available at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/arob

The application of synchrotron radiation induced X-ray emission in the measurement of zinc and lead in Wistar rat ameloblasts

Manish Arora [a,*], *Brendan J. Kennedy* [b], *Chris G. Ryan* [c], *Ross A. Boadle* [d], *D. Murray Walker* [a], *Cathy L. Harland* [e], *Barry Lai* [f], *Zhonghou Cai* [f], *Stefan Vogt* [f], *Hans Zoellner* [a], *Sheena W.Y. Chan* [a]

[a] *Cellular and Molecular Pathology Research Unit, Department of Oral Pathology, Faculty of Dentistry, University of Sydney, Westmead Centre for Oral Health, Westmead Hospital, Westmead, NSW 2145, Australia*

[b] *School of Chemistry, University of Sydney, NSW 2006, Australia*

[c] *CSIRO Exploration and Mining, Geosciences, Building 28E, Monash University, Clayton, VIC. 3168, Australia*

[d] *Electron Microscope Unit, ICPMR, Westmead Hospital, Westmead, NSW 2145, Australia*

[e] *Australian Synchrotron Research Program, Australian Nuclear Science and Technology Organisation, Lucas Heights, NSW, Australia*

[f] *Advanced Photon Source, Argonne National Laboratory, 9700 S. Cass Avenue, Argonne, IL 60439, USA*

ARTICLE INFO

Article history:
Accepted 6 April 2007

Keywords:
Synchrotron radiation
X-ray emission
Ameloblasts
Wistar rats
Trace elements
Zinc
Lead

ABSTRACT

The development of analytical techniques for the measurement of trace elements in cellular compartments of developing teeth remains an important methodological issue in dental research. Recent advances in third generation synchrotron facilities have provided high brilliance X-ray sources that can be effectively used to study trace element distributions in small spatial regions with low detection limits. The present study describes for the first time the application of synchrotron radiation induced X-ray emission (SRIXE) in measuring the distribution of zinc and lead in the ameloblasts of developing Wistar rat teeth. Wistar rats were fed a standard rat diet, containing the normal dietary requirements of zinc, *ad libitum* and exposed to 100 ppm of lead in drinking water. Resin embedded sections of first mandibular molars were analysed using a 13.3 keV incident monochromatic X-ray beam focussed to a 0.2 μm spot. Characteristic X-rays arising from the entire thickness of the sample were measured using an energy dispersive detector for quantitative analysis of elemental concentrations. The results showed that intranuclear concentrations of zinc were greater than levels in the cytoplasm. Furthermore, nuclear and cytoplasmic concentrations of zinc in the maturation stage (742 ± 27 and 424 ± 25 ppm, respectively) were significantly higher than the zinc levels observed in the nucleus and cytoplasm of presecretory stage ameloblasts (132 ± 10 and 109 ± 10 ppm, respectively) ($p < 0.05$). A clear lead signal above the background was not detected in the ameloblasts and lead concentrations could only be reliably measured in the developing enamel. Overall, SRIXE was an effective method of studying the spatial distribution of zinc in the cells of developing teeth and offered a unique combination of sub-micron spatial resolution and parts-per-million detection limits (0.8–1 and 0.6–1 ppm for zinc and lead, respectively).

* *Corresponding author*. Tel.: +61 2 9845 7892.
E-mail addresses: marora@mail.usyd.edu.au (M. Arora), b.kennedy@chem.usyd.edu.au (B.J. Kennedy), Chris.Ryan@csiro.au (C.G. Ryan), rossb@icpmr.wsahs.nsw.gov.au (R.A. Boadle), charland@aps.anl.gov (C.L. Harland), BLAI@aps.anl.gov (B. Lai), CAI@aps.anl.gov (Z. Cai), VOGT@aps.anl.gov (S. Vogt), hzoellne@mail.usyd.edu.au (H. Zoellner).
0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2007.04.003

1. Introduction

Zinc is an essential dietary element and serves numerous biological functions including protein synthesis, maintenance of neuro-sensory functions, cell-mediated immunity, thyroid function as well as bone formation and mineralisation.[1–3] There is also an increasing body of evidence supporting the role of zinc in the development and maintenance of dental tissues. Data from animal models shows that restriction of dietary zinc increases the incidence of dental caries,[4] reduces the lipid content of developing enamel, and causes morphological alterations in ameloblasts.[5] In addition, a number of zinc-finger transcription factors have recently been identified in developing mouse tooth germs and current evidence suggests that they serve an important role in cytodifferentiation of enamel epithelium and amelogenesis.[6–9] In contrast to zinc, lead exerts an adverse effect on developing teeth, with lead exposed rats showing an increased incidence of dental caries, disruption of salivary gland function, as well as delays in enamel mineralisation and tooth eruption.[10–12] The positive association of lead exposure to dental caries has also been confirmed in epidemiological studies.[13–14]

