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Identification and Characterization of a Biodegradative Form of Threonine Dehydratase in Senescing Tomato (*Lycopersicon esculentum*) Leaf

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Threonine dehydratase (TD; EC.4.2.1.16) is a key enzyme involved in the biosynthesis of isoleucine. Inhibition of TD by isoleucine regulates the flow of carbon to isoleucine. We have identified two different forms of TD in tomato (*Lycopersicon esculentum*) leaves. One form, present predominantly in younger leaves, is inhibited by isoleucine. The other form of TD, present primarily in older leaves, is insensitive to inhibition by isoleucine. Expression of the latter enzyme increases as the leaf ages and the highest enzyme activity is present in the old, chlorotic leaves. The specific activity of the enzyme present in older leaves is much higher than the one present in younger leaves. Both forms can use threonine and serine as substrates. Whereas TD from the older leaves had the same K_m (0.25 mM) for both substrates, the enzyme from the young leaves preferred threonine (K_m = 0.25 mM) over serine (K_m = 1.7 mM). The molecular masses of TD from the young and the old leaves were 370,000 and 200,000 D, respectively. High levels of the isoleucine-insensitive form of threonine dehydratase in the older leaves suggests an important role of threonine dehydratase in nitrogen remobilization in senescing leaves.

TD (also known as threonine deaminase; EC 4.2.1.16) catalyzes the first step in the Ile biosynthetic pathway. The reaction involves deamination and dehydration of L-Thr to produce α-ketobutyrate and ammonia. Two forms of the enzyme are present in microbes. One form of the enzyme that is feedback regulated by Ile, the end product of this pathway, is considered to be the "biosynthetic" form of the enzyme. The other form of the enzyme that is insensitive to inhibition by Ile is considered to be the "biodegradative" form of the enzyme (Umbarger and Brown, 1956; Leavitt and Umbarger, 1961; Datta, 1966; Whiteley and Tahara, 1966; Shizuta et al., 1969; Calhoun et al., 1973). Both forms of the enzyme use Thr and Ser as their substrates, with Thr being the preferred one. Expression of the two forms of TD in *Escherichia coli* is regulated by the availability of Thr in the growing medium (Wood and Gunsaulus, 1949; Umbarger and Brown, 1956).

Identification of plant mutants that are auxotrophic for Ile through lack of TD activity (Sidorov et al., 1981; Negrutiu et al., 1985) has demonstrated the significance of TD in Ile biosynthesis. Complementation of a TD-deficient *Nicotiana plumbaginifolia* mutant with the *ILV1* gene from *Saccharomyces cerevisiae* has confirmed the requirement of TD for Ile biosynthesis (Colau et al., 1987). Only the biosynthetic TD was found in spinach leaves (Sharma and Mazumdar, 1970) and Scarlet Rose tissue culture (Dougall, 1970). This form of the enzyme is chloroplast localized (Kagan et al., 1969), and the enzyme activity is inhibited by Ile (Kagan et al., 1969; Dougall, 1970; Sharma and Mazumdar, 1970). Biodegradative TD has been characterized in a number of parasitic and saprophitic plants (Kagan et al., 1969) and pea shoots (Tomova et al., 1969). High levels of the biodegradative TD were found in *Cuscuta* seeds, where the activity was suggested to utilize high concentrations of Thr and Ser present (Madan and Nath, 1983). Similar to the enzymes from *E. coli*, both forms of the plant enzyme can use Thr and Ser as substrates.

The reaction product of TD, α-ketobutyrate, is condensed with pyruvate to yield acetohydroxybutyrate by AHAS. The latter enzyme has been identified as the target site for several different chemical classes of herbicides (Ray, 1984; Shaner et al., 1984; Subramanian et al., 1989). It has been suggested that these compounds inhibit bacterial growth as a result of accumulation of α-ketobutyrate because of the inability of AHAS to utilize this substrate (LaRossa et al., 1990). We have been trying to determine whether or not these herbicides kill plants for the same reasons. Due to our interest in α-ketobutyrate levels, we decided to examine TD activity in various parts of tomato plants to gain a better understanding of the mode of action of herbicidal AHAS inhibitors. This is the first report of identification of a biodegradative form of TD whose expression appears to coincide with the onset of leaf senescence. The possible role of this enzyme in nitrogen remobilization in senescing leaves is discussed.

