Micro-SRIXE and Micro-XANES analyses of Cr compounds in lung cells: the "beneficial?" (dietary supplements) and the detrimental (carcinogens)

H.H. Harris,[1] C.T. Dillon,[1,2] P.A. Lay,[1] A. Levina,[1] I. Mulyani,[1] B. Lai,[3] Z. Cai.[3]

[1]School of Chemistry, University of Sydney, NSW, 2006; [2]Electron Microscope Unit, University of Sydney, NSW, 2006;
[3]Experimental Facilities Division, Argonne National Laboratory, Argonne, IL 60439, USA.

Introduction

Chromium(VI) is a known carcinogen that is metabolised to Cr(III) species, the chemical identity(ies) of which remain unknown [1,2]. The present work was performed to probe the cellular targets of Cr(VI) and the Cr(III) metabolic products to elucidate information regarding Cr(VI) carcinogenesis and Cr(III) dietary supplements.

The current work is the first study of the influence of the treatment time on elemental distributions within individual cells exposed to a xenobiotic (in this case, Cr(VI)). Other differences from the previous research included: (i) the use of a human lung epithelial cell line, A549, which is the most commonly used cellular model in the mechanistic studies of Cr(VI)-induced genotoxicity; (ii) improved resolution of micro-XANES of cells treated with Cr(VI) from 200 μm [3] to 0.5 μm; and (iii) the use of an improved spatial resolution (0.5 μm) for whole cell SRIXE. In addition, the results were combined with those obtained from XAS of bulk cell samples performed at the Australian National Beamline Faciity at the Photon Factory for structural studies of Cr(III) complexes formed in Cr(VI)-treated cells, and compared with studies on model complexes.

Methods and Materials

A549 human lung adenocarcinoma (epithelial-type) cells, were treated with Cr(VI) and freeze-dried as reported previously [4,5]. Micro-SRIXE mapping and micro-XANES spectroscopy were performed on XOR-CAT beamline 2ID-D at the Advanced Photon Source (APS), Argonne National Laboratory, Argonne, IL [6]. Individual cells were located on the alphabetised grids using a phase-contrast optical microscope (Leica DMRXE) and fluorescence-detected SRIXE elemental distribution maps were recorded at 295 K, using a 10 keV monochromatic X-ray incident beam under a He atmosphere. SRIXE images were collected simultaneously for the elements P, S, Cl, K, Ca, Cr, Cu and Zn using an incident beam focussed to 0.5 μm using a dual zone plate and order sorting aperture device as described previously [7]. The total count time at each point was one second for all images. Coarse scans at an incident energy of 10 keV, at 1-3 μm resolution and with 0.5 s per point count times were performed prior to higher resolution scans to accurately locate the cell of interest.

The total scan size of the high-resolution images range from 15×15 to 20×20 μm^2 depending on the individual cell size. The sample was raster-scanned through the incident beam. Micro-XANES spectra were recorded on points of interest within cells on the basis of observed regions of Cr localization from lower energy coarse 2D scans, with an incident energy of 6050 eV, count times of 0.5 s and a resolution range of 1-3 μm. In an effort to reduce X-ray damage to the Cr compounds present in the cells, the micro-XANES (including coarse 2D scans) were recorded prior to the high-energy high-resolution SRIXE mapping. The damage from the coarse maps at low energy is expected to be at least an order of magnitude lower than the high-resolution high-energy scans. Count times for micro–XANES spectra were 10 s per point and over a 100 eV energy range.

Results

The results in Figure 1 show obvious changes in the elemental distribution maps between the two treatment times. In three of four studied cells for the 20-min treatment Cr was localized in a small region (~10% of the total cell area), and strongly co-localized with S, Cl, K and Ca (Fig 1).

