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Sub-micron Hard X-ray Fluorescence Imaging of Synthetic Elements

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Abstract

Synchrotron-based X-ray fluorescence microscopy (SXFM) using hard X-rays focused into sub-micron spots is a powerful technique for elemental quantification and mapping, as well as microspectroscopic measurement such as μ -XANES (X-ray absorption near edge structure). We have used SXFM to image and simultaneously quantify the transuranic element plutonium at the L₃ or L₂ edge as well as lighter biologically essential elements in individual rat pheochromocytoma (PC12) cells after exposure to the long-lived plutonium isotope ²⁴²Pu. Elemental maps reveal that plutonium localizes principally in the cytoplasm of the cells and avoids the cell nucleus, which is marked by the highest concentrations of phosphorus and zinc, under the conditions of our experiments. The minimum detection limit under typical acquisition conditions for an average 202 μm^2 cell is 1.4 fg Pu/cell or 2.9×10^{-20} moles Pu/ μm^2 , which is similar to the detection limit of K-edge SXFM of transition metals at 10 keV. Copper electron microscopy grids were used to avoid interference from gold X-ray emissions, but traces of strontium present in naturally occurring calcium can still interfere with plutonium detection using its L _{α} X-ray emission.

INTRODUCTION

The transuranic actinide elements are all radioactive and naturally occur in only fleeting amounts, but the technologically important transuranic elements, neptunium, plutonium, americium, and curium, are present in a range of technological and environmental matrices associated with their anthropogenic production via nuclear reactions. Understanding their interactions with such matrices is key to modeling and predicting their behavior and protecting people and the environment from these hazardous elements. In this context, high-resolution chemical imaging of materials containing man-made actinides can provide important information about their interactions on the 10–1000 nm length scale.

A range of techniques is available for chemical imaging the transuranic elements. Because all of the actinide elements are radioactive, autoradiography can be used to image their

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distribution [1]; however chemical information is not probed by this technique, only radioactive species can be imaged, the spatial resolution of α -radiography is limited by the significant path length of α -particles in many matrices, and it is not well-suited for measuring very low-radioactivity samples. The high electron density of transuranic elements gives then excellent contrast for various forms of electron microscopy [2] and charged particle [3] or soft X-ray (< 2 keV) [4] mapping techniques. While these techniques offer high resolution and often can produce substantial chemical information regarding the samples of interest, the lower penetrating power of these radiations can make examination of thick samples difficult. Microscopic chemical mapping of transuranic elements by mass spectrometric techniques such as SIMS or laser ablation methods will have to deal with regulatory constraints on working with unencapsulated radioactive materials. In contrast to these techniques, synchrotron X-ray fluorescence microscopy (SXFM) with hard X-ray micro- or nanoprobe offers a number of advantages [5, 6] for quantitative imaging and micro-spectroscopy of actinide-containing samples. SXFM is a high sensitivity technique capable of simultaneous detection and quantification of multiple elements with $1 \mu\text{g/g}$ or better sensitivity routinely achievable. The penetrating power of hard X-rays allows examination of thick samples and it also allows encapsulation of radioactive samples for radiation protection; encapsulation that can, with appropriate caution, obviate the need for instrumentation dedicated to examining radioactive samples. The resolution of hard synchrotron X-ray microprobes is improving, and instruments with resolutions finer than 100 nm are available [7, 8].

A few X-ray microprobe studies of the elemental distribution and speciation of transuranic elements have been reported, but these studies have been principally conducted on inorganic samples with X-ray beams focused to $10 - 200 \mu\text{m}^2$ spots [9–16]. In this work we report details of submicron ($0.1 \mu\text{m}^2$) hard X-ray SXFM studies of the accumulation and distribution of the transuranic element plutonium in a complex matrix, living cells. To minimize Pu-induced radiation damage to the cells, we used the low specific activity isotope ^{242}Pu ($t_{1/2} = 3.76 \times 10^5$ years) in these experiments. Elemental maps with submicron resolution were collected while exciting the samples at the Pu L_3 or L_2 -edges (18.06 or 22.23 keV) with a $0.1 \mu\text{m}^2$ X-ray beam. Elemental mapping using the actinide L-edges instead of their M-edges reduces interference with the K-edge emissions of lighter elements such as potassium and calcium. An additional advantage is that X-ray absorption spectra at the actinide L-edges [17, 18] can also be measured in specific spots to obtain chemical information about the cell-associated Pu. Under these conditions, we can routinely detect concentrations of Pu that exceed 1.4 fg Pu in a $202 \mu\text{m}^2$ cell while mapping the elemental distribution in an area scan with 500 nm pixels that lasts $1-2$ hours. Strontium is the only significant elemental interference with the Pu L_{α} emission lines in the samples, but high concentrations of Pu can interfere with quantification of K and Ca.

