E215 The Involvement of Ca^{2+}, Protein Phosphorylation and Protein Dephosphorylation in Ethylene Signal Transduction

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To elucidate the mechanism of ethylene signal transduction resulting in induction of ACC oxidase, one of the ethylene biosynthetic genes, we determined the role of Ca^{2+} and protein phosphorylation by using pharmacological appoach. 10$\mu\ell$/L of ethylene increased ACC oxidase transcript level as well as enzyme activity. In absence of exogenous ethylene, 2,5-norbornadiene, a ethylene action inhibitor, and AVG, a inhibitor of ACC synthase reduced the basal level of ACC oxidase transcript and enzyme activity. Whereas in presence of exogenous ethylene, only 2,5-norbornadiene was appeared to reduce ACC oxidase transcript level and enzyme activity significantly. EGTA, a Ca^{2+} chelating agent, inhibited ethylene action by dose-dependent manner. Ca^{2+} depletion by EGTA pretreatment also blocked ethylene effect and when Ca^{2+} was retreated exogenously after Ca^{2+} depletion, effect of ethylene has shown to be almost completely recovered. Ca^{2+} channel blockers which block Ca^{2+} channel located in plasma membrane, were treated with or without ethylene to certify the role of extracellular Ca^{2+}. Both verapamil and lanthanum chloride inhibited ethylene action. H-7, a protein kinase inhibitor showed inhibitory effect on ethylene action at concentration of 0.5mM. Vanadate and okadaic acid which inhibit protein phosphatase 2B and protein phosphatase 1, 2A respectively, also appeared to reduce ACC oxidase transcript level and enzyme activity. The results of the present study suggest that Ca^{2+} influx from extracellular space, protein phosphorylation and protein dephosphorylation are required for induction of ACC oxidase by ethylene.

E216 Studies on the regulation of Carnation *S*-adenosylmethionine decarboxylase during petal development.

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We have generated polyclonal antibodies to carnation *S*-adenosylmethionine decarboxylase(SAMDC) to study the regulation of SAMDC gene expression during flower development. The open reading frame of SAMDC cDNA(*CSDC16*) was amplified by PCR and ligated with pMal-c2 vector. The SAMDC enzyme was translationally fused to the C-terminal end of the 42 kDa maltose binding protein(MBP) encoded in the vector. We confirmed the ligation of pMal-c2 vector with the *CSDC16* by DNA gel blot analysis with *CSDC16* gene specific probe and sequence analysis. The IPTG-induced MBP/SAMDC fusion protein was purified by affinity chromatography to crosslinked amylose resin. The identity of the affinity-purified fusion protein was determined by immunoblot analysis using anti-MBP serum and SAMDC enzyme activity assay. The size of fusion protein was about 84 kDa and had the SAMDC enzyme activity. The MBP/SAMDC fusion protein was directly used for immunization of mice. On immunoblots, the SAMDC antibody recognized the 42kDa protein from extracts of both *E.Coli* containing plasmid Mal/CSDC16 and carnation petals. Immunoprecipitation experiment showed that immune serum contained specific anti-SAMDC antibody. To determine the expression pattern of SAMDC during flower develment, immunoblots of total proteins isolated from carnation petals at seven stages(A to G) were performed using SAMDC-specific antibody. The accumulation of *CSDC16* transcripts and changes in SAMDC activities were differentially regulated during carnation petal development. These data imply that SAMDC gene expression is under transcriptional and translational control.