BioMetals **13:** 209–215, 2000.

Uptake and Retention of Selenite and Selenomethionine in cultured K-562 cells

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Key words: cell culture, ICP-MS, retention, selenite, selenomethionine, uptake

Abstract

The selenium uptake and retention have been studied in K-562 cells exposed to selenite or selenomethionine. In the uptake experiments the cells were exposed to two doses of selenite (5 or 50 μM) or selenomethionine (10 or 50 μM). In the retention study the cells were treated for 2 h with the above mentioned doses of the selenocompounds before being observed at different times. The selenium uptake in cells exposed to selenite 5 μM began to saturate at 8 h, but increased again between 48 and 96 h. In cells exposed to selenite 50 μM the selenium uptake never reached a maximum, however, at 48 and 96 h the cell viability decreased strongly. The two doses of selenite showed different retention patterns, with a relatively small cellular decrease of selenium after treatment with selenite 5 μM compared to treatment with 50 μM of selenite. The selenium uptake in cells exposed to selenomethionine 10 μM or selenomethionine 50 μM began to saturate at 24 h and 48 h, respectively. The retention patterns were similar for both selenomethionine doses with a continuous decrease of the selenium concentration during the whole observation period. The results indicated a more controlled uptake and retention pattern of selenomethionine compared to selenite.

Introduction

Selenium is an essential element in human and animal nutrition (Schwartz & Foltz 1957; Mertz 1981) and has been reported to be essential for the normal growth of cells in culture (McKeehan *et al.* 1976; Beilstein *et al.* 1987). It has been found to be a component of the mammalian enzyme glutathione peroxidase (Flohé *et al.* 1973; Rotruck *et al.* 1973), types I, II and III iodothyronine deiodinase (Valverde-R *et al.* 1997) and human thioredoxin reductase (Gromer *et al.* 1998). In the field of interactions of selenium with mercury or cadmium there are studies showing selenium protection both when given before the metal or simultaneous with the metal (Burk *et al.* 1974; Chen *et al.* 1975; Gasiewicz & Smith 1976; Chang 1983; Wahba *et al.* 1993; Lindh *et al.* 1996). Most of these studies have been performed *in vivo* and the most common pre-treatment is an injection of selenium 30 min before injection of the mercury or cadmium compound. This study was performed in an *in vitro* model (K-562) with the two selenocompounds selenite and selenomethionine and the chosen concentrations were based on results from an earlier toxicity study (Frisk *et al.*, in press). The aim of the present study was to examine the uptake and retention of selenite and selenomethionine in K-562 cells at selenium concentrations of interest for human exposure.

Materials and methods

Human erythroleukemia K-562 cells (Lozzio & Lozzio 1975; Klein *et al.* 1976) were optimally grown as stock cultures in Ham's F-10 from Biological Industries, (Beit Haemek, Israel). The growth medium was

supplemented with 10% fetal calf serum, 2 mmol/l L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin and 2.5 μg/ml amphotericin B (complete medium), all from Biological Industries.

Sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) and seleno-L-methionine ($C_5H_{11}NO_2Se$) were purchased from Merck (Germany) and Sigma (USA), respectively. Both selenocompounds were dissolved in and diluted with purified water to an original stock solution with a concentration of at least 100-fold the concentration added to the cell cultures. Before use, the original stock solutions were sterile filtered with a 0.2 μm filter (Gelman Sciences, USA). The final concentration was obtained by dilution in culture medium.

