ESRF	**Experiment title:** ***The role of selenium in DNA repair: the evaluation of benefice / risk ratio***	**Experiment number**: 30 02 858
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Introduction

Selenium is an essential trace element for higher eukaryotes. In cells, it is incorporated in many enzymes as selenocystein residue, for example in the active site of the detoxifying enzyme glutathione peroxidase. Several selenium compounds have also been shown to have inhibitory effects on mammalian cell growth, particularly cancer cells. For this reason, selenium is currently used for cancer prevention in several clinical trials. These antiproliferative properties have been attributed to the inhibition of some essential enzymes functions and to the disruption of biochemical processes. A significant loss of enzyme function may result from the synthesis of polypeptides in which some, or all, of the sulfur amino acids have been replaced by their selenium analogues. Moreover, selenomethionine has been implicated in DNA repair response in normal human fibroblasts *in vitro*, a potential mechanism for cancer-preventive action.

However selenium is toxic at high concentrations, and the range of Se concentrations delimiting essentiality, cancer-protective property and toxicity is extremely narrow. It is well known that speciation highly influences toxicity. Our aim was to evaluate the influence of Se oxidation state in toxicity and / or cancer protective effect. We studied the transformation of selenium after exposure of LnCAP prostate cancer cells to lethal or sub-lethal doses of selenite (Se(ite)), selenate (Se(ate)), selenomethionine (SeMet) or selenium dioxide (SeO_2), which enabled us to draw links between Se speciation and toxicity.

Experimental methods

Lethal doses of Se(ite), Se(ate), SeMet and SeO_2 compounds were identified on LNCaP prostate cancer cells. The concentration leading to a 50% cell mortality rate (CI_{50}) were determined to be 475 µM (Se(ate)), 525 µM (SeMet), 0.25 µM (Se(ite)) and 0.5 µM (SeO_2). For XAS experiment, cells were subsequently exposed for 24 h to 250 or 500 µM of (Se(ate)) or (SeMet), or to 0.1 or 0.5 µM of (SeO_2) or (Se(ite)). These concentrations correspond to a sublethal dose (250 µM or 0.1 µM, which do not induce any cell death) or a lethal dose of selenium compounds (50 µM or 0.5 µM, which induced the death of approximately 50% of the cells). Cells were also exposed to the lethal dose of Se compounds for 6 to 36 h to evaluate the kinetics of Se transformation in cells.

After exposure, cells were rinsed, centrifugated and freeze-dried. Dry pellets were pressed and analyzed by Se K-edge XANES/EXAFS spectroscopy, in order to follow Se transformation inside the cells. XAS spectra were recorded in fluorescence mode using a 7-element solid state Ge detector (Canberra). The monochromator was a Si(220) double crystal. In order to limit the photoreduction of Se in samples during the measurements, the experiment was conducted at -20K under inert gas, using a He cryostat. 2

to 7 spectra were recorded on each sample, depending on Se concentration. The collected scans were calibrated by setting the maximum of the white line of hexagonal (gray) elemental Se at 12.6593 keV, normalized, and simulated by linear combinations fitting (LCF) using Se reference compounds spectra [1, 2] using Athena software.

Results

Cells exposed to 0.5 µM of Se(ite) or SeO_2 did not accumulate enough selenium to permit a correct analysis of XANES spectra.

When cells were exposed to 500 µM of Se(ate) (figure 1) for 6-36 h, they accumulated Se and a time-dependent transformation of Se speciation was observed. Qualitative observation of XANES region (12650-12670 eV) reveals the presence of two distinct peaks in samples spectra. The first one, around 12660 eV, can be related to Se0 and/or SeDG references. The second one is shifted by +7.4 eV from the first one, and matches the main peak of Se(ate) reference. In the exposed cells samples, the speciation of selenium might then be a mixture of these chemical forms of Se.

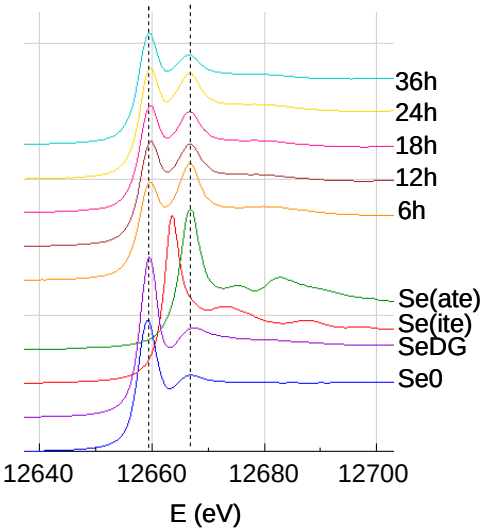

Figure 1. XANES spectra of references and cells exposed for 6-36 h to 500 µM of selenate.

