



	Experiment title: XAS study of Ruthenium anticancer drugs in tumor tissue	Experiment number: MD 678
Beamline: BM26 A	Date of experiment: from: 29.06 to: 03.07.2012	Date of report: 10.12.2012
Shifts: 12	Local contact(s): Sergey Nikitenko	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

Annette Rompel ^{a*}

Alfred Hummer ^{a*}

a) Institute of Biophysical Chemistry, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria

Report:

Aims of the experiment and scientific background

Ruthenium compounds belong to the most promising candidates of non-platinum containing metal complexes for cancer therapy. Compared to Pt drugs Ru complexes cause less side effects and resistances against the drug are less likely.^{1,2} The overall chemical and pharmacokinetic behavior of Ru is quite different from Pt compounds, as reflected in extensive protein binding³ which questions the relevance of DNA interactions *in vivo*. KP1019 (indazolium-*trans*-[tetrachlorobis(indazole)ruthenate(III)]),^{4,5} and its sodium analogue KP1339 (sodium-*trans*-[tetrachlorobis(indazole)ruthenate(III)])^{4,5} are very promising drug candidates currently under investigation. The redox potential of Ru(III)/Ru(II) is physiologically accessible and the activation of the drugs through reduction to Ru(II) is thought to play an important role in their mode of action. Therefore X-ray absorption spectroscopy was used to find out which oxidation state of the tumor-inhibiting compound and which first coordination sphere *in vitro* and *in vivo* is most likely. A database of different ruthenium compounds representing possible first coordination spheres and oxidation states *in vivo* was established. Spectra of KP1019 in BN and mice tissues (tumor S-180 and liver) from KP1019 and KP1339 treated mice were collected. The aim of the study is a fundamental understanding of the biotransformation of the ruthenium complexes KP1019 and KP1339 in biological tissue, and the underlying principle of "activation by reduction". Information about possible oxidation state/ coordination pairings will give insight into the *in vivo* metabolism of these metallodrugs and reassess the interaction with the hypothesized molecular targets.

Results

XANES and EXAFS spectra were collected at the Ru K-edge at 20 K. Ru powder was used as a reference compound for the energy calibration and to check the monochromator stability. The samples were enclosed in Kapton sealed aluminum sample holders. EXAFS spectra of Ru model compounds with mixed first coordination shells of Cl, N, O and S in oxidation states +2 and +3 were collected. K-edge spectra of KP1019 in the presence of different possible biological target molecules and of tissue samples derived from KP1339 treated mice were collected as well.

In Fig. 1 (left) and (right) the XANES spectra of the model compounds are shown. Their edge positions are clearly influenced by two phenomena, the oxidation state of the ruthenium center and the electronegativity of the surrounding ligands. The complexes with higher oxidation state and more electronegative ligands are shifted to higher energy positions. As described in ⁶ a correlation of the edge position and the calculated coordination charge can be drawn. The electronegativity values of S, Cl, N and O were taken from the Allred-Rochow scale and are 2.4, 2.8, 3.1 and 3.5, respectively. The coordination charges are plotted over the edge energy values in Fig. 2 (right), with KP1019 set as an arbitrary zero point. The edge energies were determined over the first maximum in the first derivative.

In Fig. 2 (left) the XANES spectra of a tumor and liver tissue in comparison to KP1019 in boron nitride are shown. The coordination of KP1019 in the two tissue samples seems to be the same but different from the pure Ru-drug. The edge shift of the biological samples and KP1019 in buffer in comparison to KP1019 as a solid is shown in Fig. 2 (left).

