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Original Article

Kinetic Studies on the Inhibition of Acetolactate Synthase by Pyrimidinylsalicylic Acids*

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The inhibition of acetolactate synthase (ALS) from etiolated pea seedlings by 2-(4,6-dimethoxypyrimidine-2-yloxy)benzoic acid (compound **2**), one of the pyrimidinylsalicylic acid (PS) compounds, was desensitized by SH inhibitors but not at the alkaline pH. PS compounds including pyrithiobac (2-chloro-6-(4,6-dimethoxypyrimidine-2-ylthio)benzoic acid) tested in this study inhibited ALS activity in the mixed-type manner with respect to pyruvate and in the non-competitive with respect to thiamine pyrophosphate (TPP). These compounds inhibited both of the two molecular species of ALS which were partially purified from pea seedlings and exhibited slow-binding properties to the crude preparation of ALS from pea seedlings in the extended-time-course experiments. The inhibition constants of the initial inhibition by PS compounds were 13- to 26-fold larger than those of the final steady state and the maximal first order rate constant (0.69 min^{-1}) for transition from the initial to the final steady state of the inhibition of pyrithiobac was nearly identical to those reported on other widely acknowledged ALS inhibitors. From these results, we conclude: PS compounds are slow-binding inhibitors to ALS activity, categorized in the sulfonylurea type. The binding site of PS compounds on the enzyme is considered to be located on the similar site to sulfonylureas. This binding site is judged not to be located on the regulatory center(s) but on the allosteric site in a wide sense near the catalytic center, which overlaps partially with the binding site of pyruvate and does not overlap with the binding site of TPP.

INTRODUCTION

In our previous paper,[1] we have shown that pyrimidinylsalicylic acid (PS) compounds represented by pyrithiobac-sodium (trade name; Staple), which form one group in the new class of herbicides designated "Pyrimidinyl carboxy herbicides," inhibit potently the activity of acetolactate synthase (ALS; EC4.6.3.8, also referred as acetohydroxyacid synthase; AHAS) from various plants. ALS is the enzyme which catalyzes both the formation of acetolactate from two molecules of pyruvate and hydroxybutylacetolactate from pyruvate and 2-oxobutylate in the biosynthetic pathway of branched-chain amino acids; leucine, valine and isoleucine. ALS has been shown to be the target of three structurally distinct herbicides, namely, sulfonylurea (SU), imidazolinone (IZ) and triazolopyrimidine sulfonamide (TP).[2-4] It has been demonstrated that SU and TP herbicides inhibit the activity of plants' ALS in the mixed-type inhibition with respect to pyruvate,[5,6] while IZ herbicides inhibit in the uncompetitive.[8] In the extended-time-course experiments, these herbicides have been shown to exhibit the slow-binding properties to both ALS of plants[7,8] and a bacterium.[9]

In our preceding paper,[10] we have revealed

* Action Mechanism of Herbicides, Pyrimidinylsalicylic Acids (Part III). For Part II, see Ref. 10).

that pea seedling contains only a single ALS whose molecular weight is approximately 320,000 (designated large ALS), and this molecular species changes to a lower molecular weight species (designated small ALS) which is insensitive to the feedback inhibition by leucine plus valine pair during purification.

In the present paper, we show the kinetic studies on the inhibition of ALS by PS compounds using both crude enzyme preparation and partially purified two molecular species of ALS from etiolated pea seedlings.

MATERIALS AND METHODS

1. *Chemical Compounds*

PS compounds (Fig. 1), SU and IZ herbicides tested in this study were synthesized at K-I Chemical Research Institute Co., Ltd. Purities of those chemical compounds were over 95%.

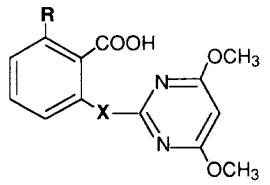
2. *Preparation of ALS from Etiolated Pea Seedlings*

Crude ALS was prepared by the method reported previously.[1] Two molecular species of ALS (large ALS and small ALS) were prepared basically by the method described previously.[10] But the hydroxyapatite column chromatography was omitted in this study.

The specific activities of crude ALS, large ALS and small ALS were 0.97, 14 to 20, 25 to 40 nmol acetoin produced per minute per milligram protein at 30°C, respectively.

3. *Assay of ALS Activity*

ALS was assayed at 30°C in a 1 ml of assay mixture containing 20 mM potassium phosphate buffer (pH 7.5), 0.5 mM $MgCl_2$, 10 μM flavin adenine dinucleotide, pyruvate, thiamine pyrophosphate (TPP) and inhibitors in different concentrations. TPP concentration was kept at 0.5 mM when pyruvate concentration was varied between 2 and 10 mM, while pyruvate concentration was kept at 40 mM when TPP concentration was varied between 0.0125 and 0.4 mM. The inhibitors except for branched-amino acids were dissolved in organic solvents and added to the reaction mixture. Branched-chain amino acids were dissolved in water and added to the reaction mixture. After incubation, the reaction was terminated by the addition of 100 μl of 6 N H_2SO_4. The amount of acetolactate produced by the enzyme reaction was determined by the method reported previously.[1]



	X	R
pyrithiobac	S	Cl
compound 1	O	Cl
2	O	H

Fig. 1 Structures of pyrimidinylsalicylic acid compounds.

