Application Forum

Isolation of Mitochondria from Cell Cultures by PCT for Proteomic Analysis

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INTRODUCTION

Proteomic profiling of mitochondria has the potential to provide insights into mitochondrial functions associated with aging, various metabolic states, and diseases such as cancer, diabetes, and cardiovascular disease [1]. Rapid and reproducible isolation of intact mitochondria is crucial for efficient enrichment and subsequent proteomic analysis of low-abundance mitochondrial proteins [2]. Here we describe a system for the isolation of intact mitochondria from rat PC12 cells using pressure cycling technology (PCT).

CELL DISRUPTION BY PRESSURE CYCLING TECHNOLOGY (PCT)

PCT destabilizes inter-molecular interactions by rapidly and repeatedly raising and lowering pressure in the reaction vessel from ambient to high levels (up to 35,000 psi [240 MPa]) [3]. High hydrostatic pressure acts preferentially on the more compressible components of the sample, such as the lipid constituents of the plasma membrane of cells. The Barocycler instrument allows the user to adjust the pressure that is applied to the sample, permitting precise control of the extent of sample disruption depending on the composition and structure of sub-cellular membrane components. Thus PCT conditions can be adjusted to disrupt cell plasma membranes while leaving organelles, such as mitochondria, intact.

METHOD

Mitochondria-enriched fractions were prepared from rat PC12 cells grown in suspension. Cells were pelleted by centrifugation and suspended in Mitochondrial Isolation Buffer (10 mM sucrose, 200 mM mannitol, 5 mM HEPES, 1 mM EGTA, pH adjusted to 7.4 with KOH and supplemented with 1 mg/mL fatty acid-free bovine serum albumin) [4]. The suspension was split into four aliquots, which were then processed using one of four sets of conditions to disrupt the cells: 1) Atmospheric pressure only ("0" kpsi); 2) 30 seconds at 5 kpsi followed by 20 seconds at atmospheric pressure, repeated for 15 cycles; 3) 30 seconds at 15 kpsi followed by 20 seconds at atmospheric pressure, repeated for 15 cycles; 4) 30 seconds at 25 kpsi followed by 20 seconds at atmospheric pressure, repeated for 15 cycles.

After cell disruption by PCT, samples were centrifuged at low speed (900× *g*) to pellet nuclei, large cellular debris, and remaining intact cells. The resulting pellet (pellet 1) was saved and the supernatant was transferred to a fresh tube and centrifuged at high speed (13,000× *g*) to separate the mitochondria (pellet 2) from the soluble cytosolic proteins. Pellets 1 and 2 were then washed with BSA-free buffer prior to analysis. Aliquots of pellet 1, pellet 2, and supernatant were separated by SDS-PAGE and were either stained with Coomasie Blue dye for total protein visualization (Figure 1), or were transferred to Immobilon-P (Millipore) for Western blot analysis (Figure 2).

To confirm that the mitochondria-enriched fractions contain intact mitochondria, blots were probed with three

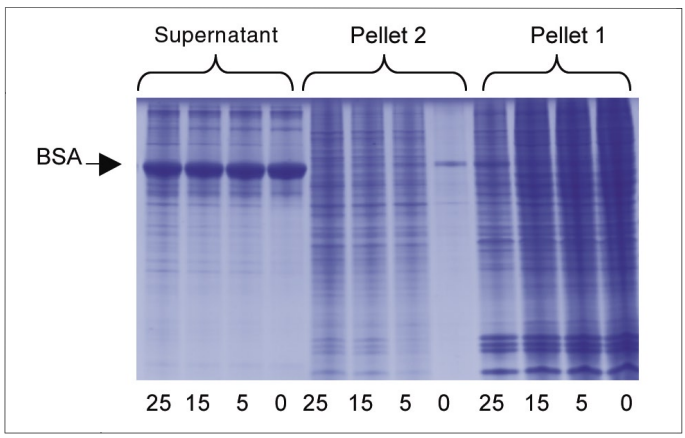

Figure 1. Coomasie blue–stained gel of pellet 1, pellet 2, and supernatant fractions from PC12 cells processed at the indicated pressure (0–25 kpsi). The BSA band in the supernatant is present due to the BSA added to the isolation buffer. Proteins were separated on an 8–16% Tris-HCl Criterion gel (BioRad).

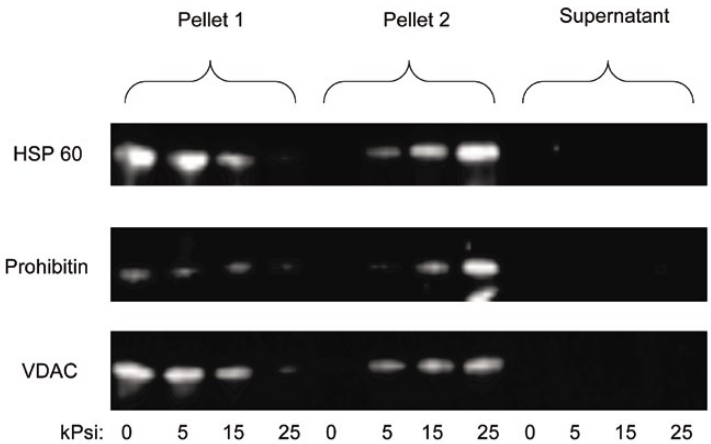

Figure 2. Western blots of pellet 1, pellet 2, and supernatant fractions from PC12 cells, pressure cycled at the indicated pressure. VDAC/Porin is a marker of the mitochondrial outer membrane; Prohibitin is a marker of the mitochondrial inner membrane; and HSP60 is a marker of the mitochondrial inner matrix space. The presence of all three proteins in the mitochondrial fraction (pellet 2) indicates that this fraction contains intact mitochondria.

antibodies (Abcam) that recognize proteins, localized to distinct mitochondrial compartments.

RESULTS AND DISCUSSION

The results shown in Figures 1 and 2 confirm that in the absence of PCT (0 kpsi control fractions) all mitochondrial markers are contained in the intact cells in pellet 1. Under initial PCT conditions cells begin to lyse, and as pressure increases, more cells lyse and release mitochondria, which are then recovered in pellet 2. It is expected that if a significantly large number of mitochondria were ruptured, HSP60 would be detected in the supernatant fraction. The absence of strong mitochondrial protein signals in the cytosolic supernatant, especially soluble HSP60, supports the conclusion that the PCT protocol is gentle enough to lyse the cells while keeping the bulk of the mitochondria intact.

These results show that PCT is an effective hands-free method for the isolation of mitochondria from cultured cells. It is possible to optimize PCT to release mitochondria from other cells types and tissues. In addition, the stringency of the pressure cycling conditions can be modified based on the requirements of downstream applications. For example, PCT conditions can be adjusted to extract a smaller number of mitochondria under relatively gentle pressure for functional studies, or a larger number of mitochondria using more intense pressure cycling for proteomic analyses.

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