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Interaction of Cisplatin with Human Superoxide Dismutase

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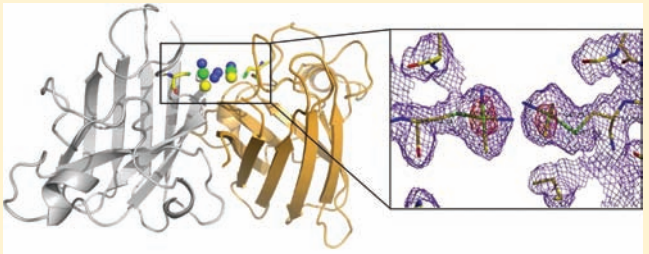
Supporting Information

ABSTRACT: *cis*-Diamminedichloroplatinum(II) (cisplatin) is able to interact with human superoxide dismutase (hSOD1) in the disulfide oxidized apo form with a dissociation constant of 37 ± 3 μM through binding cysteine 111 (Cys111) located at the edge of the subunit interface. It also binds to Cu_2−Zn_2 and Zn_2−Zn_2 forms of hSOD1. Cisplatin inhibits aggregation of demetalated oxidized hSOD1, and it is further able to dissolve and monomerize oxidized hSOD1 oligomers *in vitro* and *in cell*, thus indicating its potential as a leading compound for amyotrophic lateral sclerosis.



INTRODUCTION

Cisplatin is a chemotherapeutic drug that has been used to treat various types of cancer since the 1960s. The mode of action of cisplatin has been the topic of numerous studies and is still a target of active research.[1,2] Recently, the interaction of cisplatin with proteins involved in copper transport pathways has attracted attention, as copper chaperones, mostly containing Cys as metal ligands, could also bind and may transport cisplatin through Cys residues.[3−7]

Here we examine the interaction of cisplatin with human SOD1 (hSOD1), one of the final targets of copper chaperone. This protein has two cysteines on the surface (Cys6 and Cys111), Cys6 being only partially exposed, while Cys111 is completely solvent accessible.[8] hSOD1 is present in the cytoplasm and in the intermembrane space of mitochondria at micromolar concentrations,[9,10] as well as in the nucleus and in the peroxisomes at lower levels. It is a homodimeric metalloprotein harboring one Cu^{2+} and one Zn^{2+} ion in each of its subunits. Each subunit also contains, besides the two free Cys residues, one conserved intra-subunit disulfide bond (Cys57−Cys146). Functionally, hSOD1 is responsible for eliminating superoxide ions through disproportionation, thus protecting cells from radicals generated as byproducts of the respiratory chain and therefore from oxidative damage.[11]

Cys6 and Cys111 residues have been claimed to be involved in the aggregation of demetalated hSOD1, which may be associated with amyotrophic lateral sclerosis (ALS).[12,13] ALS is a neurodegenerative disease characterized by the progressive dysfunction and loss of motor neurons, the causes of which have yet to be understood. Insoluble protein aggregates containing hSOD1 have been found in motor neurons of ALS patients and in model mice developing ALS.[14] It is becoming evident that the molecular mechanism of ALS involves protein misfolding, aggregation, and oxidative reactions.[15−17] It is still an open question, however, whether the protein aggregates are the primary cause of ALS or a consequence of other pathogenic events.[18] A relation between hSOD1 and ALS was proposed in 1993, when point mutations were found in some familial cases of the disease.[19] It is also becoming clear that cysteine oxidation has a key role in hSOD1 aggregation when the protein is in its immature form (i.e., without the metal ions and without a stable, fully formed tertiary and quaternary structure),[8,16,20] and that Cys111 plays a major role in the formation of oligomers.[13,21] Therefore, the investigation of the interaction between cisplatin and hSOD1 is relevant for the biochemistry of cisplatin and may have consequences on ALS treatment. We show here that cisplatin binds Cys111 and not only prevents aggregation of demetalated hSOD1 but also is able to dissolve already-formed hSOD1 aggregates.

MATERIALS AND METHODS

Protein Preparation and Reagents. HSOD1 was expressed and purified according to the reported protocol.[8] Demetalation of hSOD1 was performed following the published procedure.[22] Cisplatin was

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purchased from Sigma-Aldrich (P4394). Imexon (4-imino-1,3-diazabicyclo[3.1.0]hexan-2-one) was obtained from Sigma Chemical Co. Metalated hSOD1 was obtained by adding a stoichiometric amount of $ZnSO_4$ and/or $CuSO_4$ to the apo protein. Apo hSOD1 at 0.1 mM concentration (as dimer) in 20 mM sodium phosphate buffer (pH 7.0) was incubated with cisplatin at various concentrations (0.2, 0.4, 0.8 mM).

