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Intracellular concentration map of magnesium in whole cells by combined use of X-ray fluorescence microscopy and atomic force microscopy

Stefano Lagomarsino [a,b,*], Stefano Iotti [d,j], Giovanna Farruggia [e], Alessia Cedola [c], Valentina Trapani [f], Michela Fratini [c], Inna Bukreeva [c,k], Andrea Notargiacomo [c], Lucia Mastrototaro [f], Chiara Marraccini [d], Andrea Sorrentino [c,l], Ian McNulty [g], Stefan Vogt [g], Daniel Legnini [g], Sangsoo Kim [g], Alessandra Gianoncelli [h], Jeanette A.M. Maier [i], Federica I. Wolf [f]

[a] IPCF-CNR –UOS Roma c/o Dip Fisica Universita' "Sapienza", P.le A. Moro, 2 Rome, Italy
[b] Physics Department, Universita' Sapienza, P.le A. Moro, 2 Rome, Italy
[c] IFN-CNR – V. Cineto Romano, 42 00156 Rome, Italy
[d] Dipartimento di Medicina Interna, dell'Invecchiamento e Malattie Nefrologiche Università di Bologna, Via Massarenti, 9 40138 Bologna, Italy
[e] Dipartimento di Biochimica "G. Moruzzi" Università di Bologna, Via Irnerio, 48 40126 Bologna, Italy
[f] Istituto di Patologia Generale - Università Cattolica del Sacro Cuore - Facoltà di Medicina "A. Gemelli" L.go F. Vito, 1 00168 Rome, Italy
[g] Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439, USA
[h] SINCROTRONE TRIESTE, S.S.14 km 163.5 in Area Science Park, I-34149 Basovizza – Trieste, Italy
[i] Dipartimento di Scienze Cliniche, Università di Milano, Via GB Grassi 74, 20157 Milan, Italy
[j] Istituto Nazionale Biostrutture e Biosistemi – Rome, Italy
[k] Shubnikov Institute of Crystallography, Leninskii prospekt 59, Moscow, 119333 Russia
[l] Consorzio Nazionale Interuniversitario per le Scienze Fisiche della Materia - V. della Vasca Navale, 84, 00146 Rome, Italy

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ABSTRACT

We report a novel experimental approach to derive quantitative concentration map of light elements in whole cells by combining two complementary nano-probe methods: X-ray fluorescence microscopy (XRFM) and atomic force microscopy (AFM). The concentration is derived by normalizing point-by-point the elemental (here Mg) spatial distribution obtained by XRFM, by the thickness measured using AFM. The considerable difference between the elemental distribution and the concentration maps indicates that this procedure is essential to obtain reliable information on the role and function of elements in whole cells.

1. Introduction

The intracellular distribution of various transition elements in pathophysiological conditions has been investigated by x-ray fluorescence microscopy (XRFM) [1–7]. However, when applied to whole cells, the fluorescence intensity map obtained with XRFM can be misleading because it gives information on the total element content and not on element concentration, as the probed volume is not known.

We report a novel experimental approach to derive quantitative element concentration maps in whole cells by combining XRFM with atomic force microscopy (AFM). The key idea is normalizing point-by-point the element spatial distribution obtained by XRFM, by the thickness measured using AFM. We obtained the first concentration map of magnesium in whole cells, and demonstrated that it is considerably different from the element distribution map.

Defining the intracellular concentration map of a given element is an important parameter to correlate the element with its biological regulation and function. Concentration can be obtained by XRFM on sample sections of known thickness, such as those used for electron microscopy [8,9]. However, measuring a single section does not reveal the distribution of the target element throughout the whole cell. Alternatively fluorescence tomography has been recently used to map the concentration of 17 elements from Si to Zn in a whole diatom *Cyclotella meneghiniana* [10], and to assess Cobalt distribution in keratinocyte cells [11]. However, the long time presently required for data acquisition poses serious problems of radiation damage, and limits the application of this method. The methodology we present in this paper is applicable to whole, unsectioned and unstained cells, does not need laborious sample preparation, and does not imply higher dosage than standard microfluorescence. We studied in this case magnesium, a light element which is the

* Corresponding author. Tel.: +39 0 649913138; fax: +39 0 64454816.
E-mail addresses: stefano.lagomarsino@cnr.it, s_lagomarsino@yahoo.it (S. Lagomarsino).

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most abundant divalent cation in cells, but the methodology can be applied to quantify trace metals, nanoparticles, drug delivery, and any molecule that has been suitably tagged.

Magnesium (Mg referring to both bound and free form of the cation) plays crucial structural and regulatory roles within all cells [12]. Intracellular Mg is very abundant and widely distributed; its content is finely tuned through plasma membrane transport, as well as buffering and compartmentalization in intracellular organelles, including mitochondria [13–16]. Despite recent efforts in applying new live imaging techniques to the field of magnesium research [17], an accurate characterization of Mg distribution in the cellular environment is still lacking. The novel methodological approach we present in this study has a twofold objective: 1) mapping by XRFM a low atomic number element with relatively low X-ray yields, and 2) obtaining for the first time an intracellular concentration map of Mg in a whole cell.

2. Experimental

We studied two different cell lines: osteoblast-like SaOS and HC11 mammary epithelial cells, whose total Mg content has been previously analytically determined [18].