EXPERIMENTAL SECTION

A solution of $^{242}\text{Pu}(\text{NO}_3)_4$ in nitric acid was taken from laboratory stocks and purified by anion exchange chromatography on Dowex-1 in 7.5 M HNO_3 [19]. Alpha spectrometry gave an isotopic distribution of $99.96 \text{ atom\% } ^{242}\text{Pu}$, $0.035 \text{ atom\% } ^{239}\text{Pu}$, and $0.0014 \text{ atom\% } ^{238}\text{Pu}$ and a corresponding specific α -activity of $9.29 \text{ disintegrations min}^{-1} \text{ ng}^{-1}$. The oxidation state and chemical form of the purified Pu were adjusted as previously described [20] before adding the Pu to the growth media.

Pheochromocytoma cells from a rat adrenal gland (PC12) obtained from the American Type Culture Collection were grown in a humidified $5\% \text{ CO}_2$ environment at 37°C using Ham's F12K media supplemented with 12.5% horse serum and 2.5% fetal calf serum as well as antibiotics. The cells were plated at the appropriate density in 25 cm^2 culture bottles to

achieve approximately 70% confluency the day before Pu exposure. The cells were serum-starved overnight, the the serum-free media was replaced with 2 mL of complete F12K media with serum containing between 2 and 100 μM ^{242}Pu in the desired chemical form. The cells were incubated in the presence of Pu for a predetermined amounts of time, up to 4 hours. After incubation, the adherent cells were washed three times with pH 7.4 0.1 M NaCl/0.01 M EGTA (ethyleneglycol-O, O'-bis(2-aminoethyl)-N, N, N', N'-tetraacetic acid) to remove molecular Pu complexes sorbed to cell surfaces. The cells were then suspended in 2 mL fresh NaCl/EGTA solution by scraping and pelleted by centrifugation. The cell pellet was resuspended in a known volume of fresh NaCl/EGTA, and fractions of the suspension were used to determine the total number of cells, total protein content (BCA assay, Pierce Protein Research Products), cell viability (trypan blue exclusion), or average Pu content (liquid scintillation counting of lysed cells) as needed.

Cells for SXFM measurements were deposited onto 3.05 mm diameter, 100 mesh copper electron microscopy (EM) grids with a formvar backing from a drop of the final NaCl/EGTA suspension. The grids were air dried overnight. After examination under an optical microscope, each grid was glued onto a 1 mm thick aluminum stick over a 2.5 mm hole and encapsulated between a pair of 500 nm thick SiN windows (Silson Ltd.). For additional protection from possible exposure to plutonium, each sample was sealed underneath a layer of 8 μm polyimide (Kapton) or, preferably, 6 μm polypropylene film. Finally, the sample-bearing aluminum sticks were placed in kinematic mounts adapted for light and X-ray microscopy. Individual cells on each grid were examined optically and located relative to the coordinates of grid reference points using a Leica DMXRE microscope fitted with a high-resolution digitally encoded stage.

Samples were transferred to the hard X-ray microprobe beamlines 2-ID-D [21] or 2-ID-E [22] at the Advanced Photon Source for SXFM measurements. Both beamlines use Fresnel zone plates to focus monochromatic synchrotron X-rays onto the samples. In our experiments, the beamlines always achieved a FWHM resolution of $0.3 \times 0.4 \mu\text{m}$ or better at the energies used. The beam flux on the sample for beamline 2-ID-D was typically 4×10^9 photons/sec at 18.10 keV. For beamline 2-ID-E, the flux was approximately 1×10^9 photons/sec at 23.09 keV. Both the incident and transmitted X-ray flux were monitored with N_2 -filled ionization chambers, while the complete X-ray fluorescence spectrum at each spot examined was measured using a Ge detector with a Be window. The linear detector energy scale was calibrated by elemental standards for each run. To reduce the fluorescence background from atmospheric Ar and improve detection of light elements, the sample and fluorescence detector are mounted in a He-filled chamber.