Thioflavin T (ThT) Fluorescence and Size-Exclusion Chromatography (SEC) Analysis on Oligomerization. Cisplatin-bound hSOD1 and an apo protein sample taken as reference were incubated at 37 °C in the dark. The ThT fluorescence measurements were conducted following the published procedure[21] for cisplatin and Imexon. Aliquots of 100 μL of the reaction mixture were taken at increasing time slots from 20 days up to 12 months and analyzed by gel filtration on a Superdex 200 HR 10/30 column (Amersham Biosciences) at room temperature. The column was pre-equilibrated with 20 mM sodium phosphate buffer (pH 7.0), and the flow rate was 0.6 mL/min. The chromatograms, which monitored the species formed during incubation, were obtained by measuring the absorbance at 280 nm. All experiments were repeated three times using apo hSOD1 from different batches.

Extended X-ray Absorption Fine Structure (EXAFS) Measurements. The EXAFS measurements were carried out at the GILDA beamline (ESRF, Grenoble, France). The sample was loaded in a special cell for liquids and frozen at 120 K during the test. The intensity of the incoming beam was monitored with a nitrogen-filled ion chamber, whereas the fluorescence at the Pt Lα line was measured with an energy-resolving high-purity germanium detector. The EXAFS data were extracted and fitted using the ATHENA and ARTEMIS codes.[23] The theoretical EXAFS paths were calculated using the Feff8 code[24] starting from a *cis*-$[Pt(NH_3)_2ClS]$ cluster derived from the crystalline structure determined by X-ray diffraction. Both the single (up to the C_β atom of the coordinated Cys) and the multiple (collinear N−Pt−Cl(S) clusters) scattering paths were included in the data fitting. Each path had a coordination number fixed by the structural model and variable bond length R and Debye−Waller factors σ^2. The amplitude reduction factor S_0^2 and the energy shift ΔE_0 were also fitted.

Crystallization, Diffraction Data Collection, and Structure Determination of Apo hSOD1 with Cisplatin. Apo hSOD1 crystals were obtained in 5 days according to the published method.[8] They were then soaked in 4 mM cisplatin solution for 60 h. The ratio between apo hSOD1 dimer and cisplatin in the soaking process was estimated to be around 1:6.

The crystal of the apo hSOD1-cisplatin complex was diffracted at the ID14-4 beamline (ESRF, Grenoble, France) at 100 K, reaching a maximum resolution of 2.2 Å. The data were processed, and the structure was solved as previously described.[8] Table S1 (Supporting Information) shows the data collection and refinement statistics.

Solution NMR Experiments. $^1H-^{15}N$ HSQC spectra were acquired on a ^{15}N hSOD1 sample (0.1 mM) with cisplatin (0.8 mM) at 298 and 310 K. The dynamical properties of cisplatin-bound hSOD1 were experimentally characterized through ^{15}N relaxation measurements. ^{15}N longitudinal (R_1) and transverse (R_2) relaxation rates were recorded at 298 K on a Bruker Avance 500 MHz spectrometer. R_1 and R_2 were calculated by fitting the cross peak volumes (I), measured as a function of the relaxation delay, to a single-exponential decay as described in literature.[25] A rate constant for cisplatin binding was determined by fitting the decay of the peaks intensities in the $^1H-^{15}N$ HSQC spectra as a function of time to a monoexponential decay (first-order kinetics).

Cell Culture and Transfection. Mouse motoneuronal cell line NSC-34 inducibly expressing either wild-type hSOD1 or mutant SOD1 under control of the TetON system was obtained and grown as previously described.[26] A total of 3×10^5 cells were transfected with 1 mg of the indicated plasmid DNA using lipofectamine and Plus reagents (Invitrogen), according to manufacturer instructions. After 3 h of incubation with transfection reagents, cells were shifted in normal growth medium. Induction of SOD1 expression was obtained by adding to culture medium 1 mg/mL doxycyclin for the indicated time. Cisplatin was purchased from Sigma-Aldrich (P4394), freshly dissolved in 0.9% NaCl, and added to cultured cells at the indicated concentrations and time points.

hSOD1 Solubility and Aggregation Assay. Cells were scraped off the plate in culture medium, collected by centrifugation, washed in PBS, and resuspended in 70 μL of buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0, 100 mM NaCl, and protease inhibitor cocktail) containing 0.5% Nonidet P40. Cell lysates were subjected to centrifugation at 20000g for 10 min at 4 °C. The supernatants were collected as the soluble fractions, while the pellets (insoluble fractions) were washed in PBS and resuspended in 70 μL of Laemmli sample buffer (62 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol, 0.05% bromophenol blue). Protein content was determined using the BCA protein assay (Pierce).

