ESRF	**Experiment title:** Exafs Study of Pt anticancer drugs in cells and tissues.	**Experiment number**: MD 345
Beamline:	**Date of experiment**: from: 14.03.2008 to: 20.03.2008	**Date of report**:
Shifts:	**Local contact(s)**: Sergey Nikitenko	*Received at ESRF:*

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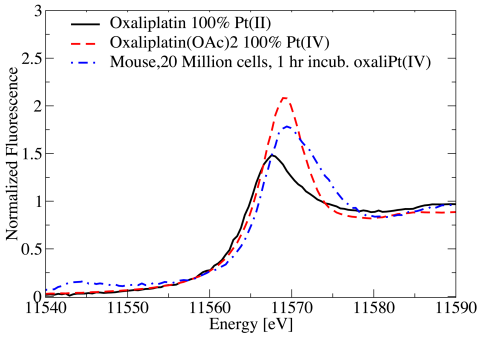
Report:

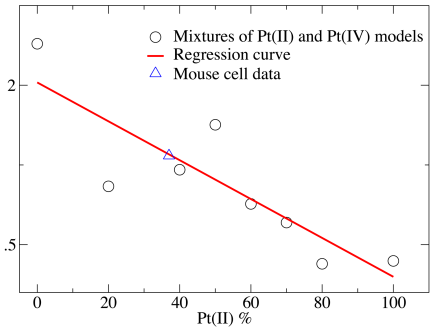
Aims of the experiment and scientific background

Cisplatin, cis-diamminedichloroplatinum(II) is among the most important chemotherapeutic agents ever developed. It is a critical component of therapeutic regimens in abroad range of malignancies [1]. However, despite an inordinate amount of work, more than three decades after its clinical introduction, the exact mechanism of cisplatin action on tumor cells is not fully understood. It is commonly accepted that cisplatin kills the cancer cell by binding covalently and essentially irreversibly to the nuclear DNA. The binding occurs in the major groove of the DNA where the two cis chloride ligands are replaced by two N7 atoms of two adjacent guanines on the same strand of the DNA. This distortion of the DNA triggers cellular responses that in favorable cases end in the induction of apoptosis [2]. It is noteworthy that only approximately 1% of the cellular platinum ends up bound to the nuclear DNA. Yet, nearly 99% of the research efforts have been directed to studying all aspects of Pt-DNA lesions.

Since the Pt-DNA adducts formed in different types of cancer cells are expected to be the same, why then is cisplatin very effective against some types of cancer (testicular, ovarian) and totally ineffective against others (colon, breast). It might be due to the adducts that the drug forms with the non-DNA cellular components, which account for 99% of the cellular Pt. Very little is known about these interactions and most of the information pertaining to the mechanism of action is derived from simplistic aqueous model inorganic chemistry. Based on model studies, and basic inorganic chemistry, it is expected that most of the cellular cisplatin will bind to sulfur containing ligands and especially to glutathione (GSH), a thiol containing tripeptide that exists in mM concentrations. There is one report claiming that 60% of the cellular platinum is bound to GSH [3]. Yet, in recent [1H, 15N] 2D NMR studies that we conducted by labeling cisplatin with ^{15}N ammine ligands, and monitoring the interactions with aqueous extracts of cancer cells, contrary to expectations, we were unable to detect any sulfur binding to cisplatin. This might be due to certain limitation of NMR spectroscopy, which would make it impossible to observe signals of cisplatin

bound to large biopolymers.
The interaction of cellular sulfur with Pt anticancer agents is believed to play a major role, and could serve to either inactivate the Pt drugs (mainly due to binding to thiols) or to activate drugs such as carboplatin (by binding to thioether).





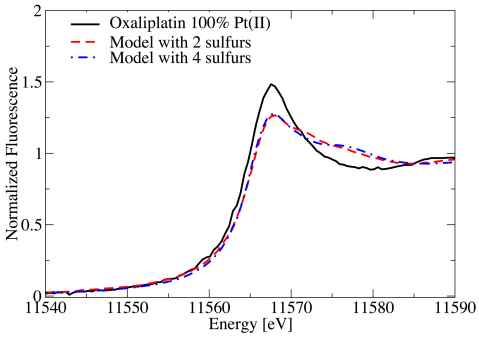


Fig. 1 (left): XANES spectra of Pt(II) Pt(IV) complexes and XANES on mouse cells.
Fig. 2 (middle): Peak height ratio extracted from Pt(IV)/Pt(II) standard mixtures (circles), a linear fit (line), and results for a mouse sample (triangle).
Fig. 3. XANES of oxaliplatin and complexes C6H18N2PtS2 and C12H24PtS4.

Results

EXAFS L_3 Pt spectra were collected for all samples at BM26A beamline (ESRF). Samples of cells, cell extracts and mice treated tumors were measured. Samples were placed in Kapton sealed holders and kept frozen at all times. Measurements were performed at 15K using a cryostat and data was collected using a multielement Ge solid-state detector.
XANES spectra of the Pt L_3 edge of therapeutically relevant Pt(II) and Pt(IV) (powder) complexes (cisplatin and oxaliplatin) were collected. The XANES spectra of solids containing varying proportions of Pt(II) and Pt(IV) complexes in 10 % steps were collected to determine whether it was possible to correlate the known proportions with the XANES edge features, as a tool for monitoring reduction of Pt(IV) in complex systems. The XANES spectra of Pt(II) and Pt(IV) differ in the height of their edges, with Pt(IV) being substantially higher. Based on the pure Pt(IV) and Pt(II) model compounds a linear relationship has been made. This is the basis for biological samples and will allow us to determine effectively the relative proportions of each oxidation state present in the biological material. To monitor the cellular reduction of Pt(IV) complexes in mouse cancer cells, the cells were treated with the Pt(IV) complexes: ct-[$Pt(NH_3)_2(OH)_2$(oxal)] and ct-[$Pt(NH_3)_2(OAc)_2$(oxal)] at a concentration of 100 μM. From height of the edge it can be concluded that 37 % reduction to Pt(II) has been occurred (see Fig. 2). The results obtained in March 2008 proved the feasibility of the experiments in cells (mouse). XANES of two compounds with sulfur bound to Pt, show a shoulder at 11577 eV, that differ from the oxaliplatin models (see Fig. 3). Sulfur coordination in mouse cells is highly unlikely.

References

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