4. *Analysis of Slow-Binding Inhibition by the Nonlinear Least Squares Method*

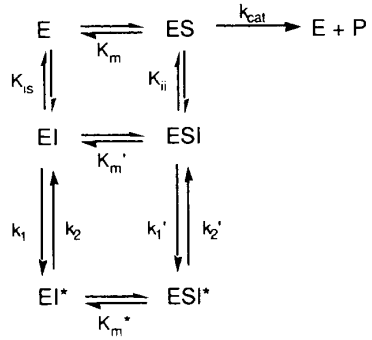
The rate equation of enzyme reaction with a slow-binding inhibitor has been shown to be

$$v = v_f + (v_i - v_f)e^{-kt} \quad (1)$$

where v is the observed velocity at any time, v_i is the initial velocity, v_f is the steady state velocity of the reaction, and k is the first order rate constant for transition from the initial to the final steady state inhibition.[11] The integrated form of Eq. (1) is given as the following equation.

$$P = v_f t - (v_f - v_i)/k + (v_f - v_i)e^{-kt}/k \quad (2)$$

We, therefore, determined the values for v_f, v_i and k by fitting the data for product (P) formation as a function of time (t) using the nonlinear least squares method devised by Yamaoka & Nakagawa.[12] And then, the inhibition constants (K_{is}, K_{is}**, K_{ii}, K_{ii}**) for



$K_m = [E][S]/[ES]$ $K_{is} = [E][I]/[EI]$ $K_{ii} = [ES][I]/[ESI]$

$K_m' = [EI][S]/[ESI]$ $K_{is}^{**} = [E][I]/[EI^*] = K_{is}(k_2/k_1)$ $K_{ii}^{**} = [ES][I]/[ESI^*] = K_{ii}(k_2'/k_1')$

$K_m^* = [EI^*][S]/[ESI^*]$ $K_{is}^* = [E][I]/\{[EI]+[EI^*]\}$ $K_{ii}^* = [ES][I]/\{[ESI]+[ESI^*]\}$

Fig. 2 Reaction scheme of enzyme inhibition for two step mixed-type slow-binding inhibitors.

the initial and the final steady state inhibition, and the maximal first order rate constant (k_1) for conversion from the initial to the final steady state inhibition were found from calculation using the following equations derived for two step mixed-type inhibition of slow-binding (Fig. 2), which we developed from those for two step competitive type inhibition derived by Schloss.[13]

$$v_i = VS/\{Km(1+I/K_{is})+S(1+I/K_{ii})\} \quad (3)$$

$$v_f = VS/\{Km(1+I/K_{is}^*)+S(1+I/K_{ii}^*)\} \quad (4)$$

$$K_{is}^{**} = 1/\{(K_{is})^{-1}+(K_{is}^*)^{-1}\} \quad (5)$$

$$K_{ii}^{**} = 1/\{(K_{ii})^{-1}+(K_{ii}^*)^{-1}\} \quad (6)$$

$$k = (k_1 Km K_{ii}+k_1' K_{is} S)/\{K_{ii} Km(k_1/k_2) + K_{is} S(k_1'/k_2')\}+(k_1 Km K_{ii}+k_1' K_{is} S)I/ \{Km K_{ii}(I+K_{is})+K_{is} S(I+K_{ii})\} \quad (7)$$

where V is the maximal velocity of enzyme reaction, S is the pyruvate concentration, Km is the Michaelis constant, I is the inhibitor concentration, K_{is} and K_{ii} are the substrate-independent inhibition constant (dissociation constant of enzyme-inhibitor complex) and substrate-dependent inhibition constant (dissociation constant of enzyme-substrate-inhibitor complex) in the initial inhibition, respectively. K_{is}^* and K_{ii}^* are the apparent substrate-independent inhibition constant and the apparent substrate-dependent inhibition constant in the final steady state inhibition, respectively. K_{is}^{**} and K_{ii}^{**} are the true substrate-independent inhibition constant and the true substrate-dependent inhibition constant in the final steady state inhibition, respectively. k_1 and k_1' are the rate constants of enzyme-inhibitor complex and enzyme-substrate-inhibitor complex for transition from the initial to the final steady state inhibition. k_2 and k_2' are the rate constants of enzyme-inhibitor complex and enzyme-substrate-inhibitor complex for transition from the final steady state to the initial inhibition.

RESULTS

1. *Effect of pH and SH Inhibitors on the Inhibition*

The inhibition of ALS activity by L-leucine was desensitized at the alkaline pH, while that by compound **2**, chlorsulfuron or imazapyr was not (Fig. 3). On the other hand, the inhibition by L-leucine was not desensitized by SH-inhibitors such as copper ion and mercury ion, while that by compound **2** was desensitized (Fig. 4, the data by mercury ion were not shown). These data indicated that the binding site of PS compounds on ALS was located on a different site from its regulatory center(s).

