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Investigation of Radiolytic Effects in Frozen Aqueous Specimens by Electron Energy Loss Spectroscopy

M.A. Aronova, A.A. Sousa, R.D. Leapman

National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD 20892

Understanding radiation damage in frozen aqueous specimens is of considerable interest in the fields of structural biology, materials science, atmospheric chemistry, and astrochemistry. This can be accomplished by characterizing the effects of the radiation on frozen aqueous solutions at a molecular level and by observing the chemical products of radiolysis [1]. In the electron microscope we can observe morphological changes, such as the formation of gaseous bubbles, and at the same time detect chemical changes to the water and organic constituents by using electron energy loss spectroscopy (EELS) to record the fine structure near the core edges of hydrogen, carbon and oxygen. In the field of structural biology there is particular interest in understanding the radiation damage process at different cryogenic temperatures because this ultimately limits the achievable spatial resolution. Here we have analyzed EELS from vitreous ice, vitreous hydrogen peroxide (H_2O_2), and frozen aqueous solutions of sucrose and protein. Thin aqueous films on EM grids were cryotransferred into an FEI Tecnai TF30 electron microscope equipped with a Gatan Tridiem Imaging Filter, and EELS data were collected at doses ranging from ~10^2 to 10^6 electrons/nm^2.

Figs. 1a and 1b show low-loss and oxygen core-edge EELS, respectively, from a frozen aqueous solution of 30% H_2O_2 recorded at a dose of 10^5 electrons/nm^2. In Fig. 1a we do not observe the generation of molecular hydrogen (K edge at 14 eV, arrow) [2], but in Fig. 1b we do find evidence for the generation of molecular oxygen (pre-edge peak at 531 eV, arrow). This oxygen K-edge spectrum is consistent with the x-ray absorption spectrum of Laffon et al. [1]. It seems likely that the H_2O_2 damages at relatively low dose to form H_2O and O_2. Having established that molecular oxygen is observable by EELS, we next consider low-loss and core-loss spectra (Figs. 2a and 2b, respectively) recorded from a frozen aqueous solution of 10% sucrose and 10% bovine serum albumin. In Fig. 2a we observe the hydrogen K-edge (14 eV) [2], but we do not find evidence in Fig. 2b for the generation of molecular oxygen. One possible explanation for this result is that hydoxyl radicals react with the carbon-hydrogen bonds of the organic components to produce molecular hydrogen while the oxygen atoms bind to the carbon. To check the calibration of energy-loss we have also recorded low-loss and core-loss spectra (Figs. 3a and 3b, respectively) from pure water containing 3% manganese chloride, which gives rise to the Mn $L_{2,3}$ white lines of known energy loss. Here there is no evidence for production of molecular hydrogen (Fig. 3a) but we do observe the molecular oxygen peak at 531 eV (Fig. 3b). A possible explanation for this finding is that hydroxyl and atomic hydrogen radicals in water recombine as soon as they are formed within the interior of the frozen layer, while near the surface hydrogen molecules can escape. This would be consistent with the observation by Dubochet et al [3] that pure water does not readily bubble under electron irradiation. Oxygen has a higher boiling point and might betrapped within the specimen.

We are continuing to perform further experiments to characterize the radiolytic products that are formed when frozen aqueous solutions are irradiated in the electron microscope with the aim of elucidating the damage mechanisms [4].

References
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[4] The authors thank Prof. Jacques Dubochet for helpful discussions. This research was supported by the intramural program of NIBIB, NIH.

