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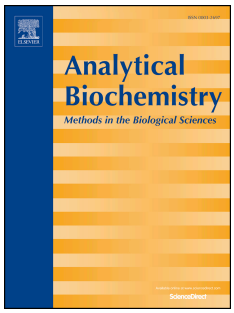

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In situ measurements of mitochondrial matrix enzyme activities using plasma and mitochondrial membrane permeabilization agents

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ABSTRACT

Activities of enzymes localized to the mitochondrial matrix of mammalian cells are often critical regulatory steps in cellular metabolism. As such, measurement of matrix enzyme activities in response to genetic modifications or drug interventions is often desired. However, measurements in intact cells are often hampered by the presence of other isozymes in the cytoplasm as well as the inability to deliver enzyme substrates across cellular membranes. Classic approaches to liberate matrix enzymes utilize harsh treatments that disrupt intracellular architecture or require significant starting material to allow mitochondrial isolation prior to sample extraction. We describe a method using permeabilization reagents for both the plasma and mitochondrial membranes to allow *in situ* measurement of matrix enzyme activities. It is applied to adherent cell monolayers in 96-well plates treated with perfringolysin O to permeabilize the plasma membrane and alamethicin to permeabilize the mitochondrial inner membrane. We present three examples validated with inhibitor sensitivity: (i) Complex I-mediated oxygen consumption driven by NADH, (ii) ATP hydrolysis by the F_1F_O complex measuring pH changes in an Agilent Seahorse XF Analyzer, and (iii) Mitochondrial glutaminase (GLS1) activity in a coupled reaction monitoring NADH fluorescence in a plate reader.

1. INTRODUCTION

Studying cellular bioenergetics often requires balancing physiological relevance with mechanistic depth [1]. Whole cells, for example, preserve changes in cell signaling, mitochondrial dynamics, and endogenous rates of energy metabolism. Although valuable for understanding global bioenergetic parameters, such as the efficiency of oxidative phosphorylation and maximal capacity for substrate oxidation, a mechanistic analysis is often prohibited in intact cells because of an inability to directly control substrate provision to mitochondria [1,2].

Experiments with isolated mitochondria, of course, provide such control; mitochondria can be offered specific oxidizable substrates to test maximal capacities of specific metabolic pathways [3]. However, not only do these experiments lack a wider cellular context, they often require large amounts of starting material and are prone to sub-selection during the isolation procedure. Furthermore, mitochondrial isolation from complex tissues such as the brain results in a heterogeneous mixture of mitochondria