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Biosynthesis of Branched Chain Amino Acids: From Test Tube to Field

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INTRODUCTION

The branched chain amino acids—valine, leucine, and isoleucine—are among the 10 essential amino acids that are not synthesized in mammals. Therefore, biosynthesis of branched chain amino acids in plants is of interest due to their importance in human and animal diets. In addition, in the early 1980s, two new classes of herbicides (imidazolinones and sulfonylureas) were introduced that inhibit the branched chain amino acid biosynthetic pathway (Levitt et al., 1981; Orwick et al., 1983; Ray, 1984; Shaner et al., 1984). These new herbicides were unique in that they control many important weeds in different crops at low-use rates. The introduction of these two classes of herbicides initiated a new era in agricultural practices worldwide due to their unique mode of action, coupled with their low mammalian toxicity and high potency. The discovery and development of these herbicides and of crops resistant to them have led to an explosion in the scientific literature on biochemical, molecular, and genetic aspects of branched chain amino acid biosynthesis. In this review, we summarize how these studies have led to our current understanding of the regulation of the branched chain amino acid biosynthetic pathway in plants.

WHAT ARE THE ENZYMES OF THE VALINE, LEUCINE, AND ISOLEUCINE BIOSYNTHETIC PATHWAY?

The biosynthetic pathway of branched chain amino acids is unique in the sense that a set of four enzymes carries out reactions in parallel pathways using different substrates leading to the biosynthesis of isoleucine or valine and leucine (Figure 1). Threonine dehydratase (TD; also known as threonine deaminase) catalyzes the first step in the isoleucine biosynthetic pathway. TD deaminates and dehydrates threonine to produce 2-ketobutyrate (2-KB) and ammonia. A form of this enzyme present predominantly in younger leaves is considered to be the "biosynthetic" form of the enzyme because it is feedback inhibited by isoleucine, the end product of the pathway (Szamosi et al., 1993, and references therein). The gene encoding the biosynthetic isozyme of TD has been isolated from tomato and potato (Samach et al., 1991; Hofgen et al., 1995a). Identification of plant mutants that are auxotrophic for isoleucine because of a lack of TD activity (Sidorov et al., 1981; Negrutiu et al., 1985) has demonstrated that TD is essential for isoleucine biosynthesis, and complementation of a TD-deficient *Nicotiana plumbaginifolia* mutant with the *Saccharomyces cerevisiae ilv1* gene, which encodes for TD, confirmed the requirement of TD for isoleucine biosynthesis (Colau et al., 1987).

Another form of TD, which is insensitive to inhibition by isoleucine, has been found in a number of parasitic and saprophytic plants (Kagan et al., 1969) and in *Cuscuta* seeds, which contain high levels of threonine and serine (Madan and Nath, 1983). This form of the enzyme, considered the "biodegradative" form, can use both threonine and serine as substrates. During senescence, amino acids are liberated as a result of protein degradation (see Callis, 1995, this issue). The biodegradative form of TD degrades threonine and serine to release ammonia, which can be converted to glutamine by glutamine synthetase (see Lam et al., 1995, this issue). Glutamine can then be transported to growing tissues or to storage tissue. Recently, a biodegradative form of TD was found primarily in older, senescing leaves of tomato (Szamosi et al., 1993; Mourad and King, 1995). The appearance of the isoleucine-insensitive enzyme during senescence thus suggests that it may play a role in remobilization of nitrogen during senescence. However, the function of this form of the protein in plants can be confirmed only by genetic studies involving isolation of appropriate mutants and characterization of the gene encoding this enzyme.

Acetohydroxyacid synthase (AHAS, also known as acetolactate synthase) carries out the first set of parallel reactions in the pathway (Figure 1). In the pathway that produces valine and leucine, AHAS catalyzes the condensation of two molecules of pyruvate to yield acetolactate. In the pathway that produces isoleucine, AHAS catalyzes the condensation of pyruvate and 2-KB to yield acetohydroxybutyrate. In the two reactions catalyzed by AHAS, an intermediate is first formed between a thiamine pyrophosphate (TPP) cofactor and pyruvate. This intermediate undergoes decarboxylation to produce a stabilized anion of hydroxyethyl-TPP, which acts as a nucleophile on the 2-keto group of a second molecule of pyruvate or 2-KB.

