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Haloxyfop Inhibition of the Pyruvate and the α-Ketoglutarate Dehydrogenase Complexes of Corn (*Zea mays* L.) and Soybean (*Glycine max* [L.] Merr.)[1]

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ABSTRACT

The grass-specific herbicide haloxyfop, ((±)-2-[4-((3-chloro-5-(trifluoromethyl)-2-pyridinyl)oxy)-phenoxy] propionic acid) has been shown to inhibit lipid synthesis and respiration, to cause the accumulation of amino acids, and not to affect cellular sugar or ATP levels. Thus studies were carried out with enzyme activities from corn (*Zea mays* L.) (haloxyfop sensitive) and soybean (*Glycine max* [L.] Merr.) (haloxyfop tolerant) to locate the possible inhibition sites among the glycolytic and tricarboxylic acid (TCA) cycle enzymes. Following along the oxidative metabolism pathway of sugars, the pyruvate dehydrogenase complex (PDC) was the first enzyme among the glycolytic enzymes that demonstrated noticeable inhibition by 1 millimolar haloxyfop. Kinetic studies with corn and soybean PDC from both purified etioplasts and mitochondria gave K_i values of from 1 to 10 millimolar. Haloxyfop also inhibited the activity of the TCA cycle enzyme, the α-ketoglutarate dehydrogenase complex (α-KGDC) which carries out the same reaction as PDC except for the substitution of α-ketoglutarate for pyruvate as one of the substrates. The K_i values were somewhat lower in this case (near 1 millimolar). The relatively high K_i values for both enzyme complexes would indicate that these may not be the herbicidal sites of inhibition, but it is possible that the herbicide could be concentrated in compartments and/or the substrate concentrations may be well below optimal. Likewise little difference was seen in the haloxyfop inhibition of the enzyme activities from the sensitive species, corn, and from the tolerant species, soybean, so the selectivity of the herbicide is not evident from these results. The inhibition of the PDC and α-KGDC as the mode of action of haloxyfop is, however, consistent with the observed physiological effects of the herbicide, and these are the only enzymic activities so far found to be sensitive to haloxyfop.

Haloxyfop is a newly developed grass-specific herbicide used for weed control in broad leaf crops as described previously (4). Because of the distinctive chemical structural linkage of two phenol groups, haloxyfop and its analogs are often called phenoxy-phenoxy compounds. Because this group of herbicides and various derivatized cyclohexene compounds display almost identical herbicidal selectivity and injury symptoms, they are regarded as being similar compounds on a biological basis.

Necrosis in meristematic tissue and necrosis and chlorosis in developing leaf tissue are the most common injury symptoms in the field following application of these herbicides (1, 5, 8). Results of biochemical and physiological experiments using these grass-specific herbicides showed the following: (a) an accumulation of free sugars in mature leaves of the injured plant (1, 5), (b) the appearance of purple leaf coloration due to the accumulation of anthocyanin (1), (c) the simultaneous inhibition of respiration and lipid synthesis in cultured cells (4), (d) no change in protein synthesis or cellular ATP content in the suspension cultured cells (4), and (e) no direct effects on net photosynthetic activity in whole plants (8). The alteration of the lipid composition of chloroplast or mitochondrial membranes due to inhibition of fatty acid synthesis by diclofop (11–14) or sethoxydim (10) was proposed to be the primary mode of action of these herbicides. With the relationship between the effect of diclofop on auxin-induced cell elongation (25) and inhibitory effects on oleic acid synthesis (14), the mode of action of diclofop was postulated to be inhibition of fatty acid synthesis. Because of the perturbation of the plasmalemma transport function by diclofop, the herbicidal activity was also suggested to be due to a proton ionophore activity (19). However, herbicides in this group do not seem to directly affect membrane function because apparent photosynthesis of sethoxydim-treated corn leaves was not changed until visible symptoms were observed in meristematic tissue (8).

The occurrence of injury symptoms in meristematic tissues and in newly developing leaf tissue with the accumulation of free sugars in mature leaves (1, 5) suggested that the inhibition sites might be located either where translocation of photosynthetic products takes place or at the site where the translocated photosynthetic products are metabolized. The results of studies using corn suspension cultures incubated with [^{14}C]sucrose indicated that haloxyfop at a sublethal concentration (0.1 μM) inhibited lipid synthesis and respiration without reducing cellular ATP and free sugar levels (4). The use of this cell culture system eliminates the possibility of blockage of translocation of photosynthate. The results obtained with the corn suspension culture (4) indicate that the site of inhibition by haloxyfop might be located somewhere either in the glycolytic or tricarboxylic acid pathway, because the cellular ATP level was not affected despite the severe inhibition of respiration and lipid synthesis.