Cells were taken from the optimally growing stock cultures and used in two types of experiments, the first to study the uptake of selenium in K-562 cells and the second studying the selenium retention in these cells. In the beginning of both the uptake and the retention study the cells were plated at a density of 1.5×10^5 cells/ml in 25 cm^2 plastic tissue culture flasks (Bibby, UK). Two doses of selenite (5 and 50 μM) and two doses of selenomethionine (10 and 50 μM) were used in each experiment. The studies were performed according to the following protocol:

In the selenium uptake study the K-562 cells were grown in 25 cm^2 plastic tissue culture flasks with a total medium volume of 30 ml. Selenite or selenomethionine was added via the medium to the cell cultures. Both selenocompounds were incubated for 0, 1, 8, 24, 48 and 96 h. At the end of each incubation, the upper part of the medium (15 ml) was removed before the rest of the medium was mixed with the cells and transferred to a 15 ml tube. From this tube an aliquot of 0.2 ml was taken for viability testing by the trypan blue dye exclusion test (Tennant 1964). The cells were then centrifuged two times, for 3 min at 470 $\times g$, with washing in 10 ml phosphate buffered saline (PBS) in between. Further 10 ml PBS was added to the cells and after mixture 0.5 ml was taken for cell counting in a cell counter (Model ZM, Coulter Counter, UK). The rest of the cell mixture was centrifuged once more for 3 min at 470 $\times g$, the PBS was removed and the trace element content in the cell pellet ($>5 \times 10^6$ cells) was determined by inductively coupled plasma mass spectrometry, ICP-MS (Houk 1994).

In the selenium retention study the K-562 cells were treated with sodium selenite or selenomethionine via the cell medium in a 15 ml test tube for two hours before being centrifuged twice, for 2 min at 230 $\times g$, with washing in 10 ml PBS in between. Then, 10 ml of complete medium was added to the tube and the cell mixture was plated for cell growth in a 25 cm^2 plastic flask with a total medium volume of 30 ml. The retention was observed at 0, 24, 48 and 96 h. The rest of the retention study was performed in the same way as described above for the uptake study with washings, viability testing, cell counting and ICP-MS analysis.

Mathematical modelling was performed both for the uptake and the retention of the two selenocompounds. The data were compared to fits from nonlinear regression.

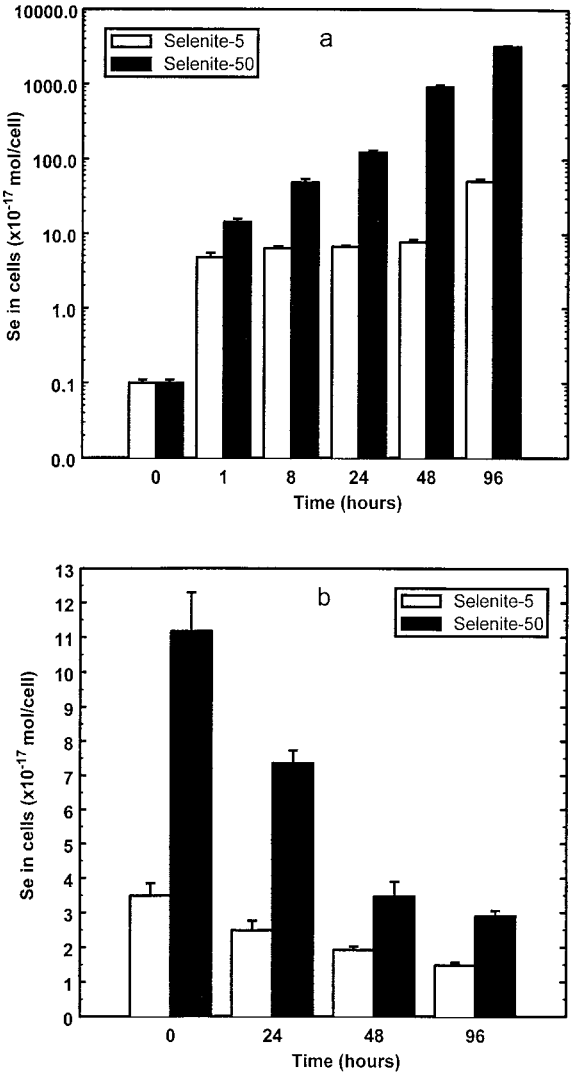

Figure 1. The cellular concentration of selenium (mol/cell) when uptake (a) or retention (b) of two doses of selenite (5 and 50 μM) has been studied. Each bar corresponds to two samples. Mean values and maximal variations are shown.

