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Molecular Mechanism of Action of Herbicides

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1. Introduction

Herbicides are the most widely used class of pesticides accounting for more than 60% of all pesticides applied in the agriculture (Zimdahl, 2002). The herbicide's mode of action is a biochemical and physiological mechanism by which herbicides regulate plant growth at tissue and cellular level. Herbicides with the same mode of action generally exhibit the same translocation pattern and produce similar injury symptoms. At the physiological level, the various herbicides control plants by inhibiting photosynthesis, mimicking plant growth regulators, blocking amino acid synthesis, inhibiting cell elongation and cell division, etc.

There are approximately 20 different target sites for herbicides (Shaner, 2003). Despite the relative importance of herbicides within crop protection products only a low number of biochemical mode of action can be shown for the marketed herbicides. Herbicides with 6 mode of action represent around 75% of herbicide sales (Klausener et al., 2007). Understanding the mode of action of herbicides has been an important tool in research to improve application methods in various agricultural practices, handle weed resistance problems and explore toxicological properties. Several enzymes and functionally important proteins are targets in these biochemical processes. Classical photosystem-II (PSII) inhibitors bind to D1 protein, a quinone-binding protein to prevent photosynthetic electron transfer. Inhibition of biosynthesis of aromatic amino acids relies on the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase. Acetohydroxyacid synthase (AHAS), a target of several classes of herbicides catalyzes the first common step in the biosynthesis of valine, leucine, and isoleucine. Several different types of herbicides apparently cause accumulation of photodynamic porphyrins by inhibiting protoporphyrinogen oxidase (PPO). Formation of homogentisate via inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme in tyrosine catabolism and carotenoid synthesis inhibited by herbicides having different structure. Lipid biosynthesis is the site of action of a broad array of herbicides used in controlling monocot weeds by inhibiting acetyl-CoA carboxylase (ACC) or very-long-chain fatty acids (VLCFA). Several compounds are potent inhibitors of glutamine synthase (GS) which catalyzes the incorporation of ammonia into glutamate.

The decreasing heterogeneity of herbicides targeting fewer mechanism of action is increasing the prevalence of herbicide resistance (Lein et al., 2004). Therefore, characterization of new modes of action by exploring novel targets is of high importance for discovery of new compound classes. Elucidation of the atomic structure of target site proteins in complex with

herbicides is important for understanding the initial biochemical response following application. Furthermore, the knowledge of molecular mechanism of action may provide a powerful tool to manipulate herbicide selectivity and resistance. *De novo* design of potent enzyme inhibitors has increased dramatically, particularly as our knowledge of enzyme reaction mechanisms has improved. Recent findings on the interaction of herbicides with target site enzymes and receptor proteins involved in their mode of action will be reviewed in this chapter.

2. Target site action of herbicides

2.1 Interaction of amino acid biosynthesis inhibitor herbicides with target site enzymes

2.1.1 Aromatic amino acid biosynthesis inhibitors

Inhibitors of biosynthesis of aromatic amino acids such as phenylalanine, tyrosine and tryptophan target the shikimic acid pathway. The first step of the synthesis of these three amino acids is the condensation of D-erythrose 4'-phosphate with phosphoenolpyruvate (PEP) to produce 3'-deoxy-D-arabino-heptulosonic acid 7'-phosphate (Figure 1). This undergoes a series of reactions, including loss of a phosphate, ring closure and a reduction to give shikimic acid, which is then phosphorylated by shikimate kinase. Shikimate phosphate is combined with a further molecule PEP to give 3-enolpyruvylshikimate 5-phosphate (EPSP). The enzyme EPSP synthase catalyzes the transfer of the enolpyruvyl moiety of PEP to the 5-hydroxyl of shikimate-3-phosphate (S3P) (Amrhein et al., 1980) has

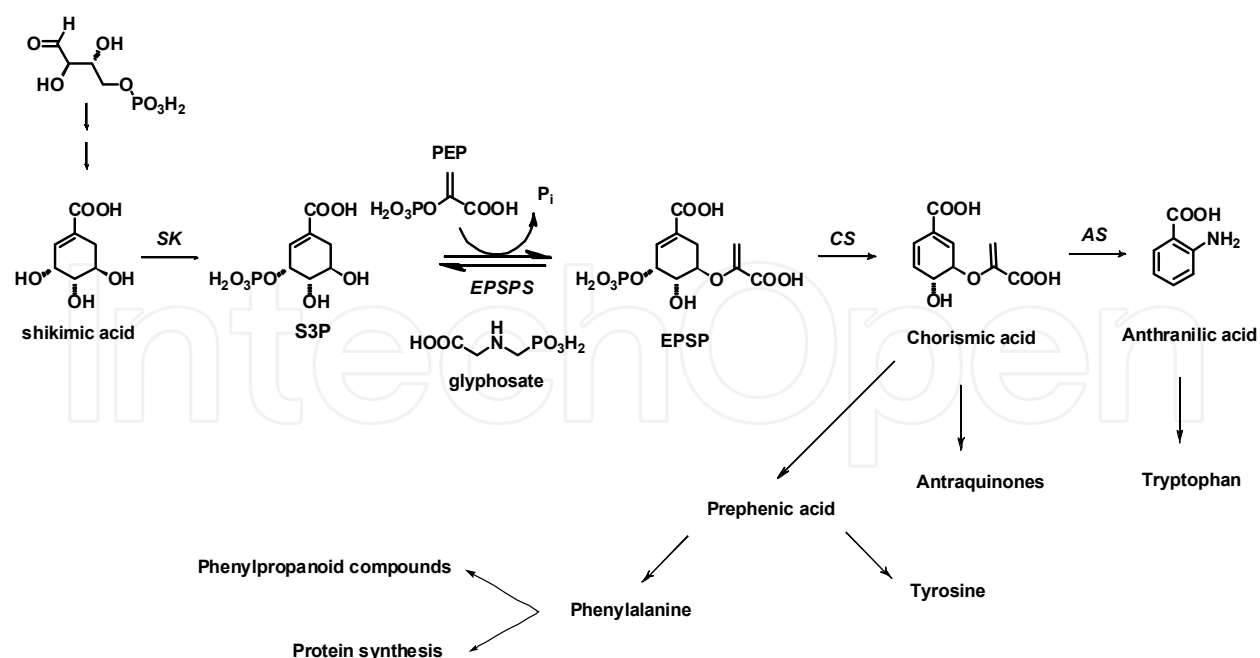


Fig. 1. Shikimic acid pathway. Biosynthesis of aromatic amino acids and action of the herbicide glyphosate. SK= shikimate kinase, EPSPS= 5-enolpyruvyl-shikimate-3-phosphate synthase, CS= chorismate synthase, AS= anthranilate synthase.

received considerable attention because it is inhibited by the herbicide, glyphosate. EPSP is converted to chorismic acid, which is at a branch point in this pathway, and can undergo two different reactions, one leading to tryptophan, and the other to phenylalanine and tyrosine. The broad-spectrum herbicide glyphosate, the active ingredient of Round-up, inhibits EPSP synthase, the enzyme catalyzing the penultimate step of the shikimate pathway toward the biosynthesis of aromatic amino acids. The extraordinary success of this simple and small molecule is based on its high specificity for plant EPSP enzymes (Pollegioni et al., 2011).