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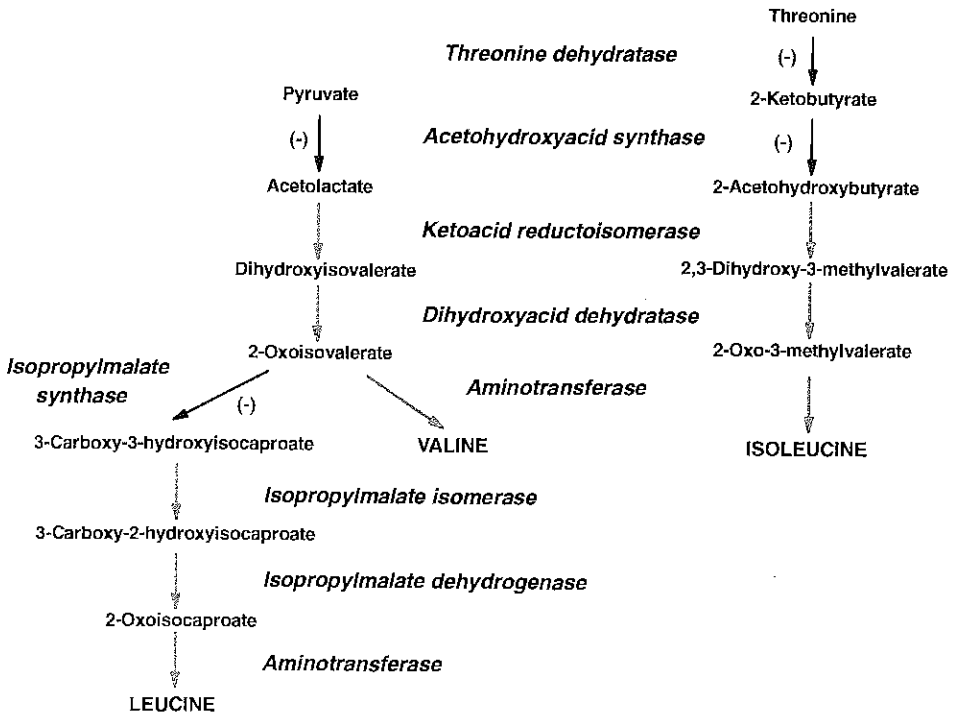

Figure 1. Biosynthetic Pathway of Valine, Leucine, and Isoleucine in Plants.

A set of four enzymes carries out parallel reactions in the pathway. The red arrows and minus signs indicate the biosynthetic steps at which the pathway is regulated due to feedback inhibition of the corresponding enzymes by the end products. Threonine deaminase is inhibited by isoleucine, acetohydroxyacid synthase is inhibited by valine and leucine, and isopropylmalate synthase is inhibited by leucine.

The result is the release of TPP and acetolactate or acetohydroxybutyrate. Although there is no net oxidation or reduction in this reaction, an unusual feature of both bacterial and plant AHAS is its dependence on flavin adenine dinucleotide (FAD) for enzyme activity (Stormer and Umbarger, 1964; Schloss et al., 1985; Muhitch et al., 1987; Singh et al., 1988). The pyruvate oxidase and AHAS genes show strong amino acid sequence similarities, which suggests that the AHAS enzymes are descended from pyruvate oxidase (Mazur et al., 1987; Chang and Cronan, 1988). Unlike AHAS, pyruvate oxidase uses FAD for redox chemistry. Thus, the flavin requirement of AHAS may be a vestigial remnant for a structural function. Other studies suggest possible functional roles of the flavin: it may shield the hydroxyethylthiamine intermediate from the solvent during enzyme catalysis (Schloss et al., 1988), or it may play a structural role in stabilizing the protein during catalysis (Muhitch et al., 1987; Singh and Schmitt 1989; Singh et al., 1989; Durner and Boger, 1990).

