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Biomedical applications of the ESRF synchrotron-based microspectroscopy platform

S. Bohic [a,b,c,*], M. Cotte [b,d], M. Salomé [b], B. Fayard [b,e], M. Kuehbacher [f], P. Cloetens [b], G. Martinez-Criado [b], R. Tucoulou [b], J. Susini [b]

[a] Inserm U-836, Team 6, Rayonnement Synchrotron et Recherche Médicales, Grenoble Institut des Neurosciences, Grenoble 38042, France
[b] European Synchrotron Radiation Facility (ESRF), BP220, Grenoble F-38043, France
[c] Université Joseph Fourier 1, Grenoble Institut des Neurosciences, Grenoble 38042, France
[d] CNRS, UMR 171, Palais du Louvre, 75001 Paris, France
[e] CNRS, UMR 8502, Laboratoire de Physique des Solides, Université Paris Sud, 91405 Orsay, France
[f] Hahn-Meitner-Institut, Department of Molecular Trace Element Research in the Life Sciences, Berlin, Germany

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ABSTRACT

Very little is known about the sub-cellular distribution of metal ions in cells. Some metals such as zinc, copper and iron are essential and play an important role in the cell metabolism. Dysfunctions in this delicate housekeeping may be at the origin of major diseases. There is also a prevalent use of metals in a wide range of diagnostic agents and drugs for the diagnosis or treatment of a variety of disorders. This is becoming more and more of a concern in the field of nanomedicine with the increasing development and use of nanoparticles, which are suspected of causing adverse effects on cells and organ tissues. Synchrotron-based X-ray and Fourier-transformed infrared microspectroscopies are developing into well-suited sub-micrometer analytical tools for addressing new problems when studying the role of metals in biology. As a complementary tool to optical and electron microscopes, developments and studies have demonstrated the unique capabilities of multi-keV microscopy: namely, an ultra-low detection limit, large penetration depth, chemical sensitivity and three-dimensional imaging capabilities. More recently, the capabilities have been extended towards sub-100 nm lateral resolutions, thus enabling sub-cellular chemical imaging. Possibilities offered by these techniques in the biomedical field are described through examples of applications performed at the ESRF synchrotron-based microspectroscopy platform (ID21 and ID22 beamlines).

1. Introduction

"*New Truths become evident when new tools become available*", a famous quote from Rosalyn Sussman Yalow (Nobel Prize in Physiology and Medicine, 1977), is exemplified today by the impressive number of techniques which have provided breakthroughs in cellular machinery, like far-field fluorescence nanoscopy (Hell, 2007) or electron tomography (Steven and Baumeister, 2008). Extending those techniques to the X-ray domain offers unique opportunities to probe sub-cellular chemical processes. For example, soft X-ray microscopes have established capabilities in absorption contrast imaging of thick hydrated biological material in their near-native environments at spatial resolutions approaching 30 nm, well beyond those achievable with conventional visible light microscopy (Carrascosa et al., 2009; Jacobsen, 1999; McDermott et al., 2009; Schneider et al., 2010). In the past decade, there has been a strong tendency in X-ray microscopy to develop alternative contrast mechanisms and spectroscopic methods, which can provide both valuable complementary information on the sample nature and/or a reduction of the necessary radiation doses (Aitken et al., 2011; Andrews et al., 2011; Bacquart et al., 2007; Chapman, 2010; de Jonge and Vogt, 2010; de Jonge et al., 2008; Heine et al., 2011; Holzner et al., 2010; Jiang et al., 2010; Lombi and Susini, 2009; Schroer et al., 2010). Simultaneously, the development of high brilliance, high energy synchrotrons, coupled with advances in manufacturing technologies of focusing optics, has led to significant improvements in sub-micrometer probes for spectroscopy, diffraction and imaging applications in the

Abbreviations: EDX, X-ray energy-dispersive spectrometry; EELS, electron energy loss spectrometry; EFTEM, energy filtered transmission electron microscopy; FTIR, Fourier transformed infrared spectroscopy; LA-ICP-MS, laser ablation inductively coupled plasma mass spectrometry; PIXE, particle induced X-ray emission; SIMS, secondary ion mass spectrometry; SR-XRF, synchrotron-radiation X-ray fluorescence; STEM, scanning transmission electron microscopy; STXM, scanning transmission X-ray microscopy; XANES, X-ray Absorption Near-Edge Spectroscopy; X-PEEM, X-ray photoelectron emission microscopy.

* Corresponding author at: Inserm U-836, Team 6, Rayonnement Synchrotron et Recherches Médicales, Grenoble Institut des Neurosciences, Grenoble 38042, France.
E-mail address: bohic@esrf.fr (S. Bohic).

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multi-keV X-ray range (>1 keV termed "hard" X-ray regime). Both by extrapolation of the experience gained in the soft X-ray regime and by the development of new techniques, "hard" X-ray microscopes now offer a unique analytical tool, which can contribute to a wide range of existing and new applications (Lombi and Susini, 2009; Ortega et al., 2009a; Paunesku et al., 2006).

Living systems, for survival, depend on their ability to accumulate, release and use certain elements, particularly metal ions, to define a certain composition that is held constant within a given homeostatic state. Several essential metal ions participate in the control of numerous metabolic and signaling pathways, but their rich coordination chemistry and redox properties confer them a propensity to randomly coordinate and catalytically react inside the cell with protein sites other than those tailored for that purpose. Indeed, about one third of all structurally characterized proteins are metalloproteins and bound metal ions or co-factors, which play a pivotal role in the structure–function relationship of proteins and other bio-molecules. In addition, all these cellular essential metals are also potentially toxic. Thus, a number of sophisticated networks of trafficking pathways are available to tightly regulate their uptake, intracellular transport and compartmentalization, and to avoid their toxic side effects. However, in spite of all the progress made, we are still merely on the brink of understanding these processes.

