Interaction of Imidazolinones with Plant Acetohydroxy Acid Synthase: Evidence for in Vivo Binding and Competition with Sulfometuron Methyl

Dale L. Shaner,* Bijay K. Singh, and Mark A. Stidham

American Cyanamid Company, P.O. Box 400, Princeton, New Jersey 08540

In vitro, imidazolinones are uncompetitive inhibitors of plant acetohydroxy acid synthase (AHAS). If plants are treated with a lethal dose of an imidazolinone, the level of AHAS activity extracted from these plants is reduced more than 80%. This reduction begins within 1 h after treatment, and the level of reduction depends on the concentration of the herbicide within the plant tissue. Sulfometuron methyl, another plant AHAS inhibitor, does not cause a decrease in extractable AHAS activity. In addition, plant AHAS activity can be protected from the inhibitory effect of an imidazolinone by treating the plants with sulfometuron methyl either prior to or simultaneously with the imidazolinone. These results indicate that the imidazolinones are interacting with plant AHAS in some way in vivo such that the herbicide does not readily separate from the enzyme during the extraction procedure or the herbicide greatly destabilizes the enzyme. Furthermore, the results suggest that while this interaction is different from that of the sulfonylureas with AHAS, the two inhibitors must bind in some competitive manner with the enzyme in vivo.

The imidazolinones are potent, broad-spectrum herbicides that kill both monocots and dicots (Los, 1987). These herbicides kill plants by preventing the synthesis of the branched-chain amino acids through the inhibition of acetohydroxy acid synthase (AHAS), the first enzyme in the pathway (Shaner et al., 1984). The imidazolinones are slow, tight-binding inhibitors of plant AHAS. Muhitch et al. (1987) reported that AHAS activity measured in crude extracts from excised maize leaves and suspension cells was reduced by incubation of the tissue with an imidazolinone prior to enzyme extraction. They suggested that the inhibitor binds so tightly to the enzyme in vivo that it is not released during the enzyme extraction procedures.

The sulfonylureas are another class of herbicides that inhibit AHAS in plants (Ray, 1984) and are slow, tight-binding inhibitors of the enzyme (LaRossa and Schloss, 1984). Schloss et al. (1988) found that the imidazolinones could compete with sulfometuron methyl for bacterial AHAS. However, the interaction of these two chemicals on plant AHAS was not determined.

The purpose of this work was to further characterize the effect of the imidazolinones on extractable AHAS activity in plants and to determine whether other AHAS inhibitors affect the plant enzyme in a similar manner. The results show that the level of extractable AHAS activity from maize rapidly decreases in a concentration-dependent manner after treatment with an imidazolinone, while sulfometuron methyl, a sulfonylurea, does not cause a loss in extractable AHAS activity. Furthermore, sulfometuron methyl supplied either prior to or simultaneously with the imidazolinone prevents the reduction in extractable AHAS normally associated with imidazolinone treatment.

MATERIALS AND METHODS

Plant Material. Maize plants (Pioneer var. 3475) were greenhouse grown in a loamy sand soil. At time of treatment, plants in the three- to four-leaf stage were transferred to a growth chamber (28/20 °C day/night; 14-h photoperiod; 350 $\mu E \cdot m^{-2} \cdot h$ light intensity) to ensure uniform conditions for the treatment period.

Growth Measurements. Elongation of the newest emerging leaf was determined by measuring the distance between the tip of the leaf and the top of the pot to the closest millimeter. Measurements were taken at least daily. All treatments had four to five replications with one plant per replicate.

Chemical Application. *Intact Plants.* Herbicides were applied to whole plants either as a foliar spray with a belt sprayer delivering 400 L/ha spray volume or as a soil drench. Herbicide solutions applied as foliar sprays were made from technical grade herbicides solubilized in a solution containing 0.25% (v/v) Tween 20, a nonionic surfactant. Root drenches were made by solubilizing technical grade compounds in water.

Excised Plants. In excised plant experiments, the shoots of greenhouse-grown maize were cut with a razor blade from their roots under degassed water, and then the shoots were placed in 25 mL of one-tenth strength Hoagland's solution plus technical grade herbicide. The solution was adjusted to pH 6.0. The amount of herbicide solution absorbed by the plant over the time course of the experiment was determined by measuring the difference in weight of the excised shoot plus nutrient solution between the beginning and end of the experiment and assuming that the change in weight was due to the water absorbed and transpired by the shoots and that all of the herbicide contained in that water was left in the shoot. Loss of weight from evaporation was corrected by measuring the change in weight of containers without plants.

