THE PRESENCE OF PHOSPHOLIPASE A2 ACTIVITY IN PLANT MITOCHONDRIA AS DEMONSTRATED BY A NEW SPECTROPHOTOMETRIC ASSAY

D. TRONO*, **, M. SOCCIO**, N. DI FONZO**, D. PASTORE*

*) Department of Animal, Plant and Environmental Sciences, Agricultural Faculty, University of Molise, Via De Sanctis, 86100 Campobasso, Italy
trono@unimol.it
**) Experimental Institute for Cereal Research, SS 16 Km 675, 71100 Foggia, Italy

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Phospholipase A2 (PLA2) is a family of enzymes that specifically hydrolyze glycerophospholipids at the sn-2 position to yield free fatty acids and lysophospholipids. The existence of plant PLA2s has been presumed for some time and several studies have been reported concerning their implication in important cellular processes, such as lipid metabolism, auxin-stimulated cell growth and plant defence response to wound stress and pathogen attack. Only recently PLA2s have been purified from different plant sources and identified as “secretory” PLA2 (sPLA2) and “intracellular” PLA2 (iPLA2), the latter being located in the cytosol or associated to plasmatic membrane, whereas no so called “cytosolic” PLA2 (cPLA2) have been so far demonstrated in plants. Moreover, to date, no information is available about the existence of any PLA2 activity in plant mitochondria. In this work we have investigated about the existence of PLA2 activity in mitochondria from durum wheat, maize, barley, spelt, tomato and lentil seedlings as well as from potato and topinambur tubers by using a new properly developed continuous spectrophotometric method based on the PLA2/lipoxygenase (LOX) coupled reactions using 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine as substrate. Measurements have been carried out at 234 nm by monitoring free linoleic acid generated by PLA2 by means of its conversion into conjugated diene hydroperoxide due to LOX reaction. The new method has been validated on the basis of an investigation carried out on a purified sPLA2 from bee venom and appears to be sensitive and more simple, accurate and reproducible than other methods already described in literature. We have evaluated the existence of a PLA2 activity in plant mitochondria on the basis of the assays, both in the homogenate and in the fraction of purified mitochondria, of phosphoenolpyruvate carboxylase and cythocrome c oxidase, marker enzymes of the cytosol and mitochondria, respectively. Mitochondrial PLA2 existence has been demonstrated in durum wheat, maize, barley, spelt and tomato seedlings, whereas it was absent in lentil seedlings and potato and topinambur tubers, thus showing differences among species/plant materials. Moreover, assays carried out using intact and osmotically ruptured mitochondria suggest that at least 60% of PLA2 activity is present either in the matrix space or at the matrix face of inner membrane. A first characterization of the mitochondrial PLA2 in plants has been carried out by using durum wheat, which displays a high mitochondrial/cellular ratio of PLA2 activity. We have observed the typical features of an enzymatic activity: heat inactivation, substrate specificity and dependence on protein content, pH and substrate concentration (with Km = 90 ± 8 (S.D.) mM and Vmax = 3.0 ± 0.08 x 10^-2 E.U. x mg^-1 of protein). The enzymatic properties resemble that of other PLA2, with pH optimum at 8.9, activation by Ca++ and inhibition by the Ca++ complexant EGTA. Moreover, we have observed competitive inhibition (Ki = 14 mM) of mitochondrial activity by...
palmitoyl trifluoromethyl ketone (PACOCF₃), a classical PLA₂ competitive inhibitor. On the other hand, mitochondrial activity was unaffected by bromoenol lactone, a typical inhibitor of the Ca⁺⁺-independent PLA₂, thus suggesting the absence of a Ca⁺⁺-independent PLA₂ in DWM.

Our data show that a mitochondrial PLA₂ exists in plants. Further investigations are required to shed some light on the physiological role of the enzyme.