The first crystal structure of EPSPS was determined for the *E. coli* enzyme in its ligand-free state (Stallings et al., 1991). EPSP synthase (M_r 46,000) folds into two globular domains, each comprising three identical $\beta\alpha\beta\alpha\beta$ -folding units connected to each other by a two-stranded hinge region. The structure upon interaction of EPSP synthase from *E. coli* with one of its two substrates (S3P) and with glyphosate was identified a decade later (Schönbrunn et al., 2001). The two-domain enzyme was shown to close on ligand binding, thereby forming the active site in the interdomain cleft. Glyphosate occupied the binding site of the second substrate PEP of EPSP synthase, mimicking an intermediate state of the ternary enzyme-substrates complex. (Figure 2). The glyphosate binds close to S3P without perturbing the structure of active-site cavity. The 5-hydroxyl group of S3P was found hydrogen-bonded to the nitrogen atom of the herbicide and the glyphosate binding site is dominated by charged residues from both domains of the enzyme, of which Lys-22 (K22), Arg-124 (R124) and Lys-411 (K411) was found in the PEP binding (Shuttleworth et al., 1999). Gly-96 (G96) residue which is not the most important in the herbicide binding plays a key role in glyphosate sensitivity of plants since replacing it an alanine residue provides the glyphosate-tolerant mutant protein (Sost and Amrhein, 1990).

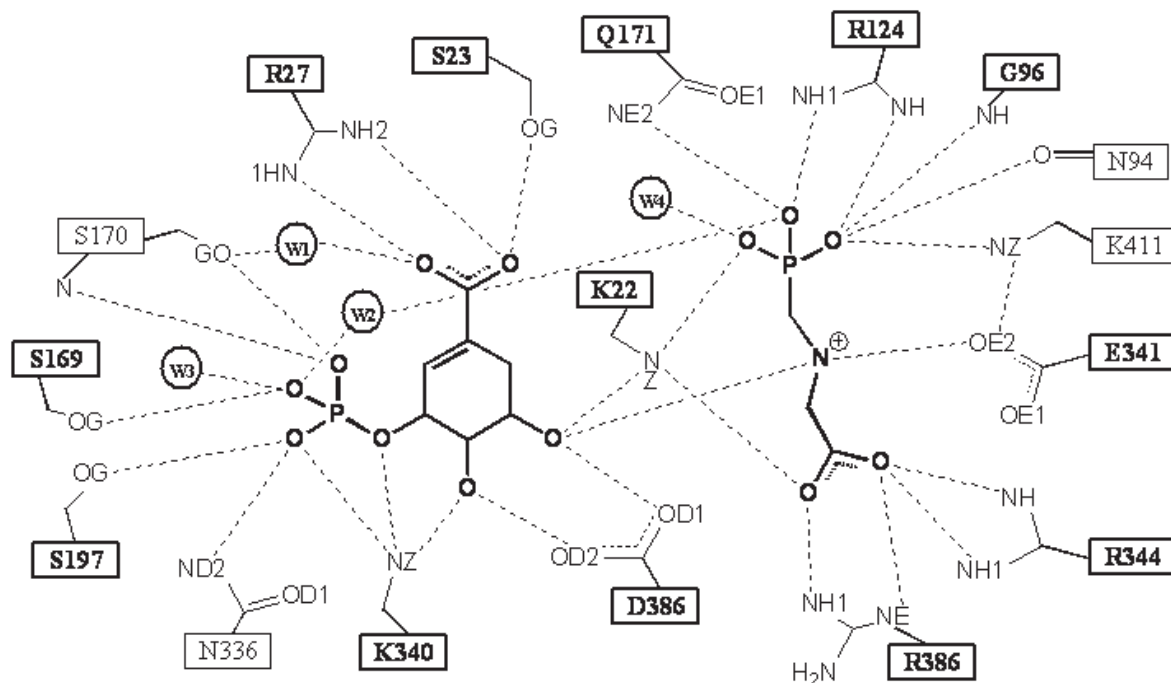


Fig. 2. Schematic representation of ligand binding in EPSP synthase-S3P-glyphosate complex (Schönbrunn et al., 2001). Ligands are drawn in bold lines. Dashed lines indicate H-bonds and ionic interactions. Strictly conserved residues are highlighted by bold labels.

Round-up ready crops such as maize, soybean, cotton and canola carry the gene coding for a glyphosate-insensitive form of EPSPS enzymes which enables more effective weed control by allowing postemergent herbicide application (Padgett et al., 1995). The genetically engineered maize lines NK603 and GA21 carry carry distinct EPSPS enzymes. NK603 maize line contains a gene derived from *Agrobacterium* sp. strain CP4 encoding a glyphosate tolerant class II enzyme, the so-called CP4 EPSP synthase. On the other hand GA21 maize was created by point mutations of class I EPSPS such as enzymes from *Zea mays* and *E. coli* which are sensitive to low glyphosate concentrations. Although these crops have been widely used, the molecular basis for the glyphosate-resistance has remained obscure.

The three-dimensional structure of CP4 EPSP synthase revealed that the enzyme exists in an open, unliganded state (Funke et al., 2006). Upon interaction with S3P, the enzyme undergoes a large conformational change suggesting an induced-fit mechanism with binding of S3P as a prerequisite for the enzyme's interaction with PEP. During interaction with glyphosate the herbicide binds to the active site of CP4 EPSP adjacent to S3P. The weak action of glyphosate on CP4 EPSP synthase can be primarily attributed to an Ala residue in position 100 of which methyl group protrudes into the glyphosate binding site and clashes with one of the oxygen atoms of the herbicide phosphonate group. As a result, the glyphosate molecule adopts a substantially different shortened conformation as interacts with the CP4 enzyme (Figure 3). Replacing Ala-100 with a Gly allows glyphosate to bind in its extended conformation positioning its N atom midway between the target hydroxyl of

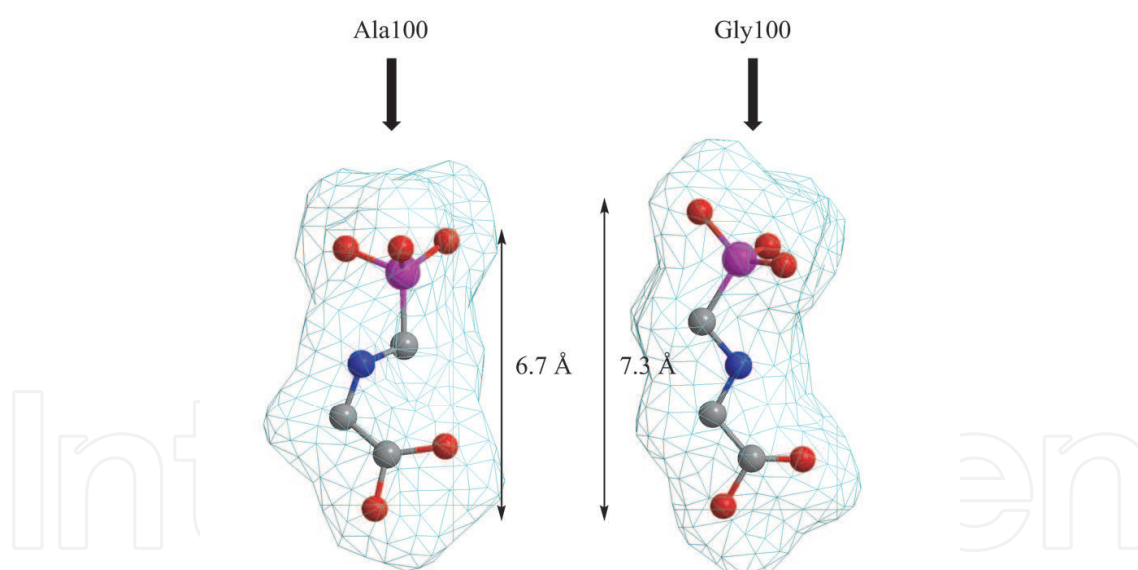


Fig. 3. Shortened and extended conformation of glyphosate (Funke et. al, 2006). Left, with Ala residue in position 100 the herbicide is ~ 0.6 Å shorter.