Cells selected by optical microscopy were located from their grid coordinates, and their suitability for SXFM was judged from rapid, coarse scans of the area around each cell. Suitable cells were then raster scanned in finer steps (usually 0.5 μm in both the x and y directions) with dwell times of 2–5 seconds per point, with 2–3 second dwell times usually giving acceptable counting statistics. The energies of the incident X-rays, 18.10 keV was sufficient to excite the L_3 -edge of Pu, as well as absorption edges of all naturally occurring elements in the samples, which allowed the simultaneous examination of all the elements from P through Pu at each spot in the samples. One set of experiments was done with an incident beam of 23.09 keV, which also excited the Pu L_2 -edge.

The areal elemental content of the cells with units of mass per unit area was determined with the program MAPS [23] from the peak area of the X-ray fluorescence spectrum for each element of interest. To determine the total amount of each element associated with each cell, the spectra obtained for every pixel of the cell were summed; the inherent X-ray fluorescence background, determined from the summed spectra of a cell-free background

area near each cell, was also considered. The peak areas were converted to area concentrations with calibration coefficients derived from thin film reference materials NBS1832 and NBS1833 measured for the particular conditions of each run. Together these standards contain elements as light as Na and as heavy as Pb. The calibration coefficients for light elements not present in the standards, for example P, S, Cl, or Cr, were estimated by linear regression. The calibration for emission lines beyond the Pb L_{α} line, most notably Sr and Pu, were determined by extrapolation of the calibration curve, taking into account element-to-element differences such as the X-ray absorption cross-sections, absorption by the detector's Be window, and the fluorescent yield of the relevant emission lines. For the elements determined from the extrapolated curve, we estimate the relative precision of the measurements to be better than $\pm 10\%$ and the absolute accuracy to be better than $\pm 40\%$. The MAPS software also accounts for overlapping peaks in the X-ray emission spectra [24], for example the Zn K_{α} (8.64 keV) and Cu K_{β} (8.90 keV) or the Sr K_{α} (14.17 keV) and the Pu L_{α} (14.28 keV) lines.

X-ray absorption spectroscopy of Pu in individual $0.1 \mu\text{m}^2$ spots within the cells was conducted on beamline 2-ID-D. The Pu L_{α} fluorescence was monitored while the incident photon energy was varied across the Pu L_3 absorption edge. The incident energy was calibrated against the first inflection point of the K-edge absorption of a Zr foil (17.998 keV) measured before the Pu spectra were collected. Repeated scans of the same spot were averaged, and the μ -XANES spectra were normalized and analyzed with the programs IFEFFIT [25] and ATHENA [26] by standard methods [27].

RESULTS AND DISCUSSION

X-ray Emission Spectra of Plutonium

The energy response and efficiency of the Ge X-ray detector was examined with a sample containing Th-232 (0.10 μg) and Pu-242 (0.12 μg) deposited as a 1.2 mm diameter thin film from an aqueous solution of 10 mg/mL bovine serum albumin onto 8 μm Kapton. In addition to the strong Th and Pu L emission lines between 13 and 19 keV (Figure 1), weak peaks below 10 keV are observed that represent a series of seven Ge detector escape peaks 9.89 keV lower than the parent L-emission line, the Pu M_{α} emission line at 3.35 keV, and K_{α} emissions from P, S, Cl, and Cu. Using these peaks, the energy response was observed to be linear over the entire energy range examined (2 – 23 keV). The mass ratio of Th/Pu in the sample also was measured from the ratios of the areas of the Th and Pu L-fluorescence lines, accounting for differences in the X-ray absorption cross-sections of Th and Pu at the incident X-ray energy (23.09 keV) and in the fluorescence yields of their L_3 (L_{α} and L_{β_2}) and L_2 (L_{β_1} and L_{γ_1}) edge emissions [28]. The Th/Pu ratio determined from the L_3 and L_2 edge emissions were 0.77 ± 0.02 and 0.80 ± 0.02 , respectively, in agreement with the value expected for this mixture, 0.83 ± 0.08 .

Elemental Mapping

Efficient examination of individual cells requires a method for identifying and locating the cells on the X-ray sample stage. At lower X-ray energies (< 10 keV), weak visual images of specific cells may be observed with an X-ray sensitive camera; however even with this system the cells are optically transparent at the higher energies required to excite the L-edges of transuranic elements. As an alternative, we locate cells of interest by optical microscopy relative to reference points on the EM grid holding each sample. Since the grids are opaque to visible light and X-rays, the grid reference points are easily relocated when samples are mounted in the X-ray beam. Rapid coarse scans of the area around a cell of interest, typically in 2–3 μm steps (Figure 2) enable the location of specific cells identified by light microscopy from the X-ray fluorescence of abundant biologically important

elements distributed throughout the cells, for example phosphorous, sulfur, or potassium. Intact cells are readily distinguished from damaged cells based on the relative amounts of these elements. After locating cells of interest, elemental maps of the individual cells can be collected with finer resolution and longer dwell times to measure the concentration and distribution of the elements of interest associated with the cells.

