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Commentary

Comment on "Mapping and Characterization of Iron Compounds in Alzheimer's Tissue"

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The recent article of Collingwood and Dobson [1] is an important article that emphasizes the importance of the characterization and mapping of iron compounds in the iron-rich brain regions with the aim contributing to our understanding of the role of pathological accumulations of iron in the regions of the brain affected by neurodegenerative disease. Nonetheless, below, I discuss ion and electron microprobe techniques for detecting and quantifying iron, not specifically cited by the authors, that allow the mapping of total iron (SIMS and XEDS) at the cellular and sub cellular level and the characterization and mapping of iron compounds (EELS) at ultra structural level.

SIMS (Secondary Ion Mass Spectroscopy) imaging technique, allows direct identification of chemical elements with high sensitivity and specificity and, as a consequence, elemental distribution can be visualized (chemical mapping) by SIMS imaging. The physical basis of the method is the following: under the bombardment of the samples by primary ions, the monoatomic or polyatomic species that composed the analyzing object are sputtered. One part of these emitted species is ionized and the SIMS instrument, with the help of a mass spectrometer, sort and maps the ejected ions by their m/e ratio. The latest generation of SIMS instruments, the NanoSIMS-50$^{\mathrm{TM}}$ instrument, operating in scanning mode, is equipped with a parallel detection system that allows the simultaneous acquisition of five elements which insures a perfect colocalization between simultaneously recorded images. This instrument is particularly useful to identify elements at a sub cellular level, because it is possible to attain resolutions of 50–100 nm with the Cs^+ source and 150–200 nm with the O^- source [2]. NanoSIMS microscopy has been already used with success for the visualization of the morphological and chemical alterations taking place in well-characterized regions in pathological brain, in particular in the study of iron distribution in Alzheimer disease tissue [3,4]. Multielemental analysis (nitrogen, phosphorus, sulphur and iron) were performed on semi-thin or ultra-thin sections of Transmission Electron Microscopy (TEM) preparations brain tissue. The possibility of using light microscopy, TEM, and SIMS on the same semi-thin and ultra-thin sections allows correlation between structural and analytical observations at sub-cellular and ultrastructural level. It has been shown that the iron-rich region mapped by nanoSIMS in the hippocampus of AD patients are ferritin and/or hemosiderin rich regions.

XEDS (X-Ray Energy dispersive Spectroscopy) and EELS (Electron Energy Loss Spectroscopy) are electron probe nanoanalysis techniques that, associated with transmission electron microscopes (ConventionalTEM, ScanningTEM or ConventionalTEM working in scanning mode, so-called AnalyticalTEM, ATEM), provide compositional maps of ultra-thin sections with nanometric resolution (1–10 nm): XEDS by detecting the element specific X-ray emission under excitation of incident electrons: EELS by detecting element specific energy loss of incident electrons. EELS provides theoretically higher detection sensitivity than EDS due to the larger number of primary events collected in the

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absence of fluorescence yield and with a larger collection angle. XEDS and EELS can be performed simultaneously by the same instrument [5]. With EELS it is possible to detect single atoms of calcium and iron in biological structures [6]. Elemental maps of iron in ferritin have been obtained by EELS since 1982 [7] because it is an ideal standard ATEM test specimen [6, 8]. EELS has been used for the analysis of iron and iron/ferritin in several human pathologies (see [9–12] for example). Recently, EELS has been combined with electron tomography to study the 3D distribution of elements in biological samples [13], particularly iron in degenerating neurons in mice with abnormal regulation of iron metabolism [14]. ATEM, with high energy resolution EELS spectra, allows the characterization of iron compounds by EXAFS and ELNES techniques (equivalent to XAFS and XANES derived synchrotron techniques) [15–20].

As we have indicated, in a recent article [4], due to the difficulty in correlating the synchrotron derived techniques with cell and tissue structure, it might be interesting to perform parallel studies, on the same samples, with microfocus XRF and multi-element nanoSIMS imaging; both being techniques suitable for total iron mapping at the cellular and sub-cellular level over a relatively large area. The study of large areas is necessary for statistical analysis and for comparison of normal and pathological tissues.

After obtaining information on the iron-rich regions and their morphology in normal and pathological brain, the XFS and XANES techniques, at micron and submicron level, and the EELS techniques, at nanometric level, can be applied to investigate the mineral iron compound in magnetic crystals and in pathological ferritin and hemosiderin.

Finally, I would like to remind Collingwood and Dobson that the hypothesis of a dysfunction of the pathological ferritin in PSP and AD, textually described "The formation of magnetite, in which ferric and ferrous ions are both present, would indicate that the enzymatic oxidation of iron inside ferritin was faulty" was already stated in our work [21]. Having knowledge of our results, Dobson suggested that the magnetite/maghemite crystals found in the brain [22–25] may originate from a ferritin precursor (cited as personal communication on page 819 of our work [21]). See also the comments of Dobson to our article [21] published in [26].

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