Tissue Harvest, Enzyme Extraction, and Measurement. Tissue was harvested and the enzyme extracted by the methods described in Muhitch et al. (1987). AHAS acitivty was measured in protein pellets after the pellets were desalted on a Sephadex G-25 column. The assay procedure described in Muhitch et al. (1987) was used. All experiments were repeated at least

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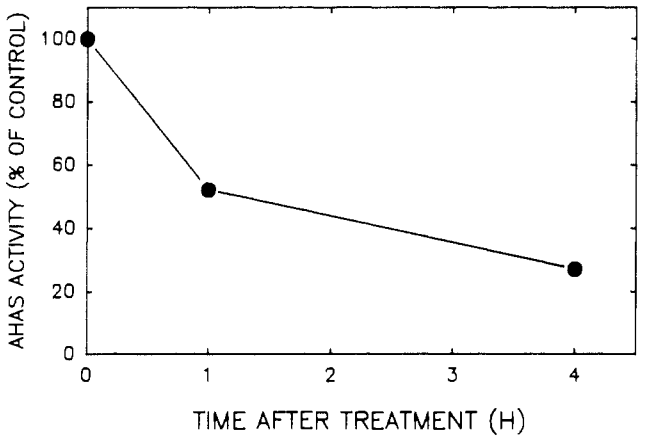

Figure 1. Decrease in extractable AHAS activity in excised corn shoots after a solution of 10 μM imazapyr was taken up in one-tenth Hoagland's solution. Control plants were fed the nutrient solution not containing herbicide. The specific activity of AHAS in control plants did not change over the time course of the experiment.

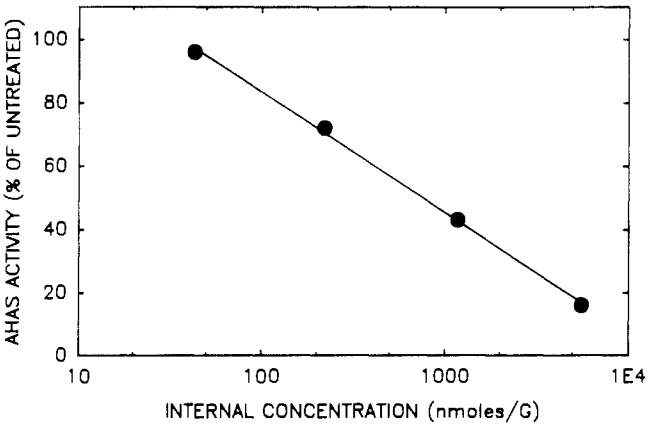

Figure 2. Relationship between internal concentration of imazapyr with extractable AHAS activity in excised corn shoots 4 h after introduction of the herbicide. The internal concentration was determined by feeding plants different concentrations of imazapyr and measuring the amount of solution taken up by the plants. The concentrations assume uniform distribution within the plant tissue.

once. Data are shown from representative experiments.

RESULTS

Feeding excised maize shoots with 10 μM imazapyr caused a rapid decrease in the level of extractable AHAS activity that was detectable within 1 h after initiation of the treatment (Figure 1). This decrease in extractable AHAS activity is not due to a mixing of the herbicide with the enzyme during the extraction procedure since spiking the extraction solution with 100 nmol of imazapyr/mL did not affect the level of enzyme activity.

The decrease in level of extractable AHAS activity was proportional to the internal concentration of the herbicide (Figure 2). If the herbicide was uniformly distributed throughout the plant tissue, the calculated internal I_{50} for imazapyr on extractable AHAS activity was 9 nmol/g of plant tissue 4 h after initiation of the experiment. This is roughly equivalent to the final I_{50} of imazapyr in an in vitro test after a 4-h incubation (Muhitch et al., 1987). The loss in extractable AHAS activity also occurred in intact maize treated with either imazaquin or imazethapyr applied either as a soil drench or as a foliar application (Figure 3).

