Safranine O as a Fluorescent Probe for Mitochondrial Membrane Potential Studied on the Single Particle Level and in Suspension

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Abstract—The permeant cationic dye safranine O is often used to measure mitochondrial membrane potential due to the dependence of both its absorption and fluorescence on mitochondrial energization, which causes its oligomerization inside mitochondria. In the present study we have used fluorescent correlation spectroscopy (FCS) to record the fluorescence changes on a micro level, i.e. under conditions permitting resolution of contributions from single particles (molecules of the dye and stained mitochondria). We have shown that the decrease in fluorescence signal from a suspension of energized mitochondria stained with a high safranine concentration (10 μM) is explained by the decrease in dye concentration in the medium in parallel with the accumulation of the dye inside the mitochondria, which results in fluorescence quenching. With 1 μM safranine O, the fluorescence rise after energization is caused by the accumulation of the dye up to a level not sufficient for full fluorescence quenching and also by the higher intensity of mitochondrial fluorescence on immersion of the dye in the hydrophobic milieu. Besides the estimation of the inner mitochondrial membrane potential, this approach also assesses the concentration of fluorescent particles. The non-monotonic dependence of the FCS parameter $1/G(\tau\rightarrow 0)$ on the concentration of mitochondrial protein suggests heterogeneity of the system with respect to fluorescence of particles. An important advantage of the described method is its high sensitivity, which allows measurements with low concentrations and quantities of mitochondrial protein in samples (less than 10 μg).

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