

Imidazolinone-Induced Loss of Acetohydroxyacid Synthase Activity in Maize Is Not Due to the Enzyme Degradation

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ABSTRACT

Acetohydroxyacid synthase (AHAS), the first enzyme leading to the biosynthesis of valine, leucine, and isoleucine, is inhibited by different chemical classes of herbicides. There is a loss in the extractable AHAS activity in imidazolinone-treated plants. Immunological studies using a monoclonal antibody against AHAS revealed no degradation of AHAS protein in imidazolinone-treated maize (*Zea mays*) plants. Therefore, the loss in AHAS activity is not due to the loss of AHAS protein.

The first enzymatic step common to the biosynthesis of the branched chain amino acids (valine, leucine, and isoleucine) is catalyzed by acetohydroxyacid synthase (also known as acetolactate synthase; EC 4.1.3.18). The enzyme catalyzes two parallel reactions: condensation of 2 mol of pyruvate to give rise to acetolactate, and condensation of pyruvate and α -ketobutyrate to yield acetohydroxybutyrate. AHAS¹ activity is inhibited by the end products of the pathway, *i.e.* valine, leucine, and isoleucine, which provides a mechanism for regulation of this pathway in higher plants (2). Biochemical and genetic studies have shown that AHAS is the target site of several classes of structurally unrelated herbicides, which include the imidazolinones, the sulfonylcarboxamides, the sulfonylureas, and the triazolopyrimidines (8).

If plants are treated with an imidazolinone herbicide and AHAS activity is then extracted from these plants and measured *in vitro*, there is a loss in extractable AHAS activity compared with untreated plants (5). This loss is specific for AHAS and is not due to a general loss in enzymatic activity from the herbicidal action of the chemical (3). Hawkes (1) speculated that this loss in extractable AHAS activity in plants treated with imidazolinones was the result of the formation of an enzyme/inhibitor complex that destabilizes the enzyme and leads to its degradation. Alternatively, the loss in AHAS activity could be due to the formation of an inactive enzyme/inhibitor complex that is not reversed during extraction and isolation of the enzyme.

Recently, a monoclonal antibody that recognizes AHAS from monocotyledonous plants has been produced (6). This antibody gives us a way to measure the levels of AHAS in monocots that is independent of enzymatic activity. The objective of our experiments was to determine if the change

in extractable AHAS activity after imidazolinone treatment was due to a rapid loss of the AHAS protein. The results show that imidazolinone treatment actually appears to stabilize the AHAS protein in relation to other proteins that are lost after herbicide treatment. Therefore, the loss of extractable AHAS after imidazolinone treatment cannot be due to a degradation of the enzyme.

MATERIALS AND METHODS

Chemicals

Technical grade imazaquin and imazethapyr were synthesized by American Cyanamid Co.

Plant Material

Three maize (*Zea mays*) varieties used in these experiments were an imidazolinone-susceptible hybrid (B73), an imidazolinone-resistant hybrid (XA17) (4), and Pioneer var 3475. Seeds were germinated on paper towels and then the seedlings grown hydroponically in a nutrient solution to the four leaf stage before herbicide treatment.

Enzyme Extraction and AHAS Assay

The procedures for the extraction and assay of AHAS have been previously described (7). Protein determinations were performed using Bio-Rad's protein assay dye reagent.

Immunological Procedures

Western blotting and solid phase ELISA procedures have been previously described (6).

RESULT AND DISCUSSION

In the first experiment, B73 and XA17 were treated via the root system with 5 μ M imazaquin in the nutrient solution for 24 h, and then plants were transferred to untreated nutrient solution. Plants were harvested 1, 2, and 3 d after initiation of treatment, and AHAS extracted and measured.

Extractable AHAS activity in the imidazolinone-sensitive maize hybrid B73 fell to less than 10% of the untreated level 1 d after treatment and remained at this level for the next 2 d (Fig. 1). There was also a loss of about 25% of the extractable AHAS activity in the imidazolinone-resistant maize hybrid XA17 by 2 d after treatment (Fig. 1). It has been shown that there are two genes expressing two different forms of AHAS activity in maize. One of these genes codes for approximately

¹ Abbreviations: AHAS, acetohydroxyacid synthase; B73, B73XMo17 maize hybrid; XA17, XA17XMo17 maize hybrid.

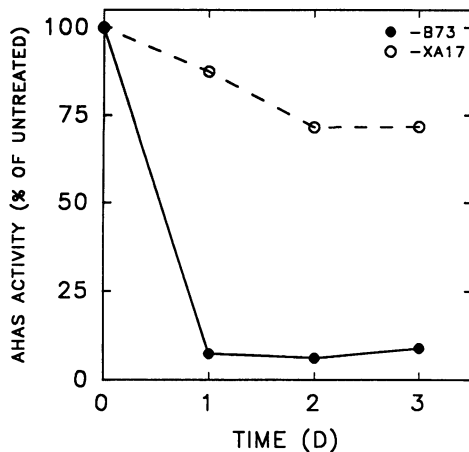


Figure 1. AHAS activity in B73 and XA17 after treatment with 5 μ M imazaquin. Plants were treated via the root system for the first day and then transferred to untreated nutrient solution. Plants were harvested on different days, as indicated in the figure, for analysis.

75% of the total AHAS activity, whereas the other gene product contributes the remaining 25% of the enzyme activity (4). The XA17 resistance is in the AHAS gene that contributes the majority of the AHAS activity. Thus, the loss in extractable AHAS activity in XA17 is due to the loss of the imidazolinone-sensitive AHAS enzyme activity.

