ROS) due to the redox cycling of copper bound to Aβ is believed to be responsible for neurodegeneration in AD.[3] His6 along with an N-terminal amino group is involved in Cu^{II} binding as a major species (structure I) in solution (Scheme 1).[4] His13 and His14 form a peculiarly stable environment around Cu^{I} (structure II).[5] According to the preorganized electron transfer (POET) mechanism, a small fraction of reactive intermediate has been predicted to exist during redox cycling of Aβ1-16-Cu^{II}.[6] The amino group of Asp1 along with His13 and His14 play a critical role in the formation of a small fraction of this reactive intermediate during ROS production by Aβ-Cu^{II}.[7] Thus, preventing the formation of the reactive intermediate is considered as an attractive target for restraining the copper-catalyzed ROS formation and the subsequent neurodegeneration in AD. However, huge challenges are associated with the design of a promising copper chelating agent for the treatment of AD.[8]

Scheme 1. Major Species Involved in Redox Cycling of Aβ1-16-Cu^{II} (A), the Structure of Cisplatin (B), and the Sequence of Aβ1-16 (C)



Cisplatin is a well-known anticancer drug used in the treatment of malignant solid tumors.[9] It targets DNA and damages it, which further leads to cell death.[10] The copper-transport proteins CTR1 and ATP7B are involved in cellular uptake and efflux of cisplatin.[11] Recently, it was shown that the binding of Cu^{I} to intracellular copper-transport proteins ATOX1 and COX17, in fact, enhances the platination of these proteins.[12] Thus, increasing evidence of the interaction of platinum with copper-transport proteins led us to hypothesize that platination of Aβ will alter its copper-binding ability and subsequently alter the ROS production by Aβ1-16-Cu^{II}. Previously, Pt^{II} complexes were shown to bind Aβ through noncovalent interactions; however, the efforts were mostly focused on using Pt^{II} complexes as inhibitors of Aβ aggregation.[13] Thus, the potential of Pt^{II} complexes to interact with Aβ and inhibit Cu^{II}-catalyzed ROS production remains undiscovered. Here, we report for the first time the inhibition of copper-catalyzed oxidation of Aβ1-16 by cisplatin and the details of structural rearrangement using mass spectrometry (MS). Throughout this study, we used Aβ1-16 as a model peptide for full-length Aβ for simplicity of our experiments. Aβ1-16 had been widely used previously because it contains all of the copper-binding residues, and it does not readily precipitate or aggregate even at high concentrations.[14,15] Initially, the effect of cisplatin on the redox behavior of Aβ1-16-Cu^{II} was studied using cyclic voltammetry [see the Supporting Information (SI) for details]. Aβ1-16-Cu^{II} exhibited a quasi-reversible redox wave with the midpoint potential ($E_{1/2}$) at −0.014 V (Figure 1). Balland et al.[6] previously studied the redox reaction for Aβ1-16-Cu^{II} and proposed the POET mechanism for this quasi-reversible wave.

In the presence of cisplatin, Aβ1-16-Cu^{II} showed a distinct positive shift in the reduction as well as in the oxidation process with $E_{1/2}$ at +0.026 V. The positive shift in the $E_{1/2}$ value upon the addition of cisplatin suggests changes in the copper-binding site

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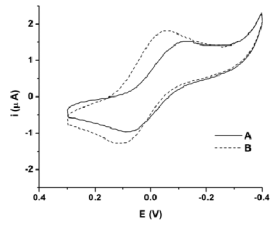

Figure 1. Cyclic voltammograms of 500 μM Aβ1-16-Cu^{II} (A) and 500 μM Aβ1-16-Cu^{II} in the presence of 500 μM cisplatin (B).