The objectives of this study were to determine if haloxyfop inhibited any of the glycolytic and TCA cycle enzymes to provide a relationship between this enzyme inhibition and prior observations of the possible mechanism of action of haloxyfop and its related analogs.

MATERIALS AND METHODS

Preparation of Organelle Enzymes. Corn (*Zea mays* L.) seeds 'Asgrow Rx 777' were germinated in the dark with 10^{-4} M $CaCl_2$ solution in vermiculite, at 30°C, in a germinator for 6 d. Soybean (*Glycine max* [L.] Merr.) seeds 'Fayette' were surface-sterilized with 10% sodium hypochlorite solution for 20 min followed by soaking in 10^{-4} M $CaCl_2$ solution overnight. The seeds were

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germinated at 23°C in the dark under a continuous spray of tap water for 5 d. Etiolated corn shoots and soybean hypocotyls were harvested under green light and were used immediately for organelle isolation Etioplasts were isolated and purified from etiolated corn and soybean shoots by the method of Leech and Leese (18) with minor modifications. Mitochondria were isolated and purified from the resulting supernatant by the method of Goldstein *et al.* (9) with the following modification: centrifugation with 20% (v/v) Percoll in isolation medium at 26,000*g* (r_{max}) using a Sorvall HB-4 rotor at 0°C. The organelle enzymes were extracted using the method of Reid *et al.* (21) with slight modifications. The purified etioplast and mitochondria preparations were resuspended in acetone at −20°C using a Teflon homogenizer followed by centrifugation at 12,000*g*, 0°C for 10 min. The process was repeated three times using the same parameters. The final pellets were dried under a stream of nitrogen gas and stored desiccated at −20°C until use for kinetic studies for PDC[3] and other TCA cycle enzymes listed below.

PDC Assay. Either mitochondrial or etioplast powders were resuspended using a Teflon homogenizer in 100 mM Tes (pH 7.5), 1 mM TPP, 5 mM DTT, 2 mM $MgSO_4$, 10 μM leupeptin, and 15% ethylene glycol (v:v); or 100 mM Bicine (pH 8.0), 1 mM TPP, 5 mM DTT, 2 mM $MgSO_4$, 10 μM leupeptin, and 15% ethylene glycol (v:v). The suspensions were cleared by centrifugation (0°C, 27,000*g*) for 15 min, and the supernatant was used for the enzyme assay. No detectable PDC activity was found in the pellet. Protein was determined by the method of Bradford (2) with BSA (Fraction V, Sigma) as the standard.

With the partially purified enzyme extracts from etioplasts and mitochondria, PDC activity was determined spectrophotometrically by the methods of Williams and Randall (30) with slight modifications. All spectrophotometric assays were performed using a Beckman DU-40 or DU-7 spectrophotometer, in 1 cm path length cells, at 30°C, and the activity was expressed as the change in absorbance at 340 nm/min. The reaction mixture for the mitochondrial PDC contained 50 mM Tes (pH 7.5), 0.2 mM TPP, 2.0 mM $MgCl_2$, 2.3 mM NAD^+, 0.12 mM Li salt of CoA, 2.6 mM cysteine·HCl, 1.5 mM sodium pyruvate, and the suspended PDC activity in a total volume of 1 ml. The reaction mixture for the etioplast PDC assay contained 50 mM Bicine (pH 8.0), 0.2 mM TPP, 2.3 mM NAD^+, 2.6 mM cysteine·HCl, 0.12 mM Li salt of CoA, 10.0 mM $MgCl_2$, 1.5 mM sodium pyruvate, and the suspended PDC activity in a total volume of 1 ml.

To investigate possible inhibitory effects of haloxyfop on other components of PDC, dihydroxylipoamide dehydrogenase activity was assayed using the method of Reid *et al.* (21). Haloxyfop solution dissolved in 25 mM Tes (pH 7.3) was added to the reaction system as the inhibitor at final concentrations of 0.1, 0.5, 1.0, and 2.0 mM. The assay mixture for the dihydroxylipoamide dehydrogenase component of the PDC contained 1 mM dihydroxylipoamide, 1 mM NAD^+, 1 mM cysteine·HCl, and 25 mM Tes (pH 7.3) in a volume of 1 ml. Dihydroxylipoamide was prepared by the method of Reed *et al.* (20). The assay mixture plus enzyme was incubated at 25°C for 1 min in the absence of NAD^+ or dihydroxylipoamide and the reaction initiated by its addition.

