Radiation Physics and Chemistry 79 (2010) 176–184

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Biomedical applications of X-ray absorption and vibrational spectroscopic microscopies in obtaining structural information from complex systems

Jade B. Aitken [a], Elizabeth A. Carter [a], Harold Eastgate [b,1], Mark J. Hackett [a], Hugh H. Harris [a,2], Aviva Levina [a], Yao-Chang Lee [c], Ching-Iue Chen [c], Barry Lai [d], Stefan Vogt [d], Peter A. Lay [a,*]

[a] School of Chemistry, The University of Sydney, NSW 2006, Australia
[b] Eastmac Pty Ltd, 8 Cassinia Close, Knoxfield, Vic 3180, Australia
[c] National Synchrotron Radiation Research Centre, No. 101 Hsin-Ann Road, Hsinchu 30076, Taiwan
[d] X-ray Science Division, Argonne National Laboratory, Argonne, IL 60439, USA

ARTICLE INFO

Article history:
Received 20 February 2009
Accepted 20 March 2009

Keywords:
X-ray absorption spectroscopy
X-ray microprobe
FTIR microprobe
Raman microprobes
Cells
Tissues

ABSTRACT

Protein crystallography and NMR spectroscopy took decades to emerge as routine techniques in structural biology. X-ray absorption spectroscopy now has reached a similar stage of maturity for obtaining complementary local structural information around metals in metalloproteins. However, the relatively recent emergence of X-ray and vibrational spectroscopic microprobes that build on these techniques has enabled the structural information obtained from the "mature" techniques on isolated biomolecules to be translated into *in situ* structural information from inhomogeneous complex systems, such as whole cells and tissues.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Most of the structural biochemical information on which our understanding of biology and medicine at a molecular level has relied is built upon structures from isolated proteins and other biomolecules. While this has been extremely important to our current understanding of such biochemistry, the local structures in crystals (protein crystallography) can be dictated, to some extent, by packing forces and the conformation that prefers to crystallise (Levina et al., 2005) and solution NMR structures are limited by the sizes of biomolecules that can be analysed. Even more important than both of these factors is that many proteins have cofactors that affect their reactivity and/or structures in cells and tissues. Thus the biomolecular and multi-molecular structural architecture present within cells and tissues can have dramatic effects on the structures of the entities taking part in *in vivo* biochemistry. In addition, there is a need to monitor changes in these architectures in response to external stimuli.

X-ray crystallography has been extended to obtain structural information on larger assemblies, such as the photosynthetic centres (Ferreira et al., 2004; Loll et al., 2005) and viruses (Stuhrmann, 2008), and NMR spectroscopy has been invaluable in determining protein dynamics and structural changes in protein/protein interactions with relatively small biomolecules, such as the interaction between α-hemoglobin and alpha hemoglobin specific binding protein (AHSP) (Feng et al., 2004). However, deductions of structural information from complex biological matrices remain at the frontiers of a molecular understanding of physiology, and understanding disease processes and optimising their treatments. Cryo-electron tomography (Lengyel et al., 2008; Zhou, 2008; Rhee et al., 1998) and small-angle scattering techniques (SAXS and SANS) (Stuhrmann, 2008) have also played an important part in obtaining structural information from complex biological particles, such as viruses and photosystems. However, all of the above scattering, diffraction and NMR spectroscopic techniques provide only low-resolution molecular structural information in larger assemblies and cannot, as yet, be applied to providing specific structural information within cells and tissues that are closer to *in vivo* conditions, except for areas of microcrystallinity, i.e., biominerals within cells and tissues, areas of regular repeating protein structures (Iwamoto, 2008), or analysis of biomaterials such as bones (Cancedda et al., 2007), using micro-diffraction techniques. The emerging techniques associated with X-ray free-electron lasers offer atomic-level

Abbreviations: AHSP, alpha hemoglobin specific binding protein; FTIR, Fourier transform infrared

* Corresponding author. Tel.: +61 2 93514269; fax: +61 2 9351 3329.
E-mail address: p.lay@chem.usyd.edu.au (P.A. Lay).

[1] Deceased.

[2] Present address. School of Chemistry and Physics, University of Adelaide, SA 5005, Australia.

0969-806X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2009.03.068

structural information on more complex structures, but destroy the sample in the process (Gaffney and Chapman, 2007). This brief review discusses the use of bulk XAS, and X-ray and vibrational spectroscopic microprobes in obtaining structural information from complex biological samples such as cells and hard and soft tissues. These techniques can reveal molecular and elemental architecture in these complex assemblies, as well as detailed structural information, at a molecular level, within them. A comprehensive review of data analysis using correlative microscopies has been published recently (Noda, 2008) and will not be discussed here.

2. Experimental

2.1. X-ray microprobe studies on teeth

The methods for the preparation of teeth cross-sections and X-ray microprobe analysis of teeth are as described in detail elsewhere (Harris et al., 2008a, 2008b).

2.2. X-ray microprobe and IR microprobe studies on A549 cells

The techniques for the preparation of A549 cells for microscopic analysis at beamlines 2I-DD and 2I-DE at the Advanced Photon Source (APS), Argonne National Laboratory (ANL) and their subsequent analyses were as reported in the literature (Harris et al., 2005) except the cells were grown on silicon nitride membranes (Duong et al., 2009). For the elemental maps obtained from X-ray microprobes, the cells was exposed to 100 μM of CrO_4^{2-} for 20 min using the same protocols described previously (Harris et al., 2005).