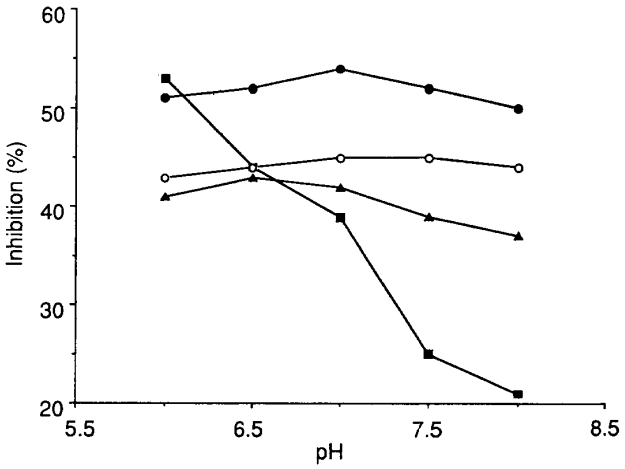


Fig. 3 Effect of assay pH on the inhibition of crude ALS of etiolated pea seedlings by compound **2**, chlorsulfuron, imazapyr and L-leucine.

●: compound **2** (500 nM), ○: imazapyr (40 μM), ▲: chlorsulfuron (12.5 nM), ■: L-leucine (0.5 mM).

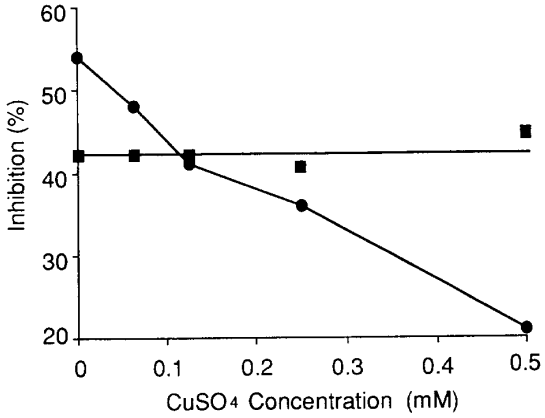


Fig. 4 Effect of $CuSO_4$ on the inhibition of crude ALS of etiolated pea seedlings by compound **2** and L-leucine.

●: compound **2** (500 nM), ■: L-leucine (0.5 mM).

2. *Analysis of the Inhibition as Rapidly Reversible Inhibitors*

2.1 *Analysis using crude enzyme*

The inhibitions of crude ALS by PS compounds, chlorsulfuron, imazapyr and branched-chain amino acids were analyzed by double-reciprocal plots (Lineweaver-Burk plots) in

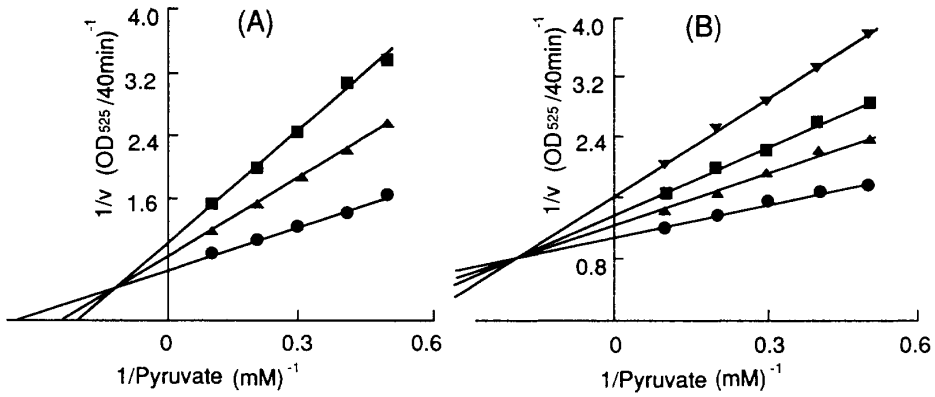


Fig. 5 Lineweaver-Burk plots of the inhibition of crude ALS from etiolated pea seedlings by pyrithiobac (A) and compound **2** (B) with variable pyruvate concentration.

(A) ●: control, ▲: 2.5 nM, ■: 5 nM. (B) ●: control, ▲: 25 nM, ■: 50 nM, ▼: 100 nM.

Table 1 Kinetics of the inhibition of acetolactate synthase from etiolated pea seedlings by pyrimidinylsalicylic acids, chlorsulfuron, imazapyr and branched-chain amino acids in the 40 min assay system.

Compound	For pyruvate[a]			For TPP[b]
	Type	K_{is} (nM)[c]	K_{ii} (nM)[d]	Type
Pyrithiobac	Mixed	4.8	17	Non-competitive
Compound **1**	Mixed	5.8	18	—[e]
Compound **2**	Mixed	124	440	Non-competitive
Chlorsulfuron	Mixed	5.6	24	Non-competitive
Imazapyr	Uncompetitive	∞	34,000	Non-competitive
L-Leucine	Partially competitive	—	—	Non-competitive
L-Valine	Partially competitive	270,000	2,300,000	—
L-Isoleucine	Partially competitive	1,200,000	14,000,000	—

[a] Pyruvate concentration was varied between 2 mM and 10 mM.
[b] Thiamine pyrophosphate (TPP) concentration was varied between 0.0125 mM and 0.5 mM.
[c] Substrate-dependent inhibition constant.
[d] Substrate-independent inhibition constant.
[e] Not tested.