S3P and Glu-354. The mutation of Ala-100 to Gly restored the CP4 enzyme's sensitivity toward glyphosate. It appears that the conformational change introduced upon glyphosate binding simple makes the EPSPS active site unavailable to PEP. Based on this molecular basis for glyphosate resistance a novel inhibitors of EPSP synthase can be designed in case of emergence of glyphosate-resistant weeds. Nevertheless, structure-activity relationships on the inhibition of EPSP synthase with analogs of glyphosate revealed that minor structural

alterations resulted in dramatically reduced potency and no compound superior to glyphosate was identified (Franz et al., 1997; Sikorski and Gruys, 1997; Mohamed Naseer Ali et al., 2005).

Molecular basis for glyphosate-tolerant GA21 maize resulting from the double mutation Thr-97→Ile and Pro-101→Ser (T97I/P101S, TIPS) and single mutation (T97I) in EPSPS from *E. coli* has recently been revealed (Funke et al., 2009). The crystal structure of EPSPS demonstrated that the dual mutation causes a shift of residue Gly-96 toward the glyphosate binding site, impairing efficient binding of glyphosate, while the side chain of Ile-97 points away from the substrate binding site, facilitating PEP utilization. The single site T97I mutation renders the enzyme sensitive to glyphosate and causes a substantial decrease in the affinity for PEP. Thus, only the concomitant mutations of Thr-97 and Pro-101 induce the conformational changes necessary to produce catalytically efficient, glyphosate-resistant class I EPSPS. Mutations of the residue corresponding to Pro-101 of *E. coli* EPSPS have been reported in a number of field-evolved glyphosate-resistant weeds (Yu et al., 2007; Perez-Jones et al., 2007). However, mutations of Thr-97 have never been observed. The decreased catalytic efficiency of the T97I mutant EPSPS with respect to utilization of PEP may explain why it has not been observed in glyphosate resistant weeds.

Detoxication of the glyphosate by oxidases and acetyltransferase has been a promising strategy to confer resistance (Pollegioni et al., 2011). However, none of these mechanisms has been shown to occur in higher plants to a significant degree. The metabolism by glyphosate oxidoreductase (GOX) and glycine oxidase (GO) resulting in the formation of aminomethyl-phosphonic acid (AMPA) and glyoxylate (the AMPA pathway) takes place only in soil by a number of Gram-positive and Gram-negative bacteria. Chemical mutagenesis and error-prone PCR were used to insert genetic variability in the sequence coding for GOX and the enzyme variants were selected for their ability to grow at glyphosate concentrations that inhibit growth of the *E. coli* methylphosphonate-utilizing control strain (Barry and Kishore, 1998). The best variants had a more basic residue at position 334. However the low level of activity and heterologous expression observed for GOX might explain the limitations encountered in developing commercially available crops based on this enzyme. Furthermore, GO can be efficiently expressed as an active and stable recombinant protein in *E. coli* (Job et al., 2002). Because of the introduction of an arginine at position 54 the crystal structure of the multiple-point variant G51S/A54R/H244A has a different conformation from the wild-type GO. The presence of a smaller alanine at position 244 eliminates steric clashes with the side chain of Glu-55 thus facilitating the interaction between Arg-54 and glyphosate (Pedotti et al., 2009). Glyphosate acetyltransferase (GLYAT) is an acetyltransferase from *Bacillus licheniformis* that was optimized by gene shuffling for acetylation of glyphosate paving the way for the development of glyphosate tolerance in transgenic plants (Castle et al., 2004). The catalytic action of GLYAT requires a cofactor AcCoA. Four active site residues (Arg-21, Arg-73, Arg-111, and His-138) contribute to a positively charged substrate-binding site (Siehl et al., 2007). His-138 functions as a catalytic base via substrate-assisted deprotonation of the glyphosate secondary amine, whereas another active site residue Tyr-118 functions as a general acid.

Despite successful efforts on developing glyphosate-resistant crops there are increasing instances of evolved glyphosate resistance in weed species (Waltz, 2010). In order to preserve the utility of this valuable herbicide, growers must be equipped with effective and

economic herbicide-trait combinations to use in rotation or in combination with glyphosate (Pollegioni et al., 2011).

2.1.2 Acetohydroxyacid synthase (AHAS) inhibitors

The endogenous AHAS gene is involved in the biosynthesis of branched chain amino acids (valine, leucine and isoleucine) catalyzing the formation of 2-acetolactate or 2-aceto-2-hydroxybutyrate (Duggleby and Pang, 2000) (Figure 4). AHAS is the site of action of several structurally diverse classes of herbicides such as sulfonylureas (La Rossa and Schloss, 1984), imidazolinones (Shaner, 1984), triazolopyrimidine sulfonamides (Gerwick et al., 1990). These herbicides are unusual inhibitors since they do not exhibit structural similarity to substrates (pyruvate, α -ketobutyrate), cofactors (thiamine diphosphate (ThDP), FAD) and allosteric effectors (valine, leucine and isoleucine) of the enzyme. When AHAS is inhibited, deficiency of the amino acids causes a decrease in protein synthesis leading to reduced cell division rate (Rost, 1984; Shaner and Singh, 1993). This process eventually kills the plants after showing symptoms in meristematic tissues where biosynthesis of amino acids primarily takes place (Zhou et al., 2007).

