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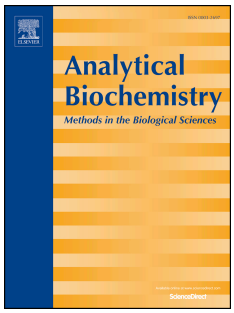

Calcein leakage as a robust assay for cytochrome *c*/H_2O_2–mediated liposome permeabilization

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Calcein leakage as a robust assay for cytochrome *c*/H_2O_2–mediated liposome permeabilization

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Abstract

Membrane-permeabilizing activity of cytochrome *c* (cyt *c*) in the presence of hydrogen peroxide associated with its functioning as peroxidase is considered relevant to initiation of the mitochondrial pathway of apoptosis. Here, we present evidence that the choice of a fluorescent dye for measuring cyt *c*/H_2O_2-induced dye leakage from liposomes by fluorescence de-quenching is of major importance. The popular fluorescent marker 5(6)-carboxyfluorescein appeared highly susceptible to cyt *c*-mediated peroxidative destruction and therefore unsuitable for the leakage assay with cyt *c*/H_2O_2. On the contrary, calcein, another conventional marker, proved resistant to oxidative stress and thus perfectly suitable for the assay. Based on the concentration dependences of the cyt *c*/H_2O_2-induced calcein leakage, the optimal conditions for the assay were found.

Key words: liposome permeabilization, calcein leakage, fluorescence de-quenching, carboxyfluorescein oxidative damage, peroxidase, cytochrome *c*.

Abbreviations: 5(6)-carboxyfluorescein (CF), bovine heart cardiolipin (CL), cytochrome *c* (cyt *c*), reactive oxygen species (ROS), mitochondrial permeability transition pore (MTP).

Introduction

Liposome leakage assay [1], based on the fluorescence de-quenching of entrapped dyes upon their release in the bulk solution, is now widely used for measuring membrane permeabilization caused by a variety of impacts (see, e.g., [2-7]). Usually, 5(6)-carboxyfluorescein (CF) and calcein, being highly fluorescent and prone to concentration quenching, are the dyes of choice for this technique, with the preference of the former due to its smaller size. Of note, unsubstituted fluorescein, being membrane-permeant, is unsuitable for this assay. Our previous study [8] of the photodynamically induced liposome leakage have revealed a substantial drawback of using CF in the assay, namely: the oxidative stress, induced by the photodynamic treatment, not only resulted in liposome permeabilization, but also led to a