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Communication

Inhibition of Acetyl-CoA Carboxylase Activity by Haloxyfop and Tralkoxydim

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ABSTRACT

Acetyl-coenzyme A (CoA) carboxylase from maize (*Zea mays* L.) is inhibited by nanomolar concentrations of both haloxyfop, an aryloxyphenoxypropionate, and tralkoxydim, a cyclohexanedione herbicide. These results suggest that acetyl-CoA carboxylase, which catalyzes the first committed step in fatty acid biosynthesis, may be the target of these herbicides, contrary to an earlier report suggesting that aryloxyphenoxypropionate herbicides do not inhibit acetyl-CoA carboxylase.

Aryloxyphenoxypropionates and cyclohexanediones are two classes of herbicides that control many monocotyledoneous species. Although these herbicides are structurally very different, there has been some conjecture based upon similarities in selectivity and symptomology that these herbicides have a similar mode of action. There have been independent reports that these herbicides disrupt lipid metabolism (2, 6–11).

Research by Hoppe (7–9) and Hoppe and Zacher (10, 11) has indicated that the aryloxyphenoxypropionate diclofop-methyl (2-[4-(2,4-dichlorophenoxy)phenoxy]-methylpropanoate) inhibits fatty acid synthesis in maize (*Zea mays* L.) root tips. Diclofop likely disrupts an early step in fatty acid biosynthesis because it inhibits acetate incorporation into fatty acids in maize chloroplasts, but not in chloroplasts of a species (*Phaseolus vulgaris* L.) insensitive to the herbicide (11). Another aryloxyphenoxypropionate, haloxyfop (2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid), reduces acetate uptake in maize suspension cells (2). The cyclohexanedione sethoxydim (2-[1-(ethoximino)butyl-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) has been shown to inhibit lipid synthesis in soybean (*Glycine max* [L.] Merr.) cell cultures (6). The exact site or mode of action of these herbicides, however, is not known.

It has been reported that an aryloxyphenoxypropionate herbicide can act as a hypolipidemic drug by reducing serum cholesterol and triacylglycerol levels in animals (4). Some hypolipidemic drugs act by directly or indirectly inhibiting acetyl coenzyme A carboxylase (acetyl-coenzyme A: bicarbonate ligase [ATP], EC 6.4.1.2) activity (1). We report here that a cyclohexanedione and two aryloxyphenoxypropionate herbicides are very effective inhibitors of ACCase[1] activity in maize.

MATERIALS AND METHODS

Plant Material. Maize (Pioneer hybrid '3780') plants were grown in a glasshouse with a 14.5 h light period.

Chemicals. Acetyl-CoA, ATP, and DTT were purchased from Sigma. $NaH^{14}CO_3$ was purchased from Amersham. Two aryloxyphenoxypropionic acids were used: (a) haloxyfop (Fig. 1), which is being developed by The Dow Chemical Company under the trademarked name of Verdict, and (b) diclofop, which is marketed by Hoescht under the trademarked name of Hoelon. For a cyclohexanedione, we used tralkoxydim (2-[1-ethoxyimino)propyl]-3-hydroxy-5-mesitylcyclohex-2-enone) (Fig. 1), which is being developed by ICI under the trademarked name of Grasp. Haloxyfop acid was supplied by N. Kurihara (Dow Chemical USA). Diclofop acid was synthesized by K. Arndt (Dow Chemical USA). The R(+) (98% enantiomeric excess) and S(−) (94% enantiomeric excess) enantiomers of haloxyfop acid and tralkoxydim were synthesized by S. Moore (Dow Chemical USA). The R(+) and S(−) enantiomers of aryloxyphenoxypropionates differ in the configuration around the chiral carbon at the 2 position of the propionate moiety of the molecule. The R(+) form is herbicidally active (14).

