Short Communication

Kinetic Characterization of the Inhibition of Plant Acetyl-CoA Carboxylase by the Grass Herbicides, Quizalofop and Sethoxydim*

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(Received April 3, 1990)

Quizalofop, a grass herbicide, has been found to be a potent inhibitor of acetyl-CoA carboxylase (ACC) in susceptible species (*Zea mays*).^{1–3)} ACC is a biotin-enzyme that catalyzes the synthesis of malonyl-CoA, an essential intermediate in both fatty acid^{4–6)} and flavonoid biosynthesis.⁷⁾

Recent studies have suggested that this enzyme is the primary target site of aryloxyphenoxypropionic acid herbicides such as diclofop, fluazifop, quizalofop and haloxyfop^{1–3, 8–13)} and cyclohexanedione herbicides such as alloxydim, sethoxydim and clethodim.^{2, 8, 9, 14, 15)} Blockage of fatty acid supply will ultimately result in a breakdown of cellular function and death of organisms.^{16–18)}

This paper describes our kinetic studies on corn ACC inhibition by *R*(+)-quizalofop and sethoxydim in three substrates, acetyl-CoA, ATP and bicarbonate. Action mechanisms of quizalofop and sethoxydim, compared to the observations reported for diclofop and haloxyfop,¹³⁾ are also discussed.

MATERIALS AND METHODS

The source and conditions of germination of corn seeds (*Zea mays* L.) have been described previously.¹⁶⁾ $\text{NaH}^{14}\text{CO}_3$ (54 mCi/mmol) was

obtained from Amersham. Acetyl-CoA, ATP and ADP were purchased from Sigma, and malonyl-CoA from P-L Biochemicals.

Acetyl-CoA carboxylase was partially purified from the homogenates of 8-day-old corn leaves by precipitation with PEG-6000 and ammonium sulfate, and column chromatography on DEAE Sepharose CL-6B and AF-Blue Toyopearl 650ML. Recoveries were approximately 15% with a 150-fold increase in specific activity. The prepared enzyme was stored at -20°C for periods of up to 2 weeks without any loss of activity. The specific activity was 2010 nmole/mg protein/min at 30°C under the conditions for the ^{14}C -carbonate fixation assay described previously.¹⁾

Data analysis: Kinetic parameters and complete inhibition patterns were determined by using FORTRAN programs of Cleland.¹⁹⁾

RESULTS AND DISCUSSION

R(+)-quizalofop was a very potent inhibitor (I_{50} , 4.5×10^{-9} M) of corn ACC, while *S*(–)-enantiomer was 1000-fold less inhibitory, indicating that the inhibition is stereoselective for *R*(+)-configuration of quizalofop. The relationship between the initial velocity, substrate concentration and inhibitor concentration for double-reciprocal plots indicated that the inhibition of *R*(+)-quizalofop was noncompetitive versus each of the substrates, acetyl-CoA, ATP and bicarbonate. The kinetic parameters in Table 1 show that corn ACC has an extremely high affinity for *R*(+)-quizalofop. Thus, the K_i values for the herbicide were approx. 10^4 times lower than the K_m value for the tightest binding substrate. The inhibition was nearly equaled in sensitivity to the level of acetyl-CoA, ATP and bicarbonate. These data fit best to the equation for noncompetitive inhibition. On the other hand, the inhibition of the enzyme by sethoxydim (I_{50} , 3.9×10^{-6} M) was competitive versus acetyl-CoA, uncompetitive versus ATP and noncompetitive versus bicarbonate (Table 2).

The reaction mechanism to plant acetyl-CoA carboxylase (ACC) has been proposed to be a hybrid (two-site) bi bi uni uni ping-pong mechanism.²⁰⁾ In this mechanism active sites of the enzyme are separated into two functionally distinct catalytic sites: The carboxybiotinyl arm formed at one site by the conversion of ATP to ADP and P_i , swings to the second site where

* Action Mechanism of the Herbicide Quizalofop-ethyl (Part 5). Part 4. See Ref. 1)

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Table 1 Estimates of the inhibition kinetic parameters of quizalofop for corn ACC.

Substrate	K _m (mM)	K _{is} (nM)	K _{ii} (nM)	Inhibition pattern
Acetyl-CoA	0.073±0.006	2.12±0.47	2.15±0.42	Noncompetitive
ATP	0.083±0.005	4.50±0.74	4.44±0.49	Noncompetitive
HCO ₃ ⁻	2.380±0.118	2.38±0.33	3.11±0.30	Noncompetitive

Table 2 Estimates of the inhibition kinetic parameters of sethoxydim for corn ACC.