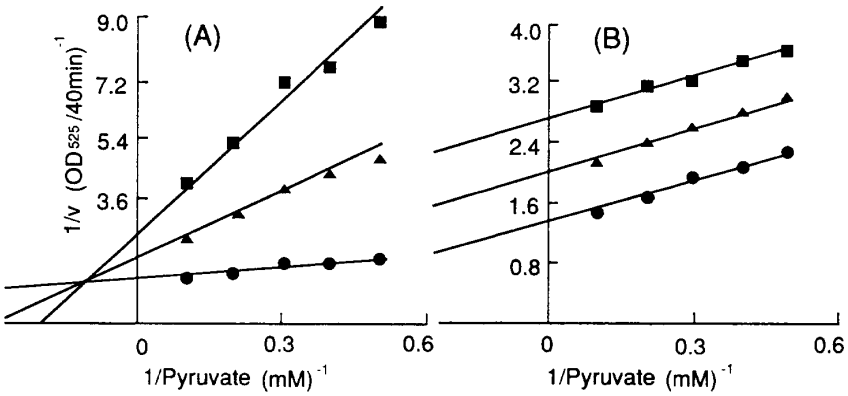


Fig. 6 Lineweaver-Burk plots of the inhibition of crude ALS from etiolated pea seedlings by chlorsulfuron (A) and imazapyr (B) with variable pyruvate concentration.

(A) ●: control, ▲: 5 nM, ■: 10 nM. (B) ●: control, ▲: 20 μM, ■: 40 μM.

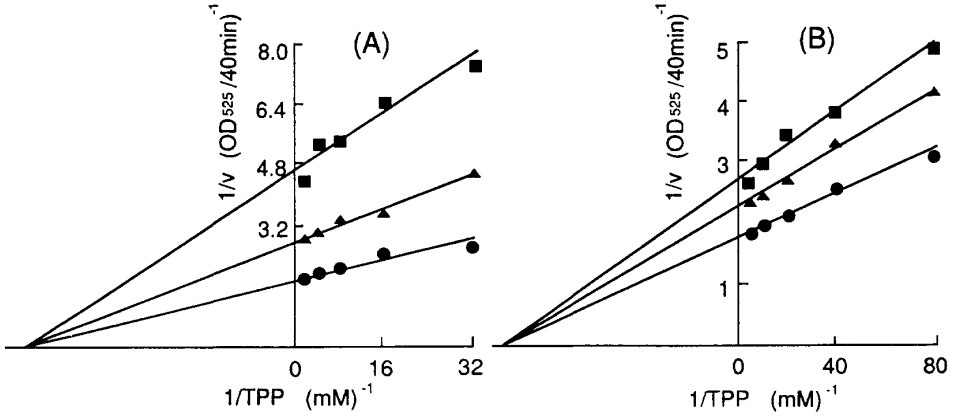


Fig. 7 Lineweaver-Burk plots of the inhibition of crude ALS from etiolated pea seedlings by pyrithiobac (A) and compound **2** (B) with variable concentration of thiamine pyrophosphate.
(A) ●: control, ▲: 10 nM, ■: 20 nM. (B) ●: control, ▲: 50 nM, ■: 100 nM.

Table 2 Inhibitions of different forms of acetolactate synthase of etiolated pea seedlings by pyrimidinylsalicylic acids, chlorsulfuron, imazapyr and branched-chain amino acids.

Compound	Crude ALS[a]				Large ALS[b]				Small ALS[c]			
	Type[d]	K_{is} (nM)	K_{ii} (nM)	I_{50} (nM)	Type	K_{is} (nM)	K_{ii} (nM)	I_{50} (nM)	Type	K_{is} (nM)	K_{ii} (nM)	I_{50} (nM)
Pyrithiobac	Mix	4 8	17	20	Mix	5 0	22	—[e]	Mix	8 0	21	—
Compound **2**	Mix	124	440	390	—	—	—	—	—	—	—	440
Chlorsulfuron	Mix	5 6	24	27	Mix	4.2	18	—	Mix	6 2	26	—
Imazapyr	Un	∞	34,000	37,000	Un	∞	33,000	—	—	—	—	40,000
Leu+Val[f]	pCo	—	—	60%[g]	—	—	—	40%[g]	—	—	—	5%[g]

[a] The fraction precipitated with ammonium sulfate (25–55%).
[b] The higher molecular weight species of ALS separated by Cellulofine GCL-2000 sf column chromatography.
[c] The lower molecular weight species of ALS separated by Cellulofine GCL-2000 sf column chromatography.
[d] Inhibition type: mixed (Mix), uncompetitive (Un), partially competitive (pCo).
[e] Not tested.
[f] 2 mM L-leucine plus 2 mM L-valine.
[g] Inhibition percentage.