Electrophoresis and Western Blot. Standard SDS-PAGE was performed as described.[26] Western blot was performed on nitrocellulose membranes (Amersham). After incubation in Tris-buffered saline (TBS) solution containing 0.1% Tween 20 and 5% nonfat milk, filters were incubated for 2 h at room temperature with a rabbit polyclonal anti-SOD1 antibody (Stressgen), which recognizes both endogenous mouse SOD1 (mSOD1) and transfected hSOD1. Following extensive washing in 0.1% Tween 20/TBS solution, filters were incubated with the appropriated peroxidase-conjugated secondary antibodies, washed in 0.1% Tween 20/TBS solution, and developed using the POD chemiluminescence detection system (Roche).

Equilibrium Dialysis. Apo hSOD1 solution (0.10 mM, 300 μL) was dialyzed against cisplatin solution (1.3 mL) of concentrations varying between 40 and 200 μM at 37 °C in the dark for 3 days, after which time equilibration was achieved. After that, inductively coupled plasma mass spectrometry (ICP-MS) analysis of metal ion (platinum) contents of the hSOD1 samples was performed in order to determine bound and free Pt(II) concentrations on a ICP-AES VARIAN 720-ES spectrometer. The data were transformed to a Scatchard plot, and the dissociation constant was then extracted from the negative reciprocal of the slope (Figure S1).

RESULTS

Solution Characterization of Cisplatin-Bound Apo and Holo hSOD1. In the $^1H-^{15}N$ HSQC spectrum of about 0.1 mM ^{15}N-labeled hSOD1 samples (either apo, Cu_2-Zn_2 and Zn_2-Zn_2 forms) in the presence of 0.8 mM cisplatin, about 20 backbone NH signals, belonging to residues located at the dimer interface, disappear. The disappearance of amide signals on $^1H-^{15}N$ HSQC spectra upon cisplatin binding can be attributed to the occurrence of conformational exchange processes at the dimeric interface. The identical behavior of the metal-free and metal-bound forms with cisplatin indicates that the same binding mode occurs in the apo and the metalated forms of hSOD1. Furthermore, addition of stoichiometric amounts of Cu(I) and Zn(II) to apo hSOD1 bound to cisplatin produced the (Cu_2-Zn_2) hSOD1 species (Table S2), indicating that hSOD1 is able to bind the physiological metals ions also after its reaction with cisplatin. Finally, the cisplatin-bound (Cu_2-Zn_2) hSOD1 protein fully retains its native activity (Table S3), consistent with the unchanged properties of the metal binding sites (Table S2) and the maintenance of the native structural features. This is a key aspect, as cisplatin binding does not impair the physiological function of hSOD1 as a superoxide radical scavenger. The dissociation constant of cisplatin to apo hSOD1 is 37 ± 3 μM. Such a value, as determined through equilibrium dialysis, allows for a rather complete (ca. 95%) conversion of apo to cisplatin-bound form in our experimental conditions.

From ^{15}N relaxation measurements, a correlation time for protein tumbling (τ_c) of 19.5 ± 2.0 ns was estimated from the

R_2/R_1 ratio[27] for the cisplatin-bound apo hSOD1. This value is comparable to that reported for apo hSOD1 alone (22.6 ± 1.9 ns).[8]

Differential scanning fluorimetry (DSF) measurements, which monitor the protein thermo-stability, provided a melting temperature of 49 °C for free apo hSOD1 which resembles the previously reported value of dimeric apo SOD1,[28] while binding of cisplatin to apo hSOD1 increases the melting temperature to 57 °C. This suggests that cisplatin binding slightly stabilizes the hSOD1 protein (Figure S2). The increase in stability of cisplatin-bound apo hSOD1 is probably related to the role of the cisplatin molecule which favors the interaction between the two hSOD1 subunits, thus reducing the tendency to monomerize.

The kinetics of cisplatin binding to apo hSOD1 was monitored indirectly by measuring the disappearance of amide signal intensity of the residues located on/close to the dimeric interface as they are not detected in the NMR spectra of the bound form. The first-order rate constant for the disappearance of apo hSOD1 signals at 310 K is $(3.7 \pm 0.4) \times 10^{-5}$ s^{-1}, which determines a half-time for the complex formation of 5.4 ± 0.5 h. This high value may be consistent with the high barrier for the complex formation.

