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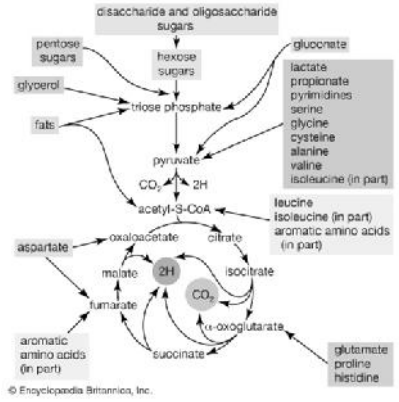
Plant Biochemistry
Lecture 11: AMINO ACIDS II



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LEARNING OUTCOMES

Students, after mastering materials of the present lecture, should be able

1. to explain the biosynthesis of aromatic amino acids including pathways, enzymes and the site of synthesis.
2. to explain the biosynthesis of glutamate-derived amino acids.
3. to explain the biosynthesis of
4. to explain the biosynthesis of histidine amino acid

LECTURE OUTLINE

3. AROMATIC AA
1. Synthesis of chorismate
2. Chorismate mutase
3. Phenylalanine and Tyrosine
4. Tryptophan Biosynthesis
5. Anthranilate Synthase
6. PAT, PAI and IGPS Enzyme
7. Tryptophan synthase
8. Biosynthesis in Plastids
9. Stress Conditions

4. ASPARTATE-DERIVED AA
1. Biosynthetic Pathways
2. Sulfur Requirement
3. Biosynthetic Regulation
4. Lysine Biosynthesis and Degradation

5. BRANCH-CHAIN AA
1. Threonine Deaminase
2. AHAS and IPMS

6. GLUTAMATE-DERIVED AA
1. Proline Metabolism
2. Metabolism Regulation
3. Arginine Biosynthesis

7. HISTIDINE

3. AROMATIC AA

- All living cells require the aromatic amino acids (**phenylalanine**, **tryptophan**, and **tyrosine**) for protein biosynthesis.
- In plants, the aromatic amino acid pathways also provide precursors for the production of numerous aromatic primary and secondary metabolites such as plant hormones (auxin, and salicylic acid), pigments (anthocyanins), volatiles, defensive phytoalexins, feeding deterrents (tannins), UV protectants (flavonoids), signal molecules (isoflavonoids), bioactive alkaloids, and structural components (lignin, suberin, and cell wall-associated phenolics) (Fig 7.16).

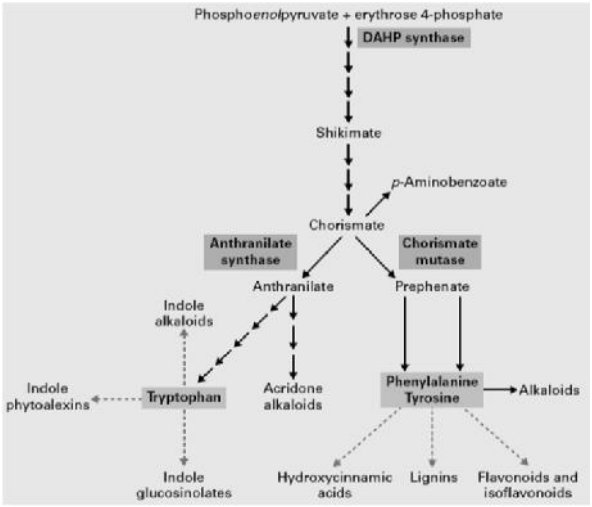


Fig 7.16 Synthesis of the aromatic amino acids in plants.
In addition to their functions in proteins, phenylalanine, tyrosine, and tryptophan serve as precursors for the synthesis of numerous primary and secondary metabolites.

- The importance of these pathways in plants is underscored by the fact that about 30% of the fixed carbon can flow through the common aromatic amino acid pathway, largely to make lignin.
- Owing to their pharmacological and biological activities, aromatic amino acid-derived plant natural products are widely used in medicine (e.g., condensed tannins and morphine) and as food supplements (e.g., flavonoids and tocopherols).
- The aromatic amino acid pathways are absent in animals; the enzymes of these pathways, therefore, became targets for the development of antibiotics against human and animal pathogens and plant herbicides.

1. Synthesis of chorismate

- Synthesis of chorismate is the common aromatic amino acid pathway.
  - Chorismic acid, the final product of the **shikimate pathway**, is the last common precursor in the synthesis of the three aromatic amino acids (**phenylalanine**, **tryptophan**, and **tyrosine**) as well as ***p*-aminobenzoic acid** (a precursor for the C carrier tetrahydrofolate), the electron transport cofactor phylloquinone, other naphtho- and anthraquinones, and **salicylic acid**.
- Plastids have a prominent function in this biosynthesis, because a full set of the shikimate pathway enzymes has been localized to these organelles.
- The seven-step synthesis of **chorismate** via the shikimate pathway (Fig 7.17) begins with the condensation of two intermediates of carbohydrate metabolism.

- The two intermediates are **phospho enolpyruvate** (PEP) from glycolysis and **erythrose 4-phosphate** from the pentose phosphate pathway.

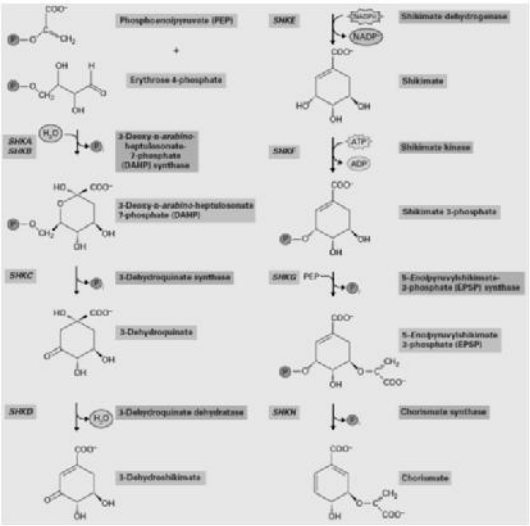


Fig 7.17 Chorismate biosynthesis from phosphoenolpyruvate and erythrose 4-phosphate.

The abbreviations SHKA through SKKH are notations proposed for the genes encoding shikimate pathway enzymes in plants.

- This reaction is catalyzed by **3-deoxy-d-arabino-heptulosonate-7-phosphate** (**DAHP**) **synthase**.
- Based on the absence or presence of certain domains in the protein as well as amino acid sequence similarity, DAHP synthases have been classified into two types (type I and II) with less than 10% sequence identity.
  - E. coli DAHP synthase belongs to the **type I DAHP** synthase family with molecular masses less than 40 kDa. Type II enzymes (≈50 kDa) were first identified in plants, but were later also identified in some microorganisms.
- The penultimate enzyme of chorismate biosynthesis, **5-enolpyruvylshikimate-3-phosphate** (**EPSP**) **synthase**, catalyzes the reversible production of EPSP and phosphate from shikimate 3-phosphate and PEP.

- EPSP synthase is the best studied enzyme of the shikimate pathway, as it is the prime target of the commercially important herbicide **glyphosate** (Fig 7.18).
- The final enzyme of the pathway, **chorismate synthase**, catalyzes the elimination of phosphate and a hydrogen atom from EPSP to produce chorismate (Fig 7.17)

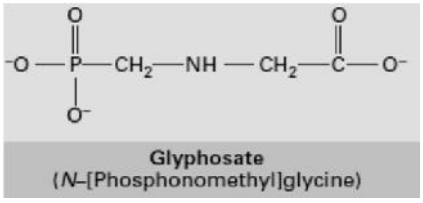


Fig 7.18 Glyphosate, herbicidal constituent of the commercial herbicide Roundup® is a competitive inhibitor of EPSP synthase

- Genes encoding chorismate synthases have been isolated from a number of plant species. The tomato (*Solanum lycopersicum*) genome contains two chorismate synthase genes that are differentially expressed.

2. Chorismate mutase

- Chorismate mutase is the committing enzyme in phenylalanine and tyrosine synthesis.
- The biosynthesis of **phenylalanine** and **tyrosine** may occur via two alternative pathways with either **arogenate** or phenylpyruvate/p-hydroxyphenylpyruvate as intermediates (Fig 7.19).
- Recent genetic evidence suggests that the arogenate route is predominant in plants. This means that plants differ from enteric bacteria and fungi, which primarily use the phenylpyruvate route.
- **Chorismate mutase** (CM), the committing enzyme for phenylalanine and tyrosine biosynthesis, catalyzes the intramolecular rearrangement of the enolpyruvyl side chain of chorismate to produce prephenate.

