One more function of cytochrome c: fusion of the inner mitochondrial membrane

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The processes of mitochondrial fission and fusion are important for the preservation of mitochondrial functionality since they can recover damaged organelles. The mechanism of mitochondrial fusion allows the association of damaged mitochondria with metabolically capable pairs. The proteins involved in mitochondrial fusion (mitofusins) are crucial for mitochondrial biogenesis and respiratory activity with repercussions for cell fate. For the fusion of the outer membrane, a diversity of proteins has been identified. Otherwise, only a dynamin-related protein orthologous to Msp1 of *Schizosaccharomyces pombe* and Mgm1p of *Saccharomyces cerevisiae* has been associated with the fusion of inner mitochondrial membrane (IMM). The fusogenic properties of respiratory cytochrome *c* (cyt *c*) previously demonstrated for liposomes points to this heme protein as a potential contributor for the mitochondrial fusion. Cyt c is a multifunctional protein that acts as a mobile electron carrier in the electron transport chain (ETC), a caspase activator during early apoptosis and an antioxidant enzyme against superoxide ions and hydrogen peroxide. All of these functions are related to the binding affinity of cyt *c* to the IMM. Two sites of cyt *c* structure are related to the binding affinity to the IMM in physiological conditions: site A and L. Site A contains lysine residues with high p*K*a values and site L is formed by a cluster of lysine residues with an observed pK_a around 7.0. Cyt *c*-mediated fusion of vesicles that are models of the IMM is dependent on ionization of the protein site L (acidic pH) and the presence of transmembrane potential. The effect of transmembrane potential on the fusogenic activity of cyt *c* is probably related to the promotion of defects in the lipid bilayer. Also, the maximal fusogenic capacity of cyt *c* was observed for liposomes with 20% of cardiolipin, the percentage present in mitochondria. The fusogenic activity decreases both at higher and lower cardiolipin percentages. In the present study, the fusogenic activity of cyt *c* for the IMM was demonstrated for mitoplasts (mitochondria depleted of the outer membrane). Isolated rat liver mitoplasts depleted of endogenous cyt *c* were trapped by optical tweezers with an inverted light microscope design in the absence and presence of added cyt *c*. Mitoplasts with endogenous cyt *c* were also tested. The laser diffraction pattern resulting from the optically trapped mitoplasts was recorded on a CCD camera. The resulting diffraction pattern depends on the relative refractive index (between the interior of the mitoplast and the dispersant), and the mitoplast size. Considering constant relative refractive index, the only change in the diffraction pattern results from the mitoplast size. As time goes by, mitoplasts diffuse into the trapping region and are squeezed against other already trapped partners, changing the diffraction pattern. Besides the diffraction pattern, bright field image is also captured by the CCD camera. After 30 min of mitoplast trapping, upon releasing the trapping beam, the mitoplasts with cytochrome c appears as a uniform spherical structure much larger than a single mitoplast. In contrast, trapped mitoplasts without cyt *c*, upon releasing the optical trap, dispersed as single mitoplasts. These results demonstrated that cyt *c* is effective to promote fusion of the IMM even in the absence of soluble components of the intermembrane space and add one more task for this mitochondrial protein.

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