Binding Mode of Cisplatin to Apo hSOD1. The crystal structure of apo hSOD1 soaked with cisplatin shows that Pt(II) is bound to the protein, whose overall conformation is absolutely maintained with respect to that of apo hSOD1 in the crystal (Figure 1). The major difference is the electron density due to the presence of two Pt(II) ions bound at the sulfur atoms of the Cys111 residues of the two subunits of the dimer. Each Pt(II) atom has an occupancy of around 0.4, as there is not enough space to allocate two metal ions between the two Cys which are facing each other at the dimer interface (Figure 1 and Figure S3). Each Pt(II) ion is coordinated to one of the two Cys 111 residues of the dimer. The coordination sphere of Pt(II), in addition to the sulfur atom of Cys 111, is completed by three ligands originating from the cisplatin complex, i.e., two NH_3 molecules and one Cl^- ion. The coordination geometry is square planar as usual for Pt(II) complexes (Figure 1).[29]

EXAFS data (Figures S4 and S5) of the cisplatin-bound apo hSOD1 are completely consistent with the crystallographic ones. In particular, the multiple scattering paths for the N−Pt−Cl(S) in collinear configurations allow to correctly reproduce the broad peak between 2.5 and 4.0 Å in the Fourier transformed spectrum. No evident deviations on the Pt−N/Cl/S bond lengths from the normal values were found (Table S4). This indicates that the coordination properties observed in solution are fully maintained in the crystal.

It is worth mentioning that the cisplatin binding of Cys111 of hSOD1 differs from what previously reported on beSOD1 (bovine erythrocyte SOD1). In beSOD1 the primary cisplatin binding site is a histidine residue (His19),[30,31] which is replaced by an Asn residue in hSOD1. Moreover, Cys111 residue, which occurs in hSOD1 and binds cisplatin, is substituted by a Ser residue in beSOD1. Therefore the binding sites of cisplatin on bovine and human SOD1 are different due to differences between bovine and human SOD sequences.

Cisplatin Inhibits the Oligomerization of Apo hSOD1. ThT is a selective probe for extended β-sheets, which is a typical structural feature of amyloids.[32] Apo hSOD1, in the absence of cisplatin, shows a progressive enhancement of the ThT fluorescence signal, consistent with our previous findings.[21] The behavior of the protein incubated with cisplatin is, on the other hand, different, as ThT fluorescence shows remarkably slower increase in intensity over time (Figure 2A). Cisplatin at 0.2 mM concentration is already able to significantly keep ThT fluorescence low. At higher cisplatin concentrations (0.4 and 0.8 mM), the quenching of ThT fluorescence is much more evident. Particularly, at the highest concentration, only a barely observable increase in the ThT fluorescence intensity is detected after 20 days. Even after 12 months, only weak ThT fluorescence has been detected in the presence of 0.8 mM cisplatin. SEC of apo hSOD1 initially shows one single peak indicating a dimeric species (32 kDa), but after the protein is incubated at 37 °C the dimeric hSOD1 peak progressively decreases and a new broad peak appears (Figure 2B). This broad peak consists of a mixture of oligomers with molecular weights ranging from 430 to 600 kDa (after 20 days). In the presence of cisplatin (0.4 and 0.8 mM) with 0.1 mM protein, the dimer peak is still very intense. After 20 days, a new peak with low intensity starts to appear, indicating the formation of a fraction of relatively small oligomers (up to about 80 kDa). Even in presence of 0.2 mM cisplatin, the molecular weight of the formed hSOD1 oligomers (ca. 120 kDa), and their relative amount with respect to that of dimeric hSOD1, is much lower than in the absence of cisplatin. Under