Elemental maps of PC12 cells incubated with Pu reveal several key spatial correlations. Treatment of the serum starved cells with soluble, molecular complexes of Pu (for example plutonium-citrate, plutonium-nitrilotriacetate, or plutonium-transferrin complexes) leads to uptake of this transuranic element (Figure 3) by a majority of cells over the course of several hours. We find the highest concentrations of Pu localized in cellular areas containing the most Fe and the lowest Pu concentrations in areas containing the cells' highest P and Zn concentrations. When the cell nucleus can be visualized in the optical micrographs, as in Figure 3a, it is obvious that the high P and Zn concentrations mark the cell nucleus, as previously reported [29–31], while the highest Fe amounts are found in the cells' cytoplasm. Thus the pattern of Pu distribution shows that most of the Pu is localized in the cytoplasm of cells. For the cell depicted in Figure 3a, 83% of the cell-associated Pu is found in the cytoplasm. Furthermore, the association of Pu with a definite intracellular structure, the cytoplasm, coupled with the low relative concentration of Pu at the edge of the cells, which is indicative of membrane bound material, demonstrates that the Pu detected by SXFM in these experiments is internal to the cells; it is not simply sorbed to the cell surface under these conditions.

Determination of Cellular Pu Content

The total cellular content of each element of interest was determined from the summed intensity of the K_{α} or L_{α} emission from each spatial pixel, normalizing for the intensity of the incident beam and using traceable thin film multi-element standards to calibrate the detector response. The average PC12 cell studied was $202 \mu\text{m}^2$ ($n = 177$ cells, range $69.25\text{--}460.75 \mu\text{m}^2$, standard deviation = $72 \mu\text{m}^2$), which corresponds to 808 spatial pixels when measured in 500 nm steps. Typical X-ray fluorescence spectra for a plutonium-containing cell and a nearby background area are depicted in Figure 4. After three hours of incubation, the total Pu content of this otherwise normal cell is 1.5 fg Pu or 6.1×10^{-18} moles ($157 \mu\text{m}^2$, 628 spatial pixels, 3 second per pixel counting time), although this is less than the cellular content of essential metals, such as Fe (3.9×10^{-16} moles) or Zn (4.6×10^{-16} moles). The average amount of cell-associated Pu for sets of individual cells measured by SXFM matched that of carefully washed cell pellets determined using liquid scintillation counting. For example, a pellet of $(3.0 \pm 0.6) \times 10^6$ cells contained 6.1 ± 0.4 ng Pu, or 2.0 ± 0.4 fg Pu/cell (propagated experimental uncertainty at 95% confidence). A set of cells from this same sample were also examined by SXFM. Each of the cells contained measurable Pu, and the average Pu content was 3.4 ± 1.3 fg ($n = 5$ cells, average area $201 \mu\text{m}^2$, with the uncertainty given as S.E.M. at 95% confidence).

The minimum detection limit of Pu in these experiments was estimated from the uncertainty in the plutonium-free background. Considering three standard deviations above the background as the lower threshold for detecting Pu, the apparent average metal content of control cells that were never exposed to Pu was examined in three separate campaigns. The standard deviation of the background detected in the Pu channel was equivalent to 0.23 ng Pu/cm^2 ($n = 23$ cells, average area $204 \mu\text{m}^2$), which corresponds to a detection limit of 1.4 fg Pu in an average cell. This is in agreement with the detection limit expected from the Poisson counting statistics governing the measurements [32], 1.3 fg Pu/cell, under normal experimental conditions (500 nm $\times$ 500 nm pixels with a 3 second dwell time and an effective fluorescence yield of $1.5 \times 10^{10} \text{ counts sec}^{-1} \text{ cm}^2 (\text{g Pu})^{-1}$). Given the average size of the PC12 cells examined, and estimating an average cell thickness of 5 μm [33] and an

average density of 1.1 g/cm^3 , this correspond to a Pu detection limit of $1.3 \text{ } \mu\text{g Pu/g}$, $5.8 \times 10^{-18} \text{ moles Pu/cell}$, or $2.9 \times 10^{-20} \text{ moles Pu/}\mu\text{m}^2$. This is comparable to the reported sensitivity of SXFM for transition metals such as Mn or Fe measured from their K-edge emissions after excitation at 10 keV [32].