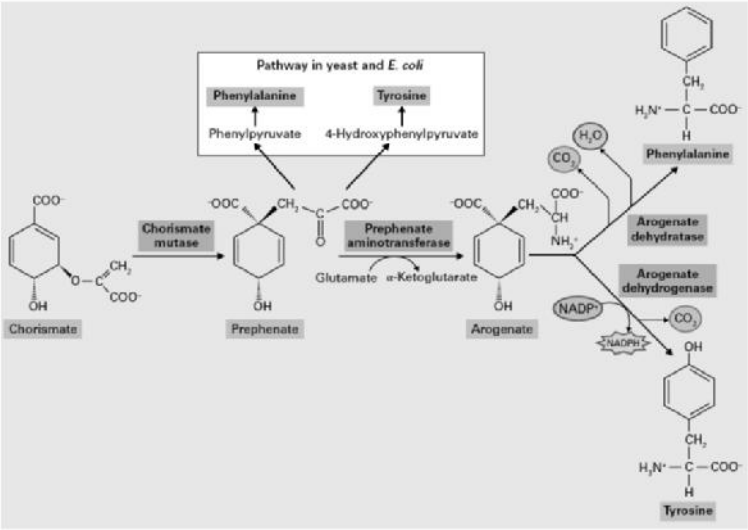


Fig 7.19 Phenylalanine and tyrosine are predominantly synthesized from arogenate in plants. This is in contrast to the major pathway in Saccharomyces cerevisiae and E. coli, where phenylpyruvate and 4-hydroxyphenylpyruvate are intermediates.

- In many plants, this activity exists in two isoenzyme forms, **CM1** and **CM2**, which are regulated quite differently from one another.
- The plastid localization and regulatory behavior of CM1 are consistent with a role for this activity as a committing enzyme in an amino acid biosynthetic pathway.
- CM1 is feedback-inhibited by each of the end products, phenylalanine and tyrosine, and is activated by tryptophan, the product of the other branch of the pathway. Tryptophan reverses the inhibition by phenylalanine or tyrosine (Fig 7.20).
- This mechanism regulates flux into the two competing pathways by increasing synthesis of phenylalanine and tyrosine when tryptophan is plentiful.

- When the supply of these amino acids is adequate, the synthesis of phenylalanine and tyrosine is and suppressed.

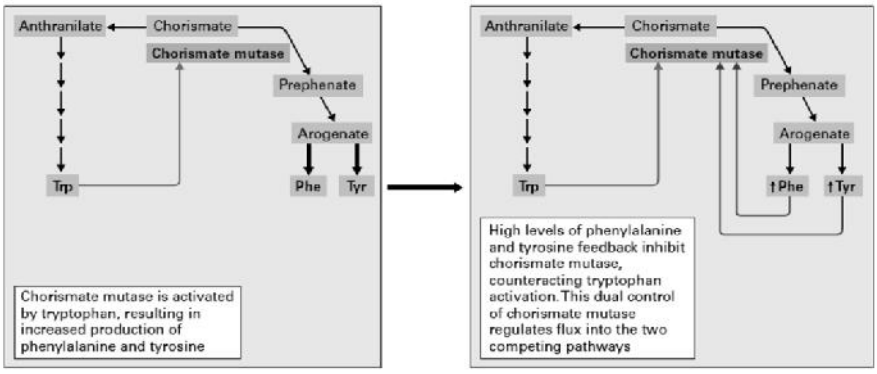


FIGURE 7.20 Allosteric regulation of chorismate mutase controls flux from chorismate into phenylalanine and tyrosine.

3. Phenylalanine and Tyrosine

- In plants, the pathway that synthesizes phenylalanine and tyrosine is regulated by its final reactions.
- The biosynthesis of **phenylalanine** and **tyrosine** in plants via the arogenate pathway represents an unusual case, wherein the committing reactions are also the last steps of the pathways.
- The final enzyme of phenylalanine biosynthesis is **arogenate dehydratase**, which catalyzes the decarboxylation and dehydration of arogenate.
  - Multiple genes encoding arogenate dehydratase have been isolated from a number of plant species.
  - These plant genes encode monofunctional dehydratases that contain two domains: a catalytic domain and a C-terminal regulatory domain that is involved in the allosteric regulation by phenylalanine.

- **Arogenate dehydrogenase** (ADH) catalyzes the oxidative decarboxylation of arogenate to tyrosine.
- This NADP-dependent enzyme lacks an additional tyrosine-binding domain, independent of the substrate binding site (unlike arogenate dehydratase), but it is feedback-inhibited by tyrosine competitively with respect to the arogenate substrate.
- ADH activities have been detected in a variety of plant species, however, genes encoding these enzymes have been isolated only from Arabidopsis and maize (two and four, respectively).
- Biosynthesis of phenylalanine and tyrosine can also occur via the alternative phenylpyruvate and p-hydroxyphenylpyruvate routes, respectively, in which aromatization precedes the transamination reaction (see Fig 7.19), which then is the final step.

4. Tryptophan Biosynthesis

- Conversion of chorismate to tryptophan has significance beyond amino acid biosynthesis.
- Plants use this pathway to produce precursors for numerous secondary metabolites, including the hormone auxin, indole alkaloids, phytoalexins, cyclic hydroxamic acids, indole glucosinolates, and acridone alkaloids (Fig 7.22).
  - These metabolites serve as growth regulators, defense agents, and signals for insect pollinators and herbivores. Some of these alkaloids, including the anticancer drugs vinblastine and vincristine, have great pharmacological value.
- The biosynthetic pathway for tryptophan was the first proven to be amenable to detailed molecular genetic analysis (Box 7.2) that results in a generally accepted in vivo pathway (Fig 7.23).
- Genes for all of the enzymes have been

- Genes for all of the enzymes have been characterized, and mutants have been identified for all but one of the seven proteins (indole-3-glycerol-phosphate synthase; IGPS; an antisense approach was chosen to downregulate this enzyme in Arabidopsis).

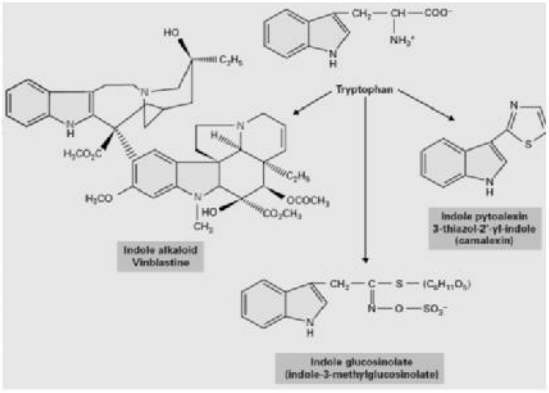


Fig 7.22 The indole ring (highlighted in yellow) derives from the amino acid tryptophan and is a common feature of many secondary metabolites in plants

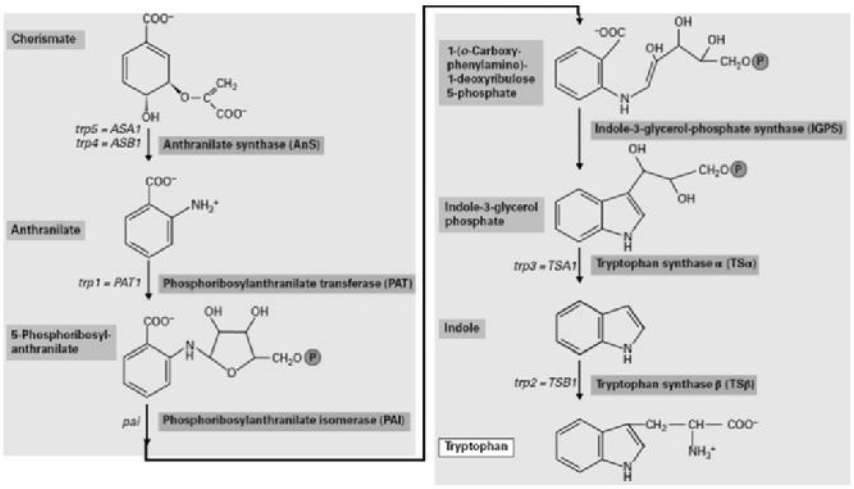


Fig 7.23 Tryptophan biosynthetic pathway of Arabidopsis. The wild-type gene names (e.g., ASA1) and mutant designations (e.g., trp5) are indicated to the left of the arrows.

5. Anthranilate Synthase

- Anthranilate synthase (AnS) is an amino-accepting chorismate- pyruvate lyase that catalyzes the first step in tryptophan biosynthesis, the formation of anthranilate. It is a two-subunit enzyme in plants that functions as an α2β2 complex.
- The α subunit catalyzes the amination of chorismate and removal of the enolpyruvyl side chain (yielding pyruvate), acting in concert with the glutamine amidotransferase activity of the β subunit (Fig 7.24).
- Analysis of the crystal structure of the bacterial enzyme suggests that the binding of chorismate to the α subunit triggers a conformational change to an active state and creates an intermolecular tunnel for ammonia transfer from the β to α subunit.