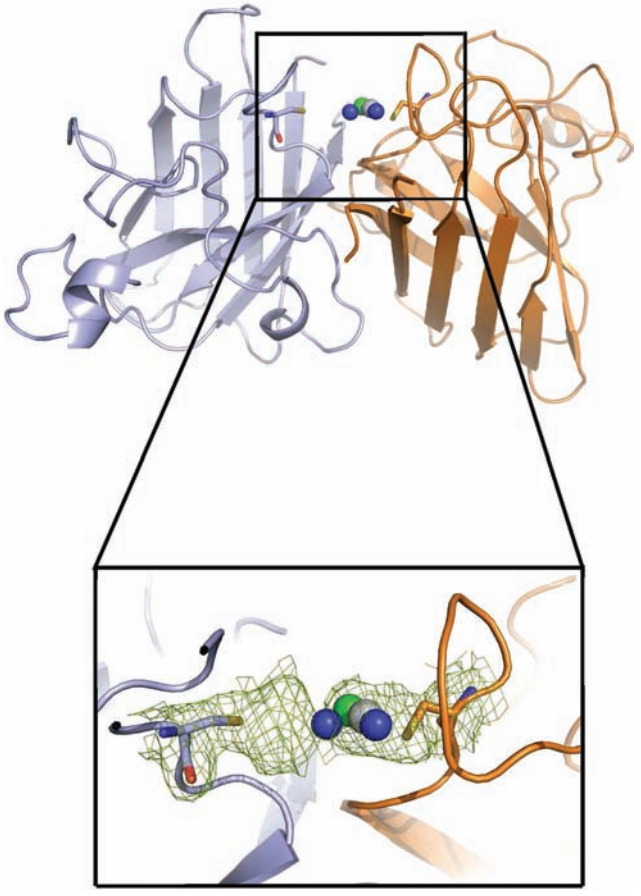

Figure 1. Crystal structure of cisplatin-bound apo hSOD1. (Top) Ribbon representation of one of the two dimers present in the asymmetric unit highlighting the two Cys111 facing each other at the interface and the cisplatin bound to one of them. (Bottom) Insight showing the two Cys111, one of the two cisplatin molecules, and their electron density. It is evident the lack of the space to host two cisplatin moieties at the same time (N, blue; Pt, gray; Cl, green).

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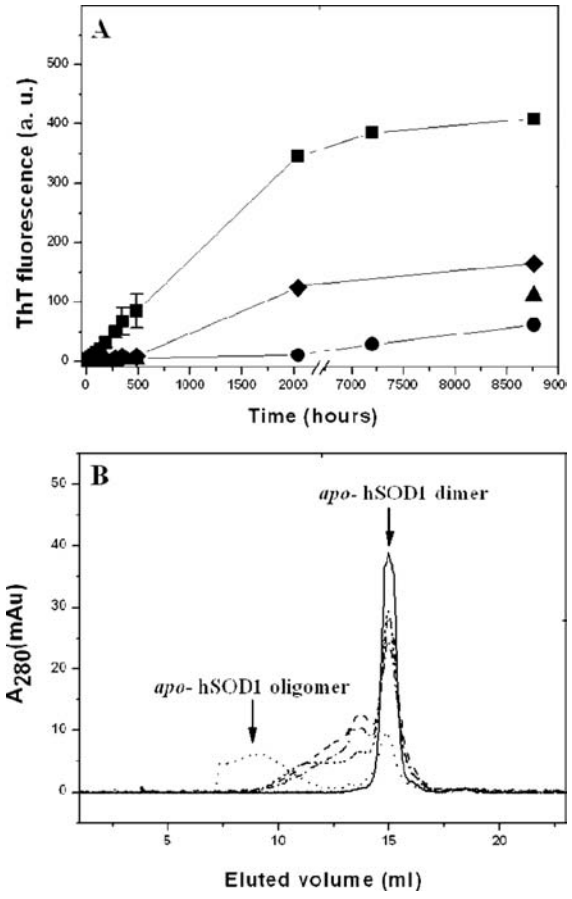

Figure 2. (A) Inhibition of formation of apo hSOD1 high molecular weight oligomers by cisplatin monitored by ThT fluorescence measurements over 9000 h. ThT fluorescence for 0.1 mM apo hSOD1 (■); 0.1 mM apo hSOD1 and 0.8 mM cisplatin (●); 0.1 mM apo hSOD1 and 0.4 mM cisplatin (▲); 0.1 mM apo hSOD1 and 0.2 mM cisplatin (◆). (B) SEC profile of apo hSOD1 oligomers formation inhibition by cisplatin. Samples containing 0.1 mM apo hSOD1 were incubated at pH 7.0 at 37 °C for 20 days. Control apo hSOD1 dimer at 0 h (solid); apo hSOD1 in the absence of cisplatin incubated at 37 °C (dot); apo SOD1 with 0.8 mM cisplatin (dash); apo hSOD1 with 0.4 mM cisplatin (short dash); apo hSOD1 with 0.2 mM cisplatin (dash dot).

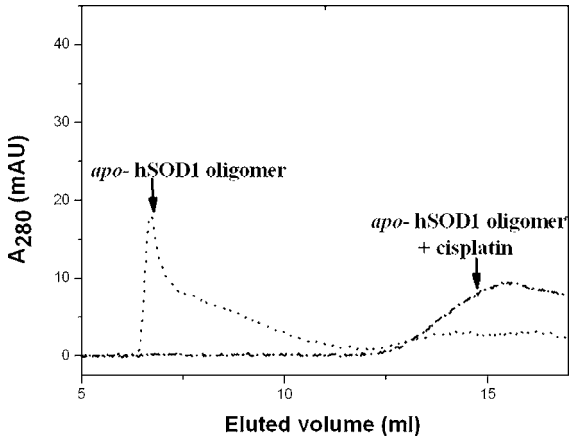

Figure 3. SEC analysis of apo hSOD1 oligomeric samples in the absence (dot) and in the presence of cisplatin (dash). The apo hSOD1 oligomers (with average molecular weight above 400 kDa) were incubated with 0.8 mM cisplatin at 37 °C for 120 h.