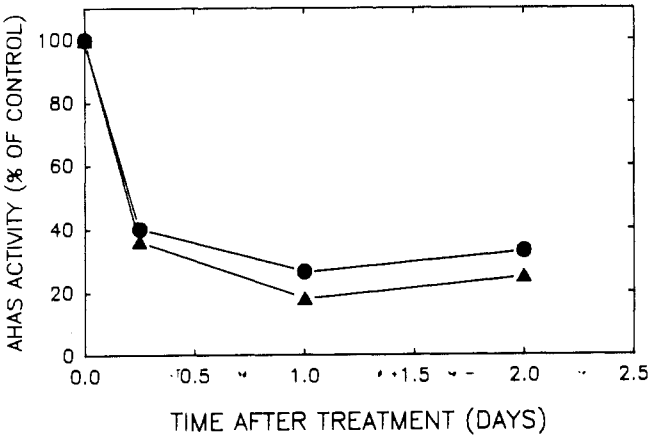

Figure 3. Effect of treating intact corn plants with either imazaquin (●) applied as a soil drench at a rate of 100 g/ha or imazethapyr (▲) applied as a foliar spray at a rate of 100 g/ha.

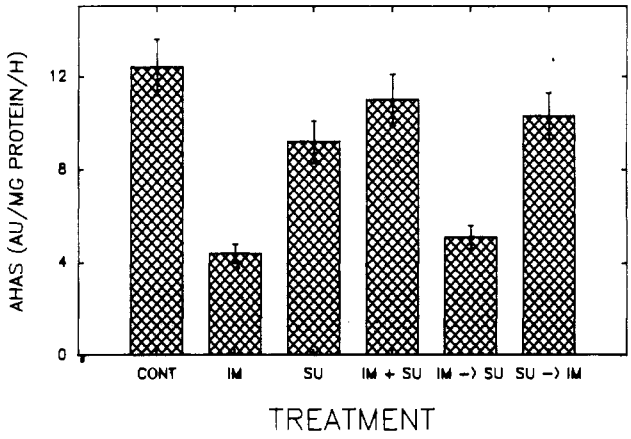

Figure 4. Effect of imazaquin, sulfometuron methyl, or combinations of imazaquin and sulfometuron methyl on extractable AHAS activity 3 days after treatment: Cont = control; SU = sulfometuron methyl; IM = imazaquin; IM + SU = imazaquin plus sulfometuron methyl; IM→SU = imazaquin followed by sulfometuron methyl 2 days later; SU→IM = sulfometuron methyl followed by imazaquin 2 days later. All herbicides were applied at a rate of 10 g/ha as a foliar spray. Bars denote ±1 standard deviation.

If maize was sprayed with herbicidal levels of sulfometuron methyl or imazaquin, either by itself or in combination, various effects on extractable AHAS occurred. As shown above, imazaquin greatly decreased the level of extractable AHAS activity (Figure 4). Sulfometuron methyl, on the other hand, had no effect on the level of extractable AHAS activity when applied alone (Figure 4), even though it had stopped growth of the plant (Figure 5). If the plants were treated with a mixture of both herbicides, growth was inhibited but there was no effect on extractable AHAS activity (Figure 4). Nor was there an effect if the plants were first treated with sulfometuron methyl and then sprayed with imazaquin 2 days later. However, plants treated first with imazaquin and then 2 days later with sulfometuron methyl did lose extractable AHAS activity (Figure 4).

This protecting effect of sulfometuron methyl could also be demonstrated when the herbicides were applied to excised plants via the transpiration stream. When applied alone, imazaquin greatly decreased the level of extractable AHAS activity (Figure 6) while sulfometuron methyl had no inhibitory effect. When the two herbicides were applied as a mixture, the level of extractable AHAS activity remained high.

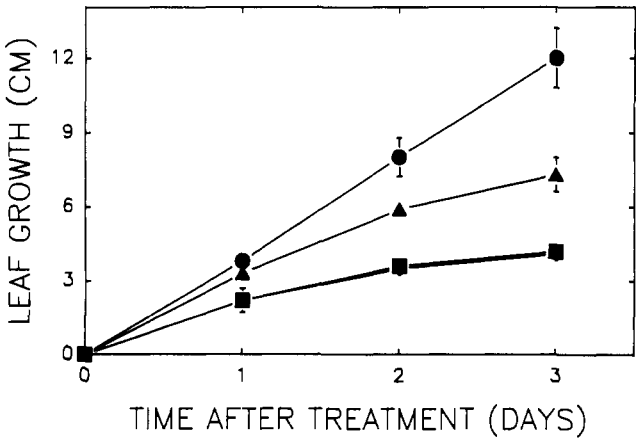

Figure 5. Effect of imazaquin, sulfometuron methyl, or combinations of imazaquin and sulfometuron methyl on leaf elongation of corn plants: (●) control; (▲) 10 g/ha imazaquin; (■) 10 g/ha sulfometuron methyl; (♦) 10 g/ha imazaquin + 10 g/ha sulfometuron methyl. All herbicides were applied as foliar sprays. Bars denote ±1 standard deviation.