MATERIALS AND METHODS

Plant Material

Tomato (*Lycopersicon esculentum* cv Tiny Tim) was grown in artificial soil in a greenhouse. Different plant parts were used for enzyme extraction as described below. For the characterization of TD in two different ages of tissue, 1- to 2-cm-long leaves are designated as the young leaves and leaves longer than 15 cm are designated as the old leaves. Only the leaf blades were used for the enzyme extraction. In the studies examining the effect of leaf age on the enzyme activity, samples for the youngest leaves included more than 20 leaves and the samples for the oldest leaves included more

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Abbreviations: AHAS, acetohydroxyacid synthase; TD, threonine dehydratase.

than 5 leaves. The intermediate-leaf-age samples included leaf numbers that were in between the numbers used for the youngest and the oldest leaves. Each experiment was conducted at least two times. Additionally, each assay was performed in duplicate in each experiment and the average of the two has been reported.

Enzyme Extraction

Plant material was homogenized in 100 mM Tris buffer (pH 9) containing 100 mM KCl. The homogenate was filtered through a nylon cloth (53-μm mesh) and centrifuged at 15,000*g* for 10 min. The supernatant was desalted on a column packed with Bio-Gel P-6 DG gel (Bio-Rad, Richmond, CA) for the quantitation of total enzyme activity. For further purification of TD, the protein in the supernatant was brought to 35% saturation with respect to ammonium sulfate. Precipitated proteins were discarded following centrifugation at 15,000*g* for 15 min. The supernatant was brought to 65% saturation with ammonium sulfate and the protein pellet containing TD activity was collected by centrifugation at 15,000*g* for 15 min. The precipitated proteins were further purified by gel permeation chromatography on a Sephacryl S-300 column or by ion-exchange chromatography on DEAE cellulose followed by gel permeation chromatography. The partially purified enzyme preparations were used for the kinetic studies.

Enzyme Assays

TD activity was determined according to published procedures (Sharma and Mazumdar, 1970). The enzyme preparation was incubated in 100 mM Tris (pH 9) containing 100 mM KCl and either 12.5 mM L-Thr or 25 mM L-Ser in a total volume of 100 μL. Following incubation of the reaction mixture at 37°C for 30 min, the reaction was stopped with 100 μL of 0.05% dinitrophenylhydrazine in 1 N HCl. The ketoacids produced were allowed to react with dinitrophenylhydrazine for 10 min at room temperature. Color was developed with the addition of 100 μL of 4 N NaOH and read at 540 nm. AHAS activity was measured according to the method described previously (Singh et al., 1988).

Protein Assay

Protein amounts were determined using the Bradford method according to the procedure furnished by the manufacturer (Bio-Rad).

Molecular Mass Determination

Desalted crude enzyme preparations (200 μL) were loaded on a Waters Protein Pak 300 SW gel filtration HPLC column (30 cm × 7.5 mm i.d.). The column was preequilibrated with 50 mM Tris (pH 9) containing 100 mM KCl. Proteins were eluted using the same buffer at a flow rate of 1 mL/min. Fractions (0.5 mL) were collected and assayed for TD activity. Proteins used for calibration included catalase (232,000 D), γ-globulin (158,000 D), ovalbumin (43,000 D), and myoglobin (17,000 D).

RESULTS

TD in Flowers and Fruits

A gene encoding TD was recently isolated from tomato (Samach et al., 1991). Expression of this gene at the mRNA level was >50-fold higher in sepals, >500-fold higher in the rest of flower, and two to five times higher in fruits and stems than in leaves or roots. However, high levels of mRNA expression did not translate into high levels of TD activity in these plant parts (very little enzyme activity data were presented). To generate this information ourselves, TD was extracted and assayed from different parts of tomato plants. Petals had no detectable levels of TD activity; however, significant levels of the enzyme activity was found in sepals, fruits, and leaves (Table I). The specific activity of TD in sepals and fruits was higher in younger tissues than in the older ones (Table I). Sensitivity of the enzyme from these tissues to Ile feedback regulation also depended upon the tissue age. The enzyme activity from younger sepals was more sensitive to inhibition by Ile than the enzyme from the older sepals. Quite opposite results were seen with TD from fruits, where Ile inhibition of TD activity was greater for the enzyme from older fruits than younger fruits.