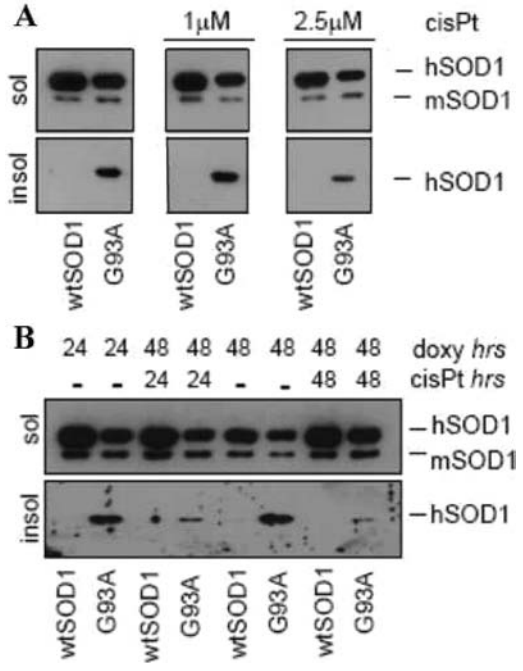

Figure 4. Effect of cisplatin on wild-type hSOD1 and G93A ALS related mutant solubility in neuronal cells. (A) Expression of wild-type hSOD1 and G93A ALS related mutant was induced in NSC-34 cells, in the absence or in the presence of the indicated concentrations of cisplatin. After 48 h of culture, insoluble (ins) and soluble (sol) fractions were isolated and analyzed in Western blot. (B) Wild-type hSOD1 and G93A ALS related mutant (mSOD1) expression in NSC-34/pTetON cells was induced for 24 h, and then 2.5 μM cisplatin was added for the next 24 h. As a control, cells tranfected for 48 h, with or without 2.5 μM cisplatin, were used. SOD1 solubility was then assayed as in panel A.

these conditions, hSOD1 is essentially present in the cisplatin bound form only, the impaired oligomerization is a direct result of cisplatin binding to Cys 111 of apo hSOD1.

A striking result is that when hSOD1 oligomers, already formed and having reached a molecular weight above 400 kDa, are incubated with cisplatin, they disappear in a process that is completed in the order of days (Figure 3).

We have also tested the cysteine reactive molecule Imexon[33] on apo hSOD1 aggregation, but no significant inhibition of protein oligomerization was observed, probably because it has larger molecular size than cisplatin (Figure S6).

Cisplatin Inhibits the Formation of and Dissolves Oligomers of Apo hSOD1 in Cells. Mouse immortalized motor neuronal NSC34 cells have been transfected with plasmids for the expression of wild-type hSOD1 and of one the most widely studied fALS-linked hSOD1 mutants, G93A, in the absence or presence of increasing concentrations of cisPt. After 48 h, cells have been lysed in a buffer containing 0.5% non-ionic detergent Nonidet P40, and the soluble and insoluble fractions have been separated. Western blot analysis (Figure 4A) showed that 2.5 μM cisplatin is strongly effective in decreasing the insolubility of G93A mutant. More striking, even when cisplatin is added to cells where the G93A mutant hSOD1 has already accumulated in an insoluble form (24 h after overexpression induced by doxycycline), the solubility of mutant G93A-SOD1 is rescued (Figure 4B), suggesting that cisplatin is able to solubilize an insoluble form of mutant hSOD1.

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DISCUSSION

In the present study we have shown that cisplatin interacts with hSOD1 and that its binding efficiently decreases the level of apo hSOD1 covalent oligomers *in vitro* even after very long period of time (12 months) as indicated by ThT fluorescence. Moreover, the higher percentage of remaining hSOD1 dimer, as well as the reduced size of the oligomers in the presence of cisplatin, indicate that cisplatin slows down the growth of apo hSOD1 oligomers. Also, cisplatin exstensively reduces the already formed oligomers.

The crystal structure of the cisplatin-bound apo hSOD1 shows that cisplatin binds covalently the sulfur atom of Cys111. The NMR data acquired on the apo hSOD1 as well as the metalated (Cu_2–Zn_2) and (Zn_2–Zn_2) hSOD1 forms suggest that a similar binding mode is present also in the metalated form, which fully retains the enzymatic activity. The EXAFS data indicated the same donor sets and equal coordination geometry for the Pt(II) ion in solution as in the crystal structure. Thus, also in solution cisplatin binds to a sulfur-containing residue of hSOD1. Given that Cys111 is the most solvent-exposed Cys residue in apo hSOD1[8] and no spectral changes around Cys6 were detected by NMR, it is reasonably expected that cisplatin primarily targets Cys111 also in solution as found in the crystal. We have previously shown that mutation of either Cys6 or Cys111 blocks hSOD1 oligomerization, indicating that both free Cys are required for hSOD1 oligomerization.[21] The coordination of the sulfur atom of Cys111 to the Pt(II) ion blocks the free thiol group in a thermodynamically and kinetically inert Pt–S bond that prevents the oxidative cross-linking of Cys111 and therefore inhibits the covalent growth of hSOD1 oligomers. Cisplatin binding has a rate of the order of 10^{-5} s^{-1}, while apo hSOD1 oligomers dissolution by cisplatin appears to be a slower process. The significant difference in the rates of cisplatin binding to apo hSOD1 dimer and of cisplatin-induced dissolution of apo hSOD1 oligomers could be due to the different solvent accessibility of the cysteine which binds cisplatin. Indeed, while in apo hSOD1 Cys111 is solvent-exposed,[8] oligomerization and formation of intersubunit disulfide bonds would result in a significant structural rearrangement that would make Cys111 less accessible in the oxidized oligomeric form.