The number of AHAS genes in plants varies from one in Arabidopsis (Mazur et al., 1987) to five in *Brassica napus* (Rutledge et al., 1991). All of the genes isolated to date contain a putative chloroplast transit peptide sequence (Mazur et al., 1987; Rutledge et al., 1991; Fang et al., 1992), which is consistent with the localization of AHAS activity in the chloroplasts (Miflin, 1974). Why some plants possess multiple forms of AHAS is not understood.

Ketoacid reductoisomerase (KARI), the next enzyme in the pathway, reduces the acetohydroxyacids from the previous step to produce dihydroxyacids. The enzyme has been purified to homogeneity from spinach and barley (Dumas et al., 1989; Durner et al., 1993), and only one gene per haploid genome was found in spinach and Arabidopsis (Dumas et al., 1991, 1993). The deduced amino acid sequence of the enzyme contains a transit peptide, which is in agreement with the localization of the enzyme in the chloroplast. Consistent with the NAD(P)H requirement of this enzyme for catalysis, the deduced amino acid sequence of the enzyme contains the "fingerprint" region of an NAD(P)H-binding site that has been reported in several NAD(P)H-dependent oxidoreductases. The spinach enzyme has been crystallized, and the crystals diffract x-rays to at least 2.5 Å (Dumas et al., 1994). Elucidation of the structure of KARI based on the crystals will aid greatly in our understanding of this enzyme.

Dihydroxyacid dehydratase carries out the next reaction in the pathway. An absolute requirement of this enzyme for the biosynthesis of the branched chain amino acids was demonstrated by isolation of auxotrophic mutants of *N. plumbaginifolia* that lack this enzyme (Wallsgrove et al., 1986). The enzyme,

which has been purified to homogeneity from spinach (Flint and Emptage, 1988; Pirrung et al., 1989), has a distinct brown color because it contains a 2Fe-2S cluster (Flint and Emptage, 1988). The Fe-S cluster is required for the enzymatic reaction, although its exact role in the reaction is not fully understood.

The last steps in the parallel pathway and the last step in leucine biosynthesis are carried out by an aminotransferase (Figure 1). Two different aminotransferase forms with different specificities for the three ketoacid substrates have been detected in barley (Wallsgrove, 1990). Similar V_{max} was observed for both forms of the enzyme when the three ketoacids were present individually. The three ketoacids in a mixture are used at comparable rates by the minor form of the enzyme. However, the major form of the enzyme uses 2-oxoisocaproate faster than 2-oxomethylvalerate and uses 2-oxoisovalerate to only a small extent. Further work on the biochemistry and molecular biology of these enzymes is needed to understand how the specificity for different substrates may regulate the biosynthesis of the three amino acids.

In contrast with what is known about valine and isoleucine biosynthesis, little is known about the biosynthesis of leucine in plants. Ketoisovalerate, the ketoacid that is transaminated to valine, is condensed with acetyl–Coenzyme A by isopropylmalate synthase in the pathway leading to leucine (Figure 1). Isopropylmalate synthase is feedback inhibited by leucine (Bryan, 1990). Isopropylmalate isomerase and isopropylmalate dehydrogenase carry out the next two reactions to produce ketoisocaproate, which is transaminated to leucine. Isopropylmalate dehydrogenase appears to be encoded by a single gene in both oilseed rape (Ellerstrom et al., 1992) and potato (Jackson et al., 1993). The genes from both species contain a putative chloroplast transit peptide, suggesting chloroplastic localization of the pathway. Future research in the leucine biosynthetic pathway should focus on characterization of the enzymes, isozyme patterns, genes encoding the enzymes, and regulation of expression of these genes.

WHERE IN THE PLANT ARE VALINE, LEUCINE, AND ISOLEUCINE SYNTHESIZED?

