Acetolactate Synthase Inhibiting Herbicides Bind to the Regulatory Site

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ABSTRACT

Acetolactate synthase from spontaneous mutants of tobacco (Nicotiana tabacum; KS-43 and SK-53) and cotton (Gossypium hirsutum; PS-3, PSH-91, and DO-2) selected in tissue culture for resistance to a triazolopyrimidine sulfonanilide showed varying degrees of insensitivity to feedback inhibitor(s) valine and/or leucine. A similar feature was evident in the enzyme isolated from chlorsulfuron-resistant weed biotypes, Kochia scoparia and Stellaria media. Dual inhibition analyses of triazolopyrimidine sulfonanilide, thifensulfuron, and imazethapyr versus feedback inhibitor leucine revealed that the three herbicides were competitive with the amino acid for binding to acetolactate synthase from wild-type cotton cultures. Acetolactate synthase inhibiting herbicides may bind to the regulatory site on the enzyme.

MATERIALS AND METHODS

Chemicals

All ALS inhibitors used in the present study were either obtained commercially or synthesized by DowElanco chemists, Walnut Creek, CA. Pyruvate, DTT, TPP, DMSO, Val, Leu, Ile, (NH₄)₂SO₄, 2-naphthol, and creatine were purchased from Sigma Chemical Company, St. Louis, MO. The media components of tissue culture were purchased from Gibco Laboratories, Grand Island, NY.

Whole Plant and Tissue Culture Materials

Wild-type cultures of tobacco (Nicotiana tabacum) and cotton (Gossypium hirsutum) were routinely maintained in MS mineral medium (10) supplemented with 3% w/v sucrose, 0.5 mg/L thiamine hydrochloride, and 0.4 mg/L (tobacco) or 4.0 mg/L (cotton) 2,4-D. Mutants of tobacco and cotton were selected for resistance to TP as described previously (21). The mutants were maintained at the maximum tolerable concentration of TP (480 ppb for tobacco mutants KS-43 and SK-53; 80, 800, and 1000 ppb for cotton mutants DO-2, PSH-91, and PS-3, respectively). A chlorsulfuron-resistant biotype of Stellaria media was collected from Stony Plain, Alberta, Canada. Kochia scoparia resistant to chlorsulfuron was obtained from North Dakota by DuPont field research personnel and made available for testing by J. Saldini, E.I. DuPont De Nemours & Company. The same two plants collected from an adjacent area not exposed to chlorsulfuron were of insufficient viability for biochemical studies. Hence, commercially available S. media and K. scoparia purchased from Herbiseed, UK, and Seeds for Research, Plentywood, Montana, respectively, were used as wild-type controls. The plants were grown in 4-inch pots containing a sandy loam soil, under greenhouse conditions (20–22°C days and 15°C nights). Twelve- to 18-d-old shoots were excised from the pots and used as the source of ALS.

Enzyme Extraction and Assay

Crude extracts of suspension cultures at mid-log phase of growth were prepared in 20 mM potassium phosphate buffer, pH 7.15, containing 1 mM DTT and 5 mM MgCl₂ as described

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1 Abbreviations: TP, triazolopyrimidine sulfonanilide; ALS, acetolactate synthase; SU, sulfonylurea; IM, imidazolinone; TPP, thiamine pyrophosphate.
RESULTS AND DISCUSSION

Effect of Valine and/or Leucine on Mutant Enzymes

Both Val and Leu (end products of the ALS pathway) inhibited wild-type cotton ALS, but the enzyme from tobacco, Kochia, and Stellaria was inhibited only by Leu (Table I). None of the enzymes showed significant feedback inhibition by Ile at 2 mM (data not shown). In comparison, all mutant enzymes showed varying degrees of resistance to feedback inhibition by Val and/or Leu (Table I). ALS from DO-2 showed a very low level of resistance to inhibition by the end products. In contrast, PS-3 cotton and SK-53 tobacco enzymes were highly resistant, and the remaining mutant ALS showed moderate resistance to inhibition by the branched chain amino acids (Table I). A PS-3 culture was also shown earlier to accumulate higher levels of Val, Leu, and Ile compared with wild-type cotton (21). The differences in the sensitivity of ALS from resistant lines to Val and/or Leu (Table I) suggest that they are distinct mutants. This was also evident from the variation in the cross-resistance pattern to different inhibitors for the enzyme from KS-43 tobacco, DO-2, and PS-3 cotton (21). The remainder of the isolates (Table I) also varied in their cross-resistance pattern to different ALS-inhibiting herbicides (data not shown), suggesting different mutations. Rathinasabapathi et al. (11) have also noted loss of feedback sensitivity to Val, Leu, and Ile in four chlorsulfuron-resistant isolates of Datura innoxia. Resistance to inhibition by Val has also been observed in ALS from sulfmometuron (a SU) resistant mutants of yeast (8). On the contrary, Creason and Chaleff (1) reported no alteration in the feedback sensitivity of mutant ALS from a SU-resistant tobacco plant. Conversely, a Val-resistant ALS from tobacco mutants was found to be not resistant to SU herbicides (13).