- As is the case in microorganisms, the α subunit can function in the absence of β subunit amidotransferase activity, provided ammonium is present at sufficient concentration (Reaction 7.1).
- However, the glutamine amidotransferase activity is likely of primary importance for AnS function in plants.

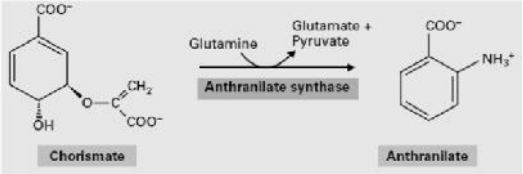


Fig 7.24 Anthranilate synthase catalyzes the first step of tryptophan biosynthesis. Note that the γ-amide group of glutamine becomes the aromatic amino group of anthranilate. This activity, often called a glutamine aminotransferase, is therefore correctly called a glutamine amidotransferase. Glutamine amidotransferases, which are PLP-independent, generally catalyze a reaction in which ammonia generated by hydrolysis of glutamine is channeled to a second active site where it acts as a nucleophile. The hydrophobic tunnel prevents protonation of NH3 and thus maintains its nucleophilicity.

6. PAT, PAI and IGPS Enzyme

- Biochemical characterizations of PAT, PAI, and IGPS lag behind molecular and genetic analyses.
- Phosphoribosylanthranilate transferase (PAT) is the second enzyme in the tryptophan biosynthesis, and catalyzes the formation of 5-phosphoribosyl anthranilate (PRA) by transferring the phosphoribosyl moiety from phosphoribosylpyrophosphate to anthranilate (Fig 7.25).
- Phosphoribosylanthranilate isomerase (PAI), the third enzyme in the tryptophan biosynthesis, converts PRA to 1-(*o*-carboxyphenyl-amino)-1-deoxyri-bulose 5-phosphate (CDRP) (Fig 7.27).
- Indole-3-glycerol-phosphate synthase (IGPS), the penultimate enzyme of tryptophan biosynthesis, catalyzes the decarboxylation and ring closure of CDRP (Fig 7.28).

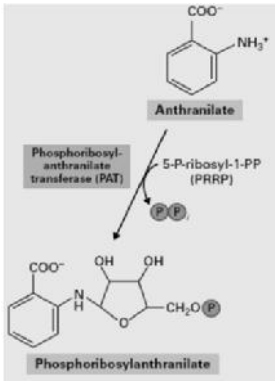


Fig 7.25 Phosphoribosylanthranilate transferase (PAT) catalyzes the second step of tryptophan biosynthesis.

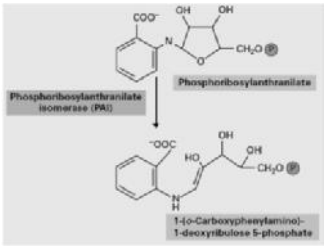


Fig 7. 27 Phosphoribosylanthranilate isomerase (PAI) catalyzes the third step of tryptophan biosynthesis.

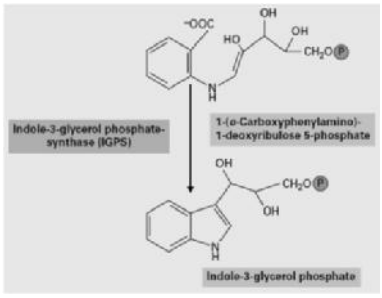


Fig 7.28 The indole-3-glycerol-phosphate synthase (IGPS) reaction produces the indole ring found in tryptophan and secondary metabolites.

- A single-copy gene encodes PAT in the Arabidopsis genome.
- PAT is constitutively expressed, and its mRNA level is post-transcriptionally enhanced by the first two introns in Arabidopsis. PAT mutants fall into two general classes: auxotrophic plants & prototrophs.
- Auxotrophic plants have such low PAT enzyme activity that seedlings cannot grow on sterile medium without the addition of tryptophan.
- Prototrophs have enough residual activity to survive without amino acid supply.
- Adult *trp1* auxotrophic mutants are small, bushy, and have greatly reduced fertility, even when tryptophan is added to their growth medium (Fig 7.26).

Fig 7.26 Morphology of the tryptophan-requiring trp1 mutants is drama-tically altered from that of wild-type plants and includes small stature and reduced apical dominance. Source: Last, Cereon Genomics LLC, Cambridge, MA; previously unpublished.

- The developmental defects could be caused by an inability to produce auxin (indole-3-acetic acid) or other metabolites derived from this pathway.
- Biochemical analysis of the prototrophic *trp1* mutants indicates the PAT enzyme activity is present in vast excess of that required for maintaining pathway flux.
- Five of the prototrophic mutants have 1% of wild-type enzyme activity or less, yet grow normally without tryptophan supplementation.
- In the case of PAI enzyme, Arabidopsis has three or four highly homologous *PAI* genes depending on ecotypes. In the Columbia ecotype, *PAI1* and *PAI2* are 99% identical including their untranslated regions.
- Owing to PAI gene redundancy, loss of any one gene does not reduce enzyme activity sufficiently to produce a distinct mutant phenotype.

- In the case of IGPS, it is the only enzyme known to catalyze the formation of the indole ring.
- One of two Arabidopsis genes encoding IGPS was isolated based on its ability to complement an *E. coli trpC*-mutation despite low amino acid identity (22–28%) with microbial enzymes.
- Plant IGPSs exist as monofunctional enzymes, in contrast to fungal and bacterial IGPSs, which are synthesized as fusion proteins containing one or two other enzymes of the tryptophan biosynthetic pathway.
- Analysis of tryptophan and IAA levels in different Arabidopsis tryptophan biosynthesis mutants, as well as in transgenic plants with antisense suppression of IGPS, suggested that indole-3-glycerol-phosphate might be the branch-point intermediate in tryptophan-independent auxin biosynthesis.

7. Tryptophan synthase

- Tryptophan synthase (TS) is a bifunctional enzyme that catalyzes the last two-step reaction of tryptophan biosynthesis, the conversion of indole-3-glycerol phosphate (IGP) and serine to tryptophan (Fig 7.29).
- TS, the best characterized of the microbial tryptophan biosynthetic enzymes, exists as an $\alpha_2\beta_2$ heterotetra-mer, and the separate subunits are capable of catalyzing independently two partial reactions of the reaction sequence:

  Reaction 1: TS α-subunit
  IGP $\rightarrow$ indole + glyceraldehyde -phosphate [IGP lyase (IGL) activity]

  Reaction 2: TS β-subunit
  Indole + serine $\rightarrow$ tryptophan + H_2O

- Conversely, the rate of cleavage of IGP at the α-subunit active site (Reaction 7.2) is increased 20-fold by binding of serine to the pyridoxal phosphate cofactor at the β-subunit active site (Fig 7.30).
- The two partial reactions are kept in phase by allosteric interactions between the two subunits, and each subunit can cause conformational changes that affect the catalytic activity of the other.

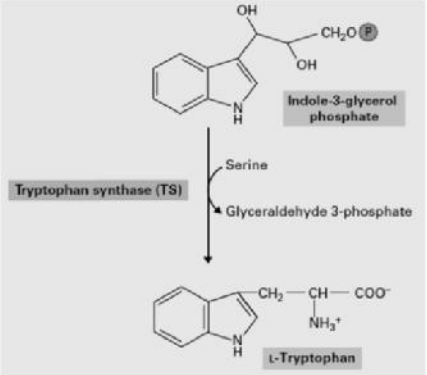


Fig 7.29 Tryptophan synthase (TS) catalyzes the final step in tryptophan biosynthesis.

- For example, α-subunit binding stimulates β activity (Reaction 2) 30-fold compared with uncomplexed β subunit.

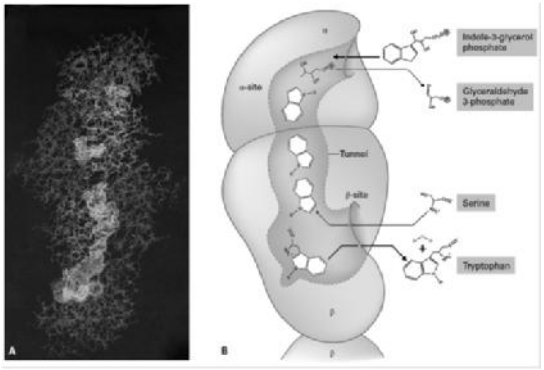


Fig 7.30 Tryptophan synthase (TS) consists of α- and β subunits that join to form two active sites with a hydrophobic tunnel between them that channels indole from the site where it is released from indole-3-glycerol phosphate to the site where it is condensed with serine.

