An X-ray microscopy perspective on the effect of glutaraldehyde fixation on cells

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Abstract

X-ray microscopy (XM) is the only microscopy technique that can provide high resolution (30 nm) imaging of biological specimens without the need to fix, stain or section them. We aim to determine the effect, if any, of glutaraldehyde fixation on algae cells from the XM perspective and thus provide beneficial information for both X-ray and electron microscopists on artifacts induced by glutaraldehyde fixation. Three species of microalgae, *Microcystis aeruginosa*, *Anabaena spiroides* and *Chlorella vulgaris*, were used in this study. XM images were obtained from unfixed and glutaraldehyde-fixed cells and cell diameter and % X-ray absorbency measured. The mean diameter of cells from fixed preparations was smaller than unfixed ones; the mean diameter of *M. aeruginosa* cells was significantly reduced from 3.92 μm in unfixed cells to 3.43 μm in fixed cells ($P < 0.05$); in *C. vulgaris* the diameter of cells was also significantly reduced from 3.50 μm in unfixed to 2.98 μm in fixed samples ($P < 0.05$); while there was no significant reduction in the diameter of *A. spiroides* cells (4.04 μm to 3.90 μm). The protein crosslinking mechanism of glutaraldehyde probably generated free water molecules, which play an important role in radiation damage induced by X-rays. This was seen as mass loss and cell shrinkage, which in the present study occurred more frequently in fixed cells than unfixed cells. In addition, we demonstrated that the uptake of glutaraldehyde by cells makes all protein constituents in the cell organise into a closely packed configuration, thus causing a rise in percent X-ray absorbency. In fixed cells, this rise was approximately by a factor of two compared to unfixed samples where protein constituents inside the cell are arranged in their native form.