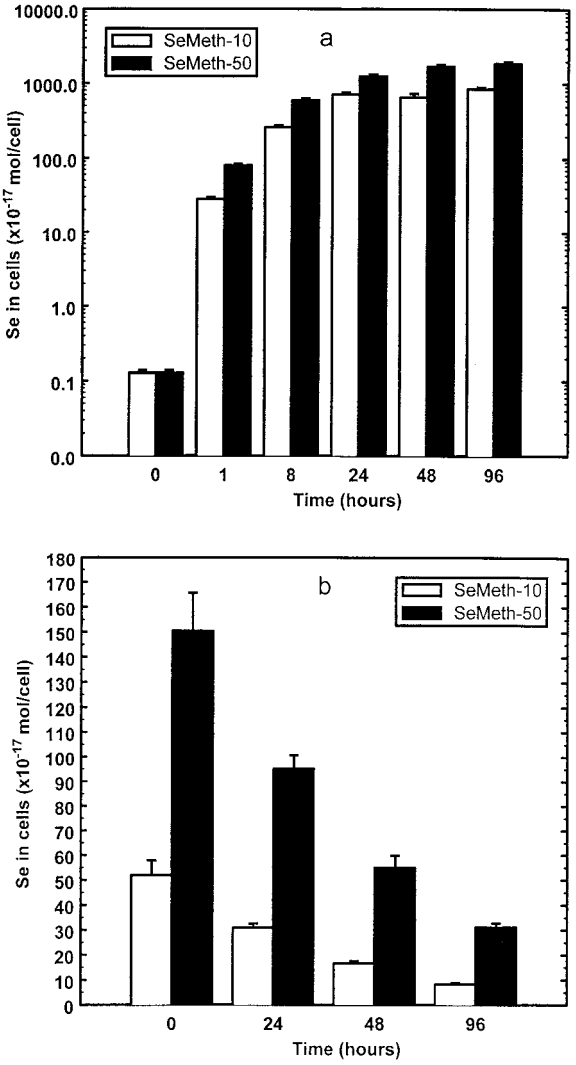

Figure 2. The cellular concentration of selenium (mol/cell) when uptake (a) or retention (b) of two doses of selenomethionine (10 and 50 μM) has been studied. Each bar corresponds to two samples. Mean values and maximal variations are shown. Abbreviation used: SeMeth, selenomethionine.

Results and discussion

Selenium uptake and retention in cells exposed to selenite

The cellular selenium concentration (mol/cell) in K-562 cells that have been treated with selenite is presented in Figure 1a. The selenium uptake in cells exposed to selenite 5 μM began to saturate at 8 h, but increased again between 48 and 96 h. Although the viability was high (>90%) throughout the observation period the late selenium increase might reflect a damage to the cells undetectable by the viability measurements. In cells exposed to selenite 50 μM the selenium uptake never reached a maximum, but at 48 and 96 h the viabilities decreased from high values (>90%) to 65 and 36%, respectively. In an acute toxicity study of selenite on rat hepatocytes there was no difference in cell viability between control cells and cells exposed to 200 μM while at 500 μM the viability was about 50% (Park & Whanger 1995), so the decreased viability in the late exposure to selenite 50 μM might indicate a strong selenite toxicity to the K-562 cells. This conclusion is also consistent with one of our earlier studies on selenite toxicity (Frisk *et al.*; in press).