(A) The structure of TS from Salmonella typhimurium with α subunits in blue, β subunit N-terminal residues in yellow, and C-terminal residues in red. A molecule of indole-3-glycerol phosphate (red) is bound to the active site of the α subunit. (B) Schematic drawing of the tunnel that guides indole from the active site of the α subunit to that of the β subunit. Source: (A) Hyde et al. (1998). J. Biol. Chem. 263:17857–17871.

8. Biosynthesis in Plastids

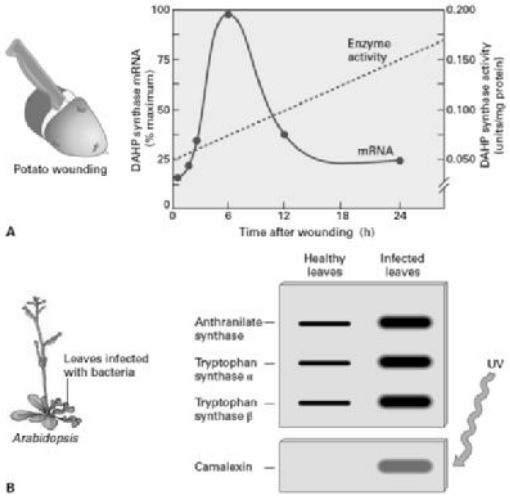
- Aromatic amino acids are synthesized in the chloroplast, and several lines of evidence show that the total set of enzymes for the biosynthesis of phenylalanine, tryptophan, and tyrosine is found in plastids.
- Isolated chloroplasts incorporate $^{14}CO_2$ into aromatic amino acids, and activities of almost all enzymes involved in chorismate and aromatic amino acid biosynthesis have been detected in chloroplast extracts.
- Consistent with this view, genes containing plastid targeting sequences have been cloned for at least one isoenzyme responsible for a given biochemical step in the plant aromatic amino acid pathway.

- Plastid localization for most pathway enzymes was confirmed using GFP (green fluorescent protein) fusion proteins. Import of precursor proteins of DAHP synthase, shikimate kinase, EPSP synthase, PAI, and TSA into isolated chloroplasts has been also been demonstrated.
  - Subcellular fractionation and localization studies have uncovered a **cytosolic DAHP synthase** activity in some plants, and cytosolic isoforms both of CM (probably all plants) and **dehydroquinate dehydratase-shikimate dehydroge-nase** (so far only found in tobacco) as well.
  - The last two enzymes were confirmed at both biochemical and genetic levels. Genes encoding cytosolic tobacco (*Nicotiana tabacum*) dehydroquinate dehydratase/shikimate dehydrogenase 2 and *Arabidopsis* and petunia (*Petunia hybrida*) CM2 have been identified, and the cytosolic localization of the corresponding proteins was demonstrated.

9. Stress Conditions

- Enzymes of aromatic amino acid biosynthesis in plants are inducible by environmental stress wounding and bacterial infection.
- The kinetics of wound-inducible DAHP synthase mRNA induction are similar to that of phenylalanine ammonia-lyase (PAL), the committing enzyme of aromatic secondary metabolism (Fig 7.31A).
- Both mRNAs and proteins for all of the enzymes of the tryptophan pathway are induced after treatment with bacterial pathogens, and the rates of induction are similar to that for camalexin accumulation.
- The timing of the responses is coordinated, and the amount of camalexin that accumulates is tightly correlated with the degree of tryptophan pathway enzyme induction in response to various bacterial pathogens (Fig 7.31B).

Fig 7.31 Plants induce the enzymes of aromatic amino acid biosynthesis under conditions that cause increases in aromatic secondary metabolism. (A) Wounding of potato tubers causes increased DAHP synthase mRNA and enzyme activity.



(B) Infection with a bacterial pathogen increases mRNAs for Arabidopsis tryptophan pathway enzymes and increases production of the antimicrobial indolic secondary metabolite camalexin, which fluoresces blue under UV light.

4. ASPARTATE-DERIVED AA

1. Biosynthetic Pathways

- Three pathways lead directly from aspartate to the amino acids lysine, threonine, and methionine (Figs. 7.2 and 7.32).
- The current understanding is that chloroplasts are fully autonomous in the biosynthesis of all aspartate-derived amino acids, including methionine (Fig 7.32).

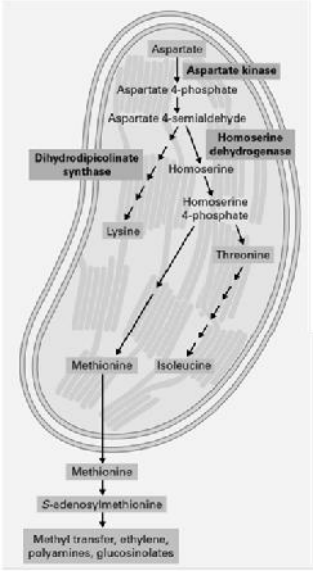


Fig 7.32 The aspartate-derived amino acids are produced in the plastid. Methionine activation to S-adenosylmethionine and methionine regeneration occur in the cytosol.

- Aspartate-derived amino acids are required in the diets of nonruminant animals, including humans.
- Some diets that rely on plant foods are deficient in one or more of these protein building blocks and can lead to health problems. The amino acid deficiencies of grains and legume seeds can be complemented by eating both in combination.
- Human vegetarians who do not eat any animal products must balance their diets with care to provide all essential amino acids in the proportions that are needed for protein synthesis.
- For example, corn has a poor amino acid content and composition, being especially deficient in lysine, but also tryptophan and methionine. On the other hand, soybean is lysine-rich but methionine- and threonine-poor.

- Aspartate provides the entire carbon skeleton for threonine, and threonine biosynthesis seems to serve as the main pathway (Fig 7.33), with branches to lysine and methionine (Figs. 7.34 and 7.35).
- Aspartate is activated by phosphorylation by **aspartate kinase** (AK; also called aspartokinase), the committing enzyme for all aspartate-derived amino acid biosynthesis.
- **Aspartate 4-phosphate** (β-aspartyl phosphate) then goes through two NADPH-mediated reductions, which are catalyzed sequentially by **aspartate-semialdehyde dehydrogenase** and **homoserine dehydrogenase** (HSDH), to yield homoserine.
- Homoserine is then phosphorylated at the 4-position by **homoserine kinase**.

- **Homoserine 4- phosphate** is at the branching point of threonine and methionine biosynthesis.

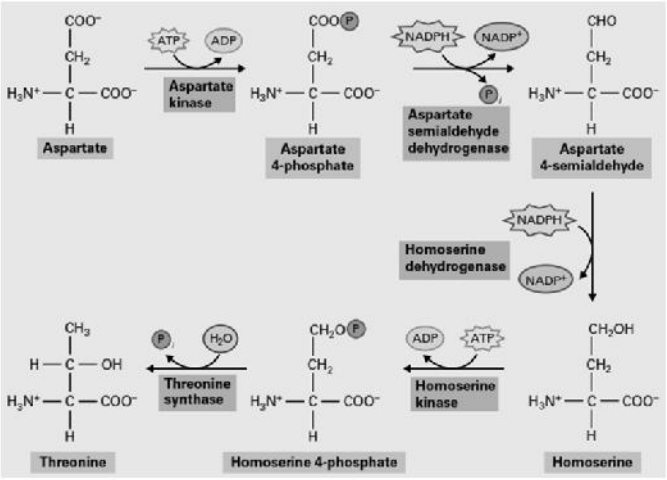


Fig 7.33 The threonine biosynthetic pathway

- In bacteria and plants, lysine is synthesized from aspartate via the diaminopimelate (DAP) pathway (Fig 7.34).
- The committing enzyme in the DAP pathway of lysine biosynthesis is dihydrodipicolinate synthase (DHPS), which condenses aspartate 4-semialdehyde with pyruvate and cyclizes the product to 2,3-dihydrodipi-colinate.

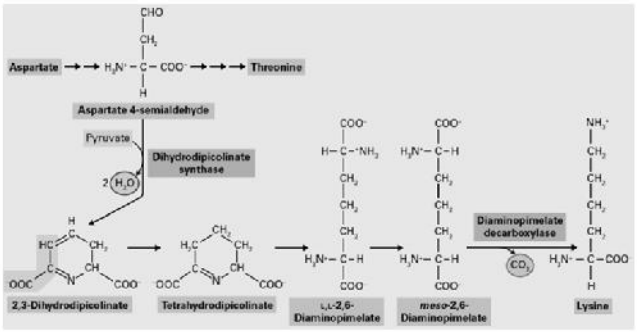


Fig 7.34 Lysine biosynthesis branches from the threonine pathway

2. Sulfur Requirement

- Amino acid Methionine containing sulphur originates from cysteine that acquires its carbon skeleton from homoserine carbon skeleton.
- The activated form of homoserine in plants is homose-rine 4-phosphate (O-phosphohomoserine), with its carbon skeleton coming originally from Aspartate, which is also the precursor of threonine (Fig 7.35).
- In the trans-sulfuration pathway, the thiol group of cysteine is transferred to homoserine to produce homocysteine, through a cystathionine intermediate.
- Cystathionine γ-synthase, another pyridoxal phos-phate-dependent enzyme, catalyzes the sulfur-linked joining of cysteine and homoserine 4-phosphate to form cystathionine (a thioether) and orthophosphate.