The synchrotron microspectroscopy techniques as developed today contribute to elucidating the distribution, concentration and chemical state of metals inside tissues and cells at the organelle level. This contribution is not only highly challenging but represents important objectives in modern analytical chemistry and an essential step towards the precise understanding of some cellular physiopathological or toxicological processes. As sketched in Fig. 1, multi-keV microspectroscopy can provide insights into the three key aspects when dealing with metallobiology. Several recent reviews have reported on biomedical applications of synchrotron-based microscopy techniques (McRae et al., 2009; Ortega et al., 2009a; Qin et al., 2011). The scope of this paper is to present the activity of the European Synchrotron Radiation Facility (ESRF) in the field of metallobiology, in particular at the two micro-spectroscopy beamlines, ID21 and ID22. The two instruments are dedicated to high spatial resolution quantitative and chemical imaging, and cover a wide energy range from 2 to 70 keV providing access to almost all metal absorption edges. This X-ray microscopy platform includes the nano-imaging station ID22NI, the ID22 microprobe and the ID21 scanning X-ray microscope (SXM). It is completed by a synchrotron-based infrared microscopy end-station, located at ID21.

The details of the instruments will be presented and the sample preparations briefly discussed. This review provides some examples of application of the ID21 and ID22 X-ray microscopy platform in the biomedical field with an emphasis on physiological and toxicological aspects of metals in cells and tissues.

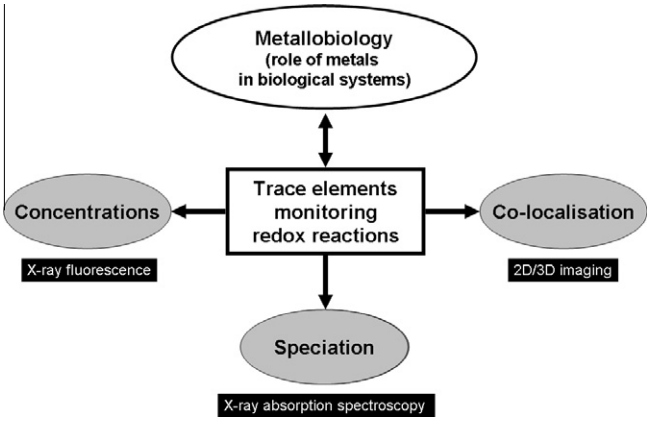

Fig.1. Multi-keV microspectroscopy approaches in metallobiology, namely metal ions concentrations (dose), element distribution (co-localization) and chemical state (speciation).

2. Materials and methods

2.1. ID21 scanning X-ray microscope

The ID21 beamline hosts two end-stations: (i) a scanning X-ray microscope, optimized for submicron X-ray fluorescence (XRF) imaging and X-ray Absorption Near-Edge Spectroscopy (micro-XANES) in the 2–9 keV range (P to Cu K-edges and L- or M-lines of heavier elements) (Cotte et al., 2007); (ii) a FTIR microscope exploiting the synchrotron emission in the mid-infrared region. The high spatial resolution and sensitivity of the SXM make it particularly suitable for the mapping and speciation of metals in cells and tissues, while the molecular information brought by the FTIR allows the study of organic matter (Walsh et al., 2008).

The SXM is located on a straight section equipped with two undulator plus one wiggler sources. A fixed exit double crystal monochromator offers an energy resolution down to $\Delta E/E = 1.5 \times 10^{-4}$ for spectroscopy. The microscope is located at 51 m from the source and housed in a chamber allowing operation in air or in vacuum (10^{-5} mbar). A load-lock system allows a fast exchange of samples under vacuum, and greatly facilitates operation under cryogenic conditions. The microscope can host two different optical focusing configurations: either Fresnel zone plates or a Kirkpatrick–Baez (KB) mirror system, which achieve a typical spot size of 0.3 (V) × 0.7 (H) μm^2 with a flux of 10^9–10^{10} photons/s. The advantages of the mirror configuration are its higher efficiency and its achromaticity, which is preferable for micro-XANES.

The SXM can be equipped with a single or a 7-element HpGe fluorescence detector (Princeton Gamma-Tech, US), which offers an increased solid angle for an optimized collection of fluorescence photons, and a large area (80 mm^2) XFlash 5100 Bruker Silicon Drift Diode (SDD). A compact X-ray wavelength dispersive spectrometer, achieving an energy resolution of a few tens of eV, has also been implemented for highly selective fluorescence detection. The sample stage can accommodate various sample environments depending on the nature of the samples (solid, liquid or frozen). A vibration-free cryo-stage, passively cooled by a LN_2 dewar, allows the analysis and preservation of hydrated samples, which is essential for biological applications, in particular for metal speciation in near native state. A visible light video-microscope allows visualization of the sample, even under vacuum, for precise alignment in the beam.

2.2. ID21 infrared microscope

This infrared end-station collects the infrared light produced by a bending magnet and a set of mirrors directs the beam toward a FT-IR Nexus Spectrometer coupled to a Nicolet Continuμm microscope. When using the synchrotron source, the beamsize can be easily reduced to ~6 × 6 μm^2, without compromising too much the spectral quality and associated acquisition time. The detection is carried out in transmission or in reflection mode, using a 50 μm Mercury Cadmium Telluride detector, in the spectral range 4000–700 cm^{-1}. Biological samples are usually prepared as thin sections deposited onto proper substrates (IR transparent windows for transmission, reflecting slides for double-transmission). By