Purification and separation of cardiac mitochondrial subpopulations for functional assays

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Abstract

Cardiac mitochondria are purified for functional assays, which contribute fundamental information on the susceptibility of ischemia/reperfusion injury. Therefore, functional and structural integrity of mitochondria needs to be preserved to maintain experimental reproducibility and sensitivity. However, deleterious effects of mitochondrial purifications are unavoidable and bias results. Removal of damaged mitochondria and other impurities by sophisticated purification procedures generally result in substantial loss of sample and/or mitochondrial function. Furthermore, studies indicate that mitochondria with different physiological states are present in cells. This heterogeneity results in additional bias on functional assays. In a proteomic approach, yeast mitochondria have successfully been purified by a technology based on deflection of particles in a laminar flow, which is perpendicular to an electrical field (ZE FFE). Therefore, the potential of ZE FFE to improve the preparation and separation of functionally intact mammalian mitochondria was analyzed.

Fresh mitochondria were extracted from cardiac tissue by homogenization and differential centrifugation, before they were subjected to fractionation by ZE FFE. Typically, the additional step adds 20min to the preparation and is compatible with assay buffers, which is important to preserve the functionality of the mitochondria. Furthermore, the technology sets virtually no limitations to increase sample amounts, since it operates in continuous flow. The rate of yield is quantitative. Analyses of the fractions by SDS-PAGE demonstrated only minor differences in the banding pattern, suggesting the purification by differential centrifugation is almost homogenous. However, identification of variances in the protein pattern of fractions indicated impurities of cytoskeleton and endoplasmatic reticulum. Immunodetection of organelle markers, such as LAMP1 and GRP78 confirmed the removal of impurities and enrichment of mitochondrial proteins after fractionation by ZE FFE. Furthermore, analysis of marker proteins for inner (ANT) and outer membrane (VDAC) proteins indicated mitochondria were not stripped of their outer membrane by the procedure. Finally, mitochondria found in different, but pure fractions, indicate alternative charge to surface ratios, and thus possibly distinct physiological states of mitochondria. Therefore, mitochondrial functionality was assayed.

Reference

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