Substrate	K _m (mM)	K _{is} (μM)	K _{ii} (μM)	Inhibition pattern
Acetyl-CoA	0.066±0.005	2.13±0.18	—	Competitive
ATP	0.091±0.007	—	2.65±0.21	Uncompetitive
HCO ₃ ⁻	2.360±0.108	2.87±0.39	3.71±0.35	Noncompetitive

Fig. 1 Hybrid (two-site) ordered bi bi uni uni ping-pong mechanism.

All substrates and products bind and dissociate in an ordered fashion. E represents all enzyme forms containing uncarboxylated biotin at the ATP-dependent biotin carboxylation site. F represents all enzyme forms containing carboxylated biotin at the transcarboxylation site.

acetyl-CoA is carboxylated to malonyl-CoA. On the basis of the substrate interaction and product inhibition studies, we recently found that corn ACC reaction was interpreted by a hybrid (two-site) ordered bi bi uni uni ping-pong mechanism.²¹⁾ The sequence can be diagrammed as shown in Fig. 1.

The present inhibition kinetic data indicate that quizalofop binds tightly to complex in both a uncarboxylated form (E) and a carboxylated form (F) with comparable affinity. That is, quizalofop interacts with ACC by interfering with both the ATP-dependent biotin carboxylation site and the transcarboxylation site. Rendina *et al.* have reported^{13,22)} that the inhibition of wheat ACC by diclofop is noncompetitive against all three substrates, and that diclofop interferes with the transcarboxylation site rather than the ATP-dependent biotin carboxylation site, because the apparent inhibition kinetic parameters of diclofop are the most sensitive to acetyl-CoA levels. On the other hands the recent report by

Cseke *et al.* has said that haloxyfop and tralkoxydim (a cyclohexanedione herbicide) show mixed type inhibition against each of the three substrates of corn ACC, and that neither haloxyfop nor tralkoxydim interacts with ACC by interfering with the binding of only one substrate.²³⁾ Although detailed kinetic data for haloxyfop are not available at present, we assume that quizalofop and haloxyfop may bind to the same region of the enzyme. In this case, noncompetitive or mixed type inhibition of ACC by aryloxyphenoxypropionic acids would be possible due to the change in binding affinity.

On the other hand, the kinetic parameters of the inhibition of sethoxydim suggest that the herbicide binds to the carboxylated form (F) of the enzyme: sethoxydim interferes with the carboxylation of acetyl-CoA at the transcarboxylation site and hardly affects the biotin carboxylation site. D. A. Winkler *et al.* have recently reported that sethoxydim and the related herbicides appear to be approximate analogues in the transition state of reaction catalyzed by ACC.²⁴⁾ Thus, sethoxydim and analogous compounds probably interfere at the transcarboxylation site in competing with acetyl-CoA for part of its binding site.

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要 約

イネ科雑草防除剤 quizalofop と sethoxydim による植物 acetyl-CoA carboxylase 阻害の速度論的特性*

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トウモロコシ葉 acetyl-CoA carboxylase (ACC) に対する quizalofop および sethoxydim の阻害形式を解析した。ACC は PEG 分画, 硫酸塩析後, DEAE-Sephacel CL-6B および Blue-Toyoparl 650 ML カラムクロマトグラフィにより約 150 倍に精製され, 回収率は約 15% であった。部分精製したトウモロコシ ACC に対して *R*(+)-quizalofop はきわめて低濃度 (I_{50} , 4.6×10^{-9} M) で阻害を示し, 一方, *S*(-) 体の I_{50} は *R*(+) 体の約 1000 倍の薬量を必要とした。一方, sethoxydim の I_{50} は 3.6×10^{-6} M を示した。Quizalofop は, acetyl-CoA, ATP および HCO_3^- に対して noncompetitive inhibitor として作用するのに対し, sethoxydim は, acetyl-CoA に対して competitive, ATP に対して uncompetitive, そして, HCO_3^- に対しては, noncompetitive inhibitor として作用した。すなわち, quizalofop は ACC の形態が uncarboxylated form および carboxylated form にかかわらず複合体を形成することにより, ATP 依存性 biotin carboxylation site および transcarboxylation site の両 site における反応に影響を及ぼし, sethoxydim は, carboxylated form と複合体を形成することにより, transcarboxylation site における反応に影響を及ぼす。

* 除草剤 quizalofop-ethyl の作用機構 (第 5 報)