The retention of selenium in K-562 cells that have been exposed to selenite for 2 h is shown in Figure 1b. The decrease in cellular selenium up to 48 h is faster for cells exposed to the higher dose of selenite, which might be due to the fact that the gradient between intracellular selenium and extracellular selenium initially is much steeper for cells treated with the high selenium dose. At 96 h the remaining cellular selenium concentration for selenite 5 μM and selenite 50 μM compared to their concentration at 0 h was 43 and 26%, respectively. The viabilities during the observation period were high (>95% at all observations). The concentration of selenium gradually decreased with time and the retention in the cells might depend on how strong selenite has been bound to different cellular proteins. In a study on canine mammary tumour cells (CMT-13) about 90% of the accumulated selenite was associated with cytoplasmic macromolecules (Hwang & Milner 1996) and non-specific selenium binding to several cytosolic proteins (74, 55, 41, 34 and 28 kDa) was detected. This non-specific selenium binding might be explained by the ability of selenite to react with free cysteine residues of proteins by a sulfhydryl-selenotrisulfide exchange reaction (Ganther & Corcoran 1969).

Selenium uptake and retention in cells exposed to selenomethionine

The cellular selenium concentration in K-562 cells that have been treated with selenomethionine is shown in Figure 2a. The selenium uptake in cells exposed to selenomethionine 10 μM or selenomethionine 50 μM began to saturate at 24 h and 48 h, respectively. The cell viabilities during the selenomethionine uptake were high (>90%) in all observations except for those cells exposed to 50 μM at 96 h (86%). This