In addition to the individual effects of lead and zinc on oral and systemic health, a number of studies suggest an interaction between these elements. Co-administration of zinc is known to reduce tissue and organ lead concentrations in animal models.[15–17] Furthermore, lead may displace zinc from a number of zinc-binding sites including δ-aminolevulinic acid dehydratase (ALAD), metallothioneins and some transcription factors.[18–20]

An important methodological issue in understanding the oral health effects of zinc, lead and other metal toxins is the development of suitable analytical techniques to measure the spatiotemporal distribution of these elements in the cellular compartments of developing tooth germs. While bulk chemical methods of analysis including inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma-optical emission spectrometry (ICP-OES) and atomic absorption spectrometry (AAS), are capable of providing detection limits in the parts-per-million (ppm) or parts-per-billion (ppb) range, these methods are not suitable for determining the spatial distribution of trace elements. Techniques such as energy dispersive X-ray analysis (EDAX), electron probe analysis (EP) and nuclear microprobe analysis (NMP) have proven useful in measuring the intracellular concentrations of trace elements in various cell types.[21–23] These methods are, however, limited by either high detection limits or poor spatial resolution. Over the past two decades, developments in third generation synchrotron sources have provided high intensity X-ray beams that can be effectively used to study trace element distribution in small spatial regions with low detection limits.

In synchrotron radiation induced X-ray emission (SRIXE) analysis, the high-energy X-rays generated by an undulator source are focussed into a sub-micron spot on the sample, where they create vacancies in the inner shells of the target atoms by photoelectric absorption. When these vacancies are filled by electrons from outer shells, characteristic X-rays are emitted that are measured in an energy dispersive detector for quantitative analysis of elemental concentrations.[24] For the purposes of trace element analysis, SRIXE offers a unique combination of sub-micron resolution and detection limits in the ppm range or better. While this technology is rapidly gaining acceptance in many areas of science, its application in the measurement of trace element concentrations in dental soft tissues remains largely unexplored. The primary aim of this study was to measure the change in intracellular distribution of lead and zinc during ameloblast differentiation from presecretory to early maturation stage. To our knowledge this paper describes for the first time the application of SRIXE in measuring the intracellular distribution of zinc and lead in ameloblasts of developing Wistar rat teeth. The advantages and limitations of this technique are also discussed.

2. Materials and methods

2.1. Animal handling

Three female Wistar rats, aged 12–14 weeks, of normal weight were mated with similarly aged male Wistar rats of normal weight.[25] The animals received 100 ppm of lead nitrate (Asia Pacific Speciality Chemicals, NSW, Australia) in the drinking water and were fed a standard rodent diet containing 40 mg/kg of zinc (AIN93G, Glen Forrest Stockfeeders, Western Australia) *ad libitum*. Lead exposure was commenced 5 days before the expected birth of the pups. The pups were housed with their mothers, fed only by lactation and had no access to the drinking water.

Groups of three rat pups were sacrificed by decapitation from each of the litters at birth and on the third and seventh day after birth. These specific time points were chosen because the presecretory and secretory stages of ameloblast development can be observed in a rat first molar tooth germ at birth and 3 days after birth, while the early maturation stage of ameloblast development can be visualised on day-7.[26] The continuously erupting rat incisor has been widely used as a model for tooth development. Rat molars were used in the present analyses, however, to permit the measurement of trace elements in radicular tissues, which was the aim of a parallel-running project. All procedures in the present study were approved by the Animal Ethics Committee, Westmead Hospital, NSW, Australia (Ethics clearance no. 124.06).

2.2. Preparation of tissue sections

Immediately after culling, the mandibles were removed using sterile surgical instruments. The samples were processed in a manner similar to that reported by Dillon et al.[27] for SRIXE analysis of hamster lung cells. First mandibular molar tooth germs were removed under a dissecting microscope and fixed overnight at 4 °C in Karnovsky's fixative, followed by washing in MOPS buffer (3-(*N*-morpholino)-propane-sulphonic acid). The samples were dehydrated with graded ethanols (50–100%) (Selby Biolab, Australia) for 15 min each at room temperature. Following dehydration, tooth germs were placed in a 50:50 mixture of low viscosity Spurr's resin (Polysciences Inc., USA) and ethanol for 1 h at room temperature. The tooth germs were then transferred to a 90:10 mixture of Spurr's resin and ethanol overnight. The final infiltration was in Spurr's resin at 70 °C for 10 min, and this was repeated three times. For