The Use of a Scanning Hard X-Ray Microprobe for the Study of Mammalian Cells to Facilitate Investigations of Chromium(VI)-Induced Cancers

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Introduction

The carcinogenic properties of Cr are largely attributed to Cr(VI) compounds, since most Cr(III) compounds are nonmutagenic.[1] While Cr(VI) complexes readily enter cells,[2-7] little is known about the intracellular distribution of Cr, i.e., does Cr induce most of its damage from the cell wall, does Cr reach the nucleus, or is it reduced so rapidly to Cr(III) by cellular intermediates that it is excluded from the nucleus?

PIXE analyses of whole cells provided evidence for Cr being distributed throughout the cell following exposure to Cr(VI), but the technique was not sensitive enough to map Cr in 1-µm-thin sections from 10-µm-diameter cells.[8-10] SRIXE is superior to its ion counterparts since the cross sections of the x-ray fluorescence generated by x-rays are typically 10-10^3 times higher than those induced by charged particles for those elements with N > 20.[11]

A scanning x-ray microprobe with submicron spatial resolution was used to provide elemental mapping of whole cells following exposure to Cr(III) and Cr(VI) compounds. Details of the intracellular spatial location of Cr were also provided following analysis of thin sections prepared from Cr(VI)-treated V79 Chinese hamster lung cells.

Methods and Materials

All solutions and cell media were prepared in milli-Q water. V79 Chinese hamster lung cells were treated in culture with the Cr complexes ($Na_2Cr_2O_7$ (0.5 µmol Cr/dish) or *cis*-$[Cr(phen)_2(OH_2)_2]^{3+}$ (2 µmol/dish)) for 4 h.[12,13] They were then freeze-dried from ammonium acetate solution.[8,9] Thin-sectioned samples were prepared by sectioning the cell pellet (1-µm thickness) after fixation in Spurrs resin.

Hard x-ray microprobe experiments were performed on SRI-CAT beamline 2-ID-D at the Advanced Photon Source. All measurements were conducted under a He atmosphere in order to eliminate the Ar K-shell fluorescence signal. Fluorescence maps were collected simultaneously for each two-dimensional scan by integrating the appropriate Kα fluorescence signal for P, Cl, K, Ca, Cr, Fe, Cu, and Zn. Whole cells were analyzed using a 1-µm-diameter focused beam. Individual cells were initially located by collecting an x-ray image of the sample with a CCD camera, followed by accurate location of the cells by collecting the fluorescence signal of known cellular elements (P and K) along a line of interest. This enabled a single cell to be centered within a scan area (generally 20 x 20 to 30 x 30 µm^2). Three to five individual cells were analyzed per sample using 1 µm steps and the emitted x-rays were detected for 3 s per point. Analysis of thin-sectioned cells was performed with a 10 keV monochromatic x-ray beam that was focused to 0.3 µm diameter using a phase zone plate. An area of 12 x 12 µm^2 was analyzed, and the x-rays were detected for 6 s per point using a Canberra Ultra-LEGe germanium x-ray detector.

Results

Figure 1 shows the two-dimensional maps of P, K, Cr, and Zn obtained from (a) a Cr(III)-treated cell and (b) a Cr(VI)-treated cell with the major elements (P, K and Zn) clearly defining the cell. Comparison of the Cr maps shows that there is high Cr uptake in the cell that had been exposed to Cr(VI) but not Cr(III).