Identification of Two Forms of TD in Leaves

Highly variable results were seen in the initial studies on TD from leaves when leaves of different sizes were pooled for the enzyme assay. It was later discovered that this variability in our observations resulted from pooling different sized leaves. Therefore, TD activity was examined in leaves from the top (young leaves) to the bottom (old leaves) of a plant. For comparison we included AHAS, an enzyme that has been well characterized in our laboratory. As observed in lima bean seedlings (Singh et al., 1990), AHAS activity per unit fresh weight of tomato leaves first increased with leaf age and then rapidly declined (Fig. 1). TD activity per unit fresh weight also increased slightly with leaf age and then decreased up to leaf 5. But then TD activity increased in older, senescing leaves (Fig. 1). An interesting pattern was seen when total activity was examined as Ile-sensitive and -insen-

Table I. *TD activity in various parts of tomato plant*

Plant Part	Specific Activity	Inhibition by 1 mM Ile
	μmol mg⁻¹ protein h⁻¹	%
Petals	N.D.[a]	N.D.
Fruits		
5–7 mm (green)	821	15
30–50 mm (red)	29	97
Sepals		
From buds and flowers	4446	47
From 5- to 7-mm fruits	4725	5
From 30- to 50-mm fruits	1086	14
Leaves		
0.5–1.5 cm	750	95
Greater than 15 cm	2246	12

[a] N.D., Not detected.

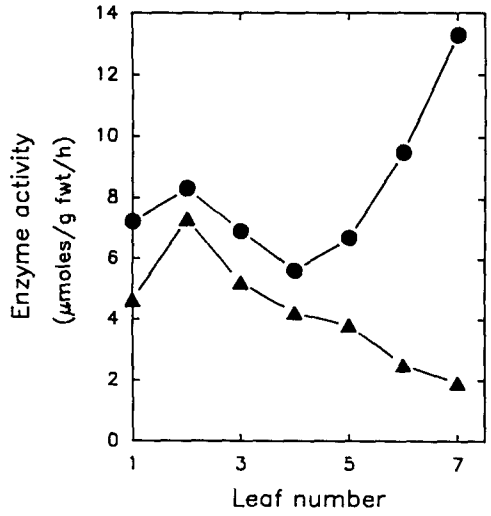

Figure 1. TD (●) and AHAS (▲) activity in the crude extracts of tomato leaves. Leaf numbers represent the leaves from the top (1 is the youngest) to the bottom (7 is the oldest) of a tomato plant.

sitive forms of the enzyme (Fig. 2). The Ile-sensitive portion of the total activity declined rapidly with leaf age. In contrast, insignificant levels of the Ile-insensitive form of the enzyme activity were present in the younger leaves. However, there was a sudden increase in the level of this form of activity in the older leaves. In this particular study, older senescing leaves were not included. It was later found that the older leaves, showing signs of senescence, had up to 10-fold higher enzyme activity than the younger leaves, as described below (Fig. 3). Therefore, the sensitivity of the enzyme activity to Ile is dependent upon the leaf age.

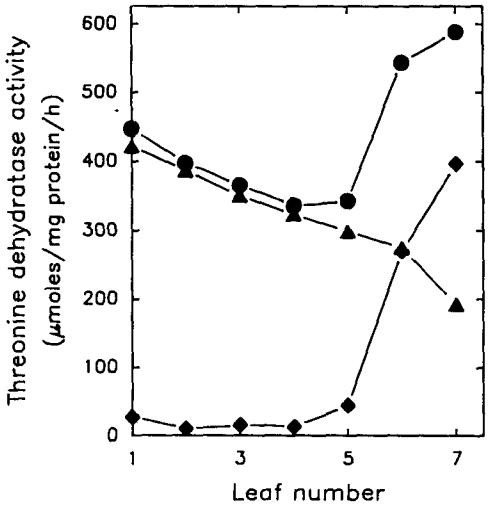

Figure 2. Specific activity of TD in the crude extracts of tomato leaves. Leaf numbers represent the leaves from the top (1 is the youngest) to the bottom (7 is the oldest) of a tomato plant. TD activity was measured in the absence and presence of 1 mM Ile. The enzyme activities shown are the Ile-sensitive form (▲), the Ile-insensitive form (◆), and the total of the two forms of activity (●).