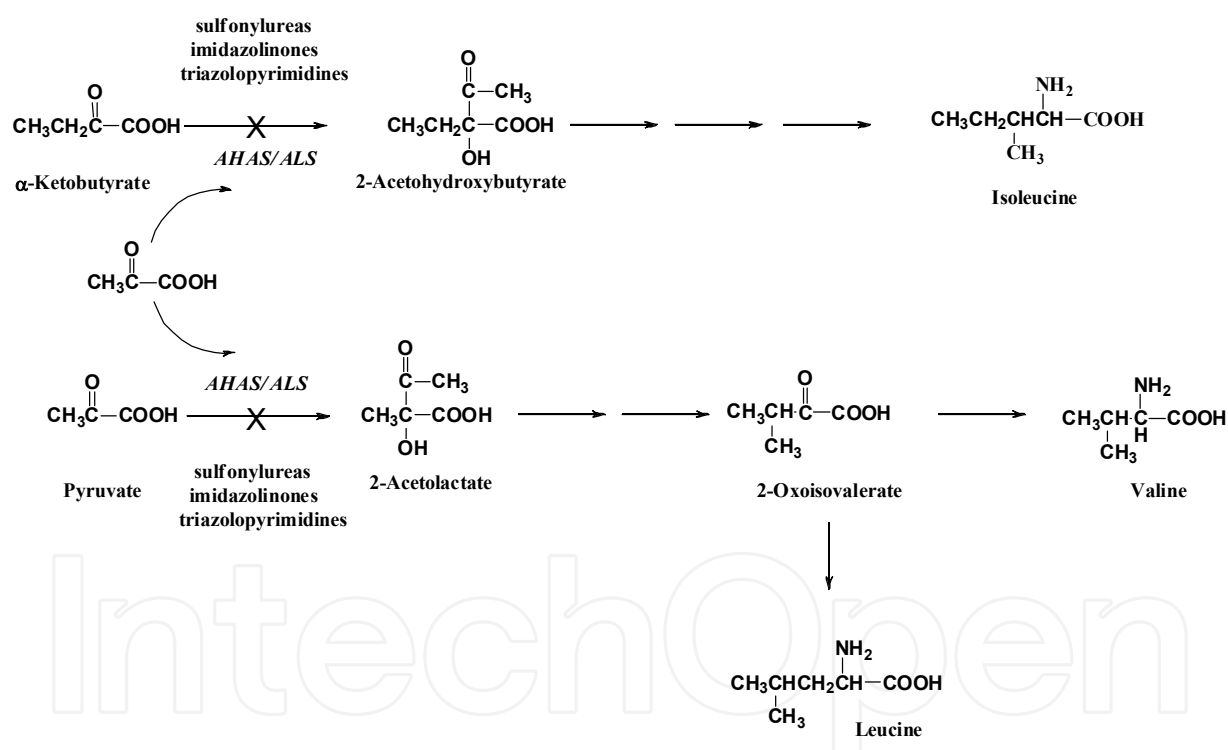


Fig. 4. Biosynthetic pathway of branched chain amino acids and the site of action of herbicidal inhibitors.

The crystalline structure of any plant protein in complex with a commercial herbicide was reported first for *Arabidopsis thaliana* AHAS in complex with the sulfonylurea herbicide chlorimuron ethyl (Pang et al., 2004). There was one monomer in the asymmetric unit and these were arranged as pairs of dimers in the crystal. The dimers form a very open hexagonal lattice, with a high solvent content of 81%. The 3D structure of *Arabidopsis thaliana* AHAS in complex with five sulfonylureas and with the imidazolinone, imazaquin has been published later by the same research group (McCourt et al., 2006). The *At*AHAS is a

tetramer consisting of four identical subunits with an overall fold. Each subunit has three domains and a C-terminal tail that loops over the active site. Associated with each subunit is FAD, Mg-ThDP, >200 water molecules and one molecule of sulfonylurea or two of imazaquin. A prolyl *cis* peptide bond observed between Leu-648 and Pro-649 at the C-terminal tail. Pro-649 is completely conserved in AHAS from 21 species (Duggleby and Pang, 2000) suggesting the critical function of this residue when the C-terminal tail changes from a disordered state in its free structure to the ordered state during the catalytic cycle. Neither sulfonylureas nor imazaquin have a structure that mimics the substrates for the enzyme, but both inhibit by blocking a channel through which access to the active site is gained. In binding of sulfonylureas to plant AHAS a bend at the sulfonyl group positions the two rings almost orthogonal to each other. The sulfonyl group and the adjacent aromatic ring are situated at the entrance to a channel leading to the active site with the rest of the molecule inserting into the channel. In *At*AHAS-imazaquin complex two herbicide molecules were found to bind to each subunit. One of these is within the channel leading to the active site, whereas a second is located around 20 Å from the active site in a pocket. Ten of the amino acid residues that bind the sulfonylureas also bind imazaquin. Six additional residues interact only with the sulfonylureas, whereas there are two residues that bind imazaquin but not the sulfonylureas. Thus, the two classes of inhibitor occupy partially overlapping sites but adopt different modes of binding. The positions of several key residues (Arg-199, Asp-376, Arg-377, Trp-574, Met-200) at the entrance of active-site channel move to accommodate the sulfonylurea chlorimuron-ethyl or imazaquin (Figure 5). Overall 28 van der Waals interactions and only one hydrogen bond contribute to the binding of imazaquin while 50 van der Waals contacts and six hydrogen bonds make a stronger binding for chlorimuron-ethyl. The higher affinity and deeper binding of chlorimuron-ethyl into the active site makes chlorimuron-ethyl more potent inhibitor ($K_{i(\text{app})} = 10.8 \text{ nM}$) to *At*AHAS as compared to imazaquin ($K_{i(\text{app})} = 3.0 \text{ }\mu\text{M}$).

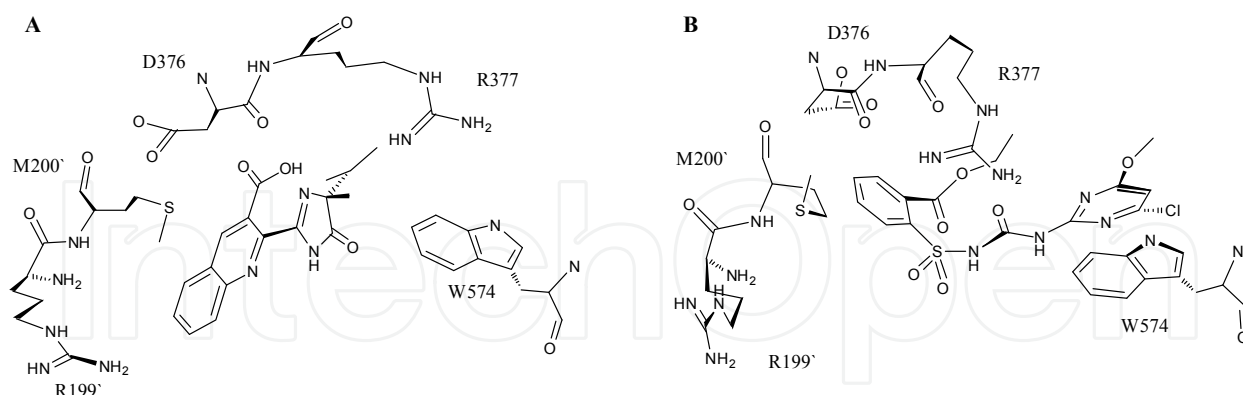


Fig. 5. Schematic representation of conformational adjustments in the *At*AHAS herbicide binding sites (McCourt et al., 2006). (A) Imazaquin. (B) Chlorimuron-ethyl.

The increasing emergence of resistant weeds due to the appearance of mutations that interfere with the inhibition of AHAS is now a worldwide problem. Knowledge of atomic resolution of the enzyme allows us to explain how the substitution of key amino acid residues by mutation results in resistance to these herbicides. Most AHAS isoenzymes resistant to the herbicides carry substitutions for the amino acid residues Ala-122 (amino acid numbering refers to the sequence in *Arabidopsis thaliana*), Pro-197, Ala-205 located at the

N-terminal end of the enzyme whereas Asp-376, Trp-574, and Ser-653 are located at the C-terminal end (Tranel and Wright, 2002). Ala-205→Val mutation resulted in resistance in eastern black nightshade (*Solanum Ptychanthum*) (Ashigh and Tardif, 2007). Eight different amino acid substitutions of Pro-197 have been found to confer herbicide resistance but only Pro-197→Leu has been implicated in strong resistance to imidazolinones (Sibony et al., 2001). It is likely that the bulky Leu residue prevents the entry of imidazolinones into the channel whereas any substitution inhibits sulfonylurea access. Ala-122→Thr (Bernasconi et al., 1995) and Ser-653→Asn (Hattori et al., 1992; Lee et al., 2011) confers strong resistance to the imidazolinones but not to sulfonylureas. Replacement of these residues by a larger one seems to impair imidazolinone binding because the steric hindrance change space where the aromatic ring situated. Substitution of Trp-574, a residue important for defining the shape of the active-site channel, by leucine changes the shape of the binding-site channel and endow high level of resistance to both both imidazolinones and sulfonylureas (Bernasconi et al., 1995).