α-KGDC Assay. The enzyme solution was prepared from the corn and soybean mitochondria powders by resuspending using a Teflon homogenizer in 100 mM Tes (pH 7.5), 1 mM TPP, 5 mM $MgSO_4$, 10 μM leupeptin, and 15% ethylene glycol (v:v). The suspensions were cleared by centrifugation at 27,000*g*, 0°C, for 15 min, and the supernatants were used for the enzyme assay. No measurable amount of α-KGDC activity was obtained from either corn or soybean etioplasts. With the partially purified mitochondrial enzyme preparation, α-KGDC activity was determined spectrophotometrically as described for PDC with one-half the amount of CoA and with α-KG in place of pyruvate. Because the corn α-KGDC activity increased proportionally up to 6 mM α-KG, the corn enzyme assays contained 6 mM α-KG, while the soybean assays contained 3 mM α-KG. The enzyme stock suspension was prepared and divided into small portions and stored in liquid nitrogen where no measurable enzyme activity was lost within 3 weeks.

The acid form of haloxyfop was dissolved in 1 ml of 1N sodium hydroxide solution, and concentrations of 10 mM in 100 mM Tes (pH 7.5) and 50 mM Bicine (pH 8.0) were prepared for mitochondrial PDC and α-KGDC and etioplast PDC assays, respectively. The final concentrations of haloxyfop in the reaction cuvette ranged from 0.1 to 5.0 mM. All assay mixture constituents including the enzyme were incubated for 1 min at 30°C before the reaction was initiated by the addition of pyruvate. In all cases, initial velocities were proportional to protein concentration over the range used and were linear with time. To minimize the interference from product inhibition of NADH and acetyl CoA, the initial velocity was measured within one minute after initiation.

Analysis of Kinetic Data. Triplicated observation data from inhibition studies were analyzed using an enzyme kinetics program by Cleland (6). All data from inhibition studies, carried out at saturating substrate concentrations as listed above, were fit to the equations (27) for competitive (1), noncompetitive (2), and uncompetitive inhibition (3) and determined by the variance.

$$v = \frac{V'_{max}}{1 + \frac{K'_A}{[A]} + \frac{K'_A [I]}{K_{is}[A]}} \quad (1)$$

$$v = \frac{V'_{max}}{1 + \frac{K'_A}{[A]} + \frac{K'_A [I]}{K_{is}[A]} + \frac{[I]}{K_{ii}}} \quad (2)$$

$$v = \frac{V'_{max}}{1 + \frac{K'_A}{[A]} + \frac{[I]}{K_{ii}}} \quad (3)$$

In these equations, A is the variable substrate, I is inhibitor (haloxyfop), K_A is the Michaelis constant, and V_{max} is the maximum velocity in the presence of the inhibitor. K_{is} and K_{ii} are the slope and intercept inhibition constants, respectively. Corresponding inhibition constants (K_i) were calculated from Dixon plots. The reversibility of inhibition was inspected by the method of Segal (24).

Chemicals. Biochemicals used in this experiment were purchased from Sigma Chemical Company unless otherwise indicated. Analytical standard grade (98%) haloxyfop (50% levo- and 50% dextro-form) was generously provided by Dow Chemical Company.

RESULTS

Survey of Glycolytic and TCA Cycle Enzymes for Haloxyfop Inhibition. In initial experiments to screen for enzymes susceptible to haloxyfop, the following glycolytic, TCA cycle, and pentose phosphate pathway enzymes were assayed mainly using methods described elsewhere: UDPG synthase, sucrose-6-phosphate synthase, glucose-6-phosphate dehydrogenase, invertase, 6-phosphate-gluconate dehydrogenase, hexose-phosphate-isomerase, phosphoglucomutase, hexokinases, phosphofructokinase, triose-phosphate isomerase, glycerate-3-phosphate kinase,

[3] Abbreviations: PDC, pyruvate dehydrogenase complex; α-KG, α-ketoglutarate; α-KGDC, α-ketoglutarate dehydrogenase complex; TCA, tricarboxylic acid; TPP, thiamine pyrophosphate.

enolase, phosphoglycerate mutase, pyruvate kinase, lactate dehydrogenase, PDC, isocitrate lyase, malate synthase, citrate synthase, aconitase, isocitrate dehydrogenase, NAD- and NADP-malic enzyme, fumarase, succinate dehydrogenase, and α-KGDC. From these studies, PDC were the first enzyme found to be inhibited by 1 mM haloxyfop in the oxidative direction of the glycolytic pathway.