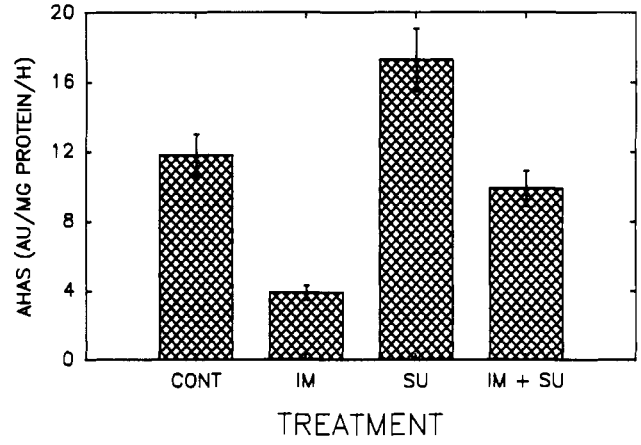

Figure 6. Effects of combinations of imazaquin and sulfometuron methyl on extractable AHAS activity in excised corn shoots 4 h after introduction of herbicides: CONT = control; IM = 1 μM imazaquin; SU = 1 nM sulfometuron methyl; IM + SU = 1 μM imazaquin plus 1 nM sulfometuron methyl. Bars denote ±1 standard deviation.

DISCUSSION

Many types of plant enzyme inhibitors decrease the level of extractable enzyme activity. Such inhibitors include tabtoxin β-lactam (Frantz et al., 1982), which decreases glutamine synthetase activity, and phaseolotoxin (Turner, 1986), which decreases ornithine carbamoyltransferase. While our data show that imidazolinones cause a rapid loss in extractable AHAS activity, these herbicides differ from tabtoxin β-lactam and phaseolotoxin, which inactivate irreversibly their respective enzymes at the active sites. Imidazolinones are uncompetitive inhibitors of AHAS (Shaner et al., 1984), and their site of action appears not to involve the substrate site directly.

The mechanism of loss in extractable AHAS activity associated with the imidazolinones is not known. This phenomenon could be explained by at least two different mechanisms. One possibility is the formation of a relatively stable imidazolinone–enzyme complex in vivo, which does not readily dissociate during the extraction procedure. Another possible explanation is that AHAS becomes very unstable when bound with an imidazolinone and is rapidly broken down or degraded either in the plant or during the extraction procedure. If antibody to the intact AHAS protein were available, one could distinguish between these two possibilities by determining whether or not the enzyme protein disappears after imidazolinone treatment or remains in an inactive form.

Schloss et al. (1988) showed that imidazolinones and sulfonylureas compete for the same binding site on bacterial AHAS. Our data suggest that these two herbicides also compete for a common site on the plant AHAS enzyme. This conclusion is further supported by the interaction of these two herbicides with AHAS from imidazolinone resistant corn. One of these corn lines, XA17, is insensitivie to both imidazolinones and sulfonylureas (Newhouse et al., 1989). This line is resistant to these herbicides due to a mutation in the AHAS gene, which results in an enzyme that is no longer inhibited by either class of herbicides. These results indicate that the mutation affects the binding of both chemical classes.

Although these herbicides appear to compete for the same site on AHAS, the binding of the two classes of compounds to the enzyme must also differ in some respect. These data show that sulfometuron methyl does not cause the same effect on extractable AHAS activity as the imidazolinones, indicating that this sulfonylurea is binding in some way different from that of the imidazolinones. Other reports also show that the binding of the imidazolinones and sulfonylureas to AHAS is not identical. Selection for imidazolinone- and sulfonylurea-resistant plants has shown that mutant lines can be selected that are resistant to only one of these classes of herbicides or to both (Saxena and King, 1988; Winder and Spalding, 1988; Newhouse et al., 1989). This phenomenon would not occur if the two classes of herbicides bind at the same site on plant AHAS. An analogous case is true for the photosynthetic inhibitors (Galloway and Mets, 1984; Ericson et al., 1985).