In a recently published paper (Le et al., 2005) the role of three well-conserved arginine residues (Arg-141, Arg-372, and Arg-376) of tobacco AHAS was determined by site-directed mutagenesis. Arg-372 and 376 residues are important for catalytic activity as they affect the binding with the cofactor FAD. The mutated enzymes such as Arg-141→Ala, Arg-141→Phe and Arg-376→Phe were inactive and unable to bind the cofactor, FAD. The inactive mutants had the same secondary structure as that of the wild type. The mutants Arg-141→Lys, Arg-372→Phe, and Arg-376→Phe exhibited much lower specific activities than the wild type and moderate resistance to herbicides such as bensulfuron methyl and AC 263222. The mutation showed a strong reductions in activation efficiency by thiamine diphosphate, while mutations Arg-372→Lys and Arg-376→Lys showed a strong reduction in activation efficiency by FAD in comparison to the wild type enzyme. Results suggested that the residue Arg-141 is located at the active site and may affect the binding with cofactors while Arg-372 and Arg-376 are located at the overlapping region of the FAD-binding site and are a common binding site for the three classes of herbicides. The molecular basis for inhibition of AHAS enzymes enables us to explain evolved weed resistance and thus allowing more sophisticated AHAS inhibitors to be developed.

2.1.3 Glutamine synthetase (GS) inhibitors

GS is one of the essential enzymes for plant autotrophy catalyzes the the incorporation of the ammonia into glutamate to generate glutamine with concomitant hydrolysis of ATP. Phosphinothricin (PPT) is a potent GS inhibitor (Lydon and Duke, 1999). Actually, PPT, a metabolite of a herbicidally inactive natural product bialaphos has been registered in many countries as a non-selective herbicide. GS plays a crucial role in the assimilation and re-assimilation of ammonia derived from a wide variety of metabolic processes during plant growth and development. The first crystal structure of maize (*Zea mays* L.) GS has recently been reported (Unno et al., 2006). The structure reveals a unique decameric structure that differs significantly from the bacterial GS structure. The GS decamer contains 10 active sites and each active site is located between two adjacent subunits in a pentamer. The active sites (20 Å deep) are formed between two neighboring monomers. The phosphorylated PPT (P-PPT) binding sites were found at the bottoms of the 10 clefts. The ADP binding sites in the ADP/P-PTP/Mn complex structures and the adenylylimido-diphosphate (AMPPNP) binding

sites in the AMPPNP/PPT/Mn complex structure are located near the openings in the 10 catalytic clefts. The P-PPT molecule is bound mainly by the main chain of Gly-245 and the side chains of Glu-131, Glu-192, His-249, Arg-291, Arg-311, and Arg-332 through hydrogen bond interactions in addition to three Mn^{2+} ions. The phosphate group of the P-PPT coordinates to the three Mn^{2+} . The structures of complexes revealed the mechanism for the transfer of phosphate from ATP to glutamate and to interpret the inhibitory action of phosphinothricin as a guide for the development of new potential herbicides.

2.2 Interaction of herbicides with 4-hydroxyphenylpyruvate dioxygenase (HPPD)

4-Hydroxyphenylpyruvate dioxygenase (HPPD) converts 4-hydroxyphenyl-pyruvate (HPP) into homogentisate (HGA) with the concomitant release of CO_2 is a target of β -triketone and isoxazole herbicides (Shaner, 2003). This nonheme, Fe^{2+} -containing, α -keto acid-dependent enzyme catalyzes a complex reaction involving the oxidative decarboxylation of the 2-oxoacid side-chain of 4-hydroxyphenyl-pyruvate, the subsequent hydroxylation of the aromatic ring, and a 1,2-rearrangement of the carboxymethyl group to yield homogentisic acid (Pascal et al., 1985) (Figure 6). The mechanism of this complex reaction has recently been revealed that the native HPPD hydroxylation reaction results in the formation of ring epoxide as the first intermediate (Shah et al., 2011). Homogentisic acid is a precursor in the biosynthesis of the plastoquinones and alpha-tocopherol. Plastoquinones are vital cofactors for phytoene desaturase (PDS) and their loss results in the inhibition of PDS and a decrease in carotenoid levels. The inability to offload electrons from the photosystems results in bleaching of the affected plants due to reduced chlorophyll levels. Triketone inhibitors exhibit structural similarity to the substrate HPP and therefore will bind bidentate to the active ferrous form of the enzyme (Prisbylla et al., 1993).

The first X-ray crystal structure of HPPD published was from *Pseudomonas fluorescens* (Serre et al., 1999) followed by structures from *Arabidopsis thaliana* (Yang et al., 2004; Fritze et al., 2004) *Zea mays* (Fritze et al., 2004), *Streptomyces avertilis* (Brownlee et al., 2004), and rat (Yang et al., 2004). However, the crystal structure of an HPPD from *Pseudomonas fluorescens* showed relatively low overall sequence homology to plant and mammalian HPPDs (21% and 29% amino acid identity, respectively) (Serre et al., 1999). The protein has a subunit mass of 40-50 kDa and typically associated to form dimers in eukaryotes (Moran 2005).

In HPPD structures the N- and C-termini fold into discrete domains and the active site is formed exclusively from the residues of the C-termini (Moran 2005). The peptide fold of HPPDs have a jellyroll fold motif (eight β -strands arranged in a barrel).

Crystal structures of *Arabidopsis thaliana*, *Zea mays* (Fritze et al., 2004) revealed that the C-terminal helix gates substrate access to the active site around a non-heme Fe^{2+} -containing center. In the *Z. mays* HPPD structure this helix packs into the active site, sequestering completely it from the solvent while in the *Arabidopsis* structure tilted by about 60° into the solvent and leaves the active site fully accessible. The crystal structures of the herbicidal target enzyme HPPD from the *Arabidopsis* with and without an herbicidal benzoylpyrazole inhibitor that potently inhibits both plant and mammalian HPPDs have been determined (Figure 7) (Yang et al., 2004). The active site of *At*HPPD is located within an open twisted barrel-like β sheet. In common with other members of this dioxygenase family, the required