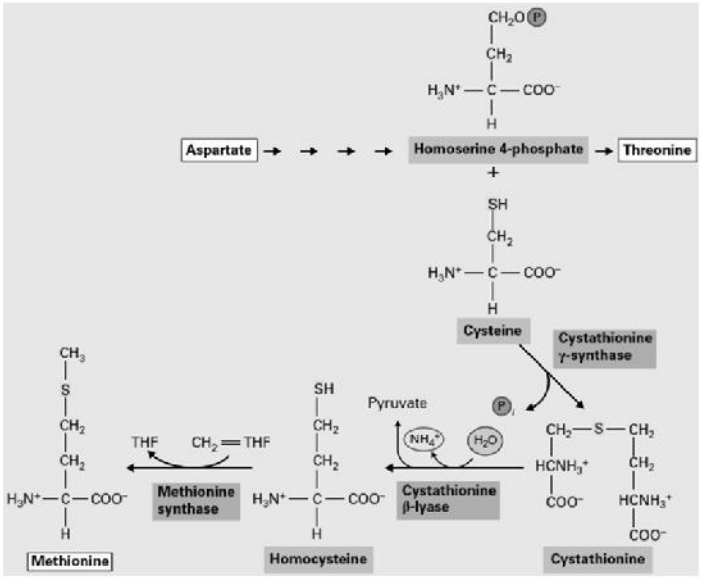


Fig 7.35 The methionine biosynthetic pathway. THF, tetrahydrofolate

- Next, the C3 skeleton of cysteine is cleaved by cystathionine β-lyase (again pyridoxal phosphate-dependent), now leaving the sulfur atom attached to the homoserine carbon skeleton; this produces homocysteine, pyruvate, and ammonium, making the reaction essentially irreversible.
- Direct sulfhydration of the activated homoserine with sulfide to give rise to homocysteine seems to be of minor physiological significance in plants.
- Homocysteine is then converted to methionine, with N^5-methyltetrahydrofolate serving as the methyl donor, in a reaction catalyzed by methionine synthase (homocysteine methylase).
- This activity not only is involved in de novo methionine synthesis, but also functions in recycling the **S-adenosyl-L-homocysteine** produced when SAM donates its methyl group in a methylation reaction (Fig

- This activity not only is involved in de novo methionine synthesis, but also functions in recycling the **S-adenosyl-L-homocysteine** produced when SAM (**S-adenosylmethionine**) donates its methyl group in a methylation reaction (Fig 7.36).

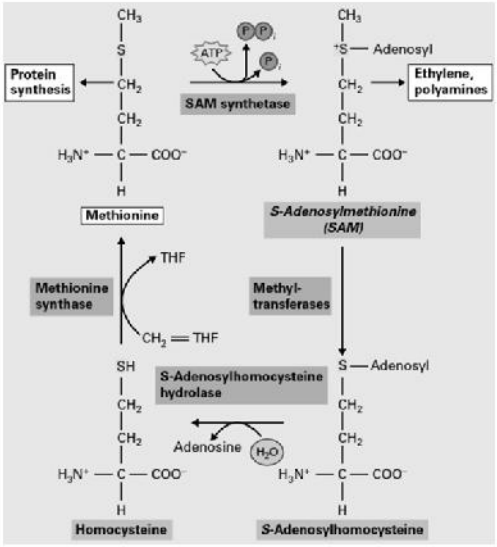


Fig 7.36 S-Adenosylmethonine is produced from methionine and can be recycled to regenerate methionine. THF, tetrahydrofolate

3. Biosynthetic Regulation

- The biochemical mechanisms regulating flux through the branches of the aspartate-derived amino acid pathway are complex.
  - First, aspartate-derived metabolism can follow three major routes, creating at least five branch point enzymes: aspartate kinase (AK), dihydrodipicolinate synthase (DHDPS), homoserine dehydrogenase (HSDS), threonine synthase (TS), and cystathionine γ-synthase (CγS). Of these, the first four are allosterically regulated in plants, and CγS is regulated at the transcriptional and, in some plants, at the posttranscriptional level.
  - Second, the products of these pathways are expected to be needed by the plant in different amounts at each stage of development.
- An overview of the types of regulatory mechanisms inferred by in vitro studies is shown in Figure 7.37.

- AK, the committing enzyme for the overall pathway, is a critical regulatory enzyme occurring in multiple classes of AK isoenzymes, which have divergent primary sequences and contrasting allosteric properties.

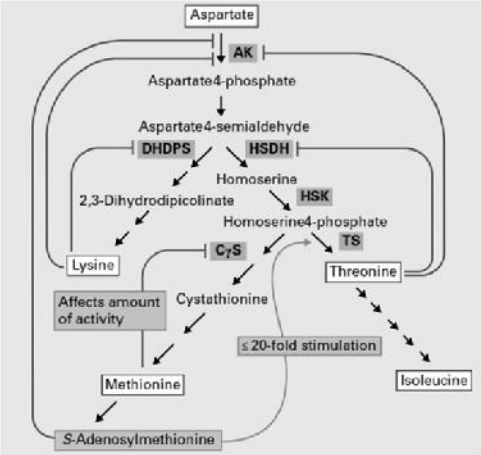


Fig 7.37 Biochemical regulatory mechanisms proposed to regulate the synthesis of amino acids derived from aspartate. Red lines indicate end-product inhibition, the purple line refers to a reduction in detectable enzyme, and the green lines indicate stimulation of activity

- If feedback regulation of AK is primarily responsible for regulating flux to all three end products (threonine, lysine, and methionine/SAM), deregulating this enzyme should increase accumulation of all three amino acids, and this is indeed the case.
- Mutants with deregulated lysine-sensitive AK were selected by taking advantage of the fact that administration of threonine and lysine causes starvation for methionine by reducing total AK activity through feedback inhibition.
- Mutants were selected with AK activities that were less sensitive to lysine inhibition (Fig 7.38).
- Wild-type plants or plant cell cultures on medium containing a mixture of lysine and threonine were death by methionine starvation due to inhibition of AK activity (Fig 7.38).

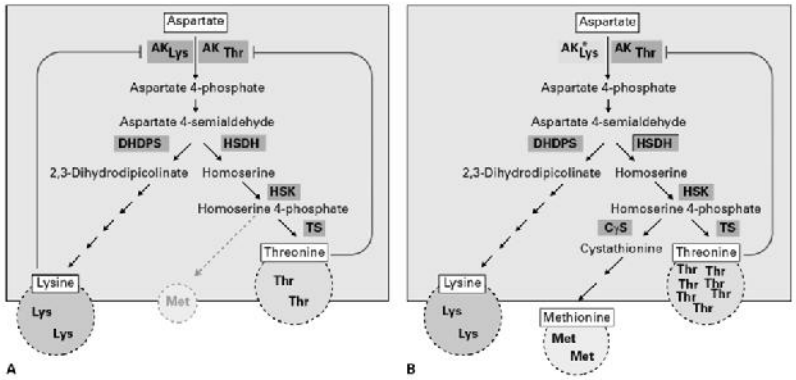


Fig 7.38 Treatment of plants with lysine plus threonine allows selection for feedback-insensitive aspartate kinase mutants. AKLys and AKThr refer to AK isoforms that are inhibited by lysine or threonine, respectively. (A) Growth of wild-type plants or plant cell cultures on medium containing a mixture of lysine and threonine causes death by methionine starvation because of inhibition of AK activity. (B) Mutants arise that are insensitive to this normally toxic amino acid mixture. These plants (AK*Lys) contain amino acid changes in their lysine-sensitive aspartate kinase activities, which make them insensitive to feedback inhibition. This restores the plant's ability to synthesize methionine.

- In plants, DHDPS activity is very sensitive to lysine inhibition (the concentration to achieve 50% inhibition, or I50, is 10–50 μM), making it a likely candidate for a key regulator of lysine accumulation.
- Plant mutants with desensitized DHDPS were identified in several species by selection for resistance to the toxic lysine analog S-aminoethyl-l-cysteine (AEC).
- This molecule competes with lysine for incorporation into proteins and allows the identification of lysine-overproducer plants.
- AEC resistance in Nicotiana sylvestris, caused by production of a lysine-insensitive mutant DHDPS enzyme with a single amino acid change, led to these plants accumulating about 10-fold more lysine than did the wild type (Fig 7.39).