These actinide concentrations represent truly low levels of radioactivity. An average cellular content of 1 fg/cell corresponds to 1 ^{242}Pu disintegration/cell every 80 days, 1 ^{248}Cm disintegration/cell every 76 days, or 1 ^{237}Np disintegration/cell every 1.2 years. For all but the shortest lived transuranic elements, large samples or long counting times would be required to quantify these isotopes accurately at similar concentrations using radioactive counting, while radiographic imaging is impractical for such low decay rates.

Spectral Interferences

SXFM imaging experiments of biologically important elements often employ gold EM grids as sample substrates. Because those measurements are typically made at incident lower energies (e.g., 10 keV), the background X-ray fluorescence from the Au is of little importance. This is not the case for measurements made at the actinide L-edges, however. Gold L_γ emissions at 13.81 keV make detection of the Np or Pu L_α lines difficult. For this reason we use Cu EM grids instead. That renders the cellular Cu content and distribution unobservable, but it allows ready detection of the transuranium actinides. Moreover, fluorescence from the Cu grids is less of a problem at the actinide L-edge energies as the X-ray absorption cross section of Cu is roughly 5–10 times lower at these energies than it is at 10 keV. Grids made of other metals, for example Ni or Mo, could also be used, though these grids generally lack an index.

For protection from the radioactive materials in our samples, the EM grids are encapsulated between SiN windows and then sealed under a layer of X-ray compatible plastic film. This raises another material compatibility issue. Our initial work used 8 μm Kapton, which is resistant to X-ray damage. Unfortunately, micron-size particles of CaHPO_4 are added in the manufacturing process to enhance slippage in Kapton film [34]. These particles can distort elemental maps (Figure 3) and cause difficulties in quantifying Pu from its L_α emission (*vide infra*). This difficulty is not encountered when high purity 6 μm polypropylene film is used in place of Kapton.

Although imaging and quantifying actinide elements using L-edge X-rays avoids interferences from light elements at the actinide M-edges, there are several common elements, in addition to gold, that may still interfere. X-ray emissions from Pb shielding can be quite intense. L_β emissions from Pb (12.61 keV) can obscure weak Th L_α emissions (Supplemental Figure S3), and its $L_{\gamma 1}$ line can interfere with the Am L_α line. Fortunately, the background intensity contributed by the Pb lines tends to be constant, as it arises from components of the apparatus, and it is readily removed. Alternately, Th or Am may be quantified with lower sensitivity at the L_3 edge from their $L_{\beta 2}$ lines (15.59 and 17.63 keV, respectively) or at the L_2 edge from their $L_{\beta 1}$ lines (16.20 and 18.86 keV, respectively). Similarly, the Pu L_α line (14.28 keV) is partially obscured by the Sr K_α at 14.17 keV (Supplemental Figure S4). Although Sr would seem to be an unusual element to encounter in many samples, as an alkaline earth element it is a common impurity (< 0.1 atom%) in Ca, which is abundant in many biological or geological samples in addition to being a component of Kapton film lubricants (*vide supra*). The MAPS software we use to analyze and display the data compensates for the overlap of these and other emission lines by peak fitting both elements to the emission line. Example Sr and Pu maps resulting from this procedure are depicted in Figure 5. The Cm L_α line (14.96 keV) also strongly overlaps the Y K_α line (14.96 keV), which makes Cm detection using its L_α emission line problematic.

High concentrations of actinide elements can also interfere with detection of the light elements K and Ca due to the M-edge emission energies (see supplemental Figure S2). For instance, the Pu M_{α} line at 3.34 keV interferes with the K K_{α} line (3.31 keV), and M-edge emissions from high concentrations of Pu can distort mapping of potassium. However, these M_{α} lines are an order of magnitude less intense than the actinide L_{α} lines and substantial interference with detection of K or Ca only occurs for cells containing micron-scale Pu particles in our studies.