The alteration in the feedback inhibition characteristics of ALS resistant to herbicides appears to be a common feature. There is also a striking similarity in the kinetic mechanism of

![SU, THIFENSULFURON](image)

![IM](image)

![SU, CHLORSULFURON](image)

![TP](image)

**Table I. Effect of Valine and/or Leucine on Wild Type and Mutant ALS**

<table>
<thead>
<tr>
<th>Source of ALS</th>
<th>Valine</th>
<th>Leucine</th>
</tr>
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<tbody>
<tr>
<td>Wild-type tobacco</td>
<td>NI</td>
<td>1 (0.068)</td>
</tr>
<tr>
<td>KS-43 tobacco mutant</td>
<td>NI</td>
<td>2.9</td>
</tr>
<tr>
<td>SK-53 tobacco mutant</td>
<td>NI</td>
<td>30.0</td>
</tr>
<tr>
<td>Wild-type cotton</td>
<td>1 (0.56)</td>
<td>1 (0.35)</td>
</tr>
<tr>
<td>PS-3 cotton mutant</td>
<td>43-350</td>
<td>29-34</td>
</tr>
<tr>
<td>PSH-91 cotton mutant</td>
<td>3-4</td>
<td>3-4</td>
</tr>
<tr>
<td>DO-2 cotton mutant</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>K. scoparia (wild type)</td>
<td>NI</td>
<td>1 (0.29)</td>
</tr>
<tr>
<td>K. scoparia (chlorsulfuron resistant)</td>
<td>NI</td>
<td>7.0</td>
</tr>
<tr>
<td>S. media (wild type)</td>
<td>NI</td>
<td>1 (0.39)</td>
</tr>
<tr>
<td>S. media (chlorsulfuron resistant)</td>
<td>NI</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* $I_{50}$, concentration of the inhibitor required to produce 50% inhibition of enzyme activity. $I_{50}$ values for wild type enzymes are given in parentheses (in mM). + NI, no significant inhibition at 0.5 to 1 mM.

**Figure 1.** Three chemical families of herbicides known to inhibit acetolactate synthase. SU: chlorsulfuron and thifensulfuron; IM: imazethapyr; and TP. SU is proprietary chemistry of DuPont while IM and TP are proprietary to American Cyanamid and DowElanco, respectively.
ALS inhibition by the branched chain amino acids (data not shown) and the herbicides. All of these compounds are linear mixed-type inhibitors with respect to both pyruvate and TPP. The relationship between the herbicides and Leu (and Val) was further examined by dual inhibition analyses of wild-type cotton ALS. The three inhibitors, TP (Fig. 2A), thifensulfuron (Fig. 2B), and imazethapyr (Fig. 2C), were varied at different fixed levels of Leu, under saturating substrate concentration. In all three cases, the kinetic pattern of inhibition best fit a family of parallel lines (Fig. 2). Similar results were obtained when Val was substituted for Leu (data not shown). This kinetic pattern, which is typical for two competitive linear mixed-type inhibitors (17), indicates that the binding of herbicides and Leu (or Val) to ALS is mutually exclusive. The herbicide binding site in the enzyme may actually overlap with that of Leu and/or Val, as is evident from the cross-resistance data presented in Table I. Alternately, the herbicides may bind to a remote site on the enzyme and still be competitive with Leu via allosteric effects. This is the first report of a direct relationship between the different families of ALS-inhibiting herbicides and its feedback inhibitor(s). Schloss et al. (16) have demonstrated that SU, TP, and IM are not only competitive with each other but also with quinone for binding to ALS. These herbicides have been proposed to bind to a vestigial quinone site on ALS that was derived during its evolution from pyruvate oxidase (16). The relationship between the ALS-inhibiting herbicides, Leu and quinone, remains to be explored further.

**LITERATURE CITED**