Extract Preparation. Extracts were prepared from entire shoots of 8 to 10-d-old maize plants. All manipulations were carried out at 4°C. The tissue was ground with a mortar and pestle in approximately two volumes of buffer (100 mM Tricine-KOH, pH 8.3, 10% [v/v] glycerol, 10 mM β-mercaptoethanol, 1 mM Na_2EDTA, and 1 mM phenylmethyl sulfonyl fluoride). After being filtered through 4 layers of cheesecloth and 2 layers of Miracloth (Calbiochem), the homogenate was centrifuged for 20 min at 30,000 *g*. Solid polyethylene glycol (Sigma P-2139) was added to the supernatant to make a 6% (w/v) solution. After being stirred for 20 min, the solution was centrifuged for 20 min at 30,000 *g*. The supernatant was made to 14% (w/v) polyethylene glycol and stirred for 20 min before being centrifuged for 20 min at 30,000 *g*. The resulting pellet was suspended in a buffer containing 10 mM Tricine-KOH, pH 7.8, and 10% glycerol and

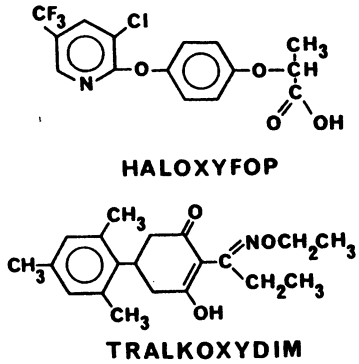

FIG. 1. The chemical structures of haloxyfop and tralkoxydim.

[1] Abbrevitations: ACCase, acetyl coenzyme A carboxylase.

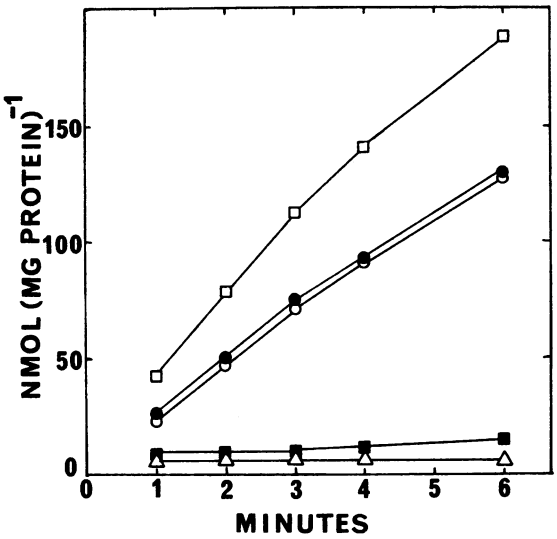

FIG. 2. Time course of ACCase activity. The treatments are control (□), 1 μM haloxyfop (●), 1 μM tralkoxydim (○), control without ATP (■), and control without acetyl-CoA (△).

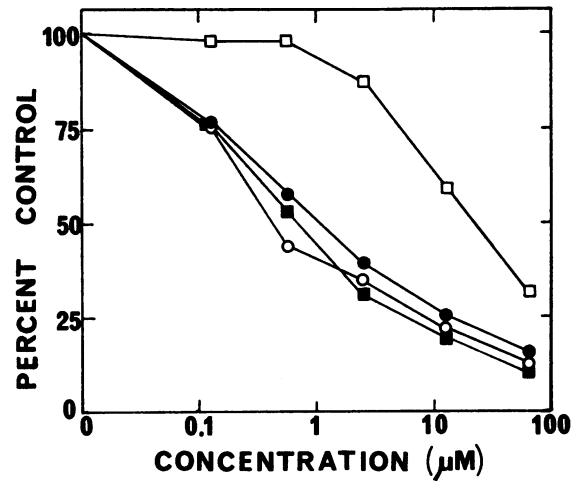

FIG. 3. Dose response of S(−) haloxyfop (□), a racemic mixture of haloxyfop (●), R(+) haloxyfop (■), and tralkoxydim (○) on ACCase activity. Reaction time was 6 min. Control rate was 20.9 nmol min^{-1} (mg protein)$^{-1}$.

then centrifuged for 5 min at 10,000 *g*. The supernatant was used for enzyme assays after being diluted to approximately 1 mg protein per ml. Fractionation by polyethylene glycol precipitation increased specific activity of the enzyme by about 10 fold while considerably decreasing acetyl-CoA independent ^{14}C incorporation.