μ -XANES Measurements

Having determined the Pu content and mapped the Pu distribution of individual cells, μ -XANES spectroscopy of specific Pu deposits within the cells can be used to obtain information about the chemical form of Pu inside the cells. The Pu L_{α} fluorescence from discrete $0.1 \mu\text{m}^2$ spots of cell-associated Pu was examined as a function of incident X-ray energy and normalized to give an edge step of 1 (Figure 5). The edge energy, defined by the first inflection point of the absorption edge, the energy of the peak (i.e. white line) maximum, the peak intensity, the peak width, and the peak shape were all invariant with repeated μ -XANES scans, indicating that the composition of the Pu deposits was not changing in the X-ray beam over the 5–20 minutes required to acquire a spectrum. Comparison with the XANES spectra of reference Pu compounds in known oxidation states [35, 36], demonstrates that the edge energy (18.061 ± 0.001 keV) and the white line width (12 ± 1 eV) of the intracellular Pu was consistent with the presence of tetravalent Pu compounds. Any other Pu oxidation state present in these samples was below the limit of detection, which we estimate to be 0.2 moles per mole Pu(IV). Neither the normalized white line intensities nor the peak widths changed with repeated scans of the same $0.1 \mu\text{m}^2$ area, but the normalized peak intensity did vary by approximately 10–20% between deposits (Figures 5a, 5b, and 5c), with more concentrated spots, for example spot A in Figure 5, typically giving less intense white lines than spots with less plutonium. The favorable counting statistics in the more concentrated Pu spots also makes rapid mapping of the Pu oxidation state feasible [16].

CONCLUSIONS

SXFM is a powerful technique for quantifying and studying trace amounts of transuranic elements in sub-micron spots. The sensitivity of the method for detecting plutonium with excitation of the L_2 or L_3 edges and detection of the L_{α} or L_{β} emissions is comparable to K-edge (10 keV) excitation of the first row transition metals, and it is much more sensitive than radiometric techniques if long-lived radioisotopes are used to avoid radiation damage prior to analysis. Unlike bulk analyses, the high resolution elemental maps also allow one to distinguish between plutonium that is associated with healthy, intact cells and cellular debris or colloidal particles in the cell suspension, and they can correlate the Pu distribution and even the chemical form to specific micron-scale intracellular structures. However, to achieve this the materials used for mounting and encapsulating samples must be carefully considered to avoid interferences in the X-ray emission spectra.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

X-ray fluorescence spectrum integrated over the full area of a mixed 0.10 μg ^{232}Th and 0.12 μg ^{242}Pu reference sample, depicted supplemental Figure S1. The incident energy, 23.09 keV, excites the Pu L $_3$ and L $_2$ edges and the Th L $_3$, L $_2$, and L $_1$ edges. The locations of the Pu and Th L emission lines as well as the associated Ge escape peaks, the Pu M α line, and the K α lines of other elements present in the sample are indicated.

Figure 2.

Locating target cells by coarse area scans with the X-ray microprobe. (a) An optical micrograph of one square of a 200 mesh copper grid containing intact and ruptured PC12 cells. (b) SXFM elemental map of potassium for locating cells in the $90 \mu\text{m} \times 90 \mu\text{m}$ area indicated on the optical image after a rapid scan in $3 \mu\text{m}$ steps.

Figure 3.

Individual rat adrenal gland cells take plutonium into the cytoplasm from media supplemented with soluble plutonium complexes. (a) False color SXFM elemental maps of a 26 $\mu\text{m} \times 26 \mu\text{m}$ area containing a single PC12 cell and the optical micrograph of the same area show iron and plutonium accumulating in the cell cytoplasm, while Zn concentrates in the area occupied by the nucleus. Calcium hydrogen phosphate spots from the Kapton encapsulation are marked with white arrows in the P map. Three element colocalization maps of other cells demonstrate (b) spatial correlation of Pu and Fe (yellow pixels in the overlap map) and (c) anti-correlation with Zn, which is spatially correlated with the highest P concentrations (magenta pixels in the overlay map).

Figure 4.

Integrated X-ray fluorescence spectrum of a 157 μm^2 PC12 cell that contains 1.5 fg ^{242}Pu (—) and a 163.25 μm^2 cell free background area near the cell (---). The Pu L_{α} line appears at 14.28 keV, and the positions of the L_{α} lines of each of the light actinide elements is indicated by a vertical line. The full spectrum is depicted in Supplementary Figure S2.

Figure 5.

Elemental maps derived from SXFM for a cell incubated with plutonium aggregates and the optical micrograph of the area imaged (top panel). μ -XANES spectra at $0.1 \mu\text{m}^2$ spots were acquired at three locations inside the cell area marked by white circles. Extracellular calcium phosphate spots are marked with white arrows in the P and Ca maps. (Spectrum a) Normalized average μ -XANES spectrum obtained at spot A from 3 scans. (Spectrum b) Normalized average μ -XANES spectrum obtained at spot B from 2 scans. (Spectrum c) Normalized μ -XANES spectrum obtained at spot C from a single scan.