40-min-fixed-time assay. Pyrithiobac, compound **1** and compound **2** showed the mixed-type inhibitions with respect to pyruvate (Fig. 5A, B, Table 1). This inhibition type was same as chlorsulfuron (Fig. 6A), but different from imazapyr (Fig. 6B) and branched-chain amino acids which showed an uncompetitive inhibition and partially competitive inhibitions, respectively (Table 1). On the other hand, pyrithiobac and compound **2** gave non-competitive inhibition as same as chlorsulfuron, imazapyr and L-leucine with respect to TPP (Fig. 7A, B, Table 1). The values of inhibition constant of pyrithiobac and compound **1** were nearly identical to that of chlorsulfuron. The K_{ii} value of pyrithiobac and compound **1** were 3 to 3.5 times larger than the K_{is} value. The K_{ii} value of partially competitive inhibitions by L-valine and L-isoleucine were approximately 10 times larger than the K_{is} value (Table 1).

2.2 Analysis using partially purified two molecular species

The inhibitions of the activities of large ALS and small ALS by PS compounds, chlorsulfuron, imazapyr and branched-chain amino acids were analyzed by double reciprocal plots. Pyrithiobac and chlorsulfuron gave mixed-type inhibition to both large ALS and small ALS as in the case of crude ALS, while imazapyr

showed an uncompetitive to large ALS as in the case of crude ALS. The inhibition constants of pyrithiobac and chlorsulfuron for large ALS and small ALS were nearly identical to those for crude ALS, and also that of imazapyr for large ALS was similar to that of crude ALS. Compound **2** and imazapyr inhibited the activity of small ALS as potently as crude ALS. Leucine plus valine pair inhibited the activities of both crude ALS and large ALS, but not small ALS (Table 2).

3. *Analysis of the Inhibition as Slowly Reversible Inhibitors (Slow-Binding Inhibitors)*

3.1 *Slow-binding*

Slow-binding properties of the inhibition to ALS activity by pyrithiobac and compound **2** were analyzed using crude ALS. Pyrithiobac and compound **2** acted as biphasic slow-binding inhibitors under the condition where the ALS activity of the sample without inhibitors was linear up to 3.3 hr in the presence of 160 mM pyruvate which showed no substrate inhibition (Fig. 8A, the data of compound **2** were not shown). On the other hand, leucine plus valine pair acted as typical reversible inhibitors (Fig. 8B).

3.2 *Inactivation of ALS during the reaction*

The activity of crude ALS of the sample in the presence of 2 mM pyruvate declined dramatically (Fig. 9A). This drastical reduction of the velocity could not be ascribed to the decrease of pyruvate concentration, for the loss of pyruvate during the reaction was approximately 10% of the initial concentration. Thus, from the plots in Fig. 9A, the velocities at appropriate time points were found through calculation and fitted to the below equation of the enzyme inactivation reaction.

$$\ln(v)=\ln\{VS/(S+Km)\}-kt \qquad (8)$$

where ln is the natural logarithm, v is the velocity, V is the maximal velocity, S is the substrate concentration, Km is the Michaelis constant, k is the first order rate constant of the enzyme inactivation and t is time.

As a result, the linear plots were yielded on the natural logarithm values of velocities as a function of time (Fig. 9B), indicating that the decline of linearity of the progressive curve was due to the inactivation of enzyme under the low pyruvate concentration. Moreover, the slope gave the k value, from which the half-time of the inactivation was found through calculation to be 77 min.

3.3 *Determination of the inhibition type of the initial inhibition*

Because of instability of ALS under the low pyruvate concentration described above, only

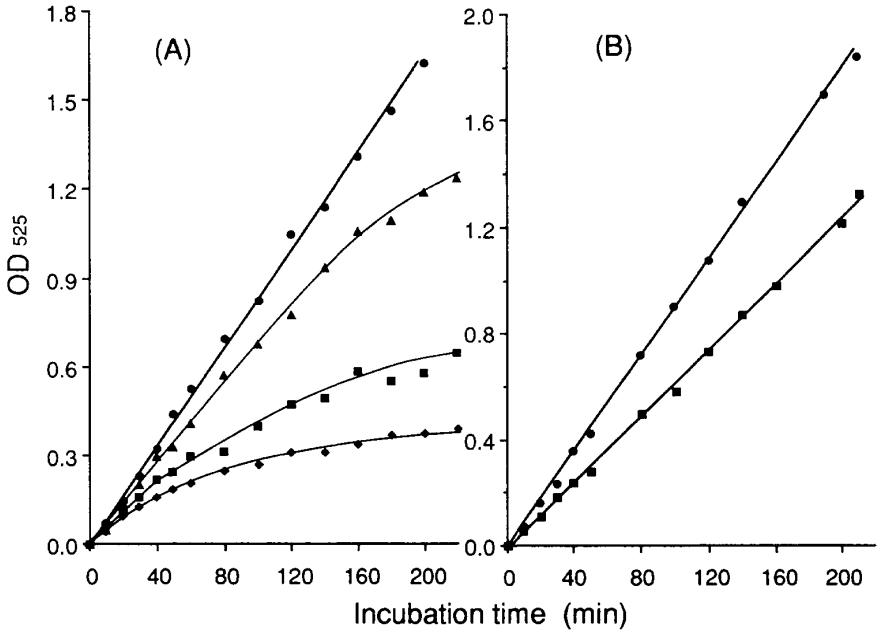


Fig. 8 Assay time-course of crude ALS of etiolated pea seedlings in the presence of pyrithiobac (A) and leucine plus valine pair (B) under 160 mM of pyruvate concentration.