favoring the reduction of Aβ1-16-Cu^{II} to Aβ1-16-Cu^{I}. Liu et al.[16] established that the $E_{1/2}$ values for copper−peptide complexes involved in neurological disorders are associated with their ability to generate H_2O_2. Human prion protein has a metal binding domain with four octarepeats consisting of the sequence PHGGGWGQ, which bind Cu^{II} with high affinity.[16] At lower copper concentrations, four histidines of the four-octarepeat domains bind a single Cu^{II} ion to form a complex (OR-Cu^{II}) that shows the $E_{1/2}$ value of +0.126 V. At higher copper concentrations, each histidine residue of the individual octarepeat domain binds one Cu^{II} to form a complex (OR-Cu^{II}_4) with the $E_{1/2}$ value of −0.025 V. H_2O_2 produced by OR-Cu^{II}_4 is much higher than that produced by OR-Cu^{II}, which is in accordance with their respective $E_{1/2}$ values.[16] We therefore speculate that the positive shift in $E_{1/2}$ upon the addition of cisplatin to Aβ1-16-Cu^{II} will also result in a decrease of H_2O_2 generation. We have carried out ascorbate consumption assay to determine the ability of Aβ1-16-Cu^{II} to generate H_2O_2 in the presence and absence of cisplatin (see the SI for details). Our results show that cisplatin reduces ascorbate consumption by Aβ1-16-Cu^{II} (Figure S1 in the SI). H_2O_2 generated from redox recycling of Aβ1-16-Cu^{II} results in extensive cellular oxidative stress, and oxidation of Aβ itself is an important consequence of this reaction.[3] We carried out copper-catalyzed oxidation of Aβ1-16 in the absence and presence of cisplatin, and the products were analyzed using matrix-assisted laser desorption ionization (MALDI) MS (see the SI for details). Aβ1-16-Cu^{II} is reduced to Aβ1-16-Cu^{I} species using ascorbic acid and then allowed to oxidize back to Aβ1-16-Cu^{II} in the presence of atmospheric oxygen. The ROS generated subsequently targets the amino acid residues in the vicinity of the copper-binding site.[17] Cisplatin, being benign toward redox reactions, does not contribute to generation of ROS. Thus, the extent of oxidation of Aβ will provide the impact of cisplatin on the redox reaction of Aβ1-16-Cu^{II}, while alteration in the extent of oxidation of the copper-binding residues of Aβ will reveal the structural rearrangements taking place in the presence of cisplatin. In the absence of cisplatin, the MS spectrum of Aβ1-16-Cu^{II} showed three oxidation peaks at m/z 1971, 1987, and 2003 (Figure 2A). Additionally, three peaks at m/z 1941, 1925, and 1910 are observed. These three peaks were assigned to deamination, decarboxylation, and decarboxylation/deamination of the Asp1 residue of Aβ1-16, respectively.[7] In the presence of cisplatin, the MS spectrum of Aβ1-16-Cu^{II} showed a significant decrease in the copper-dependent oxidation processes, as is evident from the significant lowering of the intensities of peaks at m/z 1971, 1987, and 2003 (Figure 2B).We observed weak peaks at m/z 2018, 2149, and 2184 for a metalated peptide such as Aβ1-16-Cu^{II}, Aβ1-16-Pt^{II}, and Aβ1-16-$Pt^{II}(NH_3)_2$, respectively (Figure 2B). The most remarkable feature of the MS spectrum is the absence of peaks for decarboxylation/deamination processes.

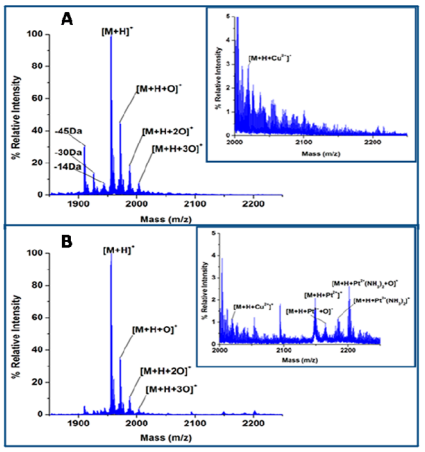

Figure 2. MS spectra for oxidized Aβ1-16-Cu^{II} (500 μM) (A) in the absence of cisplatin and (B) in the presence 500 μM cisplatin. Inset: enlarged region between m/z 2000 and 2250.

Our results showing complete inhibition of decarboxylation/deamination of Asp1 strongly imply the binding of cisplatin to the Asp1 residue of Aβ1-16. The amino groups of Asp1 and His6 residues are important for Cu^{II} binding but not Cu^{I} binding; therefore, the reduction of Aβ1-16-Cu^{II} to Aβ1-16-Cu^{I} will facilitate the interaction of cisplatin with Asp1 and His6 residues. Recently, Streltsov et al.[18] demonstrated the formation of Aβ1-16-Pt^{II} and Aβ-$Pt^{II}(NH_3)_2$ as abundant species formed during the interaction of cisplatin with Aβ1-16 in the absence of copper. The structural models suggest the involvement of histidine residues as well as the amino group of Asp1/Lys16 residues. In the presence of copper, however, the involvement of histidine residues in the binding of cisplatin will be complicated because both Cu^{II} and Cu^{I} have distinctly different binding sites (Scheme 1). However, in both cases, one or more histidine residues are available for cisplatin binding. Thus, the decrease in the oxidation can be ascribed to cisplatin binding to one of the histidine residues of Aβ1-16, most probably His6. When exposed to air, both Asp1 and His6, which are now bound to cisplatin, are unavailable for Cu^{II} binding. On the basis of this model, the shift in the redox potential, inhibition of decarboxylation/deamination of Asp1, and decrease in the oxidation of Aβ can be justified.

