Degradation of platinum based anticancer drugs by methionine: an EXAFS study

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Abstract. We characterized the structures in solution of carboplatin and oxaliplatin degradation products in presence of a large excess of methionine (Met). The reaction of carboplatin leads to the formation of cis-Pt(Met)₂ while, in the case of oxaliplatin, methionine displaces only the oxalate ligand to form Pt(diaminocyclohexane)(Met).

1. Introduction
Platinum complexes constitute a discrete class of DNA-damaging anticancer agents and their clinical uses are now widespread [1]. Three complexes are currently used in France: cisplatin, carboplatin and oxaliplatin. Carboplatin and cisplatin are used for the treatment of the same type of cancers [2]. Oxaliplatin could enlarge the types of cancer treated and overcome some resistances to the first two drugs [3,4].

Platinum-based drugs are known to react with sulfur nucleophiles, especially with the thiols and thioethers present in the cell [5]. These reactions are implied in the resistance mechanism to cisplatin [6]. Methionine (Met) inactivates the reaction of cisplatin with DNA [7]. But interaction of the drugs with Met-rich motifs of copper transporters might also promote the uptake of the drug by the cell [8,9]. Finally, L-Met is involved in the elimination of cisplatin from the organism [10]. However, carboplatin and oxaliplatin degradation products have not been structurally characterized [11].

We present an EXAFS structural characterization in solution of the degradation products of carboplatin and oxaliplatin with L-methionine.

2. Experiments
Carboplatin and oxaliplatin were obtained from pharmaceutical preparations, respectively from Bristol-Myers Squibb (“paraplatin”), and Sanofi (“eloxatine, powder”). The powders were diluted in pure water to a concentration of 10mg/mL. L-Met was directly dissolved in the drug solution, in order to saturate the solution. The solutions were studied at 2 hours, 3 days, 15 days and 1 month of reaction. All the spectra were recorded at ELETTRA (Trieste, Italy, BL11.1 station), at the Liii platinum edge, with a Si(111) double crystal monochromator and an energy-range of 11260-13000 eV. They were extracted using standard procedures available in the program Cherokee [12] including spline smoothing atomic absorption determination, energy dependent normalization and Fast Fourier transform (E₀=11568 eV, FT range: 3-15Å⁻¹). Each spectrum was recorded three times and averaged.
Since the two native drugs have already been characterized, they were used as model compounds. EXAFS modeling of the complete structures was performed as detailed elsewhere [13]: (i) determination of the first coordination sphere on filtered spectra; (ii) construction of molecular models with the Chem3D program; (iii) ab-initio EXAFS modeling with FEFF8 [14] and refinement of the parameters of the most important paths. All the fits of steps (i) and (iii) are performed with RoundMidnight code [12] by comparing the experimental spectra to the EXAFS standard formula. Several models of ligand binding are tested and, when parameters are physically acceptable, quantitatively compared using the F-test [15].

3. Results and discussion

3.1. Experimental spectra

The EXAFS experimental signals at one month of reaction and the corresponding Fourier Transform (without phase-shift correction) are presented in figure 1. All reaction products stood in solution. The evolution of the EXAFS spectra and their Fourier Transform reflects, for both drugs, the huge changes induced in the Pt(II) first coordination sphere by methionine. The first shell radial distribution functions are shifted to a greater distance, and the phases of the signals are largely changed. This suggests the apparition of sulfur atoms in the first coordination sphere.

We observe large changes in the signal beyond the first coordination sphere. In particular, the signature of the oxalate ligand of oxaliplatin (third peak of oxaliplatin FT) disappears. This suggests that this ligand has been displaced by methionine. In both cases, there is almost no evolution in the spectra between 3 days and one month. The spectra recorded at 2 hours are already very similar to the final ones. Thus reaction occurs within few hours.

Table 1. Fitting results for the first coordination sphere at one month of reaction, on the filtered spectra. AE is set to 13 eV. Left: evolution of the quality factor (QF) as a function of the number x of sulfur atoms in the first coordination sphere. Right: parameters for the best fits.

| PtN_{x}S_{x}, x: | CarboPt+Met QF | OxaliPt+Met QF | CarboPt+Met | OxaliPt+Met |
|------------------|---------------|---------------|-------------|-------------|
| 0                | 17.76         | 4.74          | N_{2}S_{2}  | N_{2}S_{2}  |
| 1                | 5.40          | 0.96          | 2.04(1)     | 2.06(2)     |
| 2                | 0.41          | 0.85          | 2.26(1)     | 2.23(1)     |
| 3                | 0.85          | 2.15          | 2.25(1)     | 2.25(1)     |
| 4                | 2.38          | 3.67          | 26(5)       | 51(8)       |

3.2. First coordination sphere modelling

We applied the method proposed by J. Penner-Hahn [16] in order to determine the number of nitrogen and sulfur atoms bound to the platinum. The results obtained on the first coordination sphere filtered spectra are presented in table 1. In the case of carboplatin, the best fit is obtained for a PtN_{2}S_{2} first
coordination sphere, with a probability of 82%. For oxaliplatin, the F-test does not permit to
distinguish PtN$_2$S$_2$ from PtN$_3$S, even if PtN$_2$S$_2$ presents a lower quality factor.