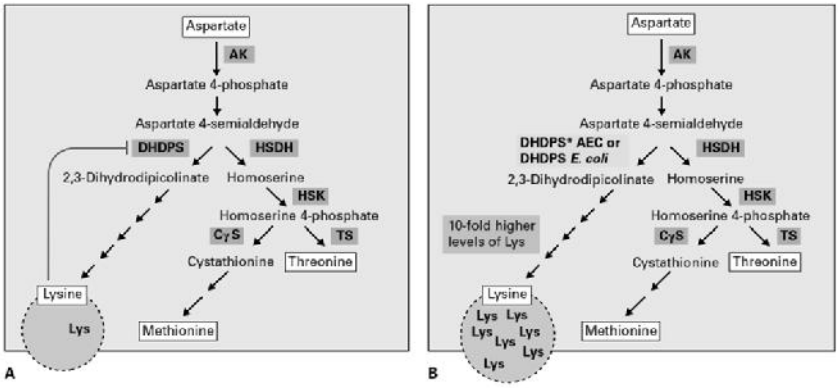


FIGURE 7.39 Dihydrodipicolinate synthase (DHDPS) regulates the accumulation of lysine in plants. (A) Feedback inhibition of DHDPS acts to control the flux from aspartate 4-semialdehyde into lysine. (B) Dominant feedback-insensitive DHDPS mutants can be selected by use of classical genetics (DHDPS* AEC) or by expression of feedback-insensitive E. coli enzyme in transgenic plants (DHDPS E. coli). These plants overproduce lysine in comparison with the plant that expresses the wild-type DHDPS activity

4. Lysine Biosynthesis and Degradation

- Plants expressing feedback-insensitive mutant DHDPS accumulate high quantities of lysine, affecting other AA, causes severe developmental defects.
- Increasing lysine biosynthesis by simply expressing mutant DHPS in a seed-specific manner may not be a apt way to increase the nutritional value of crops that requires engineering increased lysine content in seeds.
- Lysine degradation in a concerted manner with biosynthesis is necessary to maintain a certain level of lysine. The first enzyme of lysine catabolism is lysine-ketoglutarate reductase (LKR) which reduces the labile Schiff base, formed between the ε-amino group of lysine and α-ketoglutarate, to a stable opine, saccharopine (Fig 7.40).

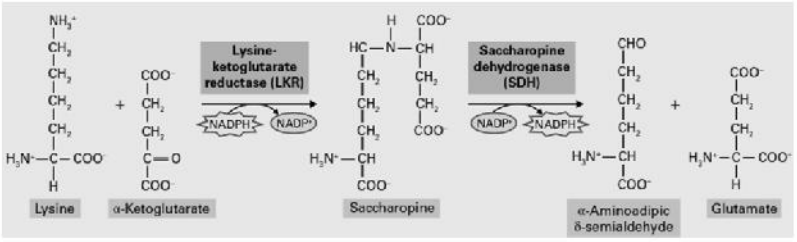


FIGURE 7.40 Initial reactions in lysine catabolism

- In the subsequent reaction, catalyzed by saccharopine dehydrogenase (SDH), the amine function is transferred to the α-ketoglutarate skeleton, giving rise to glutamate while the ε-C of the lysine C-skeleton now carries the carbonyl function (α-aminoadipic δ-semialdehyde, which is further degraded to yield acetyl-CoA).

5. BRANCHED-CHAIN AA

- Branched-chain amino acids are AA having small branched hydrocarbon residues and consist of **Isoleucine**, **leucine**, and **valine**.
- The biosynthesis of the branched-chain amino acids by plants attracts considerable attention for several reasons:
  - First, they represent three of the 10 **essential amino acids** that animals must obtain from their diet and are, therefore, nutritionally important.
  - Second, this pathway is a significant target for a variety of commercially successful herbicides.
  - Finally, the amino acid products serve as precursors for secondary metabolites (Fig 7.41).

1. Threonine Deaminase

- The biosynthesis of isoleucine, begins with deamination of threonine, while leucine and valine begin with the reaction of Pyruvate and hydroxyethyl-TPP (Fig. 7.41).
- A unique feature of branched-chain amino acid biosynthesis is that isoleucine and valine are synthesized in chloroplasts from two parallel pathways utilizing the same set of four enzymes(Fig. 7.42).

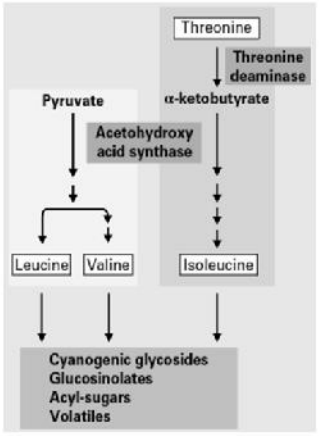


Fig 7.41 The branched-chain amino acids serve as precursors to plant secondary metabolites.

- Each of the four enzymes has dual substrate specificity, starting with either **pyruvate** (leading to valine) or **α-ketobutyrate** (leading to isoleucine).
- While pyruvate (C3) is an intermediate of glycolysis, its C4 counterpart, α-keto-butyrate, is produced by threonine deaminase (TD, also known as threonine dehydratase).
- TD is thus the committing enzyme of the isoleucine pathway and has no role in valine metabolism.

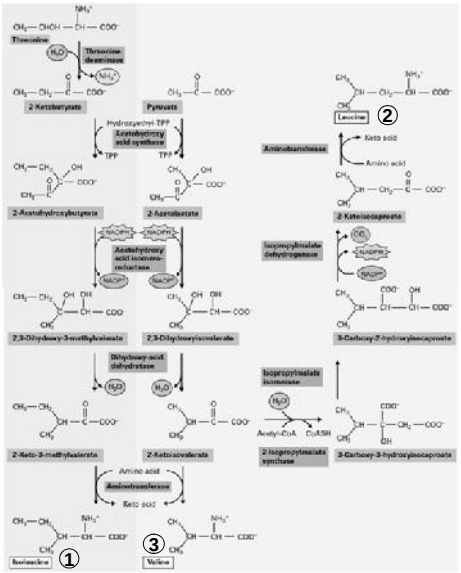


Fig 7.42 Biosynthesis of isoleucine, leucine, and valine

2. AHAS and IPMS

- The first common enzyme in the biosynthesis of the branched-chain amino acids is **acetohydroxyacid synthase** (**AHAS**; also known as **acetolactate synthase**), which catalyzes the decarboxylation of pyruvate followed by its condensation with either pyruvate or 2-ketobutyrate to form 2-acetolactate or 2-acetohydroxybutyrate, respectively (Fig 7.42).
- AHAS requires thiamine diphosphate as cofactor, which is anchored to the active site of the enzyme by a divalent metal ion such as Mg_2^+.
- AHAS also requires flavin adenine dinucleotide (FAD), even though this cofactor does not participate in the principal reactions and may have instead a structural function.

- AHAS has been intensely studied because it is the target of five commercially important and structurally highly divergent classes of herbicides: **imidazolinones**, **sulfonylureas**, **triazolopyrimidines**, pyrimidyloxy-benzoates and sulfonylaminocarbonyltriazolinones (Fig 7.43).
- **Leucine** biosynthesis branches from 2-ketoisovalerate, the last intermediate of the valine biosynthetic pathway, and evidence indicates that plants use the pathway found in microbes (Fig 7.42).
- The committing enzyme for the pathway, 2-**isopropylmalate synthase** (**IPMS**), catalyzes the transfer of an acetyl group from acetyl-CoA to 2-ketoisovalerate to produce 3-carboxy-3-hydroxyisocaproate (2-isopropylmalate or α-isopropylmalate).

