Title Characteristics of PLD-alpha C2 domain and regulation of ripening-induced gene expression in tomato fruits

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Abstract

Phospholipase D (PLD) is the key enzyme that initiates membrane phospholipid degradation during fruit ripening and senescence. To gain insight into the regulatory mechanism of PLD, the N-terminal C2 domain was cloned and expressed. The C2 domain is a calcium and lipid binding module that translocates the protein at the site of action in response to the signaling event. In a ligand binding study, recombinant C2 domain exhibited micromolar affinity towards Ca^{2+} with a maximum of two high affinity binding sites. The C2 domain had maximum affinity towards phosphatidic acid, and the binding varied based on the head groups of phospholipids. By contrast to animal systems, the recombinant C2 domain of tomato did not bind phosphatidyl choline (PC). The binding affinity of the C2 domain for phosphoinositides decreased with the degree of phosphorylation, indicating a direct role in signal transduction. Acid and chaotropic salt titrations indicated an electrostatic, rather than a hydrophobic, mode of interaction between the C2 domain and the membrane. Tertiary structure prediction and alignment revealed potential binding sites for Ca^{2+} and PIP2.

Changes in gene expression in response to the application of hexanal, an inhibitor of PLD, or 1-methylcyclpropene (1-MCP), an ethylene receptor blocker, were monitored in tomato using the TOM2 tomato oligo-array containing approximately 12000 unigenes. Key components in the ethylene biosynthetic pathway such as ACC-synthase / oxidase, and ethylene receptor and ethylene response factors were heavily down-regulated in 1-MCP-treated fruits. In addition, the main precursors of ripening and pigment development pathways, viz., geranyl pyrophosphate synthase and phytoene synthase, were also down-regulated. Hexanal treatment significantly down-regulated ACC synthase and to a lesser extent other components of the ethylene signal transduction. Similarities in the modulation of gene expression by hexanal and 1-MCP suggest that hexanal, in addition to being a PLD inhibitor, may also act as a weak ethylene inhibitor.