Talanta 161 (2016) 368–376

Contents lists available at ScienceDirect

Talanta

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

Combined in-situ imaging of structural organization and elemental composition of substantia nigra neurons in the elderly

A.D. Surowka [a,*], M. Töpperwien [b], M. Bernhardt [b], J.D. Nicolas [b], M. Osterhoff [b], T. Salditt [b], D. Adamek [c], M. Szczerbowska-Boruchowska [a,*]

[a] AGH University of Science and Technology, Faculty of Physics and Applied Computer Science, Krakow, Poland
[b] Institut für Röntgenphysik, Universität Göttingen, Göttingen, Germany
[c] Jagiellonian University, Faculty of Medicine, Chair of Pathomorphology, Krakow, Poland

ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form
2 August 2016
Accepted 4 August 2016
Available online 5 August 2016

Keywords:
Synchrotron X-ray Fluorescence Imaging
X-ray Phase Contrast Imaging
Small Angle X-ray Scattering
Mass thickness effect
Substantia nigra tissue
Neuropathology of the human brain ageing

ABSTRACT

Human dopaminergic system in general, and substantia nigra (SN) neurons, in particular, are implicated in the pathologies underlying the human brain aging. The interplay between aberrations in the structural organization and elemental composition of SN neuron bodies has recently gained in importance as selected metals: Fe, Cu, Zn, Ca were found to trigger oxidative-stress-mediated aberration in their molecular assembly due to concomitant protein (alpha-synuclein, tau-protein) aggregation, gliosis and finally oxidative stress. In the present study, we demonstrate an integrated approach to the analysis of the structural organization, assembly, and metals' accumulation in two distinct areas of SN: in the neuromelanin neurons and neuropil. By using the highly brilliant source of PETRA III and the Kirkpatrick-Baez nano-focus, large area histological brain slices are scanned at the sub-neuronal resolution, taking advantage of continuous motor movement and reduced acquisition time. Elemental analysis with synchrotron radiation based X-ray Fluorescence (SRXRF) is combined with X-ray Phase Contrast Imaging (XPCI) to correct for inherent aberrations in the samples' density and thickness, often referred to as the mass thickness effect. Based on the raw SRXRF spectra, we observed the accumulation of P, S, Cl, K, Ca, Fe, Cu and Zn predominantly in the SN neurons. However, upon the mass thickness correction, the distributions of Cl became significantly more uniform. Simultaneously with the fluorescence signal, the Small Angle X-ray Scattering (SAXS) is recorded by a pixel detector positioned in the far-field, enabling fast online computation of the darkfield and differential phase contrast (DPC). The data has demonstrated the SN neurons and neuropil produces excellent contrast which is due to their different mass density and scattering strength, indicative of differences in local structure and assembly therein. In all, the results show that combined SRXRF-XPCI-SAXS experiments can robustly serve as a unique tool for understanding the interplay between the chemical composition and structural organization that may drive the biochemical age-related processes occurring in the human dopaminergic system.

1. Introduction

Human brain aging constitutes a mixture of neuropathological structural and chemical processes that cause the progressive neuronal loss [1,2]. Current evidence points to the aberration in the structure and assembly of neurons that is thought to be due to misfolding and aggregation of proteins: α-synuclein (α-syn) [3], tau [4] and Aβ [5], considered as major driving forces underlying both the physiological ageing and the most common neurodegenerative disorders: Parkinson's and Alzheimer's disease [6]. In the affected brains, the progression in the pathology is significantly faster, although the "clinical" boundaries between the normal aging and dementias are ambiguous and often contentious [7]. The transition bio-metals (Fe, Cu, Zn, Mn) have been shown to trigger the protein aggregation that poisons the neurons and promotes their excessive apoptosis or excitotoxicity, but the nature of this interaction remains unclear [8,9]. In particular, the age-associated neurotoxicity processes that take a toll on the neuromelanin-pigmented substantia nigra (SN) dopaminergic neurons generated considerable research interest due to their elevated