To test that cisplatin interacts with Aβ1-16 in the presence of copper, we subjected cisplatin-bound Aβ1-16 to the copper-catalyzed oxidation reaction (see the SI for details). Our results show that copper competes with cisplatin for binding to Aβ1-16. The oxidation products, however, remain the same (Figure 3). In

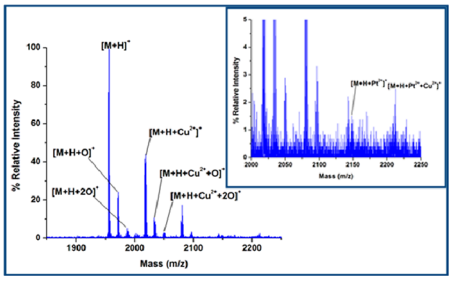

Figure 3. MS spectrum of oxidation products of 500 μM Aβ1-16 preincubated with 500 μM cisplatin. Inset: enlarged region between m/z 2000 and 2250.

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this case, however, we observed a weak peak at m/z 2213, where both copper and platinum are bound to Aβ1-16. Thus, these findings confirm that cisplatin interacts with Aβ1-16 in the presence of copper and inhibits the copper-catalyzed oxidation reaction. We further carried out MS/MS analysis of a m/z 1971 ion to understand the details of the structural rearrangement taking place during redox cycling of Aβ1-16-Cu^{II} in the presence of cisplatin. In the absence of cisplatin, the MS/MS analysis of m/z 1971 shows the most abundant peak at m/z 871 corresponding to the b7 ion (Figure 4A). Therefore, all of the peaks are

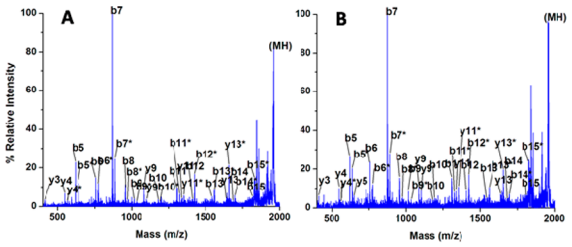

Figure 4. MS/MS spectra for a singly oxidized product of Aβ1-16-Cu^{II} at m/z 1971 in the absence of cisplatin (A) and in the presence of cisplatin (B). Asterisks indicate the corresponding oxidized peak with the addition of 16 Da.

expressed as a relative percentage assuming 100% for the b7 peak. The spectrum shows peaks at m/z 772 and 1352 corresponding to b6* and y11*, which indicate oxidation of His6 (Figure 4A). Also, peaks at m/z 1559 and 1784 correspond to b13* and y4*, respectively, indicating oxidation of His13, while the peak at m/z 1696 corresponds to b14*, indicating oxidation of His14. In the presence of cisplatin, an overall decrease in the intensities of the oxidation peaks was observed compared to that in the absence of cisplatin. In the presence of cisplatin, particularly, the ratio of b6*/b6 is decreased from 0.81 to 0.52 containing His6 residues, indicating the lowering of the oxidation reaction (Figure 4B). The ratio of y4*/y4 encompassing His13 and His14 residues decreased from 0.47 to 0.29, while the ratio b13*/b13 encompassing His13 increased from 1.77 to 2.05. These results suggest that the binding of cisplatin to Aβ1-16-Cu^{I} decreased the oxidation of His6 and His14, while the oxidation of His13 is, in fact, increased. We speculate that the binding of cisplatin to Aβ1-16-Cu^{I} results in the formation of an additional Cu^{II}-binding site involving His14 and the other donor atoms from nearby residues. These data suggest that, in the presence of cisplatin, the oxidation reaction proceeds via an alternate pathway, because of which complete inhibition of oxidation of Aβ1-16 is not observed. To confirm this, we evaluated the peroxidase activity of Aβ1-16-Cu^{II} (see the SI for details). Our results show that cisplatin moderately inhibits the peroxidase activity of Aβ1-16-Cu^{II}, indicating alternate redox pathways for ROS production (Figure S2 in the SI). Higher oxidation of His13 in the presence of cisplatin renders its key role in these alternate redox pathways.

In summary, our results suggest that the interaction of cisplatin with Aβ1-16-Cu^{II} completely inhibits decarboxylation/deamination of the Asp1 residue. MS data show complete protection of Asp1 and partial protection of His6 and His14 against oxidation. The increase in the oxidation of His13 suggests an alternate oxidation pathway. Although we have used nonphysiological concentrations of cisplatin in our studies, better platinum complexes may be designed to treat oxidative stress in AD.[13] The protective effect of Pt^{II} against copper-catalyzed oxidation can also be explored in the case of other neurological disorders involving copper such as Wilson's disease.[19]

■ ASSOCIATED CONTENT

Supporting Information

Experimental material and procedures for copper-catalyzed oxidation, MALDI MS, and cyclic voltammetry. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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