Cellular water and elemental content during stages of apoptosis : investigation by a cryo-correlative nano-imaging approach (Fluorescence/STEM)

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Cell shrinking is a structural hallmark of apoptosis that is initiated by fluxes of ions and of water. Here, we addressed the variations of ions and water concentration within different organelles during the main steps of apoptosis by applying a correlative fluorescence and cryo-STEM approach we developed (Nolin et al. Meth. Mol. Biol., 2015, 1228, 145). We induced apoptosis with actinomycin-D in stably transfected HeLa cell lines producing H2B-GFP. We first identified characteristic modifications of nucleus and of mitochondrial polarization (TMRE) during apoptosis by using biphoton excitation and time-lapse imaging. Five stages, defined on shape of nuclei and chromatin condensation, were correlated to mitochondrial depolarization, cytochrome-c diffusion and caspase-3/PARP activation. We applied our correlative method to quantify water and elemental content (N, P, S, K, Cl, Mg and Na) within mitochondria, cytosol, condensed chromatin and nucleoplasm. Cells in given stages were: i) identified on cryo ultrathin section by fluorescence imaging of chromatin, ii) positioned relatively to fiducial marks on the grid, iii) found in STEM imaging and analyzed for water and elemental contents. Water concentration in organelles is 10% higher than in control cells. At the onset of apoptosis, $[Na^+]$ and $[Cl^-]$ strongly increased in mitochondria and nucleus, whereas $[K^+]$ decreased. Conversely, $[Na^+]$ and $[Cl^-]$ strongly decreased during the following stage and finally increased again during the final stages.