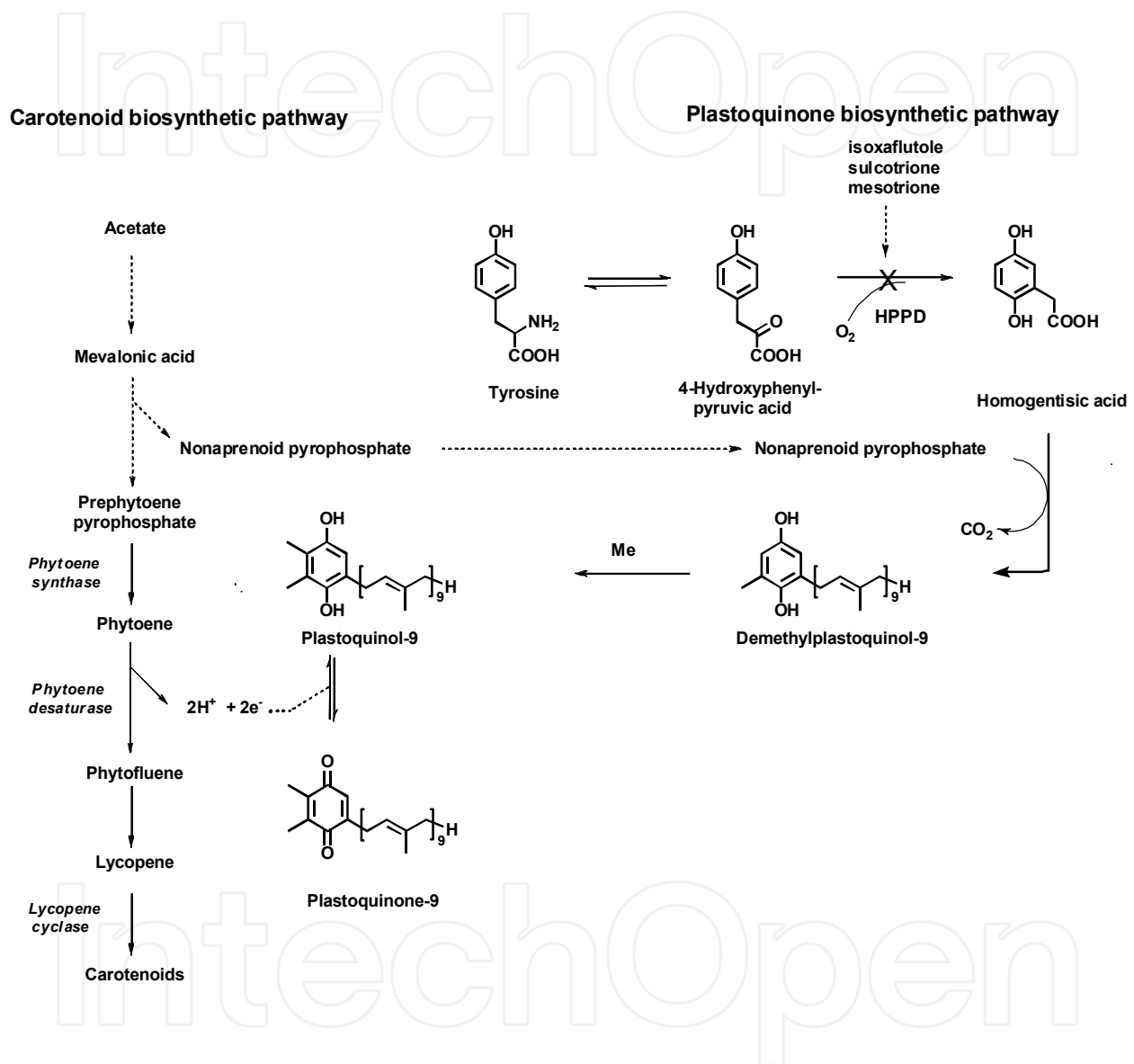


Fig. 6. Carotenoid and plastoquinone biosynthetic pathways (Pallett et al., 1998).

metal ion at the catalytic center of the active enzyme is Fe^{2+} . In the enzyme-inhibitor complex, the three amino acids coordinating to the metal ion remain the same but two coordinating water molecules have been displaced by the 1,3-diketone moiety of the inhibitor DAS869. In addition to metal coordination, the inhibitor binding site involves the side chains of several residues, most notably the phenyl groups of Phe-360 and Phe-403, which form a π -stacking interaction with the benzoyl moiety of DAS869. The N1-*tert*-butyl

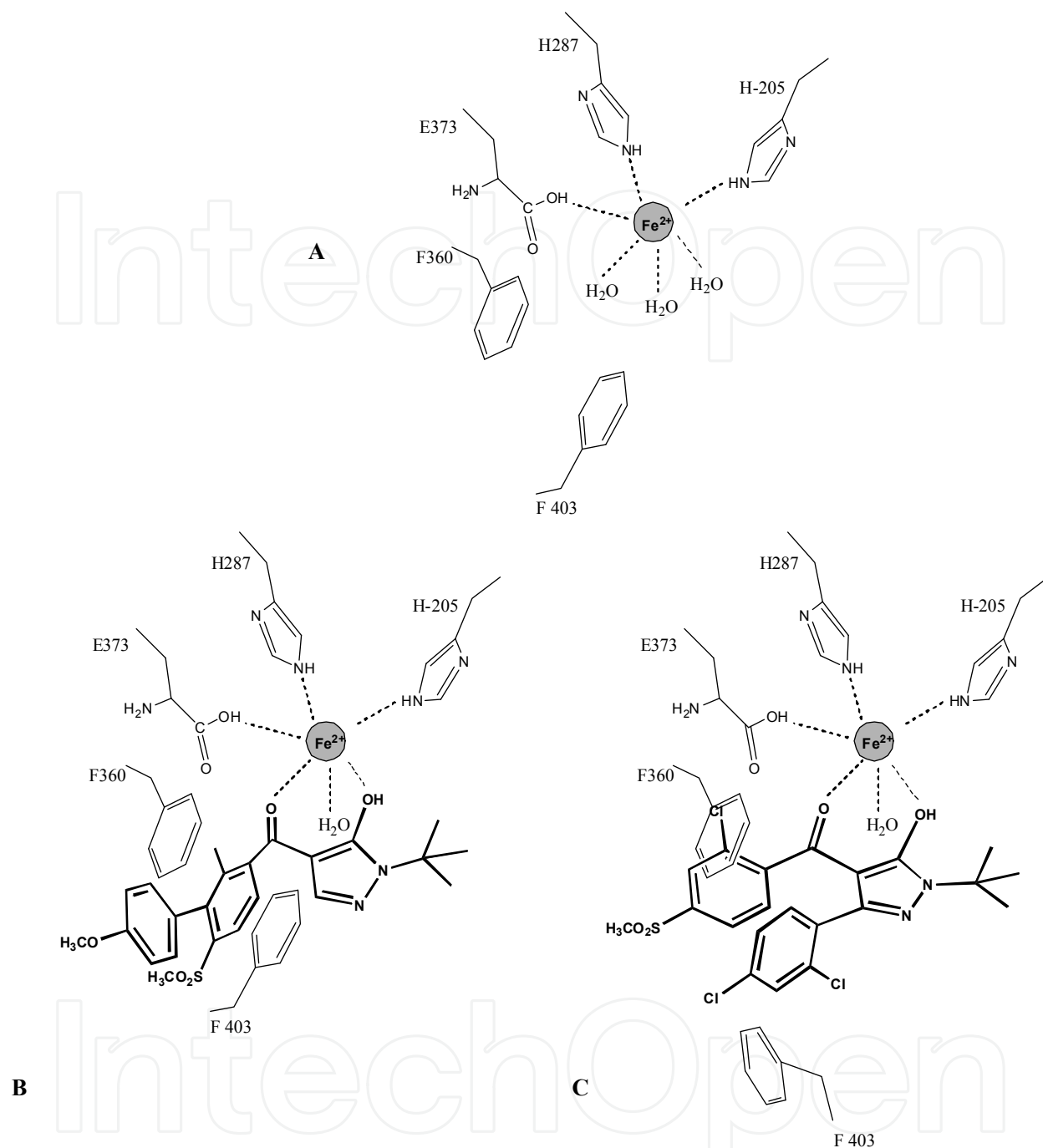


Fig. 7. Schematic representation of the active site of *AtHPPD* (Yang et. al, 2004). (A) Active site of the enzyme without herbicidal substrate. (B) *AtHPPD*-DAS869 complex. (C) *AtHPPD*-DAS645 complex.

group on the ligand pyrazole has a tight fit against Pro-259 and causes a shift of ~ 0.5 Å compared to its position in uncomplexed *AtHPPD*. No hydrogen bonding interactions with the inhibitor were detected. The structure of DAS645 a plant selective inhibitor in complex with *AtHPPD* showed similar binding pattern as it was with DAS869 but with few notable differences. Because of the steric presence of the 3-(2,4-dichlorophenyl) substitution on the pyrazole, Phe-403 has rotated away from the inhibitor.