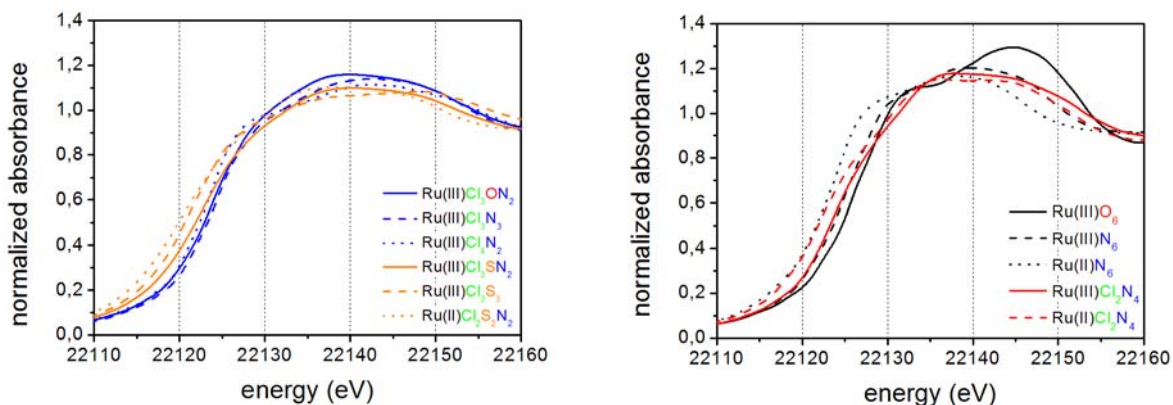


Fig. 1: XANES spectra of the chlorine and sulfur rich models (left) and the nitrogen and oxygen rich models (right).

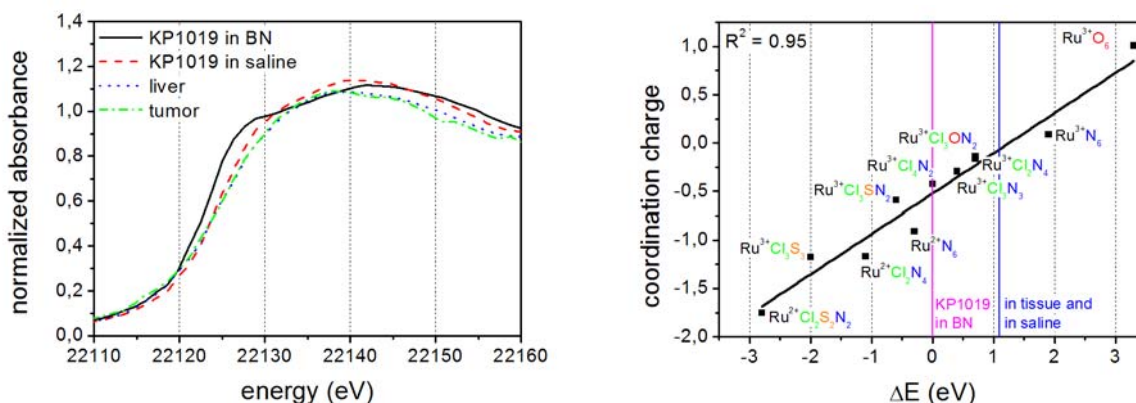


Fig. 2: XANES spectra of a tumor and liver sample (left) and the coordination charge versus energy plot (right).

References

- (1) Jakupec, M. A.; Galanski, M.; Arion, V. B.; Hartinger, C. G.; Keppler, B. K. *Dalton Trans.* **2008**, 183.
- (2) Heffeter, P.; Pongratz, M.; Steiner, E.; Chiba, P.; Jakupec, M. A.; Elbling, L.; Marian, B.; Körner, W.; Sevelde, F.; Micksche, M.; Keppler, B. K.; Berger, W. *Journal of Pharmacology and Experimental Therapeutics* **2005**, *312*, 281–289.
- (3) Heffeter, P.; Böck, K.; Atil, B.; Reza Hoda, M.; Körner, W.; Bartel, C.; Jungwirth, U.; Keppler, B.; Micksche, M.; Berger, W.; Koellensperger, G. *Journal of Biological Inorganic Chemistry* **2010**, *15*, 737–748.
- (4) Lipponer, K.-G.; Vogel, E.; Keppler, B. K. *Synthesis, Characterization and Solution Chemistry* **1996**, *3*, 243–260.
- (5) Peti, W.; Pieper, T.; Sommer, M.; Keppler, B. K.; Giester, G. *European Journal of Inorganic Chemistry* **1999**, *1999*, 1551–1555.
- (6) Cramer, S. P.; Eccles, T. K.; Kutzler, F. W.; Hodgson, K. O.; Mortenson, L. E. *Journal of the American Chemical Society* **1976**, *98*, 1287–1288.