Valine, leucine, and isoleucine are synthesized in all plant parts, as indicated by the ubiquitous presence of the mRNAs, the encoded proteins, and the activities of various enzymes of the pathway (Wiersma et al., 1989; Schmitt and Singh, 1990; Samach et al., 1991; Hattori et al., 1992; Ouellet et al., 1992; Jackson et al., 1993; Keeler et al., 1993). In each organ, the biosynthesis of these amino acids appears to take place primarily in young tissue. This conclusion is based on three observations. First, mRNAs encoding the enzymes examined thus far are most prevalent in developing, young parts of the plant (Wiersma et al., 1989; Keeler et al., 1993). Second, the activities of various enzymes of the pathway are highest in the young tissue (Singh et al., 1990; Stidham and Singh, 1991; Szamosi et al., 1993; Hofgen et al., 1995b). Third, the flux of carbon through the branched chain amino acid biosynthetic pathway occurs primarily in young tissue (Singh et al., 1994). The presence of this pathway in young tissue is consistent with the fact that actively growing tissues require high amounts of amino acids for protein synthesis.

At the cellular level, the branched chain amino acid biosynthetic pathway appears to be located primarily in the plastids. Schulze-Siebert et al. (1984) showed that isolated spinach chloroplasts synthesize all three branched chain amino acids from ^{14}C-pyruvate, whereas no other cellular component can synthesize these amino acids. Plastidic localization of the branched chain amino acid biosynthetic pathway is supported by detection of the activity of various enzymes of the pathway in the chloroplasts (Miflin, 1974; Dumas et al., 1989) as well as by immunohistochemical studies (G.K. Schmitt and B.K. Singh, unpublished data). Furthermore, all of the genes encoding various enzymes of the pathway that have been isolated thus far encode a putative chloroplast transit peptide (Mazur et al., 1987; Wiersma et al., 1989; Dumas et al., 1991, 1993; Samach et al., 1991; Ellerstrom et al., 1992; Jackson et al., 1993).

HOW IS THE BIOSYNTHESIS OF VALINE, LEUCINE, AND ISOLEUCINE REGULATED?

The branched chain amino acid biosynthetic pathway feeds carbon into three different amino acids. The flow of carbon must therefore be tightly regulated so that no one of these amino acids becomes limiting for plant growth. As in *Escherichia coli*, at least three different regulatory mechanisms appear to control flow of carbon through this pathway in plants: gene expression, substrate specificity of the enzymes, and feedback inhibition of various enzymes.

As discussed in the previous section, expression of the enzymes of the branched chain amino acid biosynthetic pathway is observed primarily in the young tissues of the plant, which is where these amino acids are most needed. In addition, expression of various genes in the pathway may be regulated in different organs to meet specific needs of the particular tissue for the end products or intermediates of the pathway. For example, the level of TD mRNA is >50-fold higher in sepals and >500-fold higher in the rest of the flower than in roots or leaves (Samach et al., 1991). Similarly, the *AHAS2* gene in *B. napus* is expressed specifically in mature ovules and extraembryonic tissues of immature seeds (Hattori et al., 1992). In other tissues, genes for isozymes of AHAS are constitutively expressed at similar levels. These results suggest that constitutively expressed isozymes may be the housekeeping proteins that produce these amino acids for protein synthesis, whereas tissue-specific isozymes may have some specific roles that are not yet defined.

Substrate affinity of the four enzymes in the parallel pathway, in which each enzyme has a choice of at least two substrates (Figure 1), is another means of controlling the flow of carbon to different branched chain amino acids. Because

the concentration of 2-KB in plant cells is very low (Shaner and Singh, 1993; Singh et al., 1994), plants must contain a form of AHAS that prefers 2-KB to pyruvate if they are to direct any carbon into isoleucine biosynthesis. Indeed, an AHAS from barley prefers 2-KB to pyruvate as the second substrate in the reaction (Delfourne et al., 1994). However, higher affinity of AHAS for 2-KB may cause preferential and uninterrupted flow of carbon to isoleucine, preventing valine and isoleucine biosynthesis. Feedback inhibition of TD by isoleucine should prevent such an imbalance (Szamosi et al., 1993, and references therein). As discussed earlier, a difference in affinity for the different substrates has also been noted for the barley aminotransferases (Wallsgrove, 1990). Regulation at the terminal step of the pathway may provide finer control of regulation of carbon flow to various end products.

