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Inhibition of 4-Hydroxyphenylpyruvate Dioxygenase by Sethoxydim, a Potent Inhibitor of Acetyl-Coenzyme A Carboxylase

Shean-woei Lin and Ding-yah Yang*
Department of Chemistry, Tunghai Christian University,
Taichung, Taiwan 40704, R. O. C.

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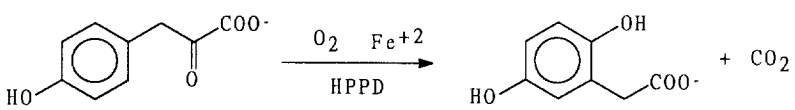
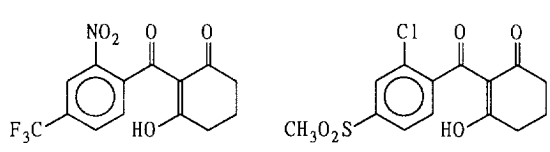
Abstract: Sethoxydim, a commercially available cyclohexanedione class herbicide by targeting the enzymatic activity of acetyl-coenzyme A carboxylase, has been found to moderately inhibit the activity of 4-hydroxyphenylpyruvate dioxygenase, a key enzyme in the biosynthesis of plastoquinones and tocopherols in plants.

4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD)[1] is a key enzyme involved in the biosynthesis of plastoquinones and tocopherols in plants[2] as well as the catabolism of tyrosine and phenylalanine in most organisms;[3] it catalyzes the conversion of 4-hydroxyphenylpyruvate and molecular oxygen to homogentisate and carbon dioxide as shown in Scheme 1.



Scheme 1

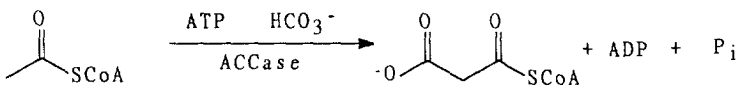
Since discovered in 1930s, HPPD has been isolated homogeneously from various sources.[4] Three transformations occur in this single enzyme conversion which include an oxidative decarboxylation, a hydroxylation of the aromatic ring, and a 1,2-shift of a carboxymethyl group. While the detail mechanism of HPPD remains unclear, several potent inhibitors have been discovered during a routine evaluation process. For example, 2-(2-(nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione[5] (NTBC) and 2-(2-chloro-4-methanesulfonylbenzoyl)-cyclohexane-1,3-dione[6] (CMBC), two derivatives of cyclohexanediones, are reversible competitive inhibitors of HPPD with an IC_{50} value of 40 and 45 nM, respectively, even though the mode of action for this inhibition is currently unresolved.



Scheme 2

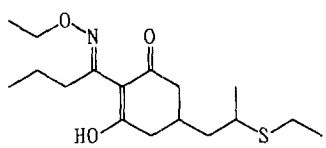
PII: S0960-894X(99)00036-0

Another important enzyme with different biological function from HPPD that can also be strongly inhibited by cyclohexanedione derivatives is acetyl-coenzyme A carboxylase[7] (EC. 6.4.1.2, ACCase) from monocotyledonous plants. ACCase is the first dedicated enzyme in the *de novo* fatty acid biosynthetic pathway that catalyzes the ATP-dependent conversion of acetyl-coenzyme A to malonyl-coenzyme A at two distinct subsites (carboxylation site and transcarboxylation site) within the active site of the enzyme, as depicted in Scheme 3.



Scheme 3

Inhibition of ACCase activity will block the fatty acid synthesis and consequently lead to cell death. For example, evidence is accumulating that ACCase is the site of action of sethoxydim,[8] 2-(1-ethoxyimino-butyl)-5-(2-ethylsulfanyl-propyl)-3-hydroxy-cyclohex-2-enone, a commercially available cyclohexanedione class of herbicide that is used for the control of grasses in broadleaf crops. Recent studies[9] have also indicated that sethoxydim is a competitive inhibitor of ACCase with respect to the substrate acetyl-coenzyme A, which suggests that binding may be at the transcarboxylation site. Unfortunately, the detail mode of action for this inhibition again remains unclear.



Sethoxydim

There are a couple of common features between HPPD and ACCase. First of all, both enzymes involve in transferring of a carboxyl group, either carboxylation or decarboxylation. Secondly, a cyclohexanedione substructure is required for a potent HPPD and ACCase inhibitor. This information prompts us to speculate that ACCase and HPPD may share some similar binding pockets in the enzyme active site, thus we expect cyclohexanedione class ACCase inhibitors like sethoxydim may also inhibit the activity of HPPD. In order to test this hypothesis, we have purified HPPD from pig liver according to the literature procedure.[10] Inhibition studies of sethoxydim with HPPD was then carried out by incubation of varying concentrations of sethoxydim[11] with HPPD in the presence of the natural substrate 4-hydroxy-

phenylpyruvate. The enzyme activity was monitored by using continuous measurements with the spectrophotometric enol-borate method as described by Lindstedt et al.[12] The time-course of the reaction of pig liver HPPD in the absence and presence of increasing amount of sethoxydim is shown in Figure 1. The activity of HPPD was obviously inhibited by the herbicide sethoxydim. Almost complete inhibition was observed at a concentration of 0.5-1.0 mM. Kinetic analysis also indicated that sethoxydim is indeed a competitive inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase with the K_i value of 300 μM.

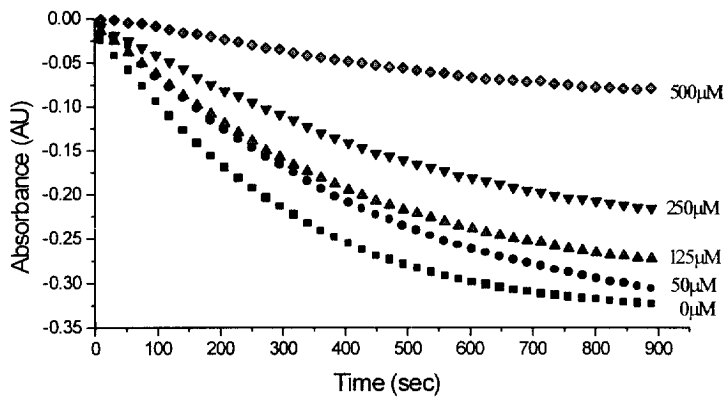


Figure 1: Time-course of the reaction of HPPD and 4-hydroxyphenyl-pyruvate (50 μM) with varying concentrations of sethoxydim (500, 250, 125, 50, and 0 μM). The protein concentration in the assay was 1 mg/mL.

In summary, the potent ACCase inhibitor sethoxydim has been found to moderately inhibit the activity of pig liver 4-hydroxyphenylpyruvate dioxygenase. This founding suggests HPPD and ACCase may share at least some similar binding pockets in the enzyme active site. Thus, understanding the mechanistic details of HPPD and the mechanism of action of NTBC and CMBC at a molecular level may shed light on the mode of action of herbicide sethoxydim to its target site ACCase and vice versa. These results may allow us to better define the specific substrate/inhibitor binding characteristics of both enzymes. Such information is important for the design of novel inhibitors to control and/or regulate tyrosine catabolism, biosynthesis of plastoquinones and tocopherols, and fatty acid metabolism as well.

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