Acetyl-CoA Carboxylase Assay. ACCase activity was assayed at 35°C in a fume hood by the acetyl-CoA dependent incorporation of $H^{14}CO_3^-$ into acid and heat stable products. The final reaction mixture contained 50 mM Tricine-KOH, pH 8.3, 5 mM $MgCl_2$, 2 mM DTT, 2 mM ATP, 15 mM $NaH^{14}CO_3$ (12.3 kBq (0.33 μCi)/μmol), approximately 0.2 μg protein/μl, 0.3 mM acetyl-CoA, and various concentrations of treatments. The reaction mixtures were incubated for 15 min at 35°C before initiating the reaction with acetyl-CoA. For multiple time point assays, 200 μl aliquots were removed from reaction mixtures and transferred to 7 ml scintillation vials containing 50 μl 6 N HCl, which terminated the reaction. For single time point assays, reactions were carried out in 7 ml scintillation vials containing 250 μl of reaction mixture and terminated by the addition of 50 μl 6 N HCl. After reactions were terminated, the contents of the vials were dried at 90°C. The dried residue was solubilized with 250 μl H_2O and 4 ml of scintillation fluid (Insta-Gel, Packard, Downers Grove, IL) was added. Radioactivity was determined using a LKB Model 1217 liquid scintillation counter.

Treatments. The compounds were made to 125 mg/L in a solution containing 1% acetone, 50 mM Tricine-KOH, pH 8.3, and 5 mM $MgCl_2$. Dilutions were made with the same solution, which was also used as the control treatment. Final acetone concentration in the assay mixture was 0.2%, which had no effect on the reaction.

RESULTS

Reaction Characteristics. The incorporation of $H^{14}CO_3^-$ into acid and heat stable products was dependent on the presence of both acetyl-CoA and ATP (Fig. 2). There was no incorporation of radioactivity when the enzyme preparation was omitted or when the extract was incubated for 5 min at 100°C before being assayed (data not shown).

Inhibition of ACCase Activity. ACCase activity was inhibited by about 40% in the presence of 1 μM haloxyfop or tralkoxydim (Fig. 2). Diclofop showed a greater inhibition (data not shown). With the exception of the S(−) enantiomer of haloxyfop, the compounds inhibited maize ACCase activity in a concentration-dependent manner (Fig. 3). Inhibition of activity ranged from about 25% at a herbicide concentration of 125 nM to more than 80% at a concentration of 75 μM. The inhibition caused by the S(−) enantiomer of haloxyfop could be accounted for by the 3% contamination in the S(−) preparation by the R(+) enantiomer, therefore the S(−) enantiomer was essentially ineffective.

DISCUSSION

To our knowledge this is the first report of an enzyme being inhibited by both an aryloxyphenoxypropionate and a cyclohexanedione. There has been some indication that haloxyfop inhibits the pyruvate dehydrogenase complex, but at relatively high (mM) concentrations (3). Therefore, it is unlikely that this effect has a significance *in vivo*. We have demonstrated that ACCase activity is inhibited at relatively low (10^{-7} to 10^{-6} M) herbicide concentrations in a dose-dependent manner.

The results of an earlier study by Hoppe and Zacher (10) suggested that ACCase activity was not inhibited by diclofop because the herbicide inhibited equally the incorporation of both [^{14}C]acetate and [^{14}C]malonate into polar lipids in maize root tips. The assumption in their work was that malonic acid was esterified to malonyl-CoA, which entered the fatty acid biosynthetic pathway. The direct conversion of malonic acid into malonyl-CoA requires a thiokinase. In many species the thiokinase is less active than is a malonate decarboxylase, which converts malonic acid to acetic acid (5). Thus, an active malonate decarboxylase would cause added malonic acid to behave like acetate. Furthermore, malonic acid is not necessarily converted directly into malonyl-CoA nor is malonyl-CoA obligatorily converted into fatty acids because there are several biosynthetic pathways utilizing malonic acid or malonyl-CoA (5, 12). In addition, there is doubt whether malonate is used at all as a precursor for fatty acid biosynthesis (13).