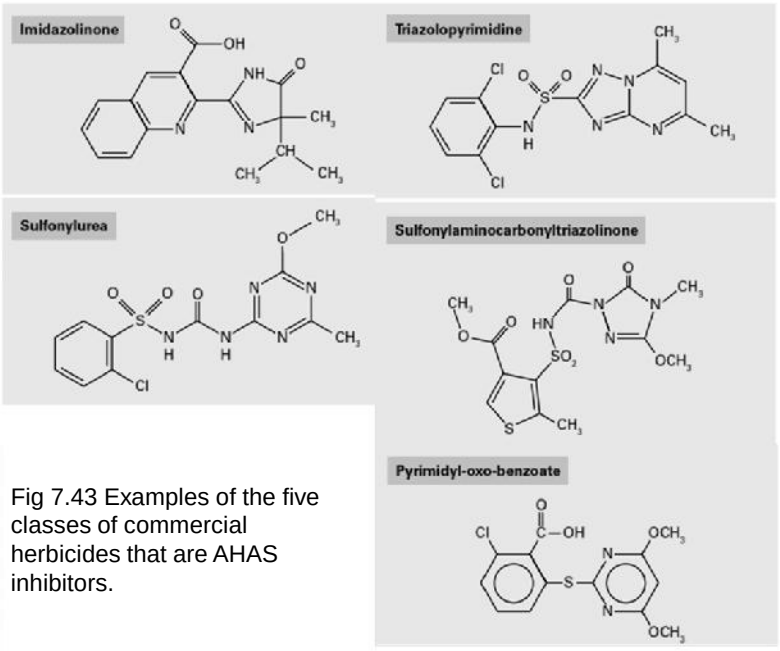


Fig 7.43 Examples of the five classes of commercial herbicides that are AHAS inhibitors.

6. GLUTAMATE-DERIVED AA

- Proline, arginine, and the (nonproteinogenic) amino acid ornithine are biosynthetically derived from glutamate.
- Proline has received major attention in plant biology because of its accumulation in responses to salt, drought, and metal stress.
- Although biochemical studies have rather neglected the synthesis of arginine in plants in the past, genomic annotations have provided missing information.

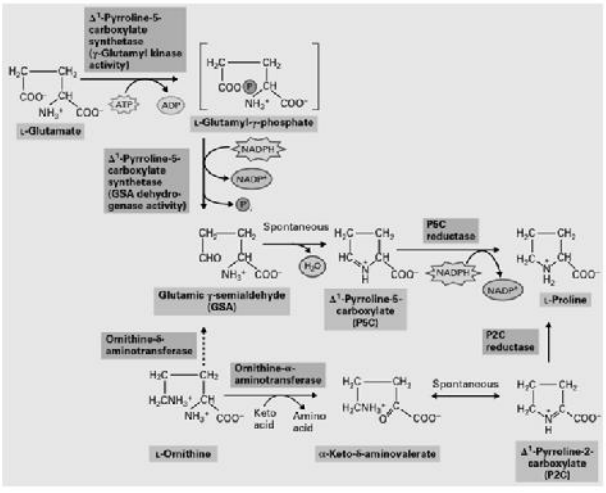
1. Proline Metabolism

- Understanding and manipulating proline biosynthesis in plants is largely motivated by observations that this cyclic secondary amino acid is one of several organic compounds that can function as compatible solutes.

- Compatible solutes are molecules that can accumulate to high concentrations in the cytoplasm without disrupting cellular activity and allow plants to lower their water potential and maintain turgor during dry or saline conditions.

- Proline also scavenges reactive oxygen species (ROS), which are formed under stressful conditions, and its function in stress situations is thus multifaceted.
- Studies of genetically engineered plants, accumulating high levels of proline, have provided clear evidence for the ability of proline to confer increased osmotic tolerance.
- Proline can be synthesized by two different pathways in plants: the first originates from glutamate, the second from ornithine, although the details of the ornithine pathway are less clear (Fig 7.44).

Fig 7.44 Multiple pathways are proposed for proline biosynthesis in plants. A glutamate-derived pathway is well established (top-down), and increasing evidence suggests the importance of ornithine as a precursor. The relative importance of the pathways through ornithine-δ-aminotransferase or ornithine-α-aminotransferase remains to be determined.



In plants, the γ-glutamyl kinase (proB in E. coli) and GSA dehydrogenase (proA in E. coli) are synthesized in a protein fusion. This activity is referred to as Δ1-pyrroline-5-carboxylate synthetase.

- The committing enzyme of the glutamate pathway, pyrroline-5-carboxylate synthetase (**P5CS**), is a bifunctional enzyme in plants.

2. Metabolism Regulation

- Regulation of proline accumulation in plants occurs both at the enzyme level and through changes in gene expression.
- The committing enzyme P5CS appears to be rate limiting in plants, and its transcript as well as free proline increase rapidly in response to desiccation, salt stress and ABA (abscisic acid) treatment, and they return to normal level when dehydrated plants are watered.

- In several plant species, PC5S overexpression (but not that of P5C reductase) leads to plants with increased soluble proline content and reduced sensitivity to osmotic stress.
- As the γ-glutamyl kinase activity of PC5S is feedback-inhibited by proline, this feedback loop paradoxically appears to counteract the effect of increased expression of the enzyme.
- Of the two *PC5S* genes of Arabidopsis, *PC5S1* responds to osmotic stress, and the loss-of-function mutant displays increased ROS levels and hypersensitivity to stress.
- Mutant *pc5s2* plants, on the other hand, have developmental defects, in particular embryo abortion during late seed development (desiccation stage).

3. Arginine Biosynthesis

- Arginine has a high N:C ratio (4:6) and serves as nitrogen storage compound in seeds, either in free form (e.g., in pea seeds) or in protein-bound form.
- In addition, it serves as a precursor in the biosynthesis of **polyamines**, certain alkaloids, and the signal molecule **nitric oxide** (**NO**).
- In terrestrial (ureotelic) vertebrates, it is the immediate precursor of **urea**, the nitrogen excretion product in urine.
- A limited number of biochemical and in silico genome mining studies have allowed elucidation of the pathway of arginine biosynthesis in plants, and these reactions are more or less identical to those in other organisms (Fig 7.45).

- The pathway can be divided into two parts: from glutamate to ornithine, and from ornithine to arginine.
- The ornithine pathway begins with the N-acetylation of glutamate by ***N*-acetylglutamate synthase** (NAGS).

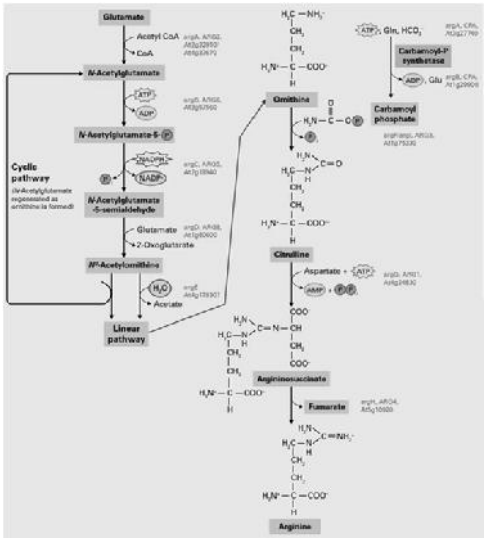


Fig 7.45 Biosynthesis of arginine. Structures are only shown for the pathway from ornithine to arginine. Structures in the ornithine pathway correspond to those also seen in Figure 7.42, with the notable difference that they are N-acetylated.

- The following γ-phosphorylation and subsequent reduction to the semialdehyde are analogous to the reactions of free glutamate in proline biosynthesis.
  - Because acetylation of the amino group prevents spontaneous cyclization to the pyrroline carboxylate, *N*-acetylglutamic semialdehyde can be transaminated to give rise to ***N*-acetylornithine**, from which ornithine is released either by hydrolysis or by transfer of the acetyl moiety to glutamate (transacetylation) within the cyclic pathway (Fig 7.45).
- Arginine synthesis is initiated by the carbamoylation of the δ-amino group of ornithine with carbamoyl phosphate as the activated intermediate in a reaction catalyzed by **ornithine carbamoyl transferase** (OCT) that produces citrulline (Fig 7.45).
- The N atom required to convert citrulline to arginine is transferred from aspartate via **argininosuccinate**, which is cleaved to yield arginine and fumarate.

7. HISTIDINE

- Histidine (His), one of the standard amino acids in proteins and plays a critical role in plant growth and development, was discovered (L-histidine) independently by Kossel and Hedin in 1896.
- Uniquely among the twenty standard amino acids, the imidazole side group of His has a pK_a of approximately 6, allowing it to alternate between the protonated and unprotonated states under physiological conditions.

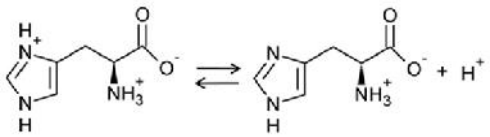


Fig 1. **L-histidine** structure.

The imidazole side group is a weak acid with a pK_a of approximately 6, allowing it to switch between the protonated and unprotonated states under cellular conditions.

- His biosynthesis in plants, occuring via the same metabolic route as in micro-organisms, begins with the condensation of 5′-phosphoribosyl 1-pyrophosphate (PRPP) and ATP (Fig. 2).
- Plant genes encoding all of the eight enzymes required for His synthesis have been identified.
- Release of pyrophosphate by hydrolysis produces 5′-phosphoribosyl AMP. The six-membered ring of AMP opens by hydrolysis followed by the isomerization of ketone.
- Ammonia provided by hydrolysis of glutamine, in a glutamine amidotransferase reaction and being guided through a tunnel, affects the production of imidazole glycerol phosphate (IGP) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR).