Protein extracts used for AHAS activity determinations were examined for the fate of AHAS protein by western blotting. Results presented in Figure 2 show a blot in which equal amounts of protein from each treatment were loaded in individual lanes. There was no indication of degradation of AHAS protein (65 kD protein band) in the extracts from untreated or imazaquin-treated seedlings of B73 or XA17. In the extracts prepared from imazaquin-treated B73 seedlings, in which degradation of AHAS protein was predicted, the intensity of the AHAS protein band detected by this antibody

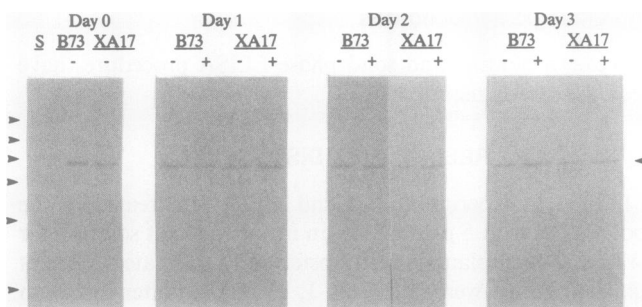


Figure 2. Western blotting using extracts of untreated and imazaquin-treated B73 and XA17 seedlings harvested at various times. Proteins (50 μ g for each sample) were electrophoresed, transferred onto a nitrocellulose membrane, and probed with a monoclonal antibody against AHAS according to the procedures previously described (6). S, molecular mass standards; -, without imazaquin; +, with imazaquin. Arrows on the left hand side indicate the molecular mass standards (200, 97.4, 68, 43, and 18.4 kD, respectively) and the arrow on the right hand side indicates the AHAS band.

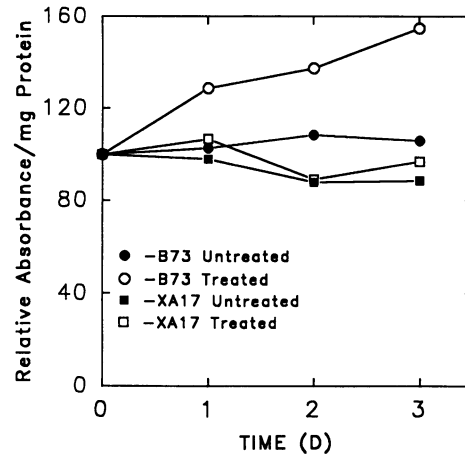


Figure 3. ELISA using extracts of untreated and imazaquin-treated B73 and XA17 seedlings harvested at various times. Equal amounts of protein from various treatments were coated in microtiter plates and the level of AHAS protein in these extracts was detected by a monoclonal antibody against AHAS according to the procedures described previously (6). The levels of AHAS protein per unit total protein present in these extracts were normalized to the level on day 0 for each treatment separately.

was even higher than in the extracts from untreated control. This observation was confirmed by quantitative determination of AHAS protein by ELISA as described below.

The levels of AHAS protein per unit total protein in the extracts were normalized to the level on day 0 for each treatment, *i.e.* the beginning of the experiment. The level of AHAS protein was relatively constant for the duration of the experiment in the extracts of untreated B73, untreated XA17, and imazaquin-treated XA17 (Fig. 3). Interestingly, contrary to the prediction, there was a progressive increase with time in the relative amount of AHAS protein in the extracts of imazaquin-treated B73.

To examine a longer-term exposure of imidazolinone on the fate of AHAS, imidazolinone-sensitive maize seedlings

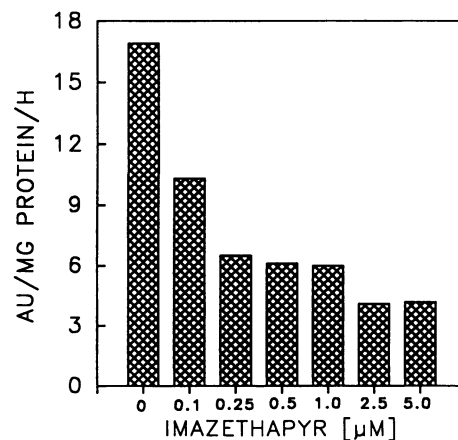


Figure 4. AHAS activity in the extracts of Pioneer 3475 seedlings after treatment with different concentrations of imazethapyr. Plants were treated via the root system for 7 d, and then harvested.

(Pioneer var 3475) were exposed to various concentrations of imazethapyr for 7 d. At the end of this period, plants were harvested and analyzed for AHAS as described above. There was an imazethapyr concentration-dependent loss of the enzyme activity (Fig. 4). Western blot analysis of the protein extracts from various treatments showed no degradation of AHAS protein (data not shown).

These results indicate that the imidazolinone-induced loss in extractable AHAS activity is due to a specific binding of the herbicide to the AHAS enzyme. Furthermore, contrary to the proposal of Hawkes (1), this binding does not appear to result in the destabilization of the AHAS protein and subsequent loss. The binding of the herbicide to the enzyme actually appears to stabilize the AHAS protein in relation to other proteins that are degraded after herbicide treatment. Therefore, the loss in extractable AHAS activity after imidazolinone treatment may be due to the formation of some type of slowly reversible or irreversible complex that is not broken during enzyme extraction and purification.

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