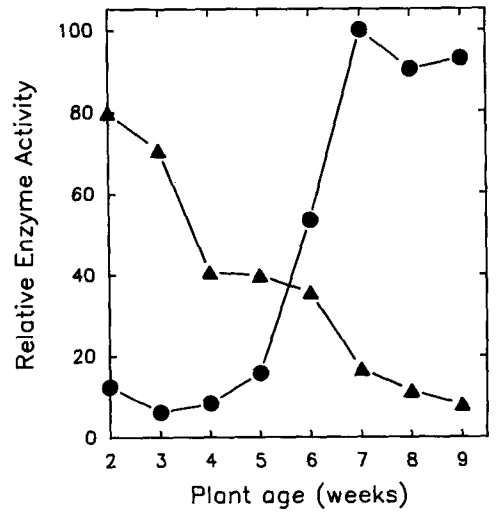

Figure 3. Specific activity of TD in the crude extracts of the third leaf from the bottom of tomato plants of different ages. TD activity was measured in the absence and presence of 1 mM Ile. The highest level of enzyme activity (344 μmol mg^{-1} protein h^{-1}) was seen in the leaves from 7-week-old plants. Results are presented as total activity in relation to the highest level of enzyme activity (●) and percent inhibition by Ile (▲).

These results were further confirmed where TD activity was examined in one particular leaf of different ages. For this study, leaf number 3 from the bottom of plants of 2 to 9 weeks old was used for TD assays. Specific activity of the enzyme initially declined and then increased with increasing leaf age (Fig. 3). Similar to the results presented in Figure 2, where leaves from the top to the bottom were examined, the increase in the specific activity of TD in older leaves was due to the appearance of an Ile-insensitive form of the enzyme. The specific activity of the enzyme was about 10-fold higher in the older leaves as compared with the younger leaves. Partially purified preparations of the two forms of TD (as described in "Materials and Methods") from younger (1–2 cm) and older leaves (>15 cm) harvested from the same plant were used for further studies.

pH Optima

TD activity from young and old leaves was assayed over a broad range of pH values using three different buffers (Mes, phosphate, and Tris). TD from young leaves had high levels of activity at pH 5.5 to 6.0 and then the activity declined rapidly with increasing pH (Fig. 4A). The activity started to rise again with increasing pH over 8.5. An interesting response to inhibition by Ile was observed (Fig. 4B). The enzyme activity was virtually insensitive to inhibition by Ile at pH 5.5, most likely due to a change in the tertiary structure of the protein at this acidic pH. However, there was increasing sensitivity of the enzyme to Ile with increasing pH. The enzyme activity was inhibited by >95% at pH 9 and above. The unusual pH optima of this enzyme are intriguing. Because this enzyme is present in chloroplasts (Samach et al., 1991, and refs. cited therein), the enzyme should be functional at neutral or slightly basic pH in vivo.