Abbreviations: SN, substantia nigra; SAXS, Small Angle X-ray Scattering; XPCI, X-ray Phase Contrast Imaging; CTF, Contrast Transfer Function; KB, Kirkpatrick-Baez mirrors; α-syn, alpha-synuclein; DA, dopamine; ROS, reactive oxygen species; PD, Parkinson's disease; OS, oxidative stress; STXM, Transmission X-ray Microscopy; NDP, nano-diffraction patterns; DPC, differential chase contrast; SR, synchrotron; DFI, dark field intensity; WG, waveguide

* Corresponding authors at: AGH University of Science and Technology, Faculty of Physics and Applied Computer Science, Krakow, Poland.
E-mail addresses: asurowka@agh.edu.pl (A.D. Surowka), magdalena.boruchowska@fis.agh.edu.pl (M. Szczerbowska-Boruchowska).

http://dx.doi.org/10.1016/j.talanta.2016.08.023
0039-9140/

propensities for accumulation of genetic mutations, improper handling of metabolic products of dopamine (DA) oxidation, and oxidative stress (OS) that result in production of cytotoxic reactive oxygen species (ROS), damage to cellular lipids/proteins, increased rate of neuronal loss, and dopamine depletion underpinning PD [10]. Notably, the nigral α-syn has recently emerged as a major causal risk factor to the pathology, since its aggregated form is particularly vulnerable to metals-driven ROS production [9]. In the basal conditions, the protein plays physiological functions, however, the presence of formidable amounts of Fe, together with DA and H_2O_2 causes the protein to fibrilize and become toxic [11]. Also note, in hyper-oxic conditions, Fe(II) was shown to be bound to the misfolded form of the nigral α-syn, oxidized to Fe(III), and released. As a matter of fact, H_2O_2 is a byproduct of this transition, which augments the OS-mediated neuronal burden [12]. Oppositely, Zn, Cu, Mn were found to participate in the anti-oxidative mechanisms that prevent the cells from the deleterious effects of OS, though some evidence argues they can also exert neurotoxic effects [9,13,14].

These and many other examples figure out the critical role of the interplay between metals and structural changes that underlie age-associated biochemical and structural burden of the neurons in the elderly [14]. However, to compromise sometimes contradictory evidence, and make the findings more integrated, a special attention must be paid to chemical/structural mapping (2D/3D) of the local extent to the neuronal burden. Currently, to probe in-situ the structural-chemical processes with the highest possible subcellular spatial resolution, highly brilliant synchrotron sources of coherent X-ray radiation have been offered for the non-invasive analyses of biological specimens in dynamic mapping and tomography modes [15]. Specifically, combined Small-Angle X-ray Scattering (SAXS) and Scanning Transmission X-ray Microscopy (STXM) allow for determination of both orientation and density of the nano-scale biological scaffolds throughout the analysis of nano-diffraction patterns (NDP) [16,17]. With the possibility for imaging of the differential phase contrast (DPC) and dark field intensity (DFI) signal, several studies demonstrated it is possible to extract the information on the scattering strength, electron density gradient, radius of gyration, and orientation of molecular assemblies (cytoskeleton, actomyosin fibers) from the NDPs of cells with excellent sub-micron resolution [18–20]. Unfortunately, to our knowledge, far too little attention has been paid to STXM studies of structural properties of SN tissue samples. Besides, there is an increasing body of inquiry on the 2D imaging of bio-metals in SN using Synchrotron Radiation-based X-ray Fluorescence spectroscopy (SRXRF) [14,21]. By using highly collimated 10–20 keV SR-beams of X-rays, aberration in the levels of selected transition metals: Fe, Cu, Zn, Mn, Se was reported in thin (10–20 μm) freeze dried autopsy SN specimens taken from PD-affected [22–24] and normally-aged [25] human individuals as well as in the cell cultures of primary DA-ergic cells [13]. However, due to variation in the sample's thickness and density, often referred to as the mass thickness effect, the quantification of elemental mass fractions is a challenging task [26]. To get rid of this issue, SRXRF experiments must be combined with methods to determine the sample's electron density which is, in turn, proportional to the mass thickness of biological elements (in the absence of large stoichiometric variations in hydrogen). One of these methods is the X-Ray Phase Contrast Imaging (XPCI), which has been proven its usefulness in the fully quantitative SRXRF imaging of biological specimens [26]. In addition, unlike to the absorption X-ray imaging, the phase contrast radiography with highly collimated beams of X-rays has provided with significantly higher sensitivity, which was recently used to unravel the morphology of synuclein-positive Levy bodies found in the SN neurons of the PD-affected human individual [27]. Unfortunately, these and many other similar experiments were not combined with SAXS, SRXRF studies to provide with more cogent chemical–structural information on the SN tissue. Therefore, there still remains a need for co-localizing the metals' fractions to any possible alterations in structure and assembly of the human SN neurons.

