ESRF	**Experiment title:** ***In vitro* study of Cd/U toxicity to renal epithelial cells** **Intracellular competition with Zn**	**Experiment number**: 30-02-662
Beamline: BM 30B	**Date of experiment**: from: 23/06/2004 to: 26/06/2004	**Date of report**: 15/10/2004
Shifts: 9	**Local contact(s)**: Olivier Proux and Vivian Nassif	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Marie CARRIERE*
Barbara GOUGET*
Laure AVOSCAN*
Richard COLLINS*
Hicham KHODJA*

CEA/DSM/DRECAM/LPS
CEA Saclay
Bât 637
F-91191 Gif sur Yvette, France

Aims of the experiment and scientific background

Nephron, the functional unit of kidney, is composed of different segments. Among these segments the glomerulus acts as filtering unit, the proximal and distal tubules as reabsorptive units. Each segment consists in distinct cell types. Epithelial cells are responsible for blood toxins filtration, water and solutes reabsorption leading to the production of concentrated urine. Their transport capacities make them frequent targets for xenobiotic toxics such as **heavy metals**.

Because of its industrial use, **cadmium** (Cd) has become ubiquitous in the biosphere and enters the food chain, thus contaminating animals. Following oral exposure, Cd is absorbed through the gastrointestinal tract and transported via the blood circulation to target tissues. **Kidneys** are one of the major sites of Cd accumulation and many studies have investigated Cd uptake, transepithelial transport and toxic effects on kidneys.

For our experiments, cell lines dog and rat were used, from distal and proximal tubular origins. Quantification of metal uptake as well as intracellular trace and major elements contents were monitored using nuclear microprobe and ICP-MS analysis. Our results proved the ability of proximal as well as distal renal cells to accumulate large amounts of Cd (1000 ppm of Cd per dry weight, as a function of toxic metal concentration and time of exposure) and a strong competition between Cd and the endogen Zn was demonstrated. The aim of the present experiment was to determine the local structure of intracellular Cd and Zn after cadmium exposure. X-ray absorption spectroscopy (EXAFS) of lyophilized cells at the Cd and Zn K-edges was performed on BM30B.

Experimental method

MDCK (dog, distal tubule) and NRK52E (rat, proximal tubule) cultured cells were continuously grown at 37 °C, 5% CO_2 in DMEM (Dulbecco's Modified Eagle's Medium) cell culture medium supplemented with 10% (v/v) fetal calf serum. For metal exposure, cells were grown in 175 cm^2 flasks. The cells were washed twice with serum free cell culture medium, and 0-1 mM Cd was administered as $CdCl_2$ solutions diluted in serum free culture medium. Following 0-24 h incubation periods, cells were rapidly washed with PBS/EDTA 2 mM and trypsin was used to detach cells from their support. After centrifugation, the pellet was frozen and then lyophilized by highering temperature from –10 °C to 20 °C in 3h under a 0.37 mbar vacuum. The samples were dispersed in boron nitride and pressed as 5-mm diameter pellets. The amount of sample was calculated to give a jump of one across the K-edges. Zn and Cd K-edges EXAFS spectra were recorded in fluorescence mode using a 30 elements solid state Ge detector (Canberra). The monochromator was a Si(220) double crystal. To avoid evolution of samples during the measurements, acquisitions were

conducted using a cryostream. At least 6 spectra for each sample were recorded and averaged to improve the statistics. The EXAFS oscillations were isolated from the raw, averaged data by removal of the pre-edge background, approximated by a first-order polynomial, followed by μ_0-removal *via* spline fitting techniques (SEDEM and Athena). Curve-fitting amplitudes and phases will be calculated in a second step.

Results

We expected to elucidate the intracellular speciation (XANES and EXAFS) of Cd accumulated in renal epithelial cells exposed to 20 or 50 µM $CdCl_2$ concentrations during 1-24 h. The local structure of the endogen Zn after exposure of the cells to Cd should allow to confirm the hypothesis that Cd substitutes to Zn on metallothionein. 3 shifts of experiments were conducted at the Zn K-edge and 6 shifts at the Cd K-edge. Data are still under process. However, the first results prove that the sensitivity of the beamline BM30B is particularly well suited to the study of diluted samples containing Cd or Zn concentrations as low as 100 ppm. Spectra for intracellular endogen Zn and for Cd accumulated for longer than 2 h could be recorded without any difficulty.

Fig. 1 shows the Zn K-edge XANES spectra (A) and the corresponding EXAFS oscillations (B) of MDCK cells exposed (MCd20-8h) or not (MCd0) to 20µM $CdCl_2$ for 8h compared to the metallothionein (MT). XANES and EXAFS signals obtained for MCd0 and MCd20-8h at this edge are strictly identical: the Zn local structure after Cd intoxication remains stable. The comparison of EXAFS oscillations obtained with cells and with the protein of reference (MT) clearly shows that the Zn local structure in our samples is similar to that of Zn in the metallothionein, with a probable slight contraction of the distances. In comparison to the XANES spectrum obtained for Zn-metallothionein (MT), the white line intensity is strongly decreased and double for cellular samples. The average position of the edges for cells and MT is comparable: the Zn valence may be the same.