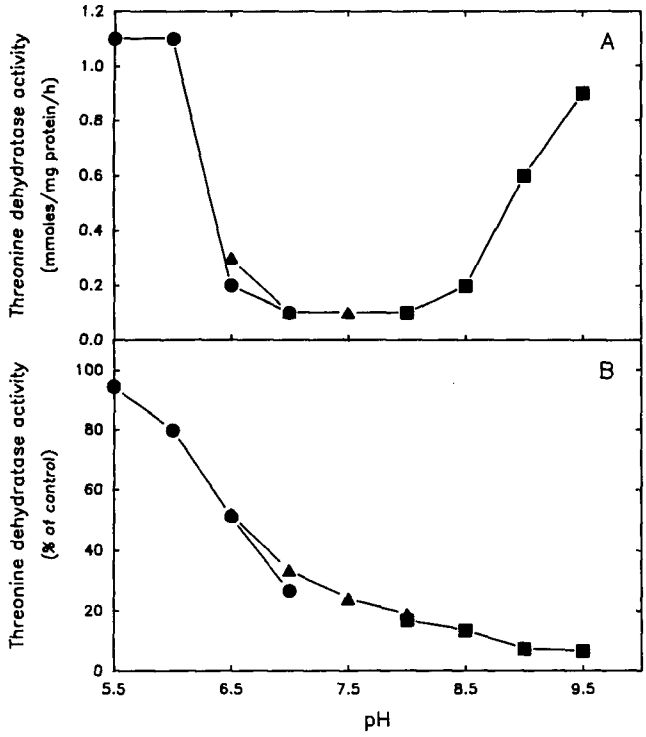

Figure 4. TD activity from young leaves at different pH (A) and its sensitivity to inhibition by Ile (B). Enzyme preparation following 35 to 65% ammonium sulfate preparation was used for the assay. ●, Mes; ▲, Tris; ■, phosphate.

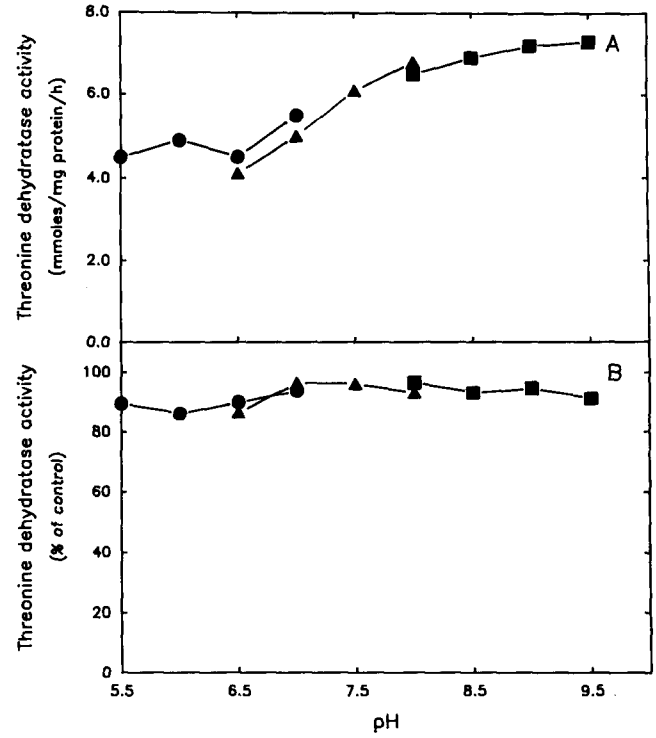

Figure 5. TD activity from old leaves at different pH (A) and its sensitivity to inhibition by Ile (B). Enzyme preparation following 35 to 65% ammonium sulfate preparation was used for the assay. ●, Mes; ▲, Tris; ■, phosphate.

Activity of TD from old leaves increased as the pH was raised, reaching a maximum between pH 9.0 and 9.5 (Fig. 5A). This enzyme activity was insensitive to Ile at every pH value (Fig. 5B). For further studies, TD activity from both sets of leaves was measured at pH 9, because the enzyme activity is high and the sensitivity of the enzyme to Ile is unaffected at this pH, and also because plant TD activity at this pH has been studied previously by other laboratories (Sharma and Mazumdar, 1970; Samach et al., 1991).

Substrate Specificity and Saturation

It has been claimed in several previous studies that both Thr and Ser are used by TD; however the reaction products were not identified (Kagan et al., 1969; Sharma and Mazumdar, 1970; Madan and Nath, 1983). Appearance of very high levels of TD activity in the older leaves prompted us to identify the reaction products to make sure that we were indeed measuring TD activity. An HPLC assay was developed that showed that TD from both young and old leaves produced α-ketobutyrate and pyruvate from Thr and Ser, respectively (Singh et al., 1993).

To determine the specificity of the enzyme for the two known substrates, Thr and Ser, three additional structurally similar amino acids, Leu, Lys, and Val, were examined as substrates for the enzyme. As expected, high levels of the enzyme activity were observed with Thr and Ser. However, there was no utilization of Leu, Lys, and Val as substrates by TD from either young or old leaves (data not presented). This result demonstrates that both forms of TD from tomato have high substrate specificity for Thr and Ser.

The enzyme from the two sources, young and old leaves, showed a hyperbolic saturation curve with Thr (data not shown). Similar saturation curves were obtained with Ser. K_m for Thr was identical (0.25 mM) for the enzyme from both young and old leaves (Table II). TD from old leaves had similar affinity for Ser (K_m = 0.25 mM). In contrast, the enzyme from young leaves preferred Thr over Ser (K_m for Ser = 1.7 mM).