The sulfonylureas are much more tightly bound to plant AHAS than the imidazolinones (Ray, 1984; Shaner et al., 1984). Although these data would indicate that sulfometuron methyl potentially is much more herbicidally active than the imidazolinones, the differences in the effective dose of these two chemicals at the whole plant level are relatively small. One possible explanation for this phenomenon is that the imidazolinones bind more tightly to the plant enzyme in vivo than in vitro data would indicate. Additionally, some differences in the predicted versus actual herbicidal activity of these two types of herbicides may also be a function of differences in the absorption, translocation, and metabolism of the herbicides. These data are the first to show an effect of inhibitors on the levels of extractable plant AHAS activity. They also clearly show there are differences between the binding of the two different classes of inhibitors to this plant enzyme. While the mechanisms involved in this effect still need to be elucidated, research on this area will provide additional information on the herbicidal activity of the imidazolinones. Other areas of research include the effects on AHAS from imidazolinone-resistant crops and on crops that tolerate the imidazolinones due to their ability to metabolize the herbicides as well as the mechanisms causing this loss in extractable AHAS activity.

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Received for review July 11, 1989. Accepted January 18, 1990.

Inhibition of Acetyl-Coenzyme A Carboxylase by Two Classes of Grass-Selective Herbicides

Alan R. Rendina,*,‡ Adrienne C. Craig-Kennard, Jacqueline D. Beaudoin, and Mary K. Breen

Chevron Chemical Company, P.O. Box 4010, Richmond, California 94804

The selective grass herbicides diclofop, haloxyfop, and trifop ([(aryloxy)phenoxy]propionic acids) and alloxydim, sethoxydim, and clethodim (cyclohexanediones) are potent, reversible inhibitors of acetyl-coenzyme A carboxylase (ACC) partially purified from barley, corn, and wheat. Although inhibition of the wheat enzyme by clethodim and diclofop is noncompetitive versus each of the substrates adenosine triphosphate (ATP), HCO_3^-, and acetyl-coenzyme A (acetyl-CoA), diclofop and clethodim are nearly competitive versus acetyl-CoA since the level of inhibition is most sensitive to the concentration of acetyl-CoA ($K_{is} < K_{ii}$). To conclusively show whether the herbicides interact at the biotin carboxylation site or the carboxyl transfer site, the inhibition of isotope exchange and partial reactions catalyzed at each site was studied with the wheat enzyme. Only the [^{14}C]acetyl-CoA–malonyl-CoA exchange and decarboxylation of [^{14}C]malonyl-CoA reactions are strongly inhibited by clethodim and diclofop, suggesting that the herbicides interfere with the carboxyl transfer site rather than the biotin carboxylation site of the enzyme. Double-inhibition studies with diclofop and clethodim suggest that the [(aryloxy)phenoxy]propionic acid and cyclohexanedione herbicides may bind to the same region of the enzyme.

There are two major chemical classes of postemergence herbicides that are used for the control of annual and perennial grasses (monocots) in a large variety of broad-leaved crop plants (dicots): substituted 1,3-cyclohexanediones (alloxydim, sethoxydim, clethodim) (for reviews see Iwataki and Hirono (1979) and Ishikawa et al. (1985)) and various derivatized phenoxypropionic acids (diclofop, haloxyfop, trifop) (for a review see Nestler (1982)). The structures and common names for representatives of each class are shown in Table I.

Studies on the uptake, translocation, and metabolic fate of both classes of herbicides in tolerant and susceptible plants have shown that the susceptibility of monocotyledenous species is probably not due to differential metabolism of the compounds to nonherbicidal forms or to differential uptake or transport (Swisher and Corbin, 1982; Veerasekaran and Catchpole, 1982; Buhler et al., 1985). A number of recent studies have shown that representatives of both classes of herbicides inhibited de novo fatty acid biosynthesis in isolated chloroplasts, cell cultures, or leaves of susceptible grasses such as corn, wheat, and wild oats but not in tolerant broad-leaved plants such as soybean, spinach, and sugar beet (Burgstahler and Lichtenthaler, 1984; Hoppe, 1985; Hoppe and Zacher, 1985;

‡ Present address: Agricultural Products Department, E. I. du Pont de Nemours and Co., Inc., Wilmington, DE 19898.

0021-8561/90/1438-1282$02.50/0