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Chloroplast biogenesis 91: Detection of δ-aminolevulinic acid esterases activity in higher plant and insect tissues ☆

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Abstract

δ-Aminolevulinic acid (ALA) esterase(s) is an enzyme or a family of enzymes that regenerate ALA from ALA esters by hydrolysis. These enzyme(s) are highly active in cancer cells. As a consequence ALA esters have been used to advantage in ALA-dependent photoradiation therapy, since ALA esters translocate better to sites of metabolism in cancer cells and tissues than free ALA. In this work, it is shown that ALA esterase(s) also occur in insect and plant tissues, but are less active than in cancer cells. In plant cells ALA esterase activity is observed in the cytosol as well as in the plastids where most of the activity is observed in the plastid stoma. The ALA esterase activity appears to be sensitive to the nature of the esterifying alcohol as well as to components of the incubation medium. The observed lower activity of ALA ester conversion to tetrapyrroles in insect and plant cells, in comparison to free ALA, suggests that the use of ALA esters in photodynamic insecticidal and herbicidal applications may not be as advantageous as their use in cancer photodynamic therapy treatments. It is proposed that ALA esterase(s) may be involved in the mobilization of sequestered and esterified ALA. Esterification and sequestering of excess ALA may be visualized as a mean of cellular detoxification.

Keywords: Amino acid esterases; Photodynamic herbicides; Photodynamic insecticides; Photodynamic cancericides; Amino acid esters; δ-amionolevulinic acid

1. Introduction

Tetrapyrrole-dependent photodynamic herbicides [1,2], insecticides [3,4], and their extension to cancericides [5–9] are compounds that force biological tissues to accumulate undesirable amounts of metabolic intermediates of the chlorophyll (Chl) and/or heme metabolic pathways,

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namely tetrapyrroles. In the light, the accumulated tetrapyrroles photosensitize the formation of singlet oxygen which kills treated tissues by oxidation of their cellular membranes. Tetrapyrrole-dependent photodynamic pesticides and cancericides usually consist of δ-aminolevulinic acid (ALA[1]), a 5-carbon amino acid, with or without one of several chemicals referred to as modulators. ALA and the modulators act in concert [4,10]. The amino acid serves as a building block of tetrapyrrole accumulation, while the modulator alters quantitatively and qualitatively the pattern of tetrapyrrole accumulation.

During the past two decades, Rebeiz and collaborators have developed excellent herbicidal formulation for use under greenhouse conditions. Unfortunately, extrapolation to field conditions has been less successful. Preliminary investigations have indicated that the thicker cuticle of plants grown under field conditions interferes with penetration of ALA to site of metabolism inside living cells [11]. Recently, it has come to our attention that several cancer photoradiation therapy researchers have solved the problem of ALA penetration in mammalian cancer cells by using apolar, esterified derivatives of ALA [12,13]. Once inside the cell, the ester groups are apparently hydrolyzed by putative ALA-esterases, which paves the way for the conversion of regenerated ALA to damaging tetrapyrroles.

The success of using ALA esters to improve ALA penetration in cancer cells raised the possibility of observing the same phenomenon in plant and insect tissues. In this work, we demonstrate that ALA esterase activity is detectable in plant and insect tissues, but to a lesser extent than in cancer cells.

2. Materials and methods

2.1. Plant material

Barley (*Hordeum vulgare*, Hi Barley Brand) seeds were purchased from Illini FS (Urbana, IL). Cucumber (*Cucumis sativus* var. Beit alpha) seeds were purchased from Hollar Seeds (Rocky Ford, CO). Germination was carried out in plastic trays containing wet vermiculate either in darkness or in a growth chamber illuminated with 1000-W metal halide lamps (211 $W m^{-2}$) under a 14-h light/10-h dark photoperiod. Etiolated or green tissues were harvested after 5–7 days of growth at 28 °C.

2.2. Insects

Trichoplusia ni (*T. ni*) larvae were provided by Dr. A.R. Zangerl, Department of Entomology, University of Illinois at Urbana-Champaign. The larvae were maintained in two ounce plastic containers (Solo Cup, Urbana, IL) on artificial diets as described in [14].

2.3. Cancer cells

Gibbon monkey lymphoma cell line MLA 144 was kindly provided by Dr. K.W. Kelly, Department of Animal Sciences, University of Illinois at Urbana-Champaign. Cells were cultured in RPMI 1640 (Gibco, Grand Island, NY) with 5% fetal bovine serum albumin (FBS) at 37 °C, 7% CO_2 and 95% relative humidity. Cells were maintained in the logarithmic phase of growth by passage 24 h prior to assay.

2.4. Chemicals

δ-Aminolevulinic acid was purchased from Biosynth International (Naperville, IL), δ-aminolevulinic acid methyl ester (ALAME) (Fig. 1), 1,10-phenanthroline (Oph), 2,2′-dipyridyl (Dpy), ninhydrin, and *p*-dimethylaminobenzaldehyde were purchased from Sigma Chemical (St. Louis, MO). ALA ethyl ester (ALAEE) and ALA butyl ester (ALABE) were synthesized as described below.

2.5. Synthesis of ALA derivatives

ALAEE and ALABE (Fig. 1) were synthesized from ALA and the corresponding alcohol as described by Kloek and van Henegouwen [12]. Essentially, the syntheses involve reactions of the alcohols with thionyl chloride at 4 °C. The purity of

[1] *Abbreviations used*: ALA, δ-aminolevulinic acid; ALAME, ALA methyl ester; ALAEE, ALA ethyl ester; ALABE, ALA butyl ester; Oph, 1,10-phenanthroline; Dpy, 2,2′-dipyridyl; TLC, thin layer chromatography; Proto, protoporphyrin IX; Mpe, Mg-Proto monomethyl ester; Pchlide *a*, protochlorophyllide *a*.