Feedback inhibition provides a third mode of regulation. Inhibition of TD by isoleucine is observed in vitro (Dougall, 1970; Sharma and Mazumder, 1970; Szamosi et al., 1993) and clearly operates in vivo, based on measurements of intermediates and end products in vivo (Giovanelli et al., 1988; Szamosi et al., 1994; Singh et al., 1995). Moreover, mutants of both *Rosa* callus culture and Arabidopsis that contain an isoleucine-insensitive form of TD accumulate high levels of isoleucine, corroborating the notion that isoleucine biosynthesis is regulated by feedback inhibition of TD (Strauss et al., 1985; Mourad and King, 1995). Leucine biosynthesis is also regulated by feedback inhibition, in this case of isopropylmalate synthase by leucine (Oaks, 1965).

A more general mechanism of regulation of carbon flow to all three amino acids by feedback inhibition is due to the inhibition of AHAS by valine and leucine (Miflin, 1969, 1971; Miflin and Cave, 1972; Stidham and Singh, 1991). The in vitro effects of these amino acids are seen in vivo, as measured by changes in the pools of various amino acids and effects of the amino acids on plant growth (Miflin, 1969; Borstlap, 1972). Further evidence of feedback regulation of AHAS comes from valine-resistant mutants. Valine inhibits plant growth because it inhibits AHAS (Borstlap, 1972). This inhibitory effect of valine on growth was used to isolate mutants of tobacco and Arabidopsis that can grow in the presence of normally lethal concentrations of valine or valine and leucine (Bourgin et al., 1985; Relton et al., 1986; Wu et al., 1994). Evaluation of AHAS activity from these mutants indicated that resistance to these amino acids is due to decreased sensitivity of the enzyme to valine and leucine (Relton et al., 1986; Wu et al., 1994). In Arabidopsis, valine resistance was found to be due to a single dominant mutation in a nuclear gene tightly linked to the gene encoding AHAS (Wu et al., 1994).

Interestingly, a synergistic inhibition of AHAS is observed in the presence of both valine and leucine (Miflin, 1969, 1971), suggesting that there are two separate binding sites for these amino acids (Miflin, 1971). It is not yet known where on the enzyme these amino acids bind. AHAS from *E. coli* consists of two subunits—a large subunit that contains the catalytic machinery, and a small subunit that is responsible for feedback inhibitor sensitivity (Weinstock et al., 1992, and references therein). So far, plant homologs have been detected for the large subunit only (Mazur et al., 1987; Wiersma et al., 1989; Singh et al., 1991a, 1991b; Fang et al., 1992; Hattori et al., 1992). AHAS in maize is present in various oligomeric forms with different regulatory properties. At least two different forms, a tetramer (AHAS I) and a monomer (AHAS II), have been characterized (Singh et al., 1988). Whereas AHAS I shows normal sensitivity to valine and leucine, AHAS II is insensitive to inhibition by these amino acids. These results led to the proposal that feedback inhibitor binding sites are formed by oligomerization of a single subunit of the enzyme (Stidham, 1991). This hypothesis may explain synergistic feedback inhibition of AHAS by valine and leucine, because interaction between separate subunits would be necessary for such cooperativity. However, a dimeric form of the enzyme obtained from *E. coli* expressing the Arabidopsis AHAS is insensitive to feedback inhibitors, whereas a dimeric form of the native Arabidopsis enzyme is inhibited by valine and leucine (Singh and Schmitt, 1989; Singh et al., 1992). Therefore, feedback inhibitor binding sites may not be formed simply by oligomerization of a large subunit of AHAS.

Although no small subunit of AHAS has been detected in plants, three lines of evidence imply that one may exist. First, although the catalytic function of *E. coli* isozymes is entirely contained within their large subunits (Weinstock et al., 1992), the small subunits are required for stabilization of an active conformation of the large subunits and for valine sensitivity. Because the mature AHAS protein from plants is approximately the same size as the large *E. coli* subunit, and the plant and *E. coli* enzymes share conserved structural domains (Mazur et al., 1987), it might be predicted that the two enzymes share the requirement of a small subunit for valine sensitivity. Second, the lack of sensitivity of the *E. coli*–expressed Arabidopsis AHAS to inhibition by valine and leucine could be due to the requirement for a small subunit (Singh et al., 1991a, 1991b). Third, transformation of tobacco with the Arabidopsis AHAS gene resulted in a >25-fold increase in the mRNA level but only a twofold increase in the specific activity of the enzyme (Odell et al., 1990). Because a small subunit of AHAS is required for the stabilization of an active conformation of the catalytic large subunit (Weinstock et al., 1992, and references therein) in *E. coli*, it is possible that lack of correspondence between mRNA levels and the enzyme activity in the studies of Odell et al. (1990) is also due to insufficient levels of the small subunit of AHAS in plants. However, whether or not plants contain a second subunit of AHAS can be resolved only by future work.

