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Research review paper

Reaction mechanisms of the bacterial enzyme 1-aminocyclopropane-1-carboxylate deaminase

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Abstract

The enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase promotes plant growth by sequestering and cleaving plant-produced ACC thereby lowering the level of ethylene in the plant. Decreased ethylene levels allow the plant to be more resistant to a wide variety of environmental stresses. Here the biochemical reaction mechanisms involved in ACC deaminase activity are critically reviewed.

Keywords: ACC deaminase; Plant growth-promoting bacteria; Direct β-hydrogen abstraction; Nucleophilic addition

Contents

1. Introduction

Plant growth-promoting bacteria include a diverse group of free-living soil bacteria that can stimulate the growth of plants by one or more of a number of different direct or indirect mechanisms (Glick, 1995). Indirect stimulation of plant proliferation includes preventing phytopathogens from inhibiting plant growth and development while direct stimulation includes providing plants with compounds such as fixed nitrogen, phytohormones, or solubilized iron from the soil (Glick, 1995; Glick et al., 1999).

Many plant growth-promoting bacteria contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and this enzyme can cleave the ethylene precursor ACC to α-ketobutyrate and ammonium and thereby lower the level of ethylene in developing or

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Table 1
Biochemical characteristics of some ACC deaminases

Enzyme source	*Pseudomonas putida* UW4	*Pseudomonas putida* GR12-2	*Pseudomonas* sp. ACP	*Hansenula saturnus*	*Penicillium citrinum*
Subunit molecular mass (daltons)	36,874	35,000	36,500	40,000	41,000
Estimated subunits	3	3	3	2	2
pH optimum	8.0	8.5	8.5	8.5	8.5
T_m	60.2 °C	nd	nd	nd	nd
K_m	3.4 mM	nd	1.5 mM	2.6 mM	4.8 mM
$\Delta G^{\#}$ at 298 K	69.6 kJ/mol	nd	nd	nd	Nd
$\Delta H^{\#}$	46.8 kJ/mol	nd	nd	nd	Nd
$\Delta S^{\#}$	−78 J/mol K	nd	nd	nd	Nd
Crystal structure	No	No	Yes	Yes	No
References	Hontzeas et al. (2004b)	Jacobson et al. (1994)	Karthikeyan et al. (2004a)	Minami et al. (1998), Yao et al. (2000)	Jia et al. (2000)

nd = not determined.

stressed plants (Glick, 1995; Glick et al., 1998; Jacobson et al., 1994). When ACC deaminase-containing plant growth-promoting bacteria are bound to a plant, they act as a sink for ACC ensuring that plant ethylene levels do not become elevated to the point where root growth is impaired (Glick, 1995). This activity is important during normal plant development and also protects plants from the deleterious effects of numerous environmental stresses, including flooding (Grichko and Glick, 2001), phytopathogens (Wang et al., 2000), salt (Mayak et al., 2004b), drought (Mayak et al., 2004a) and heavy metals (Belimov et al., 2001, 2005; Burd et al., 1998). Moreover, ACC deaminase-containing plant growth-promoting bacteria up-regulate genes involved with plant growth and protein production while down-regulating plant genes involved with ethylene stress and defence signaling pathways (Hontzeas et al., 2004a). The ACC deaminase-containing plant growth-promoting bacteria, in part, alleviate the need for the plant to actively defend itself against various environmental stresses (Hontzeas et al., 2004a; van Loon and Glick, 2004). The crystal structure has been determined for the yeast (Minami et al., 1998), and recently for the bacterial (Karthikeyan et al., 2004a,b) ACC deaminase enzymes; the biochemical and thermodynamic properties of the ACC deaminase from *Pseudomonas putida* UW4 have been measured (Hontzeas et al., 2004b). Here, we present and analyze recent results that focus on mechanistic studies that delve into the biochemical mechanism used by this important enzyme.

2. Primary structure and evolution of ACC deaminase

Until quite recently only a small number of bona fide ACC deaminase genes had been isolated and characterized. However, in the past few years a large number of bacterial ACC deaminase genes have been isolated and shown to encode ACC deaminase activity (summarized in Glick, 2005). Alignment of the amino acid sequences from some of the genes indicates that there is conservation of key amino acid residues (i.e. Lys^{51} Cys^{162}), both thought to be important for enzymatic activity. In addition, phylogenetic analysis of ACC deaminase genes indicates that many of these genes are inherited through horizontal gene transfer (Hontzeas et al., 2005), possibly because many of them are plasmid encoded. Very few ACC deaminase proteins have been purified and biochemically characterized; and crystal structures have been produced for two (Table 1). Characteristic of all ACC deaminase enzymes is their low affinity for the substrate ACC, always in the millimolar range.

2.1. Pyridoxal 5′-phosphate dependent enzymes and ACC deaminase

ACC deaminase is a member of a large group of enzymes that require pyridoxal 5′-phosphate (PLP) for enzymatic activity. PLP and pyridoxamine 5′-phosphate (PMP) are the two forms of vitamin B_6 that are cofactors for enzymes, many of which are involved in amino acid metabolism (Christen and Metzler, 1985). Jansonius (1998) classified PLP enzymes based on their three dimensional structure, into four folding types. These are i) tryptophan synthase, ii) aspartate aminotransferase, iii) D-amino acid aminotransferase and iv) alanine racemase (Jansonius, 1998). According to this classification scheme, ACC deaminase fits into the tryptophan synthase (TRPSβ) family. Initially, the association of PLP with its target enzyme and the mechanistic studies of PLP-mediated catalysis were thoroughly investigated