(A) ●: control, ▲: 2 nM, ■: 10 nM, ◆: 20 nM. (B) ●: control, ■: 1 mM each.

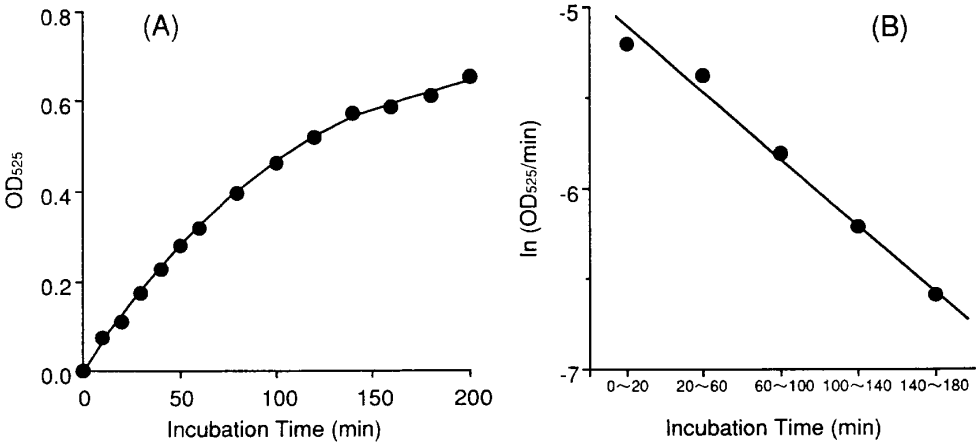


Fig. 9 Assay time-course of crude ALS of etiolated pea seedlings under 2 mM of pyruvate concentration (A) and the plots of the natural logarithm values of velocities at appropriate time points in (A) as a function of time (B).

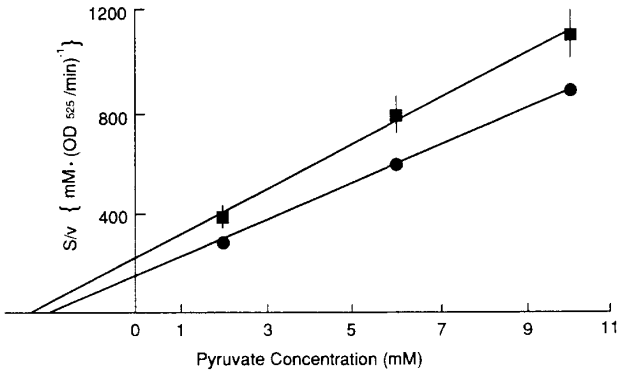


Fig. 10 Hanes-Woolf plots of the initial inhibition of crude ALS of etiolated pea seedling by pyrithiobac with variable pyruvate concentration.
●: control, ■: 10 nM.

the initial velocities (v_i) could be determined from fitting the data for product formation as a function of time to the Eq. (2), when low pyruvate concentrations were employed. With pyrithiobac using crude ALS, three pyruvate concentrations (2, 6, 10 mM) were tested to find three initial velocities. And the resultant velocities were fitted to S/v *vs.* S plots (Hanes-Woolf plots) to know the inhibition type of the initial inhibition. Consequently, a mixed-type inhibition was obtained (Fig. 10), which was identical to that obtained by the foregoing 40-min-fixed-time assay.

3.4 Determination of the inhibition constants and the k_i value

Since the initial inhibition type of pyrithiobac was proved to be a mixed-type, we determined the inhibition constants of the initial inhibition and the final steady state inhibition from fitting the velocities with 160 mM

Table 3 Inhibition constants of the initial and the final steady state inhibition, and maximal first order rate constant for transition from the initial to the final steady state inhibition, of the slow-binding inhibition of acetolactate synthase of etiolated pea seedlings by pyrimidinylsalicylic acids.