Inhibition by Ile

TD from the two sources were further characterized to examine sensitivity of the enzyme to Ile in the presence of

Table II. *Summary of the properties of TD from the young leaves (fresh weight = 31 mg/leaf; Chl = 6.9 mg/g fresh weight) and the old leaves (fresh weight = 1450 mg/leaf; Chl = 0.5 mg/g fresh weight) of tomato*

Property	Young Leaves	Old Leaves
pH optima	5.5–6.0; >9.5	9.0–9.5
K_m for Thr (mM)	0.25	0.25
K_m for Ser (mM)	1.7	0.25
Inhibition by Ile (%)	>80	<10
Molecular mass (D)	370,000	200,000

Thr or Ser. The enzyme activity from young leaves was inhibited by Ile in the presence of both substrates (Fig. 6A). In contrast, TD from old leaves was insensitive to inhibition by Ile in the presence of either substrate (Fig. 6B).

Molecular Mass

To avoid any effects of steps involved in the purification procedure on the native state of TD, molecular mass determinations were performed by gel-permeation chromatography of the crude extracts. The elution profile of crude extracts from the young leaves yielded one peak of enzymic activity that was totally inhibited by 1 mM Ile (data not shown). Chromatography of the extracts from old leaves produced a major peak of the enzyme activity that was insensitive to Ile inhibition. A smaller peak of Ile-sensitive enzyme activity was also observed that corresponded to the enzyme from the young leaves. Therefore, consistent with the results described in Figure 2, tomato leaves contain two different forms of the enzyme that are separated by gel-permeation chromatography. Calibration of the column with several protein standards gave an estimated molecular mass of 370,000 and 200,000 D for TD from young and old leaves, respectively (Table II).

DISCUSSION

The Ile-sensitive form of TD activity is present in flowers, fruits, and leaves, which signifies a role of this enzyme in Ile biosynthesis in all of these plant parts. The specific activity of this enzyme was about 6-fold higher in young sepals than in young leaves (Table I). However, this difference does not account for the 50- to 500-fold higher mRNA levels previously reported in flower parts (Samach et al., 1991). Indeed, protein expression quantified by antibodies against the enzyme or the enzyme activity did not correlate with the levels of mRNA in the work of Samach et al. Our results confirm their enzyme assay results and demonstrate that high levels of mRNA and protein expressed in flower parts are enzymically inactive.

In general, higher concentrations of amino acid biosynthetic enzymes are present in young, growing tissue, where demand for metabolites is greater compared to older tissue.

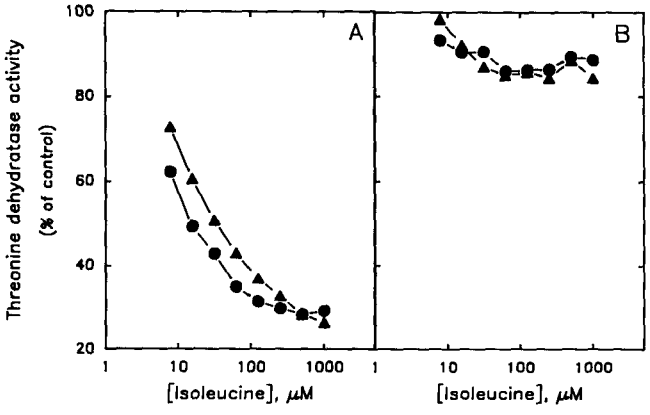

Figure 6. Inhibition of TD activity by Ile in the presence of Thr (●) or Ser (▲). A, Young leaves; B, old leaves.