The structural similarity between apo hSOD1 and its cisplatin-bound form, as observed from their crystal structures, reveals that cisplatin binding to Cys111 does not induce structural changes to the protein fold. NMR data show that, also in solution, the global folding and the metal binding sites of hSOD1 are not affected by cisplatin binding. The disappearance of the ^{1}H–^{15}N HSQC signals of residues located at or close to the dimeric interface suggests the presence of conformational exchange processes in solution occurring on the millisecond to microsecond time range when cisplatin is bound. The presence of such processes is supported by the fact that more amide signals can be detected at higher temperature (310 K). It is also consistent with the crystallization failure of the preformed cisplatin-hSOD1 adduct. In contrast to the dynamic behavior on the subunit interface in solution, no significant variations in the disorder, with respect to the apo hSOD1, was observed in the crystal structure when cisplatin is diffused in preformed crystals, as confirmed by the low *B*-factor values of the dimer interface residues.[34]

This finding highlights that (small) molecules, which introduce limited stereohindrance, could be selected as potential leading compounds for blocking hSOD1 oxidative oligomerization. Indeed, Imexon, which is known to also bind cysteines and sulfhydryl groups in general but has larger molecular size than cisplatin, is not able to inhibit the formation of SOD aggreggates. In addition, our approach to block Cys111 is consistent with the stabilizing effects observed when the two Cys111 are cross-linked at the dimer interface.[35,36]

Overall, the present study points to a unified picture of the mechanism by which cisplatin modulates apo hSOD1 oligomerization *in vitro* and *in cell*. On one side, the binding of cisplatin to the solvent exposed Cys111 in apo hSOD1 blocks this site and prevents the formation of intermolecular disulfide bonds. One the other side, the high affinity binding of cisplatin to the cysteine sulfur drives the break of the intermolecular disulfide bonds in the already formed oligomers. Thus, cisplatin is able to block and revert the formation of hSOD1 oligomers through selectively targeting Cys111, without affecting the normal functional pathway of hSOD1 maturation and enzymatic activity. This work benefits from the detailed information on the chemical reactivity of cisplatin as well as on the mechanism of apo hSOD1 oligomerization at the site-specific level. From this work it appears that cisplatin is a promising lead compound for the rational design of ALS treatments.

ASSOCIATED CONTENT

Supporting Information

X-ray data collection, crystal structural refinement statistics, EXAFS data and quantitative analysis, ICP analysis of hSOD1 proteins, enzymatic activity of cisplatin-bound (Cu_2–Zn_2) hSOD1, ThT results on Imexon, data analysis of equilibrium dialysis and the DSF data, and complete ref 19. 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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REFERENCES

(1) Wang, D.; Lippard, S. J. *Nat. Rev. Drug Discov.* **2005**, *4*, 307.
(2) Kelland, L. *Nat. Rev. Cancer* **2007**, *7*, 573.
(3) Arnesano, F.; Scintilla, S.; Natile, G. *Angew. Chem., Int. Ed.* **2007**, *46*, 9062.
(4) Abada, P.; Howell, S. B. *Metal Based Drugs* **2010**, 317581.
(5) Crider, S. E.; Holbrook, R. J.; Franz, K. J. *Metallomics* **2010**, *2*, 74.