We do not present any direct evidence that these herbicides kill plants by inhibiting ACCase activity *in vivo*. However, in the case of the aryloxyphenoxypropionates, there is some circumstantial evidence for implicating ACCase as the site of action. Physiological processes related to lipid metabolism have been inhibited at herbicide concentrations similar to the range reported here for inhibition of ACCase activity. For example, Cho *et al.* (3) showed that [^{14}C]acetate incorporation into lipids of maize cells was inhibited 43% after 24 h of a treatment of 0.1

μM haloxyfop. At concentrations as low as 0.1 μM, several aryloxyphenoxy propionate herbicides and analogs inhibit [^{14}C] acetate incorporation into fatty acids in maize chloroplasts after a 60 min incubation (11). Other evidence suggesting that ACCase is the site of action of aryloxyphenoxypropionates is that ACCase activity is inhibited only by the R(+) and not the herbicidally inactive S(−) enantiomer. It has been proposed that the source of differences in activity between the two enantiomeric forms lies in the recognition of the active form by a receptor (or binding site) since there seem to be little or no differences between uptake and subsequent translocation of the two enantiomeric forms of aryloxyphenoxypropionates (15). Finally, data from our laboratory indicate that selectivity of aryloxyphenoxypropionate and cyclohexanedione herbicides among resistant and susceptible species are correlated to sensitivity of ACCase to these herbicides.

LITERATURE CITED

1. Beyen AC, MJH Geelen 1982 Short term inhibition of fatty acid biosynthesis in isolated hepatocytes by mono-aromatic compounds. Toxicology 24: 184–197
2. Cho H-Y, JM Widholm, FW Slife 1986 Effects of haloxyfop on corn (*Zea mays*) and soybean (*Glycine max*) cell suspension cultures. Weed Sci 34: 496–501
3. Cho H-Y, JM Widholm, FW Slife 1987 Inhibitory effects of haloxyfop on pyruvate dehydrogenase complex and carbon metabolism. Weed Sci Soc Am Abstr 27: 62
4. Granzer E, H Nahm 1973 HCG 004, a new highly potent hypolipidaemic drug. Arneim Forsch 23: 1353–1354
5. Hatch MD, PK Stumpf 1962 Fat metabolism in plants. XVII. Metabolism of malonic acid and its α-substituted derivatives in plants. Plant Physiol 37: 121–126
6. Hatzios KK 1982 Effects of sethoxydim on the metabolism of isolated leaf cells of soybean [*Glycine max* (L.) Merr.]. Plant Cell Rep 1: 87–90
7. Hoppe HH 1980 Veränderungen der Membranepermeabilität, des Kolenhydratgehaltes, des Lipidgehaltes und Lipidzusammensetzung in Keimwurzelspitzen von *Zea mays* L. nach Behandlung mit Diclofopmethyl. Z Pflanzenphysiol 100: 414–426
8. Hoppe HH 1981 Einfluß von Diclofop-methyl auf die Protein-, Nukleinsäure- und Lipidbiosynthese der Keimwurzelspitzen von *Zea mays* L. Z Pflanzenphysiol 102: 189–197
9. Hoppe HH 1985 Differential effect of diclofop-methyl on fatty acid biosynthesis in leaves of senstive and tolerant plant species. Pest Biochem Physiol 23: 297–308
10. Hoppe HH, H Zacher 1982 Hemmung der Fettsäurebiosynthese durch Diclopfop-methyl in Keimwurzelspitzen von *Zea mays*. Z Pflanzenphysiol 106: 287–298
11. Hoppe HH, H Zacher 1985 Inhibition of fatty acid biosynthsis in isolated bean and maize chloroplasts by herbicidal phenoxy-phenoxypropionic acid derivatives and structurally related compounds. Pest Biochem Physiol 24: 298–305
12. Nikolau BJ, ES Wurtele, PK Stump 1984 Tissue distribution of acetyl-coenzyme A carboxylase in leaves. Plant Physiol 75: 895–901
13. Roughan PG, R Holland, CR Slack, JB Mudd 1978 Acetate is the preferred substrate for long-chain fatty acid synthesis in isolated spinach chloroplasts. Biochem J 184: 565–569
14. Sakata G, K Makino, K Morimoto, T Ikai, S Hasebe 1985 Synthesis and herbicidal activity of optically active ethyl 2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]propanoate. J Pest Sci 10: 69–73
15. Uchiyama M, N Washio, T Ikai, H Igarashi, K Suzuki 1986 Stereospecific responses to (R)-(+) and (S)-(−) quizalofop-ethyl in tissues of several plants. J Pest Sci 11: 459–467