ESTIMATION OF MITOCHONDRIAL NADH/NAD$^+$ RATIO FROM DOUBLE CHANNEL FLIM

Petr Ježek[1], Hana Engstová[1], Andrea Dlasková[1], Olexandr Chernyavskiy[2], and Lydie Plecitá-Hlavatá[1]

[1]Dept.75, [2]Dept.60, Institute of Physiology, Czech Academy of Sciences v.v.i., Vídeňská 1083, Prague 4, Czech Republic; E-mail: jezek@biomed.cas.cz

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The increasing mitochondrial matrix NADH/NAD$^+$ ratio determines elevated superoxide formation at the proximity of Complex I flavin site. To estimate this ratio within intact cells, we employed double-channel confocal fluorescence lifetime imaging (2chFLIM) with 140 fs pulse width, Leica TSC-SP8 confocal microscope plus a novel Becker & Hickl attachment for double-channel FLIM. Imaging of HepG2 and INS-1E cells, within mitochondrial region of interests yielded parameters of NAD(P)H autofluorescence decay at 467–499 nm and FAD decay at 500–550 nm, while two-photon excitation was at 700 nm. At each 4×4 pixels, the iterative reconvolution yielded two lifetimes for free and bound molecules τ_F, τ_B, of 0.4–0.5 ns, ~2.6 ns and their normalized amplitudes α_F and α_B. Reconstructed FLIM images provided average parameters for each location. A ratio of bound NADPH/NADH was estimated from the measured τ_B magnitudes, while using empirical term according to Duchen and colleagues [1]. Free NADH was approximated from Scatchard equation. Free NAD$^+$ was derived from its Stern-Volmer quenching of FAD (longer FAD lifetime of free molecules). Approximating association constants, we estimated absolute NADH/NAD$^+$ ratios being higher e.g. in HepG2 cells forced to oxidative phosphorylation. Without constants, one can obtain exact changes in NADH/NAD$^+$ ratios between any two conditions. HepG2 cells yielded different responses to rotenone in remaining unfragmented tubules of mitochondrial network and co-existing small fragments. Investigating changes upon glucose addition leading to insulin secretion in model pancreatic β-cells, INS1E cells, we found decreasing mitochondrial NADH/NAD$^+$ ratios, but not with blocked metabolic shuttles exporting redox equivalents. Thus the effective consumption of matrix NADH by mitochondrial shuttles (providing cytosolic NADPH) decreases the matrix NADH/NAD$^+$ ratio in INS1E cells.

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[1] T.S. Blacker, Z.F. Mann, J.E. Gale, M. Ziegler, A.J. Bain, G. Szabadkai, M.R. Duchen, Separating NADH and NADPH fluorescence in live cells and tissues using FLIM. Nat. Commun. 5 (2014) 3936.