Fig 2. The histidine biosynthetic pathway in Arabidopsis. Abbreviations of enzyme names are indicated in parentheses, and the corresponding Arabidopsis gene names and AGI codes are shown in blue. Allosteric inhibition of ATP-PRT activity by L-His is indicated in red. PRPP (5′-phosphoribosyl-1-pyrophosphate), PRATP (*N*′-5′-phosphoribosyl-ATP), PRAMP (*N*′-5′-phosphoribosyl-AMP), ProFAR (*N*′-[(5′-phosphoribosyl) formimino]-5-aminoimidazole-4-carboxamide) ribonucleotide, PRFAR (*N*′-[(5-phosphoribulosyl)formimino]-5-aminoimidazole-4-carboxamide) ribonucleotide, IGP (imidazole glycerol-phosphate), IAP (imidazole acetol-phosphate), AICAR (5′-phosphoribosyl-4-carboximide-5-aminoimidazole) and 2-OG (2-oxoglutarate). Hyperlinks to chemical structures and TAIR locus pages are provided

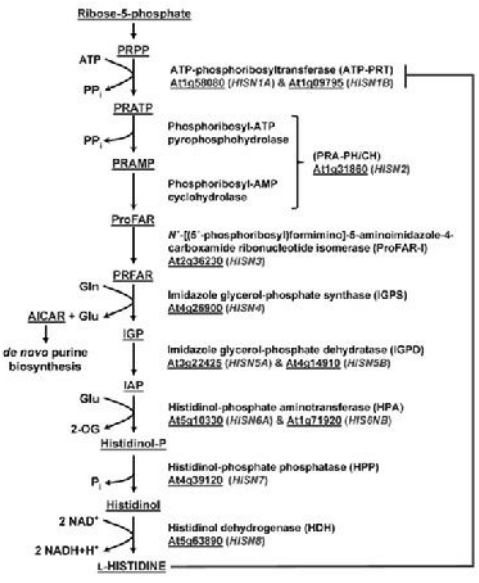
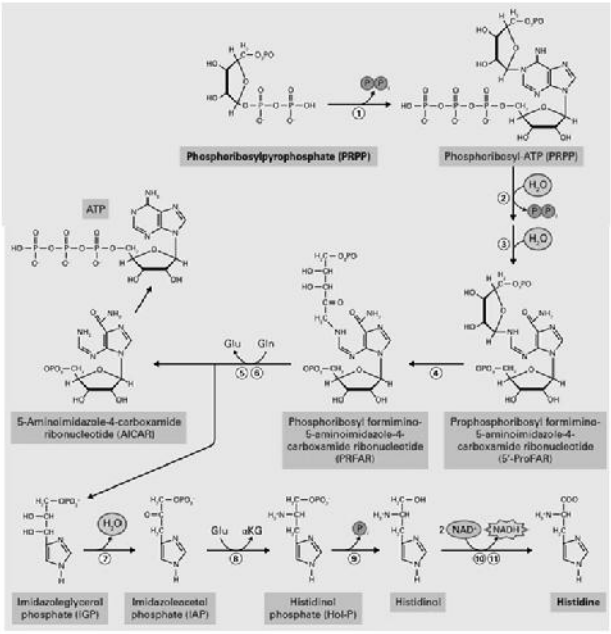


Fig 7.46 Biosynthesis of histidine

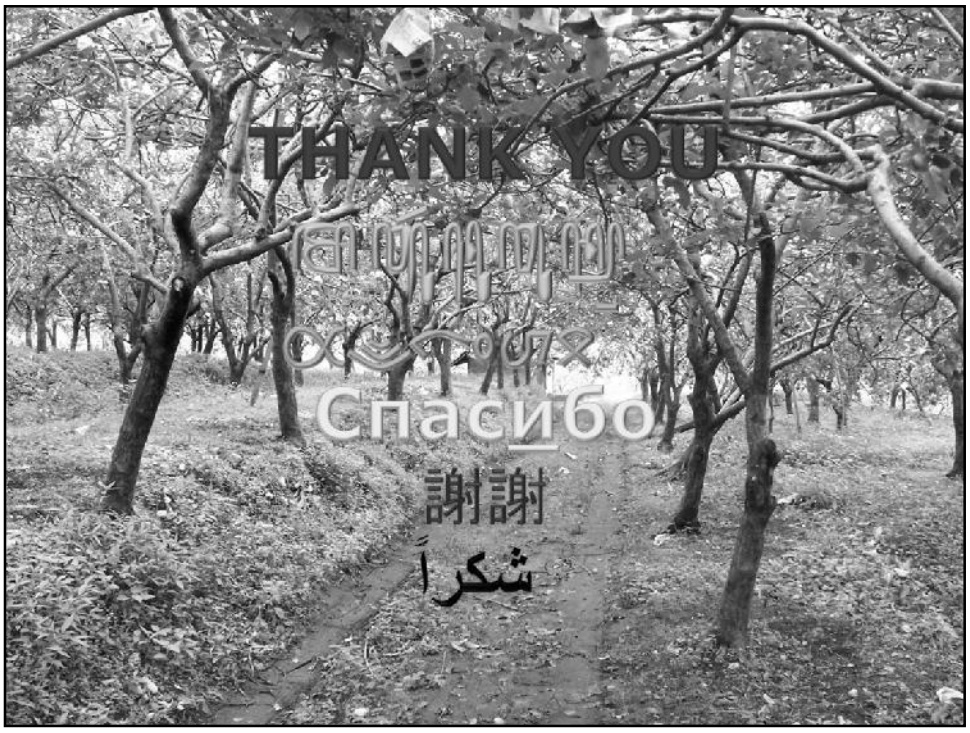


- AICAR is used in a salvage pathway to regenerate ATP, while IGP is converted by consecutive dehydration, transamination, hydrolysis of the phosphate group, and two dehydrogenation steps to histidine (Fig 7.46)
- All proteins involved in the histidine pathway carry an N-terminal extension either proven or assumed to direct the proteins to the plastids.
- It is generally accepted that the complete pathway operates in the **plastid**.
- His is the fourth most metabolically expensive amino acid to synthesize (after the aromatic amino acids Phe, Trp and Tyr), with estimates of 31- 41 ATP molecules required per molecule of His produced.

SUMMARY

1. Amino acids, the building blocks of proteins in all organisms, play additional roles in plants.
2. In plants, amino acids serve as precursors to a plethora of natural products that provide defense against pathogens and herbivores and tolerance to abiotic stress.
3. They also store or transport nitrogen from sources to sinks. The control of amino acid synthesis in plants, therefore, affects many aspects of growth, development, and survival.
4. The synthesis of essential amino acids in plants and the amino acid composition of seeds relate indirectly to animal and human nutrition.
5. Thus, understanding the pathways that allow and control amino acid synthesis in plants has significance with regard to basic research on the control of metabolic pathways as well as practical implications.
6. Although amino acid biosynthetic pathways have been well defined in microbes, the situation in plants is still less defined, in part because of additional unique complexities.

7. For example, in many instances, plants have multiple isoenzymes that catalyze the same biosynthetic reactions. These isoenzymes may be localized in distinct organelles or distinct cell types, or may be present at different developmental stages.
8. Defining each step in an amino acid biosynthetic pathway and determining how each step is regulated, not only within the context of the respective pathway but rather in the context of the general metabolic network, are some of the key aspects of current research in amino acid biosynthesis in plants.
9. Molecular, genetic, and biochemical approaches have been combined to elucidate the steps of these pathways in plants and to understand the regulation of these pathways at the level of gene regulation and beyond.
10. Plant mutants in amino acid biosynthetic enzymes, which can now readily be identified in T-DNA insertion lines, have shown that the synthesis of amino acids in vivo affects numerous diverse processes, including photorespiration, hormone biosynthesis, and plant development.
11. Thus, while being products of primary metabolism, amino acids also control many diverse aspects of plant growth and development.



5. Research of AA Pathways

- Uncovering the features of amino acid biosynthesis unique to plants should stimulate both fundamental and applied research aimed at understanding;
  - the genes that control growth-limiting processes (e.g., the assimilation of inorganic nitrogen into amino acids) and
  - the factors that regulate the synthesis of plant secondary compounds from amino acid precursors.
- Such studies should also provide a framework for manipulating amino acid biosynthesis pathways in transgenic plants.
- For example, enzymes in several pathways have been identified as targets for herbicides, and in some cases, the genes encoding these enzymes have been used for engineering herbicide resistance (Box 7.1).