TD activity followed a similar trend, in which the enzyme activity was high in the young leaves (Fig. 1) and the activity declined with leaf age. However, there was a sudden rise in the specific activity of the enzyme in the older senescing leaves. This behavior was quite different from that of AHAS, the next enzyme in the pathway. Further characterization revealed that this TD activity is due to the appearance of an Ile-insensitive form of the enzyme. Besides Ile insensitivity, the enzyme from old leaves differed from the enzyme from young leaves in pH optima, substrate saturation, and molecular mass (Table II). Differences in the molecular masses of the two forms of enzyme led us to suspect that the two proteins are different aggregation states of the same subunit(s). However, we never got any indication of this possibility in numerous gel filtration studies performed. Insensitivity of the enzyme from young leaves to Ile at low pH (Fig. 4B) pointed toward a possible conversion of this enzyme to the Ile-sensitive form. However, we could not find any evidence for such a conversion either. In repeated studies on the enzyme from the two age groups of leaves, we always found >95% of the Ile-sensitive form of the enzyme in the young leaves and 80 to 95% of the enzyme activity as the Ile-insensitive form in the old leaves. A greater variability in the results with the enzyme from old leaves is due to greater heterogeneity in the age of older leaves sampled than in the age of younger leaves.

Similar to the results with leaves, there are indications of two different forms of the enzyme in sepals and fruits (Table I). The enzyme activity from young sepals (separated from buds and flowers) was more sensitive to inhibition by Ile than the enzyme activity from the old sepals (separated from fruits). A different pattern was seen in the enzyme activity from fruits, where the enzyme activity from older tissue was more sensitive to Ile inhibition than the enzyme activity from younger tissue. We did not characterize the enzymes from these sources any further, but it would be interesting to identify the origin of different forms of TD activity in these tissues.

Whether or not the two forms of TD in tomato leaves are two different gene products remains to be seen. Because there is precedence for a separate gene for biodegradative TD in microorganisms (see the refs. cited in the introduction), our results support the notion that the two forms of enzyme identified in tomato are two separate gene products. This hypothesis is supported by the presence of biodegradative form of TD in parasitic and saprophitic plants that must metabolize Thr and Ser obtained from the host plant (Kagan et al., 1969). Our results are also supported by a previous report of the identification of a biodegradative form of TD in shoots of seedlings of pea, a flowering plant (Tomova et al., 1969). However, Tomova et al. did not separate the Ile-sensitive and -insensitive form of the activity and also did not demonstrate the regulation of expression of the two forms of enzyme activity.

A sharp increase in specific activity, a higher affinity for Ser, and Ile insensitivity are properties of TD extracted from aging leaves. All these changes in TD suggest that new reactions are taking place in older leaves of tomato. It is possible that there is a separate pathway for utilization of α-ketobutyrate and pyruvate that does not lead to amino acid

synthesis; thus TD, as the enzyme involved in that process, would not be inhibited by Ile. We propose and support another plausible explanation for the emergence of the biodegradative form of TD in older leaves. Proteins break down during leaf senescence, and ammonia from liberated amino acids is transferred to Gln, which is then transported out of the senescing leaves (Thimann, 1980). However, the mechanism of deamination of amino acids in the older leaves has not been elucidated. A new form of TD described here will degrade Thr and Ser to release ammonia, which can be used by Gln synthetase to produce Gln. It has been shown that Gln synthetase activity in the senescing leaf is sufficient to produce Gln from all of the N released during protein hydrolysis (Storey and Beevers, 1978). Therefore, the biodegradative form of TD may metabolize both Thr and Ser to release N for use in young, growing tissues. Similar forms of other enzymes for release of ammonia from other amino acids may be present in senescing leaves, which may complete the missing link of how ammonia is released from amino acids before incorporation into Gln in senescing leaves.

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LITERATURE CITED

Calhoun DH, Rimerman A, Hatfield GW (1973) Threonine deaminase from *Escherichia coli*. I. Purification and properties. J Biol Chem **248:** 3511–3516

Colau D, Negrutiu I, Van Montagu M, Hernalsteens J-P (1987) Complementation of a threonine dehydratase-deficient *Nicotiana plumbaginifolia* mutant after *Agrobacterium tumefaciens*-mediated transfer of *Saccharomyces cerevisiae* ILV1 gene. Mol Cell Biol **7:** 2552–2557

Datta P (1966) Purification and feedback control of threonine deaminase activity of *Rhodopseudomonas spheroides*. J Biol Chem **241:** 5836–5844

Dougall DK (1970) Threonine deaminase from Paul's Scarlet rose tissue cultures. Phytochemistry **9:** 959–964

Kagan ZS, Sinelnikova EM, Kretovich WL (1969) L-Threonine dehydratases of flowering parasitic and saprophytic plants. Enzymologia **36:** 335–352