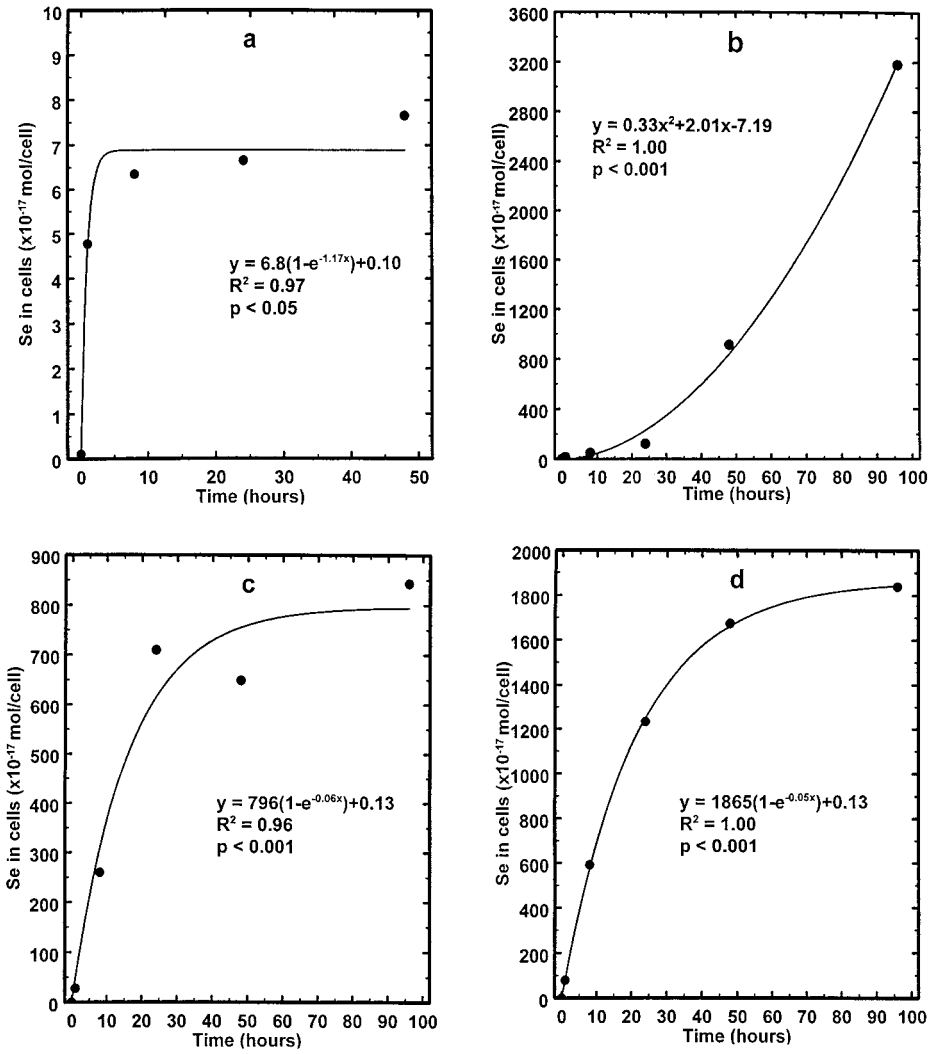

Figure 3. Regression analysis of the uptake of (a) selenite 5 μM, (b) selenite 50 μM, (c) selenomethionine 10 μM and (d) selenomethionine 50 μM. Each data point corresponds to the mean of two samples.

slight decrease in viability seems reasonable compared to a study on K-562 cells showing that a 72 h exposure of selenomethionine 93 μM resulted in a 50% growth inhibition (Kajander *et al.* 1990). The uptake of selenomethionine may reflect that the cells can metabolise it similar to the amino acid methionine. Kajander *et al.* (1991) found selenomethionine to be effectively metabolised to a selenium analogue of S-adenosylmethionine. The selenium analogue was further metabolised in transmethylation reactions and in polyamine synthesis, similarly to the corresponding sulphur metabolites of methionine. Increased methionine supplementations have revealed reduced cellular selenomethionine incorporation (Beilstein & Whanger 1987) and less cytotoxicity of selenomethionine (Kajander *et al.* 1990).

The retention study of selenomethionine in these cells is shown in Figure 2b. The decrease of cellular selenium was very similar for cells exposed to both selenomethionine doses during the observation period with a remaining cellular selenium concentration for selenomethionine 10 μM and selenomethionine 50 μM at 96 h of 16 and 20%, respectively. The viabilities during the observation period were high (>95% in all observations).