WHAT ARE THE INHIBITORS OF ENZYMES IN THE PATHWAY?

Research on the branched chain amino acid pathway in plants was greatly stimulated with the discovery in the early 1980s

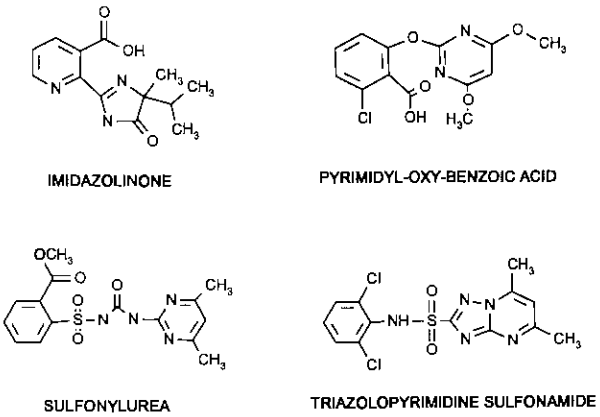

Figure 2. Structures of Inhibitors of Acetohydroxyacid Synthase.

These inhibitors and close analogs of them have been developed or are being developed as commercial herbicides.

that AHAS is the site of action of two highly active and commercially successful classes of herbicides, the imidazolinones and the sulfonylureas (Ray, 1984; Shaner et al., 1984). Several additional classes of compounds that inhibit AHAS have since been discovered (Stidham, 1991). Compounds representing imidazolinones, sulfonylureas, triazolopyrimidine sulfonamides, and pyrimidyl thio-benzoates are all being used or developed as commercial herbicides (Figure 2). These chemicals are uncompetitive inhibitors of AHAS, indicating that they bind to the enzyme–pyruvate complex (Shaner et al., 1984; Schloss, 1989). Imidazolinones and sulfonylureas are slow, tight-binding inhibitors of the enzyme (LaRossa and Schloss, 1984; Muhitch et al., 1987; Schloss, 1989). For example, imazaquin, an imidazolinone, has a K_i (initial inhibition constant) of 0.8 mM and a K_i^* (final inhibition constant) of 20 μM. The maximal rate of transition between initial and final inhibition is 0.6/min, and the half-time for reversal of the enzyme-inhibitor complex is 0.8 hr (Schloss, 1989). Sulfometuron methyl, one of the sulfonylureas, has a K_i of 1.7 μM and a K_i^* of 82 nM; the maximal rate of transition between initial and final inhibition is 0.15/min, and the half-time for reversal of the enzyme-inhibitor complex is 1.6 hr (Schloss, 1989). Sulfometuron methyl binds to the enzyme in the absence of pyruvate, TPP, and metal, but a slowly reversible complex of the enzyme and inhibitor is formed only in the presence of all cofactors and pyruvate (LaRossa and Schloss, 1984; Schloss, 1984; Schloss and Van Dyk, 1988).

Since the discovery that inhibitors of AHAS are potent herbicides nontoxic to mammals, researchers have searched for and identified inhibitors of other enzymes in the branched chain amino acid pathway (Schulz et al., 1988; Pirrung et al., 1989; Schloss and Aulabaugh, 1990; Wittenbach et al., 1992, 1994; Hawkes et al., 1993; Szamosi et al., 1994). All of these inhibitors kill plants, although the amounts needed to control weeds are relatively high as compared with inhibitors of AHAS.

WHAT HAVE WE LEARNED FROM THE INHIBITORS AND MUTANTS THAT ARE RESISTANT TO THEM?