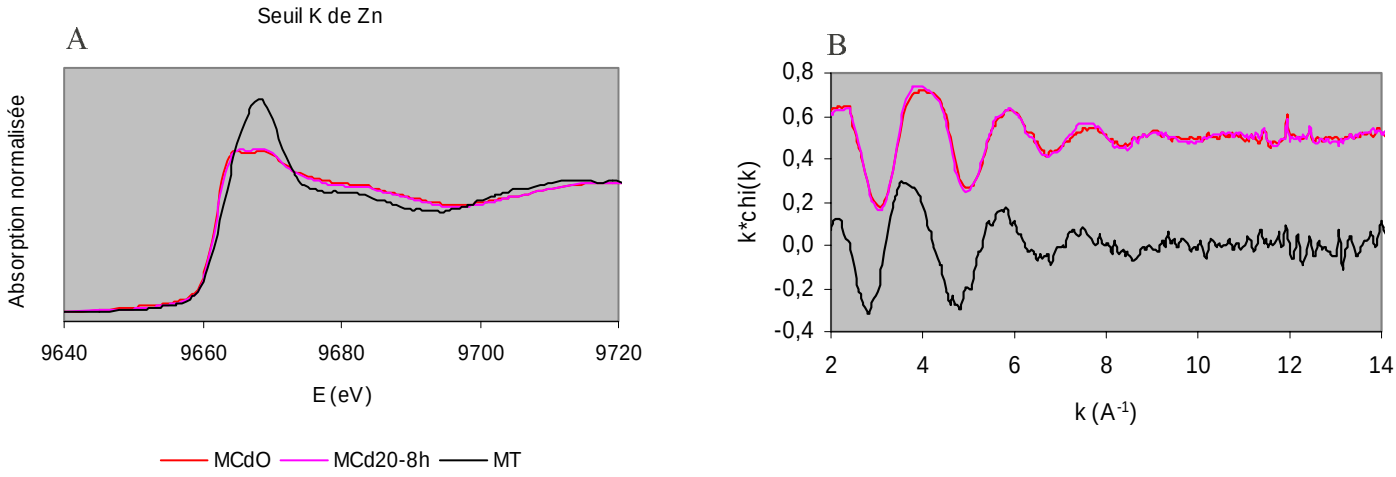

Fig. 1: Zn K-edge XANES spectra (A) and the corresponding k-weighted EXAFS spectra (B) of MDCK cells exposed to 20µM $CdCl_2$ for 8h (MCd20-8h) compared to cells not exposed to Cd (MCd0) and Zn-metallothionein (MT).

Fig. 2 shows the Cd K-edge XANES spectra (A) and the corresponding EXAFS oscillations (B) of MDCK cells exposed to 20µM $CdCl_2$ for 4, 6 or 8 h (MCd20-4, 6, 8 h respectively) compared to references (CdCl, CdO, CdS). The spectra for our reference metallothionein could not be recorded at this edge since its content in Cd was too low compared to the detection limit. An enriched Cd-metallothionein will be analysed during the next experiment. Metallothionein is a protein able to fix 7 atoms of Cd by Cd-S bonds and the reference Cd-S is used here for the comparisons.

Examination of our data confirms the evolution of the Cd local structure in MDCK cells in course of the exposure to the toxic. After 4 h of Cd exposure, the local structure of Cd in cells is similar to that of Cd-S (EXAFS) with a possible mixture of Cd-Cl and Cd-S (XANES). At time 6 h, the local order is strongly comparable to that of Cd-O (the amplitudes and the frequency of oscillations are similar). After 8 h, the Cd local structure is once again comparable to Cd-S.

On the contrary, as shown in Fig. 3, at 50 µM $CdCl_2$ the local order of Cd accumulated in NRK52E cells is similar to Cd-S whatever the time (2 or 4 h). When cells were exposed to 20 µM $CdCl_2$ for 8 h, the data seem to be relative to a mixture of Cd-S, Cd-Cl and Cd-O. The results should be confirmed after modelization of the EXAFS oscillations obtained from these data.