(6) Arnesano, F.; Banci, L.; Bertini, I.; Felli, I. C.; Losacco, M.; Natile, G. *J. Am. Chem. Soc.* **2011**, *133*, 18361.
(7) Palm, M. E.; Weise, C. F.; Lundin, C.; Wingsle, G.; Nygren, Y.; Bjorn, E.; Naredi, P.; Wolf-Watz, M.; Wittung-Stafshede, P. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6951.
(8) Banci, L.; Bertini, I.; Boca, M.; Calderone, V.; Cantini, F.; Girotto, S.; Vieru, M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 6980.
(9) Field, L. S.; Furukawa, Y.; O'Halloran, T. V.; Culotta, V. C. *J. Biol. Chem.* **2003**, *278*, 28052.
(10) Rakhit, R.; Crow, J. P.; Lepock, J. R.; Kondejewski, L. H.; Cashman, N. R.; Chakrabartty, A. *J. Biol. Chem.* **2004**, *279*, 15499.
(11) Fridovich, I. Superoxide and superoxide dismutase. In *Advances in Inorganic Biochemistry*; Elichorn, G. L., Marzilli, L. G., Eds.; Elsevier North Holland: New York, 1979; Vol. *1*, pp 67−90.
(12) Niwa, J.; Yamada, S.; Ishigaki, S.; Sone, J.; Takahashi, M.; Katsuno, M.; Tanaka, F.; Doyu, M.; Sobue, G. *J. Biol. Chem.* **2007**, *282*, 28087.
(13) Cozzolino, M.; Amori, I.; Pesaresi, M. G.; Ferri, A.; Nencini, M.; Carri, M. T. *J. Biol. Chem.* **2008**, *283*, 866.
(14) Forsberg, K.; Jonsson, P. A.; Andersen, P. M.; Bergemalm, D.; Graffmo, K. S.; Hultdin, M.; Jacobsson, J.; Rosquist, R.; Marklund, S. L.; Brannstrom, T. *PLoS ONE* **2010**, *5*, e11552.
(15) Valentine, J. S.; Hart, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3617.
(16) Furukawa, Y.; Kaneko, K.; Yamanaka, K.; O'Halloran, T. V.; Nukina, N. *J. Biol. Chem.* **2008**, *283*, 24167.
(17) Nordlund, A.; Leinartaite, L.; Saraboji, K.; Aisenbrey, C.; Grobner, G.; Zetterstrom, P.; Danielsson, J.; Logan, D. T.; Oliveberg, M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 9667.
(18) Chattopadhyay, M.; Valentine, J. S. *Antioxid. Redox. Signal.* **2009**, *11*, 1603.
(19) Rosen, D. R.; et al. *Nature* **1993**, *362*, 59.
(20) Bosco, D. A.; Morfini, G.; Karabacak, N. M.; Song, Y.; Gros-Louis, F.; Pasinelli, P.; Goolsby, H.; Fontaine, B. A.; Lemay, N.; McKenna-Yasek, D.; Frosch, M. P.; Agar, J. N.; Julien, J. P.; Brady, S. T.; Brown, R. H., Jr. *Nat. Neurosci.* **2010**, *13*, 1396.
(21) Banci, L.; Bertini, I.; Girotto, S.; Martinelli, M.; Vieru, M.; Whitelegge, J.; Durazo, A.; Valentine, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 11263.
(22) Banci, L.; Bertini, I.; Cramaro, F.; Del Conte, R.; Viezzoli, M. S. *Biochemistry* **2003**, *42*, 9543.
(23) Ravel, B.; Newville, M. *J Synchrotron Radiat.* **2005**, *12*, 537.
(24) Ankudinov, A; Ravel, B.; Rehr, J.; Conradson, S. *Phys. Rev. B* **1998**, *58*, 7565.
(25) Marquardt, D. W. *J. Soc. Ind. Appl. Math* **1963**, *11*, 431.
(26) Ferri, A.; Cozzolino, M.; Crosio, C.; Nencini, M.; Casciati, A.; Gralla, E. B.; Rotilio, G.; Valentine, J. S.; Carri, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13860.
(27) Fushman, D.; Ohlenschlager, O.; Ruterjans, H. *J. Biomol. Struct. Dyn.* **1994**, *11*, 1377.
(28) Furukawa, Y.; O'Halloran, T. V. *J. Biol. Chem.* **2005**, *280*, 17266.
(29) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; Wiley: New York, 1990.
(30) Weidt, S. K.; Mackay, C. L.; Langridge-Smith, P. R.; Sadler, P. J. *Chem. Commun.* **2007**, 1719.
(31) Calderone, V.; Casini, A.; Mangani, S.; Messori, L.; Orioli, P. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 1267.
(32) Krebs, M. R.; Bromley, E. H.; Donald, A. M. *J. Struct. Biol.* **2005**, *149*, 30.
(33) Iyengar, B. S.; Dorr, R. T.; Remers, W. A. *J. Med. Chem.* **2004**, *47*, 218.
(34) Hörnberg, A.; Logan, D. T.; Marklund, S. L.; Oliveberg, M. *J. Mol. Biol.* **2007**, *365*, 333.
(35) Ray, S. S.; Nowak, R. J.; Brown, R. H.; Lansbury, P. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3639.
(36) Auclair, J. R.; Boggio, K. J.; Petsko, G. A.; Ringe, D.; Agar, J. N. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 21394.