Imidazolinones and sulfonylureas cause many different physiological responses in plants (Shaner, 1991; Shaner and Singh, 1992). However, most of the research on the physiological effects of these inhibitors has focused on two processes, mitosis and photosynthate transport. One of the first responses to inhibition of AHAS is a cessation of mitosis. This inhibition occurs between the G2 and M phases of mitosis (Rost, 1984; Reynolds, 1986). Imidazolinones and sulfonylureas also inhibit thymidine incorporation into DNA. This inhibition is not due to a direct effect of chlorsulfuron on plant nuclei, DNA polymerase, or kinase (Ray, 1982). The inhibition of mitosis and DNA synthesis is reversed by exogenously added supplies of the branched chain amino acids (Rost and Reynolds, 1985; Shaner and Reider, 1986), demonstrating a clear connection between mitosis and branched chain amino acid synthesis. The nature of this connection remains unexplained.

AHAS inhibitors also reduce the rate of photosynthate transport. Soon after an AHAS inhibitor is applied, neutral sugars accumulate in the treated leaves (Shaner and Reider, 1986; Bestman et al., 1990). This inhibition of sugar transport out of the leaves appears to be due to interference with sucrose loading and can be partially alleviated by supplying branched chain amino acids to the plant. Hall and Devine (1993) found that chlorsulfuron rapidly inhibits sucrose uptake into leaf discs of susceptible Arabidopsis but not into those of sulfonylurea-resistant Arabidopsis mutants. However, chlorsulfuron treatment has no effects on H^+-ATPase activity or total plasmalemma protein content, which supports the hypothesis that the inhibition of photosynthate transport is due to loss of an essential protein. As with the effects of AHAS inhibitors on mitosis, there is still no clear explanation for this effect on photosynthate transport. However, these results do show that the branched chain amino acid pathway may be important for other processes in the plant in addition to being a supplier of precursors for protein and other metabolites.

A number of mutants in Arabidopsis, canola, maize, soybean, wheat, and other plants have been isolated that are resistant to imidazolinone and sulfonylurea herbicides (Figure 3; Haughn and Somerville, 1986; Anderson and Georgeson, 1989; Sebastian et al., 1989; Swanson et al., 1989; Newhouse et al., 1991a, 1991b, 1992). The mutants in crop species have been developed (for example, maize and soybean) or are in the process of being developed (for example, canola and wheat) for commercial use. These mutants appear to have the following similarities: a single gene confers resistance, resistance appears to result from herbicide-resistant AHAS activity, and the in vivo whole plant herbicide-resistance spectrum and the in vitro AHAS activity herbicide-resistance spectrum are directly correlated. In all cases examined, resistance has been attributed to mutations in the coding region of the AHAS gene (Haughn et al., 1988; Mazur and Falco, 1989; Sathasivan et al., 1991; Hattori et al., 1995). Furthermore, transformation of

Figure 3. Phenotype of an Imidazolinone-Resistant Maize Mutant.

A segregating population of an imidazolinone-resistant maize line treated with 70 g/ha of imazethapyr. Sensitive plants in the population (arrowheads), which were killed by the herbicide, can be seen in the front row, in the midst of healthy resistant plants. Growth of the resistant plants is unaffected by herbicide treatment. The resistant trait was backcrossed into several elite lines by Pioneer Hi-Bred International (Johnston, IA), and the imidazolinone-resistant maize varieties have been sold since 1992.

different plant species with the mutant AHAS genes confers high levels of resistance to various herbicides (Haughn et al., 1988; McHughen, 1989; Miki et al., 1990; Li et al., 1992; Vermeulen et al., 1992). These results provide conclusive evidence that AHAS is the sole site of action of imidazolinone and sulfonylurea herbicides.

The diversity of chemicals that inhibit AHAS is intriguing. The mutant lines resistant to inhibitors appear to shed some light on why AHAS seems so indiscriminate. The herbicide-resistant mutants of different plant species have three different patterns of resistance to imidazolinone and sulfonylurea herbicides: selective resistance to the imidazolinones (Haughn and Somerville, 1990; Newhouse et al., 1991a, 1991b, 1992), selective resistance to the sulfonylureas (Haughn and Somerville, 1986; Mourad and King, 1992), and cross-resistance to both classes of herbicide (Newhouse et al., 1991a, 1991b). Comparison of whole-plant responses and AHAS activity in these mutants indicates that the binding sites for imidazolinones and sulfonylurea herbicides are distinct but lie close to one another.