Compound	Initial[a]		Final[b]		Initial/Final ratio[c]	Rate[d] (min^{-1})
	K_{is} (nM)	K_{ii} (nM)	K_{is} (nM)	K_{ii} (nM)		
Pyrithiobac	28	82	1.1	3.1	26	0.069
Compound **2**	280	810	22	63	13	—[e]

[a] Initial inhibition.
[b] Final steady state inhibition.
[c] Ratio of the initial inhibition constant to the final steady state inhibition constant.
[d] Maximal first-order rate constant for transition from the initial to the final steady state inhibition.
[e] Not tested.

pyruvate and inhibitors to the Eqs. (3)–(6) which are derived for two step mixed-type inhibition. Moreover, three rate constants (k) for transition from the initial to the final steady state inhibition were determined with pyrithiobac by employing three different concentrations of pyrithiobac, and the maximal first order rate constant for transition from the initial to the final inhibition (k_1) was evaluated from the double-reciprocal plots of the reciprocal of pyrithiobac concentrations ($1/I$) *vs.* the reciprocal of the rate constants ($1/k$) under the assumptions of $k_1=k_2$ and $k_1 \gg k_1'$ in the Eq. (7). As results, the initial inhibition constants of pyrithiobac and compound **2** were 26- and 13-times larger, respectively, than those of the final steady state inhibition. The k_1 value of pyrithiobac was evaluated to be 0.069 min^{-1} (Table 3). From these values, the association and dissociation half-time of the final enzyme-inhibitor complex were found to be 10 min and 260 min, respectively.

DISCUSSION

It has been shown that SU herbicides such as chlorsulfuron[5] and bensulfuron-methyl,[14] and one of the TP herbicides[6] inhibit ALS activity of plants in the mixed-type manner with respect to pyruvate, while one of the IZ herbicides, imazapyr, inhibits it in the uncompetitive.[3] We showed the same inhibition types with PS compounds as those of SU and TP herbicides. These inhibition types are different from those by feedback inhibitors; leucine[10] and valine,[15] whose inhibition types are partially competitive (Table 1). These results indicate that the binding sites of PS and SU herbicides on the enzyme are different from those of feedback inhibitors, though there is a report which has shown that the binding sites of SU, IZ, TP herbicides and feedback inhibitors are identical.[16] This idea could be supported by the results that the sensitivity of the ALS inhibitions by PS, SU and IZ to the assay pH and the SH inhibitors differed from that by L-leucine (Figs. 3 and 4), and that the small ALS which lost their sensitivities to the feedback inhibition was potently inhibited by those ALS-inhibiting herbicides (Table 2). It would seem that the reports by LaRossa & Schloss[9] and Singh *et al.*[17] also advocate this idea, which have shown that isozyme II of ALS from *Salmonella typhimurium* having no sensitivity to the feedback inhibition *a priori*, and the molecular species of ALS insensitive to feedback inhibition, are both inhibited by ALS-inhibiting herbicides. Nakata,[18] Singh *et al.*[19] and Subramanian *et al.*[20] have reported that the inhibition types of SU, IZ and TP herbicides are not competitive with respect to TPP. We showed that PS compounds, SU and IZ herbicides were non-competitive inhibitors for TPP, whereas PS and SU are mixed type ones and IZ was an uncompetitive one for pyruvate (Table 1). Schloss *et al.*[21] have demonstrated that imazapyr competes to the binding of sulfometsuron methyl (one of SU herbicides) to the enzyme. After taking these results and information into consideration, it is suggested that the binding sites of PS compounds, and also SU and TP herbicides on the enzyme might be located on the allosteric site in a wide sense near the site where pyruvate binds, and IZ herbicides bind to the site which differs from but overlaps with that of these three type herbicides. This site is considered not to the regulatory site which is called the allosteric site in a narrow sense, but to be the ubiquinone binding site on the enzyme, which lost its role in the enzymatic reaction during the evolutionary process.[21]

On the other hand, it has been shown that SU and IZ herbicides are slow-binding inhibitors[7,9] which inactivate ALS irreversibly after the gaining of the final steady inhibitions.[5] And the irreversible inactivation of the enzyme has been found only when pyruvate exists in the reaction mixture.[22] We, therefore, examined slow-binding properties of PS compounds. In consequence, pyrithiobac was proved to be a slow-binding inhibitor which showed a mixed-type inhibition in the initial inhibition. This inhibition type was in contrast with that reported on one of the pyrimidinylpicolinic acid compounds,[23] which is an analog of PS compounds. Moreover, the maximal first order rate constant for transition from the initial to the final steady state inhibition (k_1) of pyrithiobac was nearly identical to those of other ALS-inhibiting

herbicides.[8] This result might support the hypothesis of Hawkes[8] that the apparent slow phase of ALS inhibition by ALS-inhibiting herbicides is due to slow irreversible inactivation of the enzyme rather than isomerization of the enzyme-inhibitor complex to a more tightly bound form as proposed originally.[9] For it seems more reasonable to consider that ALS is inactivated by different kinds of ALS inhibitors with the same k_1 value from its labile nature, rather than to consider that different kinds of ALS inhibitors have the same k_1 value. The binding assay between ALS inhibitors and ALS at different time points of incubation shall give an important clue for further understanding of this point. A different reaction scheme from Fig. 2 might be required depending on the result of further studies. In every event, these slow-binding properties are assumed to be one of the major factors for the extremely high herbicidal abilities of PS compounds as well as other generally acknowledged ALS inhibitors.