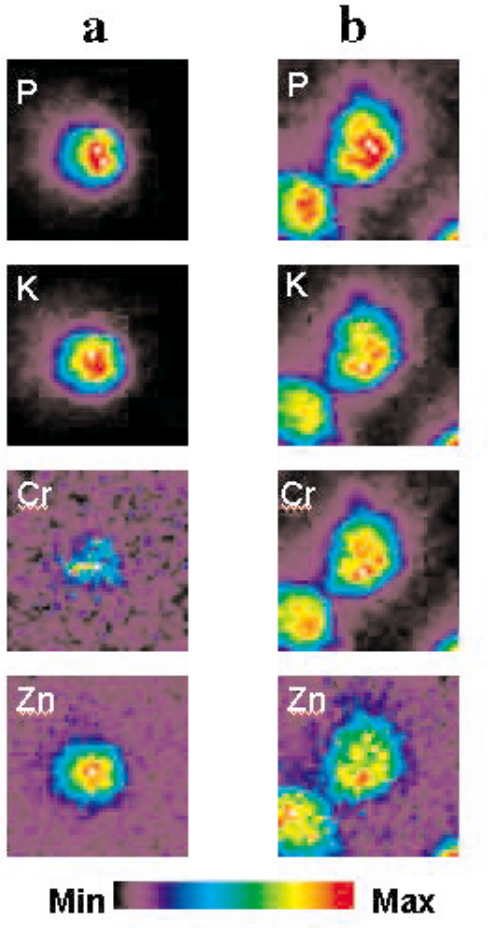

FIG. 1. Two-dimensional maps of P, K, Cr and Zn obtained from a representative cell that had been treated with (a) Cr(III) ($[Cr(phen)_2(OH_2)_2]^{3+}$, 2 µmol/dish), and (b) Cr(VI) ($[Cr_2O_7]^{2-}$, 0.5 µmol Cr/dish). The scan dimensions were 20 x 20 µm and the beam diameter was approximately 1 µm.

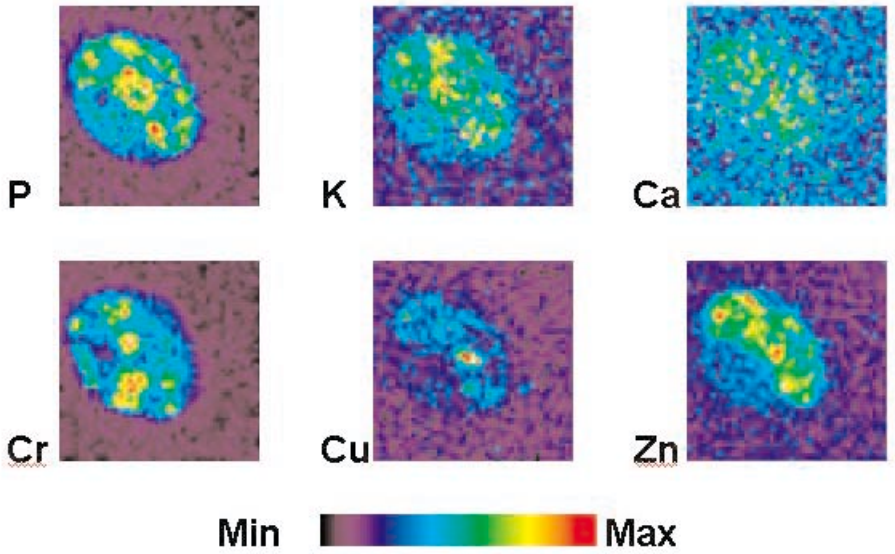

FIG. 2. Two-dimensional maps of P, K, Ca, Cr, Cu and Zn obtained from a thin section of a cell that had been treated with Cr(VI) ($[Cr_2O_7]^{2-}$, 0.5 μmol Cr/dish). The scan dimensions were 10 x 10 μm^2 and the beam diameter was approximately 0.3 μm.

Figure 2 shows the results of the elemental mapping of a thin section obtained from a Cr(VI)-treated cell, which is defined by the P, K, Ca, Cr, Cu, and Zn maps. The high incidence of P, K, and Zn in the upper right portion of the cell indicates the position of DNA and hence probably defines the nucleus of the cell. There are two distinct localizations of Cr within the cell. The smaller of the two Cr localizations, in the center of the cell, overlaps with the region of greatest P and Zn concentrations. The second larger localisation of Cr, at the lower right side of the cell, occurs in a region of relatively low P, K, Ca, Cu, and Zn.

Discussion

Excellent elemental maps were produced from whole mammalian cells for the major cellular elements, P, K, Ca, Cu, and Zn, using hard x-ray imaging. The technique also provided qualitative information on the relative uptake of Cr following treatment with Cr(VI) and Cr(III) complexes. This was consistent with findings from atomic absorption spectroscopy, radioactive tracer analysis, gas-liquid chromatography, and micro-PIXE analysis.[2-5,7] The high sensitivity imparted by the synchrotron x-ray based technique, in combination with excellent submicron spatial resolution, allowed, for the first time, direct determination of the locality of Cr within a cell from thin sections showing that Cr enters the cell. Therefore, it can be concluded that Cr does not exert damage from the cell wall but from direct interactions with intracellular components. Accumulation of Cr in the nucleus indicates that Cr binds to DNA and other nuclear biomolecules. Also evident in the cell is a localization of Cr in the lower left portion of the cell that may correspond to an organelle, possibly a further site of damage, or an acidic vacuole in the cell.

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