The interaction between the β -triketones and the catalytic site of *At*HPPD was modeled by docking of inhibitors into the active site plant HPPD (Dayan and Duke, 2007). The 1,3-diketone moiety of all the docked inhibitors coordinated Fe^{2+} ion still formed an octahedral complex with three strictly conserved active site residues (Glu-373, His-287 and His-205) and a critical binding site H_2O molecule, providing a strong ligand orientation and binding force. The observed interactions were consistent with those established with several classes of potent 1,3-diketone-type HPPD inhibitors. The β -triketone-rich essential oil of manuka (*Leptospermum scoparium*) and its components leptospermone, isoleptospermone, and grandiflorone were inhibitory to HPPD. Structure-activity relationships indicated that the size and the lipophilicity of their side-chains affected the potency of the compounds. Both the the exceedingly tight association of HPPD inhibitors and the relatively slow onset of inhibition are consistent with such inhibitors acting as transition state analogs (Kavana et al., 2003).

Identification of catalytic residues in active site of the Carrot HPPD protein has also been disclosed (Raspail et al., 2011). The results highlights a) the central role of Gln-272, Gln-286, and Gln-358 in HPP binding and the first nucleophilic attack, b) the important movement of the aromatic ring during the reaction, and c) the key role of Asn-261 and Ser-246 in C1 hydroxylation and the final ortho rearrangement steps (numbered according to *At*HPPD crystal structure).

2.3 Interaction of acetyl-CoA carboxylase (ACC) inhibitors with the target site enzyme

Acetyl-CoA carboxylases (ACCs) are crucial for the biosynthesis of fatty acids. They catalyze the production of malonyl-CoA from acetyl-CoA and CO_2 , a reaction that also requires the hydrolysis of ATP (Shaner 2003) (Figure 8). Cyclohexanediones such as sethoxydim and the

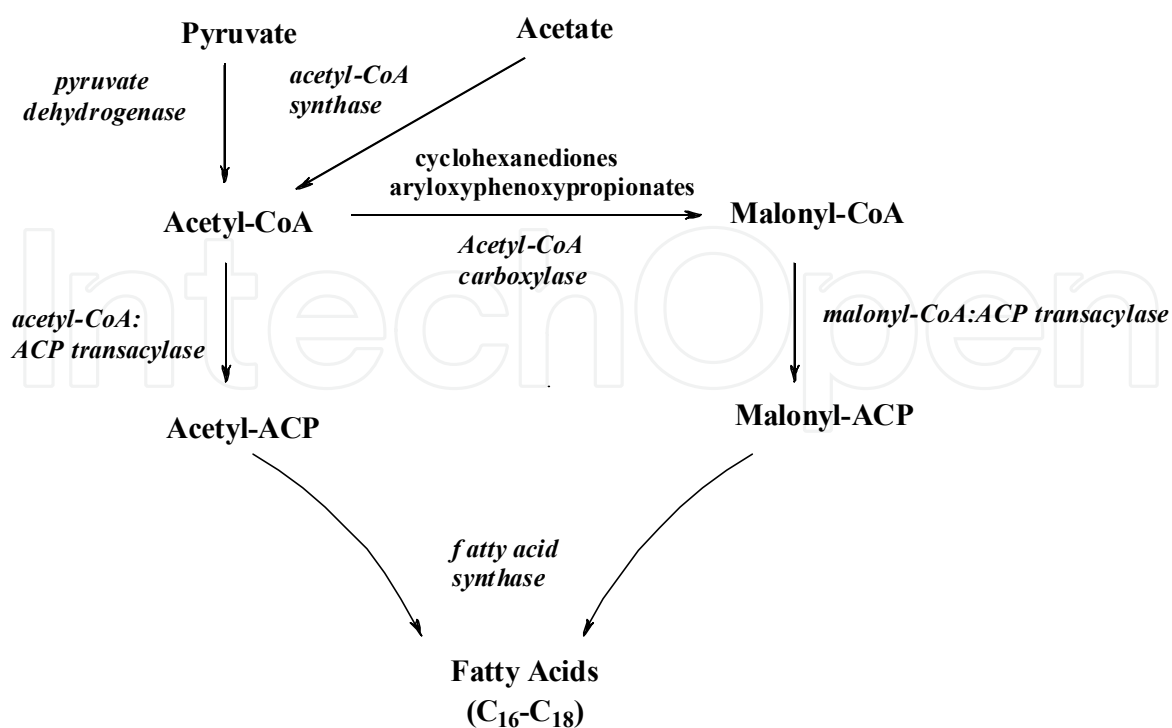


Fig. 8. Schematic representation of fatty acid biosynthesis.

aryloxyphenoxypropionates such as haloxyfop and diclofop, two different classes of widely used commercial herbicides are known inhibitors of ACCs (Burton, 1997). In grasses, such as wheat and maize, ACC is a high molecular weight, multi-domain enzyme, whereas in broadleaf species ACC exists as a multi-subunit enzyme. The cytosolic form of ACCs is a multi-subunit enzyme. The herbicidal ACC inhibitors specifically inhibit the multi-domain enzyme that is in the Gramineae and therefore they can be selectively used to control grasses in broadleaf crops. The molecular mechanism for the inhibition of the carboxyltransferase (CT) domain of ACC by haloxyfop and diclofop herbicides was established by analyses of crystal structure of a complex of the yeast enzyme with the herbicides (Zhang et al., 2004). Haloxyfop is bound in the active site region, at the interface between the N domain of one monomer and the C domain of the other monomer of the dimer (Figure 9). The pyridyl ring of the inhibitor is sandwiched between the side-chains of

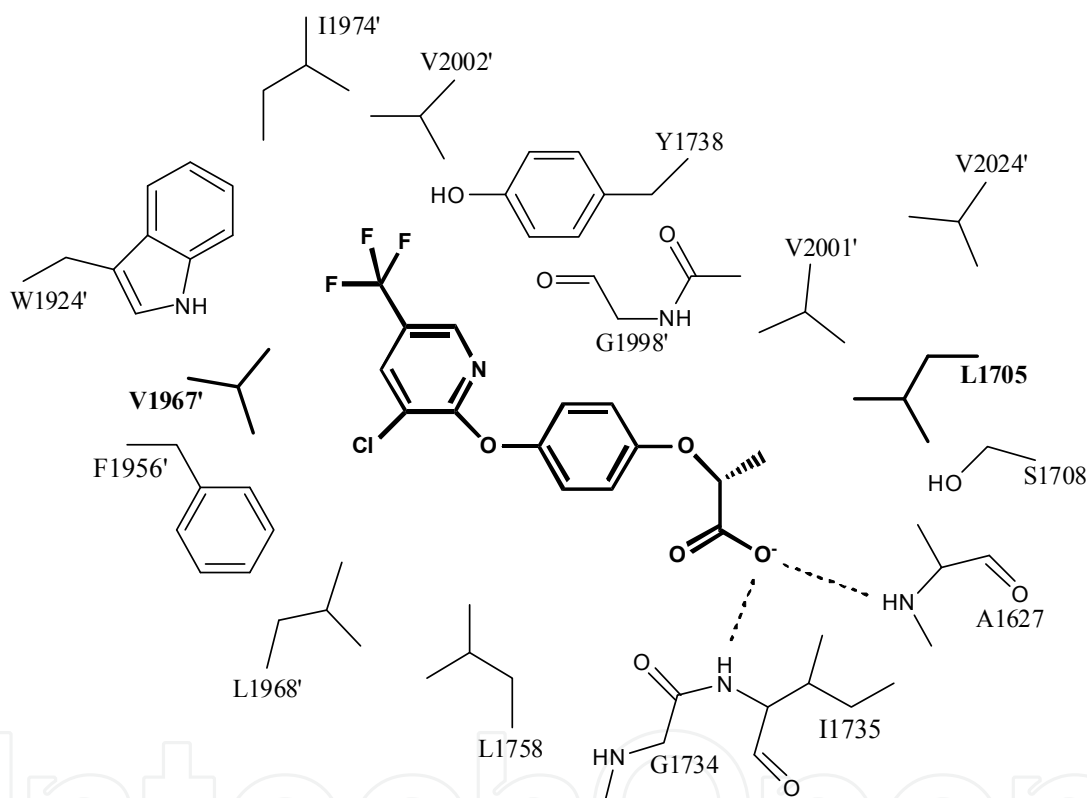


Fig. 9. Schematic representation of the interaction between haloxyfop and the CT domain (Zhang et al., 2004).