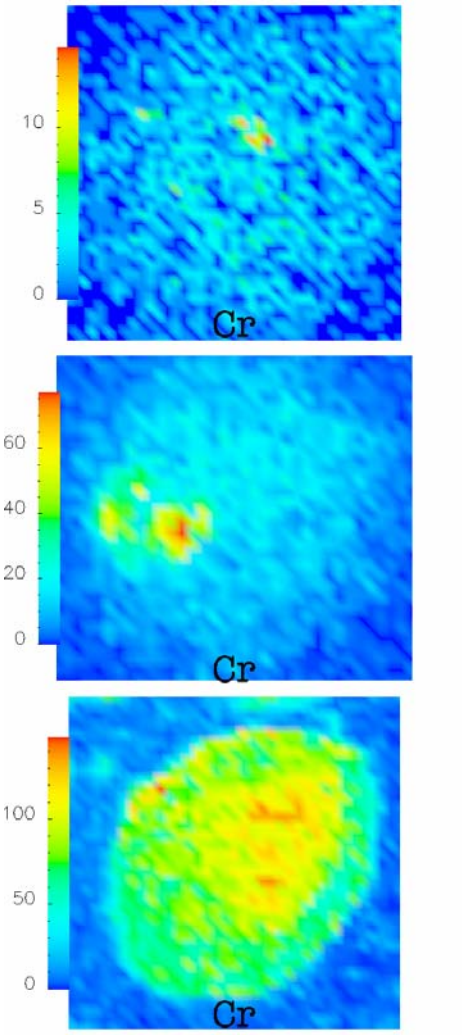

Fig 1. SRIXE Cr distribution maps of single A549 human lung carcinoma cells treated with 100 μM Cr(VI) in comparison with untreated cells. From top: control, 20–min treatment, 4–hr treatment.

In the untreated cells (Fig 1), the Cr levels were almost indistinguishable from the background except for a few small areas, and no co-localization of the light elements with Cr was observed. After the 4-hr treatment, Cr was distributed throughout the cells (Fig 1), but higher Cr concentrations

occurred in the region of Zn localization, suggesting the presence of Cr in the cell nuclei (rich with Zn-finger proteins). Unlike the 20–min treatment, no obvious co-localization of the light elements with Cr was observed. Relatively high concentrations of Cr, Cu and Zn, but not Fe or light elements, were observed in the cell membrane region for two cells in the 4-hr treatment. No such localization of the heavy metals on the membrane was obvious for untreated cells, or for the cells treated for 20 min.

Figure 2 shows a comparison of Cr K-edge XANES of bulk cell pellets, with micro-XANES spectra collected for Cr hotspots of several individual cells, and model Cr complexes. No obvious changes in micro-XANES spectra were observed between Cr hotspots in the cells treated for either 20 min or 4 hr and the shapes of the micro-XANES spectra were similar to those for a bulk cell sample.

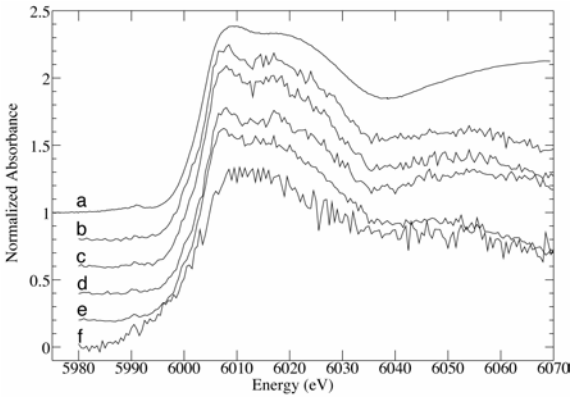

Fig 2. Cr K-edge XANES spectra of A549 human carcinoma cells, from (a) bulk cell pellet recorded at ANBF, (b-f) single freeze dried cells recorded at APS 2-ID-D, (b-e) spectra from Cr hot spot of three different cells treated with Cr(VI) for 4 hr (from Cr hot spots indicated with arrows in Fig 1), (f) from Cr hot spot of Cr treated with Cr(VI) for 20 min (indicated by arrow in Figure 1).

Discussion

The predominance of Cr(III) was confirmed by micro-XANES spectroscopy of intracellular Cr hot spots. Analysis of the XANES spectra obtained from ANBF indicated that these species were polynuclear complexes (probably with a combination of carboxylato and hydroxo bridging groups and O-donor atoms of small peptides or proteins) [8].

The current work provides the first insight into chemical mechanisms of cellular defence against Cr(VI), which may be similar for other toxic compounds. This may also be important in potential toxic effects of Cr(III) dietary supplements where recent studies have indicated that they exert their biological activities by oxidation to Cr(VI) [9]. At low concentrations of dietary supplements, this may not be a serious issue as the protective mechanisms of the cells may prevent the movement of the genotoxin to the cell nucleus. However, if such defence mechanisms are exhausted, then such oxidation of Cr(III) to Cr(VI) may present a greater cancer hazard.

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