

ABSTRACT

4-Hydroxybenzoic acid (4-HBA) is a widely occurring plant secondary metabolite often used in food, pharmaceutical and polymer industries. Biosynthesis of hydroxybenzoates is poorly understood. Therefore this thesis aims at exploring the enzymatic route of 4-HBA formation in *Daucus carota*, a widely used model plant in studying plant metabolism. Hairy root cultures were established in *D. carota* to address this issue. Metabolic profiling of phenolics by HPLC//ESI-MS/GC-MS from these hairy roots showed a substantial amount of 4-HBA accumulation in the cell wall as well as a detectable amount in the cytosol. However, cytosolic 4-HBA was found to be in unconjugated form. These hairy root cultures were further treated with methyl-jasmonate or chitosan to uplift the levels of 4-hydroxybenzoic acid accumulation. Inhibitor and precursor feeding experiments were carried out with chitosan-elicited hairy roots to probe the enzymatic route(s). Supplementation of aminooxyacetic acid (a competitive inhibitor of phenylalanine ammonia-lyase) and piperonylic acid (a mechanism-based inhibitor of cinnamate 4-hydroxylase) in the culture media resulted in the reduced accumulation of wall-bound 4-HBA in the hairy root lines. However addition of 3, 4-methylenedioxycinnamic acid (a potent inhibitor of 4-hydroxycinnamoyl-CoA ligase, 4CL) did not inhibit 4-hydroxybenzoic acid accumulation but suppressed lignin and total flavonoid accumulation, suggesting that 4CL enzyme activity is not required for 4-HBA formation. Evidence of an unusual 4-hydroxybenzaldehyde synthase (HBS) that catalyzes the penultimate step of 4-HBA biosynthesis by making phenylpropanoid side-chain cleavage of 4-coumaric acid was demonstrated for the first time in cell-free extract of methyl-jasmonate-treated hairy roots of *D. carota*. The last step of the biosynthetic pathway was catalyzed by a NAD⁺-dependent 4-hydroxybenzaldehyde dehydrogenase (HBD). The accumulation of 4-HBA was preceded by an increase in the activities of 4-hydroxybenzaldehyde synthase and 4-hydroxybenzaldehyde dehydrogenase activities in methyl-jasmonate treated hairy roots. A rapid 5.6-fold enhancement of phenylalanine ammonia-lyase (PAL) activity, the first enzyme of the phenylpropanoid pathway was also observed. HBS and HBD enzymes were partially characterized from cell-free extract. Both the enzymes showed activity in a relatively broad pH range (7 to 8.5) and the temperature optimum around 35 °C. The preferred substrate of HBS and HBD was 4-coumaric acid and 4-hydroxybenzaldehyde, respectively.

Keywords: *Daucus carota*, Hairy root, 4-Hydroxybenzoic acid, 4-Hydroxybenzaldehyde dehydrogenase, 4-Hydroxybenzaldehyde synthase