3.3. Complete modelling of the spectra at one month of reaction

In order to model the whole signal for both degradation products, we tested different models. All our
previous studies proved that it is easier to displace the carboxylate ligand than the amine [13]. For
oxaliplatin, we observe that the oxalate signature vanishes completely for the reaction product. Thus
we assume that, after reaction, there is no more oxygen in the first coordination sphere. With this
hypothesis, three models correspond to a PtN$_2$S$_2$ first coordination sphere: Pt(NH$_3$)$_2$(Met)$_2$ or
Pt(Dach)(Met)$_2$ in the case of a monodentate chelation of methionine (respectively for carboplatin and
oxaliplatin; Dach: diaminocyclohexane), cis-Pt(Met)$_2$, or trans-Pt(Met)$_2$, for a bidentate chelation. For
oxaliplatin, a PtN$_3$S first coordination sphere corresponds to a Pt(Dach)(Met) complex.

The analysis of the theoretical signals shows that, when methionine is bound to platinum in a
bidentate way, the signal beyond the first coordination sphere is mainly due to single scattering by the
carbon atoms: carbon bound to nitrogen (C$_N$), bound to sulfur (C$_S$) or carbon C' of methionine (C'),
and to multiple scattering through the platinum (figure 2). For a monodentate fixation, the flexibility
of methionine induces very large radial distance distribution for all atoms, except for the two carbon
atoms bound to sulfur. Thus depending on the structural model, the fit includes two shells for the
mixed N/S first coordination sphere, one or three shells for the carbon atoms and multiple scattering
through the platinum. In order to limit the correlations, we fitted a unique Debye-Waller factor.

The results of the different fits are presented in table 2. As the signal of the first coordination
sphere largely predominates, all model lead to acceptable fits. We observe that correlations induce in
cases a reduction of the PtC distances range, and a slight underestimation of the PtC' distance. The
presence of different PtC distances is essential to reproduce the very low signal around 3 Å in R-
space: for both Pt(NH$_3$)$_2$(Met)$_2$ and Pt(Dach)(Met)$_2$ models, the theoretical signal presents a large peak
(20% of the main peak) while the experimental signal is lower than 5%. The main difference between
the models with a bidentate fixation of methionine stands in the signal around 4Å: higher and shifted
to larger distances for trans-PtN$_2$S$_2$ compared to cis-PtN$_2$S$_2$, lower and shifted to smaller distances for a
PtN$_3$S first coordination sphere (figure 2). On the basis of these observations and of the quality factor
values, it is possible to discriminate between the different models: In the case of carboplatin, the best
fit is obtained for the cis-Pt(Met)$_2$, model with a probability of 75% (cPM2). For oxaliplatin, the best fit
corresponds to Pt(Dach)(Met) with a probability of 72%. These models give the best reproduction of
the signal around 4Å.

### Table 2. Fit parameters for the different models: PN2M2: Pt(NH$_3$)$_2$(Met)$_2$, cPM2: cis-Pt(Met)$_2$, tPM2: trans-Pt(Met)$_2$, PDM2: Pt(Dach)(Met)$_2$, PDM: Pt(Dach)(Met). N is the number of equivalent paths, σ

|          | CarboPt+Met | OxaliPt+Met |
|----------|-------------|-------------|
| PtN (Å)  | 2.05(1)     | 2.05(1)     |
| PtS (Å)  | 2.26(1)     | 2.26(1)     |
| PtCN (Å) | 3.10(4)     | 3.13(3)     |
| PtCS (Å) | 3.12(4)     | 3.13(3)     |
| PtC' (Å) | 3.27(4)     | 3.29(4)     |
| PtNPtS (Å)| 4.32(2)     | 4.33(3)     |
| N        | 33(4)       | 28(3)       |
| Residue  | 3.786%      | 1.331%      |
| QF       | 1.82        | 0.70        |
| ρ$_{TF3}$| 16.803%     | 11.70%      |

The values in parentheses are the uncertainties in the last significant digit.
These results are in agreement with other theoretical [17] and experimental evidences. Cisplatin and carboplatin proved to have similar degradation products with different sulfur nucleophiles [11,13]. Murdoch et al [10] showed that, for the cisplatin degradation product Pt(L-Met)₂, the cis-isomer was predominant. Mass spectrometry experiment suggested that for oxaliplatin, the Dach ligand remained after reaction [11]. Our results give the first structural evidence for carboplatin and oxaliplatin degradation products.

**Figure 2.** Experimental and theoretical signals for: carboplatin cis-Pt(Met)₂ model (left), carboplatin trans-Pt(Met)₂ model (middle) and oxaliplatin Pt(Dach)(Met) model (right).

### 4. Conclusion

We have followed the EXAFS spectra evolution of carboplatin and oxaliplatin in the presence of a large excess of methionine. In both cases, the reaction is complete within a few hours, the reaction products stand in solution and we observe the apparition of sulfur atoms in the first coordination sphere. The degradation products of carboplatin and oxaliplatin are respectively cis-Pt(Met)₂ and Pt(Dach)(Met). This experimental structural characterization corroborates previous non-structural experimental works on degradation of these drugs by methionine.

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