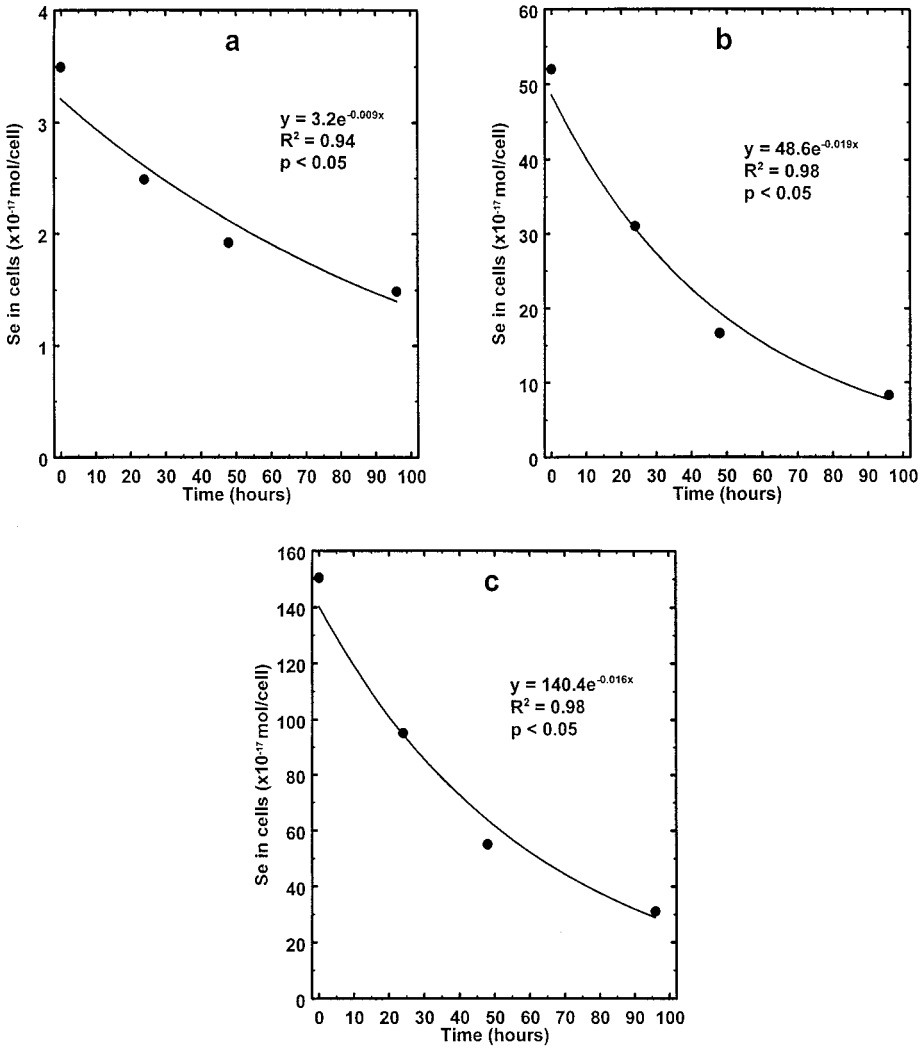

Figure 4. Regression analysis of the retention of (a) selenite 5 μM, (b) selenomethionine 10 μM and (c) selenomethionine 50 μM. Each data point corresponds to the mean of two samples.

Comparison of selenite and selenomethionine with respect to kinetics of uptake and retention

The two selenocompounds showed different kinetic patterns of uptake. For selenite a rapid uptake saturation was found followed by some unexplained cellular toxicity phenomenon at the end of the observation period, measured as a decreased viability and a strong increase of the selenium concentration. Selenomethionine exhibited a strong uptake from the beginning which saturated with time and no strong decrease in cell viability was noticed throughout the observation period. The different uptake patterns were clearly seen when fitting the uptake data to regression models. The uptake of the low selenite dose could be fitted by a function with asymptotic behaviour when considering the observations up to 48 h (Figure 3a). However, the last observation with a very high concentration indicates an uncontrolled uptake. The uptake of the high selenite dose seemed to increase during the whole observation period fitting well to a polynomial (Figure 3b). The uptake of both selenomethionine doses saturated and could be fitted by a function with asymptotic behaviour (Figure 3c and 3d). A comparison of the uptake of the low selenite dose (0–48 h) with the uptake of both selenomethionine doses showed a low uptake of selenite compared to selenomethionine. Similar results as reported here were seen in two other studies, one on Chang liver cells, mouse my-

oblasts and human fibroblasts showing that all three cell types took up significantly more selenomethionine than selenite during a 48 h incubation; (Beilstein & Whanger 1987); the other on human lymphocytes in culture (Karle *et al.* 1983). The different uptakes of selenite and selenomethionine might be explained by differences in metabolic routes. Selenium in both forms can be incorporated into glutathione peroxidase, but selenomethionine may also be incorporated in other proteins probably in substitution for methionine (Beilstein & Whanger 1986a, b).

The retention patterns from the 2 h selenium exposure seemed more similar for both selenocompounds. When performing a regression analysis on the retention data it was found that selenite 5 μM and both doses of selenomethionine fitted to an exponential decrease in selenium (Figure 4a–c). However, selenite 50 μM seemed to express a biphasic exponential decrease which unfortunately not could be fitted due to too few data points. Again, the cellular reaction on selenomethionine seems more controlled than on selenite with very similar retention patterns of the two selenomethionine doses. From this conclusion one should be aware of that the factor between the two selenomethionine doses is 5 compared to 10 between the selenite doses which of course might contribute to the obvious difference in retention of the selenite doses.

Although the difference in cellular uptake and retention between the compounds selenite and selenomethionine remains to be explained these data may contribute valuable information necessary for further studies on for example selenium interactions with other elements as cadmium and mercury and for the understanding of the role of selenium in the protection against metal toxicity.

Acknowledgements

The authors thank Ulla Johansson, Biomedical Radiation Sciences, Uppsala University for valuable technical assistance. Financial support was given by the Swedish Cancer Society.

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