Synchrotron-based FTIR spectra were recorded at the infrared beamline (BL14A) at the National Synchrotron Radiation Research Centre (NSRRC), Taiwan using a Nicolet Magna 860 FTIR spectrometer equipped with a Continuum IR microscope (Spectra Tech), mapping stage controller, ×32 objective (0.65 numerical aperture) and a MCT detector. The bench was configured with a collimated synchrotron light, which served as an external input to the spectrometer. The modulated light was directed into the infrared microscope for spectroscopy. The beam current was 250 mA (now operating at 300 mA) and the synchrotron was running in top-up mode.

The spectra were collected in the mid-IR range of 4000–700 cm^{-1} using a spectral resolution of 4 cm^{-1}, with the co-addition of between 64 and 128 scans, aperture of either 10 μm × 10 μm or 12 μm × 12 μm, and step size of between 8 and 10 μm. The optics were purged using dry N_2 and the automatic atmospheric suppression function in the OMNIC™ software was used to minimise infrared absorption by CO_2 and water vapour in the ambient air. A single-beam background spectrum was collected from an area free of sample. Stage control and data collection were performed using OMNIC™ (OMNIC, 2001). The IR data were collected using the OMNIC software coupled with a gating system for eliminating the noise during electron injection of 60 s periods for synchrotron top-up operation. Optical images were obtained using a camera linked to the Continuum microscope.

SR-FTIR functional group maps were produced using OMNIC™ Atlμs™ (2005). In order to produce the maps, data were first truncated then baseline corrected and finally the area under the band of interest was measured. The two diagnostic regions chosen for analysis were the CH stretching region from 3000–2800 cm^1 and the amide I band from 1713–1603 cm^1.

3. Results

3.1. XRF imaging of teeth

Ingestion of Hg from dental amalgam, with potential negative health effects, has generally been considered to occur directly from filling surfaces. Recently, we examined the importance of mercury exposure by direct migration through the tooth by X-ray fluorescence imaging of Hg, Ca, Zn and Cu in human teeth filled with amalgam for at least 20 years (Harris et al., 2008b).

An example of elemental maps from a cross section from an unfilled extracted tooth at 100 μm spatial resolution that was extracted is given in Fig. 1A and a higher resolution map of an area of dentine at 1 μm spatial resolution, which is marked by the square, is given in Fig. 1B. The enamel layer is clearly identified by the higher Ca intensity, which corresponds to the outer layer in the optical image and this, in turn, is covered by a thin layer of calculus, which is more obvious in the elemental maps than it is in the optical images. The area where the pulp was present is the area in the centre as defined by the optical and elemental images. Interestingly, there is relatively little Ca and a high level of Cu in a mineral deposit on the right of the pulp and this may be indicative of tooth decay (Harris et al., 2008a), but this is undergoing further investigation (Harris et al., unpublished results, 2008). Even more surprising was that Hg was near areas of the tooth that once contained an active bloodstream (pulp) and in calculus (on the outside of the tooth). The Hg around the pulp and in the calculus appeared to have come from other teeth in the mouth of the patient, i.e., those filled with Hg amalgam, via the bloodstream and Hg within the oral cavity, respectively. The phase-contrast image in Fig. 1B clearly shows the presence of dentinal tubules and other morphological features on the surface of the tooth section, which can be correlated with the elemental distributions.

3.2. X-ray and FTIR microprobe imaging of cells

A variety of research has been conducted within our group on X-ray imaging of cells and tissues with respect to Cr-induced cancers (Cholewa et al., 2001; Dillon et al., 2002; Harris et al., 2005; Levina and Lay, 2008), the potential risks of Cr dietary supplements (Levina and Lay, 2008), cardiovascular disease (Witting et al., 2006), strokes (Duong et al., 2009), metal anti-inflammatory drugs (Dillon et al., 2004; Weder et al., 2002) and many other areas.

An example of an elemental map of a Cr(VI)-treated (100 μM, for 20 min) A549 lung cell is given in Fig. 2. As has been reported previously (Dillon et al., 2002; Harris et al., 2005), Cr is dispersed throughout the cell and has entered the nucleus where it can cause the genotoxic effects that ultimately lead to Cr-induced cancers but, in the current example, the Cr had entered the cell nucleus at a somewhat earlier stage of treatment than was found previously (see Discussion). This is important because it has been shown previously that there is a direct relationship between the clonogenic survival of cells and the amount of Cr entering the nucleus (Harris et al., 2005). However, an additional interesting aspect of this cell is that it is binucleating and has shown features that have not been seen before. In particular, there is a clearly defined ring of Fe around each nucleus of the binucleating cell, which is presumably in the nuclear membrane and has not been observed in cells that are not binucleating. The cell nuclei that are encircled by Fe and are marked by the high concentrations of P (DNA) and Zn (zinc finger proteins), which correspond to the nuclei observed in the optical image (not shown). At this stage it is

Download English Version:

https://daneshyari.com/en/article/1886697

Download Persian Version:

https://daneshyari.com/article/1886697

Daneshyari.com