LaRossa RA, Van Dyk TK, Smulski DR (1990) A need for metabolic insulation: lessons from sulfonylurea genetics. *In* Z Barak, DM Chipman, JV Schloss, eds, Biosynthesis of Branched Chain Amino Acids. VCH Publishers, Weinheim, Germany, pp 109–121

Leavitt RI, Umbarger HE (1961) Isoleucine and valine metabolism in *Escherichia coli*. X. The enzymatic formation of acetohydroxybutyrate. J Biol Chem **236:** 2486–2491

Madan VK, Nath M (1983) Threonine(Serine) dehydratase from *Cuscuta campestris* Yunck. Biochem Physiol Pflanzen **178:** 43–51

Negrutiu I, De Brouwer D, Dirks R, Jacobs M (1985) Amino acid auxotrophs from protoplast cultures of *Nicotiana plumbaginifolia* Viviani. Mol Gen Genet **199:** 330–337

Ray TB (1984) Site of action of chlorsulfuron. Plant Physiol **75:** 827–831

Samach A, Harevent D, Gutfinger T, Ken-Dror S, Lifschitz E (1991) Biosynthetic threonine deaminase gene of tomato: isolation, structure and upregulation in floral organs. Proc Natl Acad Sci USA **88:** 2678–2682

Shaner DL, Anderson PA, Stidham MA (1984) Imidazolinone: potent inhibitors of acetohydroxyacid synthase. Plant Physiol **76:** 545–546

Sharma RK, Mazumder R (1970) Purification, properties and feedback control of threonine dehydratase from spinach. J Biol Chem **245:** 3008–3014

Shizuta Y, Nakazawa A, Tokushige M, Hayaishi O (1969) Studies on the interaction between regulatory enzymes and effectors. III. Crystallization and characterization of adenosine-5′-monophosphate-dependent threonine deaminase from *Escherichia coli*. J Biol Chem **244:** 1883–1889

Sidorov V, Menczel L, Maliga P (1981) Isoleucine-requiring *Nicotiana* plant deficient in threonine deaminase. Nature **294:** 87–88

Singh BK, Newhouse KE, Stidham MA, Shaner DL (1990) Acetohydroxyacid synthase-imidazolinone interaction. *In* Z Barak, DM Chipman, JV Schloss, eds, Biosynthesis of Branched Chain Amino Acids. VCH Publishers, Weinheim, Germany, pp 357–372

Singh BK, Stidham MA, Shaner DL (1988) Assay of acetohydroxyacid synthase from plants. Anal Biochem **171:** 173–179

Singh BK, Szamosi IT, Shaner DL (1993) A high performance liquid chromatography assay for threonine/serine dehydratase. Anal Biochem **208:** 260–263

Storey R, Beevers L (1978) Enzymology of glutamine metabolism related to senescence and seed development in the pea (*Pisum sativum* L.). Plant Physiol **61:** 494–500

Subramanian MV, Loney V, Pao L (1989) Mechanism of action of 1,2,4-triazolo[1,5-a]pyrimidine sulfonamide herbicides. *In* LG Copping, J Dalziel, AD Dodge, eds, Prospects for Amino Acid Biosynthesis Inhibitors in Crop Protection and Pharmaceutical Chemistry, Monograph No 42. British Crop Protection Council, Surrey, UK, pp 97–100

Thimann KV (1980) The senescence of leaves. *In* KV Thimann, ed, Senescence in Plants. CRC Press, Boca Raton, FL, pp 85–116

Tomova WS, Kagan ZS, Blekhman GI, Kretovich WL (1969) Biodegradative L-threonine dehydratase from pea seedlings. Biokhimiya **34:** 266–272

Umbarger HE, Brown B (1956) Threonine deamination in *Escherichia coli*. II. Evidence for two L-threonine deaminases. J Bacteriol **73:** 105–113

Whiteley HR, Tahara M (1966) Threonine deaminase of *Clostridium tetanomorphum*. I. Purification and properties. J Biol Chem **241:** 4881–4889

Wood WA, Gunsalus IC (1949) Serine and threonine deaminases of *Escherichia coli*: activators for a cell-free enzyme. J Biol Chem **181:** 171–182