Haloxyfop Inhibition of PDC. When kinetic studies were carried out and displayed on a Lineweaver-Burk double reciprocal plot of substrates *versus* absorbance of the PDC activity, in most cases noncompetitive inhibition was seen except for corn etioplast PDC (Figs. 1 and 2; Tables I and II; and data not shown). Therefore, a plot of V_{max} *versus* different levels of enzyme was prepared according to the method of Segal (24) to determine if the haloxyfop inhibition was due to irreversible inhibition or reversible noncompetitive inhibition. The results show that haloxyfop was a reversible noncompetitive inhibitor of PDC since the slopes were altered and all of the lines intersect at the origin (Fig. 3 shows data for soybean mitochondrial PDC as an example). Therefore, all the reciprocal plots of haloxyfop enzyme inhibition kinetics of corn mitochondrial PDC (Fig. 1), soybean mitochondrial PDC (Fig. 2), and corn and soybean etioplast PDC (data not shown) were determined to show reversible noncompetitive inhibition when plotted as in Figure 3. The apparent K_m values of the corn mitochondrial PDC for pyruvate, CoA, and NAD^+ were 79.3, 4.1, and 60.4 μM, respectively (Table I). The K_i values of haloxyfop for corn mitochondrial PDC *versus* pyruvate, CoA, and NAD^+ were 5.2, 3.0, and 3.1 mM, respectively, as determined from the Dixon plots in Figure 1.

The soybean mitochondrial PDC was also inhibited by haloxyfop noncompetitively, and the apparent K_m values for pyruvate, CoA, and NAD^+ were 68.2, 5.0, and 94.0 μM, respectively (Table I). The calculated K_i values for soybean mitochondrial PDC for pyruvate, CoA, and NAD^+ were 3.7, 2.8, and 3.0 mM, respectively (Fig. 2).

The apparent K_m values of corn etioplast PDC for pyruvate, CoA, and NAD^+ were 120.4, 3.8, and 16.0 μM, respectively (Table II). Haloxyfop inhibited corn etioplast PDC as an uncompetitive inhibitor with respect to pyruvate, and as a noncompetitive inhibitor with respect to CoA and NAD^+. The K_i values of haloxyfop for pyruvate, CoA, and NAD^+ were 10.5, 2.1 and 6.9 mM, respectively. The K_m values of soybean etioplast PDC for pyruvate, CoA, and NAD^+ were 21.5, 3.9, and 12.2 μM, respectively (Table II). The calculated K_i values of haloxyfop for soybean etioplast PDC were 10.2, 4.0, and 1.2 mM, respectively.

The inhibitory effect of haloxyfop was also measured on the dihydroxylipoamide dehydrogenase component of the pyruvate dehydrogenase complex. The inhibition of the dihydroxylipoamide dehydrogenase activity from both corn and soybean mitochondria by 1.0 mM haloxyfop was less than 2%, although the enzyme activity of dihydroxylipoamide dehydrogenase was about 15 times greater than the PDC activity per unit protein content even though the assay was carried out at a lower temperature (25°C) than that for PDC (30°C).

Haloxyfop Inhibition of α-KGDC. The enzyme preparation from purified corn and soybean mitochondria also contained α-KGDC activity. The initial rate of the α-KGDC activity was usually less than 20% of the PDC activity and was proportional to the amount of protein used.

Haloxyfop inhibited the corn α-KGDC as a competitive inhibitor with respect to α-KG, as an uncompetitive inhibitor with respect to CoA, and as a noncompetitive inhibitor with respect to NAD^+ (data not shown). The apparent K_i values of haloxyfop for a α-KGDC with respect to α-KG, CoA, and NAD^+ were 0.58, 1.15, and 1.24 mM (Table III). The K_m values of corn α-KGDC with respect to α-KG, CoA, and NAD^+ were 3.744, 0.016, and 0.295 mM, respectively.

Although the soybean enzyme was inhibited by haloxyfop as a noncompetitive inhibitor for α-KG, CoA, and NAD^+ (Table III), the apparent values of K_m and K_i of the enzyme were almost identical to that of the corn enzyme. The apparent K_i values of haloxyfop for soybean α-KGDC with respect to α-KG, CoA, and NAD^+ were 0.8, 1.10, and 1.26 mM. The apparent K_m values of soybean α-KGDC with respect to α-KG, CoA, and NAD^+ were 3.641, 0.014, and 0.245 mM.