Mutants of Datura and cotton have been isolated that contain a form of AHAS insensitive to various AHAS-inhibiting herbicides as well as to the feedback inhibitors (Rathinasabapathi et al., 1990; Subramanian et al., 1990). Identification of these mutants led to the proposal that inhibitors of AHAS bind to the valine/leucine feedback regulatory site on the enzyme (Subramanian et al., 1991). However, the existence of both valine-resistant AHAS from mutants of Arabidopsis and tobacco that have unaltered sensitivity to herbicides (Relton et al., 1986; Wu et al., 1994) and herbicide-resistant AHAS with unchanged sensitivity to valine and leucine (Creason and Chaleff, 1988; Subramanian et al., 1990; Newhouse et al., 1991a, 1992) suggests that the herbicide and feedback inhibitors bind to separate sites on the enzyme.

The similarity between plant AHAS and pyruvate oxidase has led to the proposal that the herbicide binding domain of AHAS is an evolutionary vestige of the ubiquinone binding domain of pyruvate oxidase (Schloss et al., 1988; Schloss, 1989). This proposal was supported by the finding that ubiquinone homologs Q_0 and Q_1 are potent inhibitors of AHAS. Based on kinetic studies of the normal and herbicide-resistant forms of AHAS, Butler and Siehl (1992) proposed that the herbicide binding pocket on AHAS is quite large and contains distinct domains to accommodate the negative charge, hydrophobic portion, and electron-deficient heterocycles of the inhibitors. The heterocycle portion of the inhibitors may bind, at least partially, to the quinone binding area. The leucine binding domain was proposed to include the binding area for a portion of the anionic and/or hydrophobic regions of the herbicides. These proposals for the herbicide binding domain are appealing, but a definitive picture will emerge only after the crystal structure of the enzyme–inhibitor(s) complex is solved.

The imidazolinone-resistant mutant lines of maize have been useful in elucidating the number of maize isozymes of AHAS and their level of expression. Two maize lines that are resistant to imidazolinone herbicides, XA17 and XI12, show a high degree of tolerance to imidazolinones, whereas a third resistant line, QJ22, has a much lower tolerance (Anderson and Georgeson, 1989; Newhouse et al., 1991a, 1991b). The three alleles (XA17, XI12, and QJ22) were found to map to two unlinked loci (Newhouse et al., 1991b), a finding consistent with the fact that maize appears to possess two AHAS genes (Fang et al., 1992). The locus that XA17 and XI12 define is on the long arm of chromosome 5, and the locus that QJ22 defines is on chromosome 4 and may be close to the centromere (Newhouse et al., 1991b). Evaluation of the resistant fraction of the enzyme activity in different mutants indicates that the XI12 locus contributes ~80% of the enzyme activity, whereas the QJ22 locus contributes the remaining 20% of the enzyme activity (Newhouse et al., 1991a). This difference in the level of expression of the two genes may explain the differences in the whole plant tolerance of XA17, XI12, and QJ22 to the imidazolinone herbicides (Newhouse et al., 1991a).

CONCLUSION

Since the discovery that imidazolinone and sulfonylurea herbicides inhibit AHAS, great strides have been made in our understanding of the branched chain amino acid biosynthetic pathway. Inhibitors and inhibitor-resistant mutants have been useful in deciphering the enzymes involved, isozyme patterns, enzyme mechanisms, localization of the pathway, and regulation of carbon flow into various amino acids synthesized through this pathway. On the practical side, the new herbicides and the herbicide-resistant crops have provided important tools for better crop management. However, despite our increased knowledge of the pathway, it is not yet clear why only AHAS inhibitors have been successful commercial herbicides. With regard to AHAS, many questions remain, such as the subunit composition of the native enzyme and the locations of inhibitor and feedback regulator binding sites. The resolution of these questions about AHAS and further studies of the other enzymes of the pathway will provide a better understanding of branched chain amino acid biosynthesis in plants.

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