2.1. Cell culture and fixation

HC11 mammary epithelial cells and osteoblast-like SaOS cells were cultured in MEM or RPMI medium (Sigma), respectively, supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulphate. For XRFM measurements, cells were plated on 1×1 mm^2, 200-nm thick silicon nitride (Si_3N_4) membrane windows, mounted on a 5×5 mm^2 Si frame (Silson, UK), previously sterilized in ethanol. Cells were incubated at 37 °C in 5% CO_2 for at least 24 h before fixation. At 50–80% confluency, cells were briefly rinsed in 150 mM KCl, then fixed in ice-cold methanol/acetone 1:1 and air dried.

2.2. XRFM measurements

XRFM measurements on HC11 and SaOS cells were carried out at two different X-ray microscopes in large-scale facilities for synchrotron radiation: at the 2-ID-B beamline at Advanced Photon Source (APS, Argonne, IL, USA) [19,20] and at the TwinMic beamline at Elettra (Trieste, Italy) [21,22]. In both cases Fresnel zone plate lenses were used to focalize the incoming 1.5 keV beam, monochromatized by grating monochromators. The main differences between the APS and the Elettra installations concerned the fluorescence detector geometry and the environment. At the 2-ID-B beamline (APS) the whole apparatus was in air, filled with He gas to reduce absorption by air. The geometric arrangement of sample, fluorescence detector, and transmission detector is illustrated in Fig. 1.

The sample was slightly tilted (about 10°) with respect to the plane perpendicular to the incoming beam, to improve the fluorescence signal. The fluorescence detector, which was oriented in the horizontal plane at about 90° from the incident beam to reduce elastic scattering, was a SII model Vortex EX-60 silicon drift diode. The zone plate, the sample and the detector were in air. In order to reduce absorption, the air paths between sample and detectors were filled with flowing He gas. X-ray fluorescence spectra from this detector were acquired and digitized by a XIA Saturn DXP multichannel analyzer. The transmitted flux was measured with a custom-built 9-element configured detector which allows for both absorption and differential phase contrast imaging. The signals from the various detector elements of this system were read out in parallel with charge-sensitive amplifiers, then digitized by computer.

At the TwinMic beamline (Elettra) any components (zone plate, sample, detectors) were in vacuum, thus avoiding any absorption by air. Six Si-drift detectors (SDD) were disposed radially with respect to the sample, and measured simultaneously the fluorescence intensity emitted by the sample. The angle formed by the SDDs and the sample plane was 20°. Fig. 2 shows schematically the fluorescence detector geometry. Data analysis in this paper was carried out on spectra obtained as the sum of all the detectors, but data from single detectors can be analyzed as well.

Taking into account all the optical elements, we evaluated a final spot size onto the sample of about 50 nm at APS, and of either 500 nm or 1 μm at Elettra, depending on the experiment (see Figs. 2 and 4, respectively). The samples were transversally scanned in the zone plate focus under computer control, and at each scan step a full fluorescence spectrum and selected energy regions-of-interest were acquired with energy dispersive fluorescence detectors. The transmitted flux was also measured by multi-element configured detectors. Fluorescence spectra were analyzed with the PyMca platform [23] and deconvoluted into their components, in particular the elastic scattering peak centred at 1.5 keV and the Mg K fluorescence peak at ~1.253 keV. The integrated intensity under the Mg fluorescence peak was then obtained and normalized with respect to the incident flux, which was measured by beam intensity monitors.

2.3. AFM measurements

AFM topography maps were collected by using a Digital Instruments D3100 AFM equipped with a Nanoscope IIIa controller. The AFM measurements were carried out in air in Tapping Mode at a resonance frequency of about 260 kHz. The Tapping Mode AFM technique operated with typical scanning parameters usually produces a lower energy transfer to the sample, i.e. a lower chance of inducing damage, than the contact mode. Indeed imaging our dried cell samples in contact mode resulted in a slight damage, though much lower respect to living (or still hydrated) cells (not shown). Consequently, the Tapping Mode was chosen in order to minimize and possibly avoid cell damage. To this aim the amplitude feedback set-point was set as close as possible to that of the free oscillating cantilever: this required to reduce significantly the scan rate and to increase the gain parameter, in order to ensure an optimal image quality.

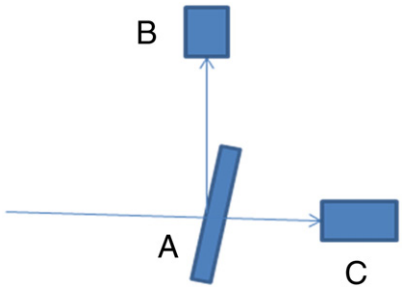

Fig. 1. Schematic lay-out of the experiment at the 2-ID-B beamline (APS, Argonne, USA). A: Sample; B: fluorescence detector; C transmission detector.

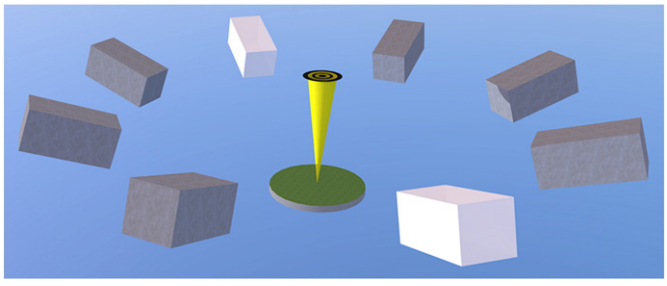

Fig. 2. Fluorescence detector geometry at the TwinMic beamline at Elettra (Trieste, Italy) The 8 Silicon Drift Detectors (SDD) are located in an annular configuration and their axis is tilted of 20° with respect to the sample plane. The 2 SDDs represented in white were not available during the measurements.