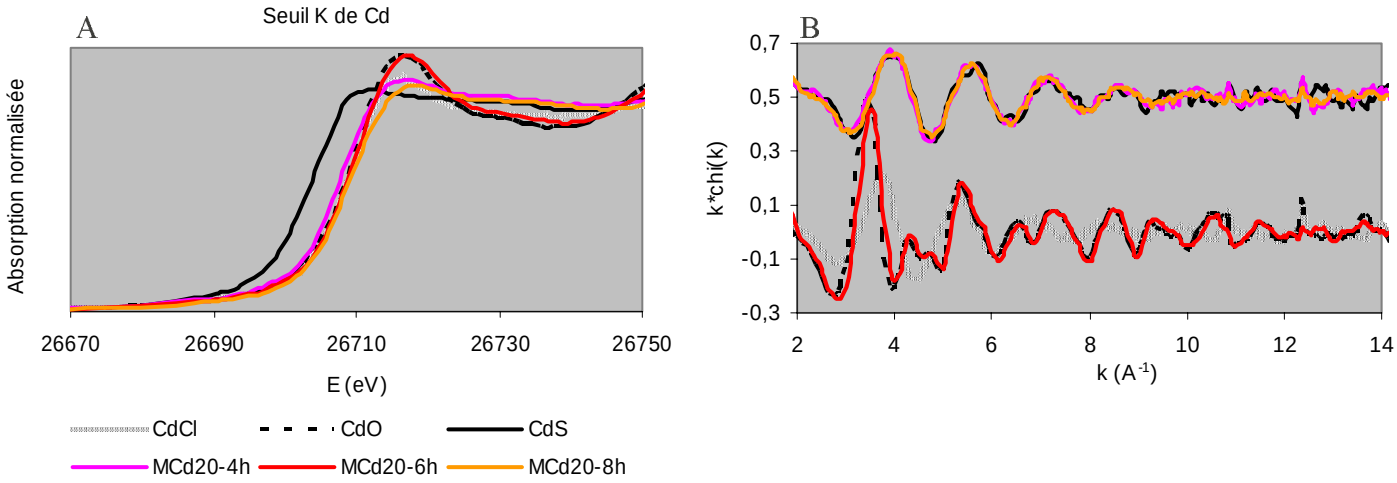

Fig. 2: Cd K-edge XANES (A) and the corresponding k-weighted EXAFS spectra (B) of MDCK cells exposed to 20 µM $CdCl_2$ for 4, 6 or 8 h (MCd20-4, 6 or 8 h) compared to references (CdCl, CdO, CdS).

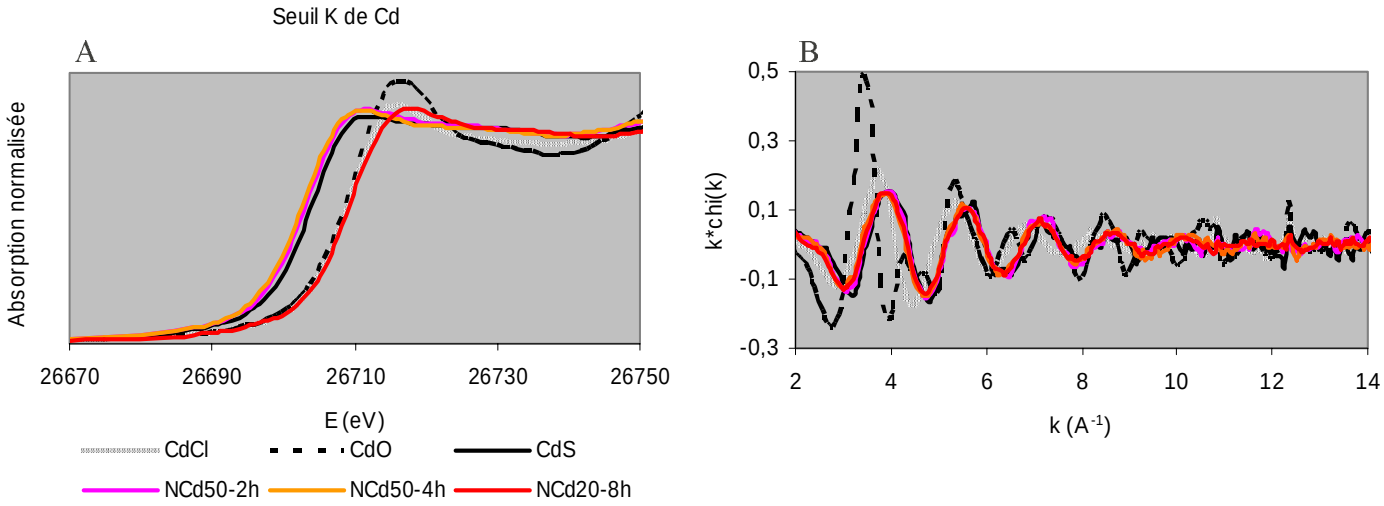

Fig. 3: Cd K-edge XANES (A) and the corresponding k-weighted EXAFS spectra (B) of NRK52E cells exposed to 20 or 50 µM $CdCl_2$ for 2, 4 or 8 h (NCd50-2 or 4h, NCd20-8h) compared to references (CdCl, CdO, CdS).

Conclusions and perspectives

Fitting of the measured data using a structural model of shells has not been processed yet. However, the first results obtained from XANES spectra and examination of EXAFS oscillations are promising. They should be confirmed by analysis of more samples ranging the whole course of Cd intoxication for both cell lines.

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