Besides the different patterns of inhibition described above, the activity of corn α-KGDC was stimulated by up to 6 mM α-KG while the soybean α-KGDC was increased only by up to 3 mM α-KG. While the α-KG saturation concentration for α-KGDC activity was higher than the pyruvate concentration needed for PDC, CoA concentrations above 0.05 mM caused substrate inhibition of both corn and soybean α-KGDC activity.

DISCUSSION

The haloxyfop inhibition of PDC activity is consistent with the previous observations of inhibition of lipid biosynthesis and the subsequent loss of membrane integrity (11–14) since the role of the PDC in mitochondria is to provide acetyl-CoA for the TCA cycle and possibly for fatty acid synthesis via decarboxylic oxidation of pyruvate (21, 22, 27, 30), and the plastid PDC activity provides acetyl-CoA for lipid synthesis (7, 30).

The listed roles of PDC are in agreement with the haloxyfop injury symptoms of corn suspension cultured cells reported previously (4): (a) simultaneous inhibition of lipid synthesis and respiration, indicating that haloxyfop inhibition is taking place before the TCA cycle and fatty acid synthesis; (b) no significant changes of cellular sugar content and ATP content, indicating that the cells utilized the glycolytic pathway until membrane disruption occurred; (c) an increase in free amino acids, which may indicate that the cells utilize the accumulated organic acids and convert them into the corresponding amino acid via transamination. Also, the lactate content was increased by haloxyfop during incubation with corn and soybean suspension cultured cells (HY Cho, unpublished data), which shows that blocking the entry of carbon into the TCA cycle causes the accumulation of pyruvate and NADH or NADPH in the cells. This could lead to the conversion of cellular metabolic intermediates into ethanol and lactate and into amino acids by transamination of α-keto organic acids. These effects would be analogous to the effect of hypoxia due to the flooding of plants, which prevents TCA cycle activity (17).

Except that PDC catalyzes the conversion of pyruvate to acetyl-CoA and α-KGDC catalyzes the conversion of α-ketoglutarate to succinyl-CoA, both complexes are very similar; they both utilize α-keto acids, CoA, and NAD^+ in a ping-pong mechanism, and they consist of similar subunits such as dihydrolipoic transacetylase and dihydrolipoic dehydrogenase (16, 20). PDC activity is, however, found both in mitochondria and plastids including chloroplasts while α-KGDC is found only in the mitochondria, being part of the TCA cycle. Thus, since the α-KGDC reactions are similar to those of PDC, it is not surprising that haloxyfop also inhibits activity of α-KGDC. However, when one compares the enzyme kinetics, the K_m values of α-KGDC were higher than the PDC values with respect to the corresponding substrates, and the K_i values of haloxyfop for the α-KGDC were lower than those for the PDC with respect to the corresponding substrates. These results suggest that α-KGDC might be the primary inhibition site by haloxyfop, since it is more easily inhibited.

If we assume that the mode of action of haloxyfop is the inhibition of these two enzymes, the cause of accumulation of amino acids in the haloxyfop treated cells can be explained as a result of the accumulation of cellular α-keto acids. The accumulated α-keto acids are subject to transamination with expenditure of NADH and conversion into the corresponding amino