	Se0	Se(ite)	Se(ate)	R^2
6 h	64	16	20	0.021
12 h	71	17	12	0.012
18 h	73	16	11	0.011
24 h	74	18	8	0.017
36 h	81	14	5	0.003

	SeDG	Se(ite)	Se(ate)	R^2
6 h	60	15	25	0.007
12 h	64	16	20	0.002
18 h	66	16	18	0.002
24 h	68	17	16	0.003
36 h	71	15	14	0.004

Table 1. Distribution of Se species (%) in cells exposed for 6-36 h to selenate, determined by linear combination fitting of references: Se0, elemental selenium; Se(ite), selenite; Se(ate), selenate.

The reconstruction of experimental spectra by linear combination of Se0 or SeDG, Se(ite) and Se(ate) references, that we previously published [1, 2] gave two sets of selenium species distribution data (Table 1). Fits using Se0, Se(ite) and Se(ate) as references (first set of data, Table1) are poorer than fits using SeDG, Se(ite) and Se(ate) as references (second set of data, Table1), as shown by the increase of the residual (R^2). For this reason we rather think that samples contained Se(ate), Se(ite) and SeDG. However this hypothesis need to be confirmed, for example by transmission electron microscopic observation of exposed cells, since Se0 can be easily identified as electron-dense precipitates in cells.

From 6h to 36 h of exposure, an evolution of Se species concentrations was observed: Se(ate) cell content decreased from 25 to 14% of total Se, while SeDG concentration increases from 60 to 71%. The concentration of Se(ite) was almost stable, around 15% of total Se content. Se(ate) might then be internalized by cells and transformed to Se(ite) which then reacts with reduced glutathione (GSH) to form SeDG. This Se compound has already been described as being the major metabolite of inorganic selenium in mammalian tissues *in vivo*, and suggested to be the most effective Se compound in inhibiting the growth of neoplastic cells [3]. The observed toxicity of Se(ate) in LNCap cells might then be explained by the increase of SeDG intracellular concentration.

When cells were exposed to 250 or 500 µM of SeMet, this compound was accumulated but no Se speciation transformation occurred (Figure 2).

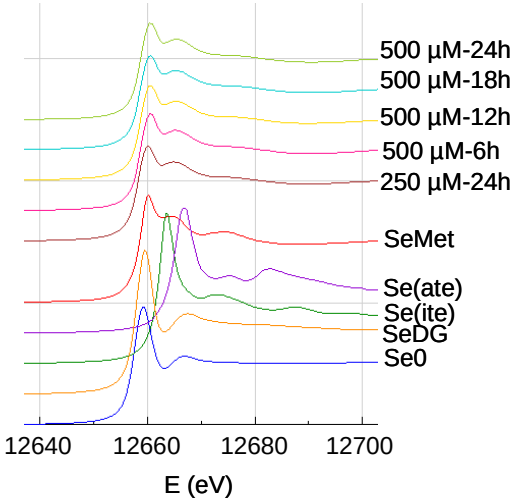

Figure 2. XANES spectra of references and cells exposed for 6-36 h to 500 µM of selenomethionine.

However, it cannot be excluded that selenomethionine is included in proteins, such as enzymes, and consequently reduce their activity.

Conclusions and perspectives

During this experiment, selenium speciation after cell exposure to Se(ate) and SeMet was checked. While Se(ate) was internalized in cells and transformed to Se(ite) and Se(DG), SeMet was internalized but not transformed. The metabolization of Se(ate) to SeDG can be an explanation of Se(ate) toxicity, since this compound has been shown to be implicated in the inhibition of the activity of several enzymes. Still, after 6 h, 60% of Se had already been transformed to SeDG but no cell death was observed. These first results, obtained from XANES analyses, are promising but incomplete. Still the possible evolution of Se speciation after cell exposure to Se(ite) and SeO_2 need to be assessed, and the early kinetics of Se(ate) transformation needs to be examined.

References

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