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REFERENCES

1) T. Shimizu, I. Nakayama, T. Nakao, Y. Nezu & H. Abe: *J. Pesticide Sci.* **19**, 59 (1994)
2) T. B. Ray: *Plant Physiol.* **75**, 827 (1984)
3) D. L. Shaner, P. C. Anderson & M. A. Stidham: *Plant Physiol.* **76**, 545 (1984)
4) B. C. Gerwick, M. V. Subramanian, V. I. L. Gallant & D. P. Chandler: *Pestic. Sci.* **29**, 357 (1990)
5) J. Durner, V. Gailus & P. Böger: *Plant Physiol.* **95**, 1144 (1991)
6) M. V. Subramanian & B. C. Gerwick: "ACS Symposium Series, Biocatalysis in Agricultural Biotechnology," ed. by J. R. Whitaker & P. E. Sonnet, No. 389, American Chemical Society, Washington, D.C., pp. 277–288, 1989
7) M. J. Muhitch, D. L. Shaner & M. A. Stidham: *Plant Physiol.* **83**, 451 (1987)
8) T. R. Hawkes: "BCPC Monograph, Prospect for Amino Acid Biosynthesis Inhibitors in Crop Protection and Pharmaceutical Chemistry," ed. by L. G. Copping, J. Dalziel & A. D. Dodge' No. 42, Lavenham Press, Lavenham, Suffolk, pp. 131–138, 1989
9) R. A. LaRossa & J. V. Schloss: *J. Biol. Chem.* **259**, 8753 (1984)
10) T. Shimizu, I. Nakayama, N. Nakao & H. Abe: *J. Pesticide Sci.* **19**, 185 (1994)
11) J. W. Williams & J. F. Morrison: "Methods in Enzymology," ed. by D. L. Purich, Vol. 63, Part A, Academic Press, New York, San Francisco, London, pp. 449–453, 1979
12) K. Yamaoka & T. Nakagawa: *J. Pharm. Dyn.* **6**, 595 (1983)
13) J. V. Schloss: "Target Sites of Herbicide Action," ed. by P. Böger & G. Sandmann, CRC Press, Boca Raton, Florida, pp. 193–198, 1989
14) K. Segnil, K. Usui & K. Ishizuka: *Weed Res. Jpn.* **38** (Suppl.), 198 (1993)
15) M. E. Davis: *Plant Physiol.* **39**, 53 (1964)
16) M. V. Subramanian, V. L. Gallant, J. M. Dias & L. C. Mireles: *Plant Physiol.* **96**, 310 (1991)
17) B. K. Singh, M. A. Stidham & D. L. Shaner: *J. Chromatogr.* **444**, 251 (1988)
18) M. Nakata: *J. Pesticide Sci.* **16**, 583 (1991)
19) B. K. Singh, K. E. Newhouse, M. A. Stidham & D. L. Shaner: "BCPC Monograph, Prospect for Amino Acid Biosynthesis Inhibitors in Crop Protection and Pharmaceutical Chemistry," ed. by L. G. Copping, J. Dalziel & A. D. Dodge, No. 42, Lavenham Press, Lavenham, Suffolk, pp. 87–95, 1989
20) M. V. Subramanian, V. Loney & L. Pao: "BCPC Monograph, Prospect for Amino Acid Biosynthesis Inhibitors in Crop Protection and Pharmaceutical Chemistry," ed. by L. G. Copping, J. Dalziel & A. D. Dodge, No. 42, Lavenham Press, Lavenham, Suffolk, pp. 97–100, 1989
21) J. V. Schloss, L. M. Ciskanik & D. E. V. Dyk: *Nature* **331**, 360 (1988)
22) T. R. Hawkes & S. E. Thomas: "Biosynthesis of Branched Chain Amino Acids," ed. by Z. Barak, D. M. Chipman & J. V. Schloss, VCH Verlagsgesellschaft, Weinheim, pp. 373–389, 1990
23) K. P. Gerbling & C. Kötter: *Pestic. Sci.* **30**, 329 (1990)

要　　約

ピリミジニルサリチル酸系除草剤の作用機構*

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棉用除草剤ピリチオバックに代表されるピリミジニルサリチル酸系化合物のエンドウアセト乳酸シンターゼ(ALS)阻害はpH変化により変動しなかったが，SH

基阻害剤の同時添加により回復した．本系統化合物はエンドウ ALS をピルビン酸に対して混合型，チアミンピロリン酸に対して非拮抗型で阻害し，さらに，フィードバック阻害に非感受性となった分子種に対しても同じ阻害形式を示した．本系統化合物の ALS 阻害は slow-binding であり，最終定常状態の阻害は初期状態の阻害よりも 13 から 26 倍強く，初期から最終状態への最大転移速度定数はすでに報告されている他の ALS 阻害剤と同様な値となった．この結果，本系統化合物はスルホニルウレア系除草剤のカテゴリーに属す slow-binding な ALS 阻害剤であり，その ALS 結合部位はピルビン酸結合部位に近接した広義でのアロステリック部位であると考えられる．

* ピリミジニルサリチル酸系除草剤のアセト乳酸シンターゼ阻害様式の解析 (第3報)