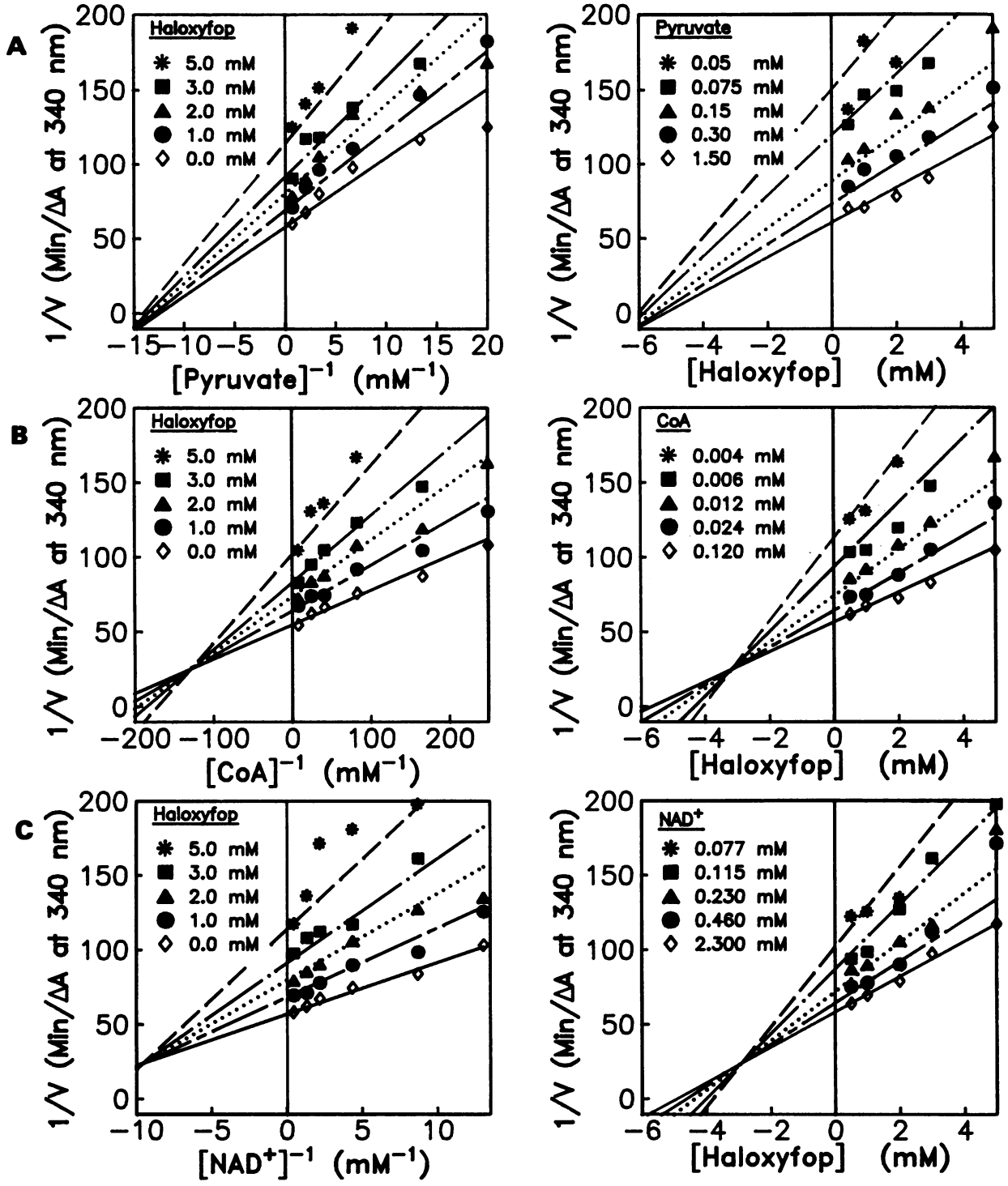

FIG. 1. Lineweaver-Burk double reciprocal plots (left) and Dixon plots (right) of the pyruvate dehydrogenase complex from corn mitochondria with respect to (A) pyruvate, (B) CoA, and (C) NAD^+ in the presence of haloxyfop. Each reaction mixture contained 0.42 mg protein from a preparation with 4.4 μmol NADH formed min^{-1} mg^{-1} protein, specific activity.

acids (15). Similar results could be obtained by increasing the cellular pyruvate levels (23), by placing the plant cells under anaerobic conditions (17, 26), or by treating the plant with certain inhibitors (26). A similar phenomenon is also possible in animals where the simultaneous malfunction of these two enzymes in mammals causes 'maple syrup disease' due to the accumulation of amino acids (15). Further *in vivo* studies are needed to determine if indeed haloxyfop does block cell metabolism at the sites proposed.

Although the location of the inhibition sites and the relationship between inhibition symptoms are compatible, certain observations raise questions about the site of action: (a) the calculated K_i values of haloxyfop for corn (susceptible plant) and soybean (tolerant plant) were not significantly different, (b) the