Ramifications arising from exploring the interplay between chemical and structural age-associated changes within the human dopaminergic system may substantially boost our knowledge on the extent to the neuronal burden, necessary for understanding the processes that drive the human brain aging, Parkinson's disorder, and other dopamine-related alterations [13,14,21,22,24,36]. Recently, synchrotron X-ray based techniques: SRXRF [13,21,22,24–26,37], XPCI [26,27,38], SAXS [18–20] and many other have offered an in-situ analysis of biological specimens with the sub-cellular spatial resolution and ultra-low sub-ppm sensitivity for detection of chemical elements. However, despite huge technical advances in the state-of-the-art of instrumentation and methodologies, the integrated approach to tackling this critical problem by a single measurement is still pending [21]. Besides the instrumental issues, one has to stress the matrix-effects-related problems due to the inherent heterogeneity of biological specimens when the goal is the quantification of elements. So far, the problem has been addressed in our latest work, and the effect was found to significantly affect the elemental fractions in SN computed without correction [36]. In order to address current technical and analytical challenges in the combined structural/chemical analysis of thin (heterogeneous) biological specimens, the major objective of this study was to design, optimize and test a combined setup enabling quasi-simultaneous implementation of SRXRF, XPCI and scanning SAXS techniques. In addition, by combining the first two methods, our work extends the previous studies on the influence of the mass thickness effect and overcomes this artifact by a correction scheme.

In our present study, we demonstrate for the first time simultaneous high-resolution XPCI-SRXRF-STXM experiments using the highly brilliant source of PETRA III. Unlike to previous studies, this unique combination extends the chemical information obtainable from sole SRXRF imaging by structural information which can be derived from SAXS nano-diffraction patterns. We also show that by applying combined SRXRF-XPCI, a significant mass thickness effect can be avoided, yielding a corrected elemental ratio between bio-metals in the neurons with respect to the surrounding tissue. In addition, we also demonstrate the usefulness of the approach for possible structural and chemical analysis of submicron cellular deposits.

2. Sample preparation

The substantia nigra tissue specimens were taken during routine autopsy section from the aged individual not affected by any persistent neurological alterations. Following the autopsy, the tissue samples were rapidly frozen in −80 °C. Just before the experiment, the SN samples were cryosectioned in −18° onto either 20 or 25 μm thick serial sections (n=2 sections used in this study), mounted onto 200 nm-thick silicon nitride membranes (Si_3N_4, Silson Ltd., UK) and dried in a deep freezer at −80 °C in darkness. Neither any paraffin embedding and chemical fixation were utilized to end up with a specimen in a pristine state. More details on the sample preparation protocol could be found elsewhere [24,25].

3. The beamline

All the experiments have been performed on the P10 coherence beamline at PETRA III (Hamburg, Germany) using the nano-focus