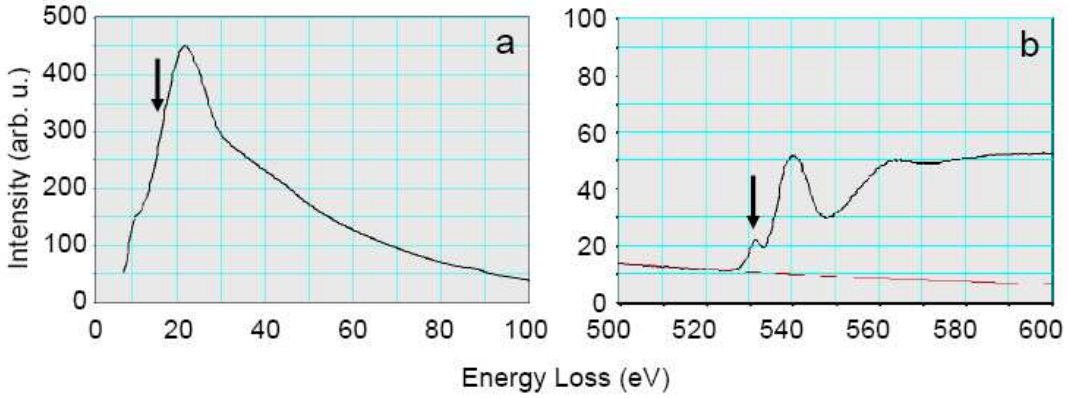

Fig. 1. EELS from frozen solution of 30% hydrogen peroxide at a dose of 10^5 electrons/nm^2.

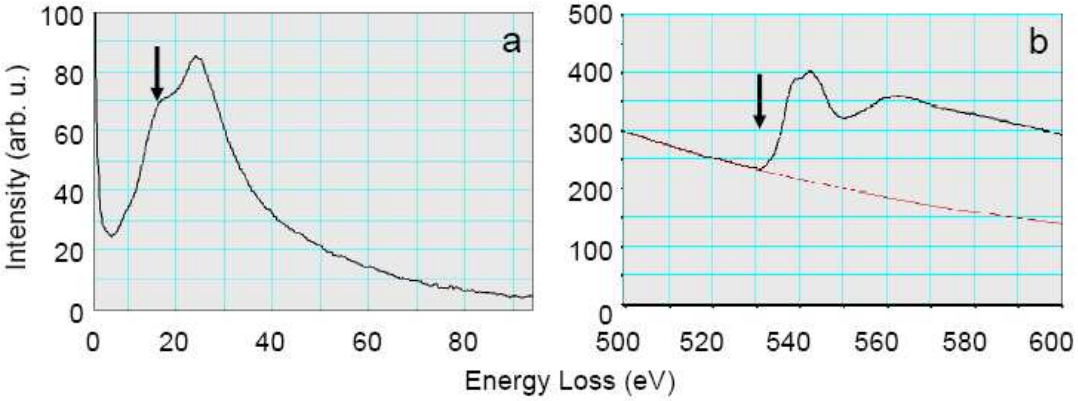

Fig. 2. EELS from frozen solution of 10% sucrose and 10% BSA at a dose of 10^5 electrons/nm^2.

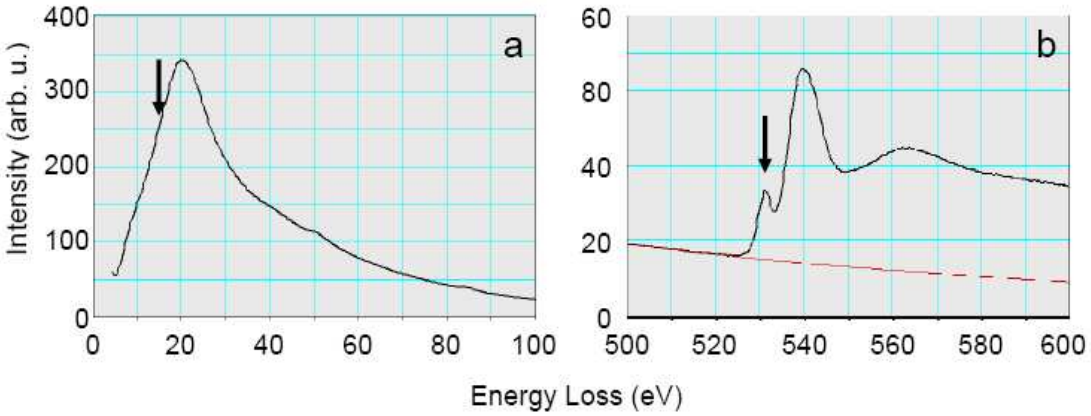

Fig. 3. EELS from vitrified aqueous solution of 3% $MnCl_2$ at dose of 10^5 electrons/nm^2.

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