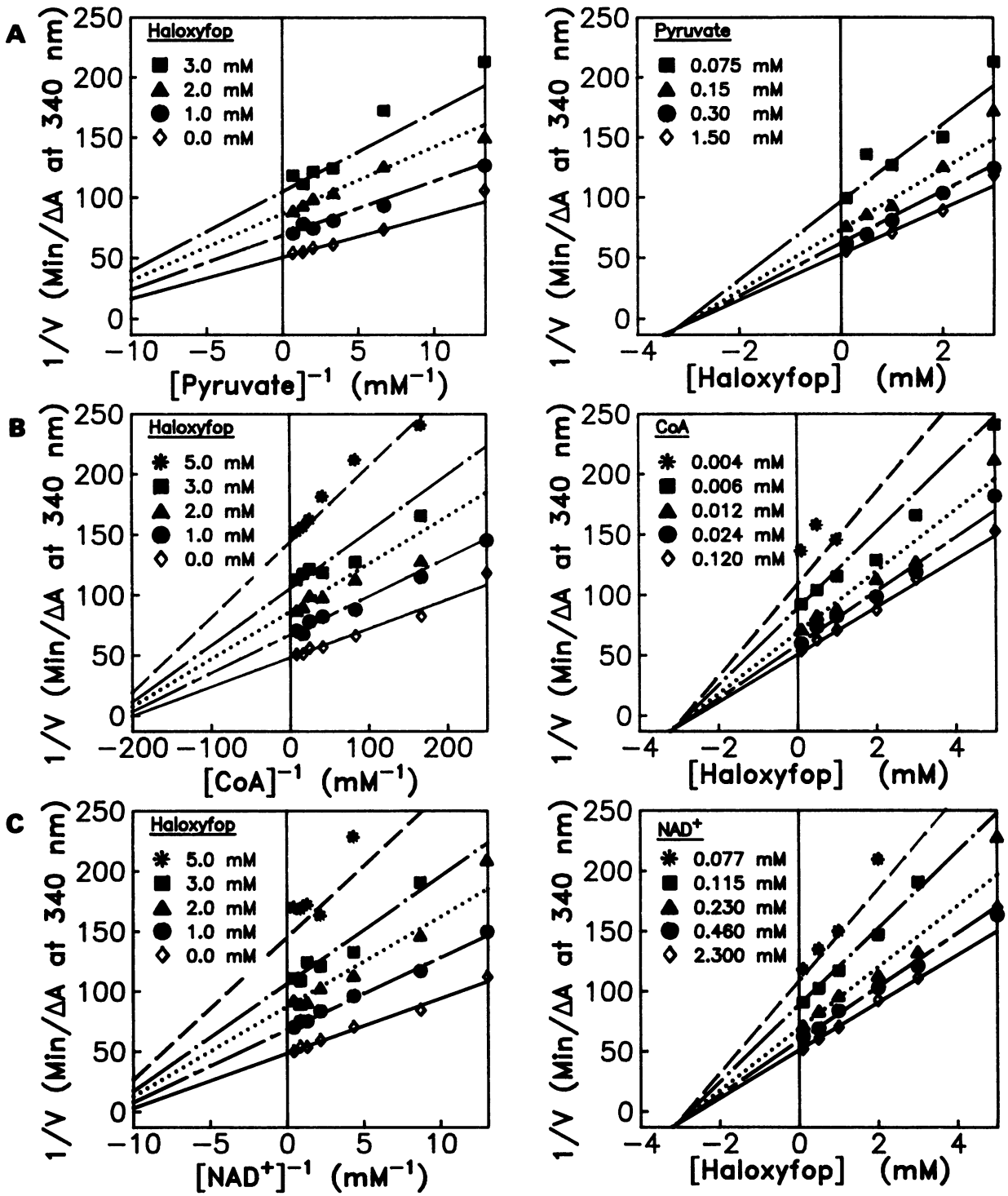

FIG. 2. Lineweaver-Burk double reciprocal plots (left) and Dixon plots (right) of the pyruvate dehydrogenase complex from soybean mitochondria with respect to (A) pyruvate, (B) CoA, and (C) NAD^+ in the presence of haloxyfop. Each reaction mixture contained 0.40 mg protein from a preparation with 5.9 μmol NADH formed min^{-1} mg^{-1} protein, specific activity.

leaf cells did not display injury symptoms despite foliar application and inhibition of lipid synthesis as was reported in the isolated soybean leaf cells (10), and (c) the K_i values of haloxyfop for the PDC or α-KGDC enzymes are much higher than the LC_{50}s (herbicide concentration causing 50% death) for the corn and soybean cell cultures, 0.05 and 2.4 μM, respectively (4).

One can speculate that haloxyfop could be concentrated at the active site, due to the translocation from the site of application of plants to the active site (*i.e.* meristematic tissue), and could be concentrated still further by the mitochondria or plastids. This has not been investigated as yet. However, in a related example, a fungicide, *sec*-butylamine, is known to be a PDC competitive

Table I. *Kinetic Values for the Effects of Haloxyfop on Mitochondrial Pyruvate Dehydrogenase Complex of Corn and Soybean as Calculated from the Data of Figures 1 and 2 by the Methods of Cleland (6)*

	Corn			Soybean		
	Pyruvate	CoA	NAD^+	Pyruvate	CoA	NAD^+
			mM			
K_A	0.0793	0.0041	0.0604	0.0682	0.0050	0.0940
K_{is}	5.18	3.03	2.93	4.22	3.18	3.13
K_{ii}	5.23	5.97	5.14	2.49	2.54	2.52
K_i	5.2	3.0	3.1	3.7	2.8	3.0

Table II. *Kinetic Values for the Effects of Haloxyfop on Etioplast Pyruvate Dehydrogenase Complex from Corn and Soybean with Respect to Pyruvate, CoA and NAD^+*

	Corn			Soybean		
	Pyruvate	CoA	NAD^+	Pyruvate	CoA	NAD^+
			mM			
K_A	0.120	0.0038	0.0160	0.0215	0.0039	0.0122
K_{is}		2.39	6.02		3.32	1.11
K_{ii}	9.94	21.8	27.6	9.27	14.9	9.51
K_i	10.5	2.1	6.9	10.2	4.0	1.2

Table III. *Kinetic Values for the Effects of Haloxyfop on α-KGDC from Corn and Soybean with Respect to α-KG, CoA, and NAD^+*

	Corn			Soybean		
	α-KG	CoA	NAD^+	α-KG	CoA	NAD^+
			mM			
K_A	3.74	0.0161	0.295	3.64	0.0144	0.245
K_{is}	0.511		1.18	0.688	2.76	1.62
K_{ii}		0.952	1.39	1.22	1.05	1.06
K_i	0.58	1.15	1.24	0.8	1.10	1.26

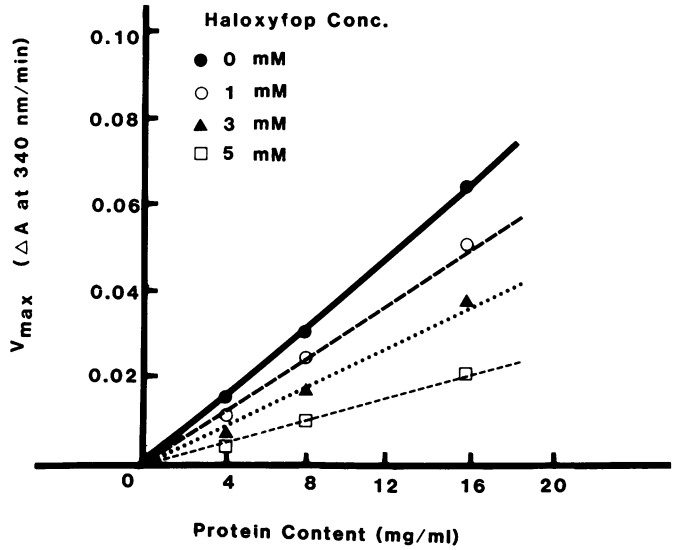

FIG. 3. Effect of haloxyfop on different levels of soybean mitochondrial pyruvate dehydrogenase complex activity.

inhibitor of pyruvate with a K_i of 13.8 mM. *Penicillium* concentrates *sec*-butylamine from 0.5 mM in the medium to 15 mM in the mitochondria within 30 min (28, 29). However, there is a report that more [^{14}C]haloxyfop was taken up by soybean suspension-cultured cells than by corn suspension-cultured cells, and there was also no clear indication of degradation or detoxification of haloxyfop by the soybean cells (3). These cell culture results indicate that the selectivity of the herbicide is not due to differential rates of uptake. The lessened uptake by the corn cells might, however, be due to their decreased metabolic activity during incubation since corn cells are more sensitive to haloxyfop than are soybean cells (4). It is also possible that the substrate concentrations for the PDC and α-KGDC activities are lower than the K_m values, so the haloxyfop concentrations needed for significant enzyme inhibition would be lower than the *in vitro* determined K_i values.

These studies indicate that PDC and α-KGDC are possible sites of inhibition by haloxyfop. However, further studies are required to determine if haloxyfop accumulates in mitochondria and plastids and what the mechanism is for selectivity between tolerant and susceptible plants.

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