Tyr-1738 and Phe-1956' (primed residue numbers indicate the C domain of the other monomer), showing π - π interaction. The trifluoro-methyl group is positioned over the plane of the Trp-1924' side chain, as well as near the side-chains of Val-1967', Ile-1974', and Val-2002'. The phenyl ring in the center of the inhibitor is situated between the Gly-1734-Ile-1735 and Gly-1997'-Gly-1998' amide bonds. One of the carboxylate oxygen atoms of the inhibitor is hydrogen-bonded to the main-chain amides of Ala-1627 and Ile-1735 whereas the other is exposed to the solvent. The methyl group of haloxyfop has van der Waals interactions with the side chains of Ala-1627 and Leu-1705. In contrast, this methyl group in the *S* stereoisomer of haloxyfop will clash with one of the carboxylate oxygens of the inhibitor explaining the selectivity for the *R* stereoisomer of this class of compounds. There

are substantial conformational changes in the active site of the enzyme upon herbicide binding. Most importantly, the side chains of Tyr-1738 and Phe-1956' assume new positions in the inhibitor complex to become π -stacked with the pyridyl ring of haloxyfop. A similar binding pattern was shown for diclofop. Most of the residues that interact with the herbicides are either strictly or highly conserved. Only two residues show appreciable variation among the different CT domains, Leu-1705 and Val-1967'. Variation/mutation of these residues can confer resistance to the herbicides in plants (Zagnitko et al., 2001). The residue that is equivalent to Leu-1705 in the CT domains of wheat and other sensitive ACCs is Ile and the Ile→Leu mutation renders the enzyme resistant to both haloxyfop and sethoxydim. The residue that is equivalent to Val-1967' in sensitive plants is Ile and the Ile→Asn mutation makes the plants resistant to FOPs but not to the DIMs. The Ile→Val mutation can confer resistance to haloxyfop and does not affect the sensitivity to clodinafop (Delye et al., 2003).

2.4 Interaction of auxin herbicides with auxin receptors

A plant hormone, naturally occurring auxin (indole-3-acetic acid, IAA) regulates plant growth by modulating gene expression leading to changes in cell division, elongation and differentiation (Woodward and Bartel, 2005). IAA coordinates many plant growth processes by modulating gene expression which leads to changes in cell division, elongation and differentiation. The perception of auxin signal by cells has been a topic of research. A receptor for auxin was identified as the F-box protein TIR1 (transport inhibitor response 1) is a component of cellular protein complex (SCF^{TIR1}) (Tan et al., 2007). TIR1 was reported to recognize synthetic auxin analogues such as 1-naphthalene acetic acid (1-NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D). Similarly to IAA, both compounds are able to promote the binding of Aux/IAA proteins to the TIR1 F-box protein. Auxin-induced genes are regulated by two classes of gene-transcription factors, auxin/response factors (ARFs) and the Aux/IAA repressors. The auxin signalling pathway includes ARFs binding to auxin-response promoter elements in auxin-response genes. When auxin concentrations are low, Aux/IAA repressors associate with ARF activators and repress the expression of the genes. When auxin concentrations increase, auxin binds to TIR1 receptor in the SCF^{TIR1} complex, leading to recruitment of the Aux/IAA repressors to TIR1. Once recruited to SCF^{TIR1} complex, the repressors enter a pathway that leads to their destruction and the subsequent activation of the auxin/response genes. A recently published crystalline structure of TIR1 complex showing how auxins fit into the surface pocket of TIR1 and enhance the binding Aux/IAA repressors to TIR1. In contrast to an allosteric mechanism, auxin binds to the same TIR1 pocket that docks the Aux/IAA substrate. Without inducing significant conformational changes in its receptor, auxin increases the affinity of two proteins by simultaneously interacting with both in the cavity at protein interface functioning as a 'molecular glue'. The synthetic auxins bind to TIR1 in a similar manner, but with affinities determined by how effectively their ring structures fit into and interact with the promiscuous cavity of the receptor.

2.5 Research for finding new target sites

Demand for new herbicides having with novel mode of action is a continual challenge stimulated by several reasons such as weed resistance evolved to several classes of herbicides as well as strict environmental and safety requirements.

Adenylosuccinate synthase (AdSS), an essential enzyme for *de novo* purine synthesis was found as a promising herbicide target site for hydantocidin (Siehl et al., 1996), a naturally occurring spironucleoside isolated from *Streptomyces hygrosopicus* (Haruyama et al., 1991). Hydantocidin was shown to be a proherbicide that, after phosphorylation at the 5' position, inhibits adenylosuccinate synthase (Fonné-Pfister et al., 1996). The mode of binding of hydantocidin 5'-monophosphate (HMP) to the target enzyme from *E. coli* was analyzed by determining the crystal structure of the enzyme inhibitor complex. The binding site was found at one end of a crevice across the middle of the enzyme. It was shown that AdSS binds the phosphorylated hydantocidin at the same site as it does adenosine 5'-monophosphate, the natural feedback regulator of this enzyme. The phosphate group is very important for binding to the enzyme and involves most of the direct contacts that the inhibitors have with the protein, including hydrogen bonds to Arg-143 from the other monomer in the dimer. The 2' and 3' hydroxyl groups of the ribose moiety have hydrogen bonds with Arg-303 and the main-chain carbonyls of Gly-127 and Val-273. The sugar groups have slightly different positions in the binding sites, which may be favorable in the case of HMP due to the internal hydrogen bond between the hydantoin and the phosphate groups. In the region where the structures of the two ligands differ, most of the contacts with the protein are made in both cases via water molecules. The hydantoin moiety is coplanar with the adenosine group and many of the water molecules lying in the same plane.

Embryonic factor 1 (*FAC1*) is one of the earliest expressed plant genes and encodes an AMP deaminase (AMPD), which was identified as a herbicide target (Dancer et al., 1997). Coformycin (Isaac et al., 1991) produced by various microbes and carbocyclic coformycin (Bush et al., 1993) isolated from a fermentation of *Saccharothrix* spp. have previously been reported to have herbicidal activity. AMPD catalyzes deamination of AMP to inosine-5'-monophosphate and together with the adenylosuccinate synthase (AdSS) and adenylosuccinate lyase it forms the purine nucleotide cycle. The N-terminal transmembrane domain in *Arabidopsis FAC1* was indentified using a recombinant enzyme (Han et al., 2006). The active site of *FAC1* with bound coformycin 5'-phosphate, a herbicidally active form of coformycin, is positioned on the C-terminal side of the imperfect (α/β)₈-barrel, surrounded by multiple helices and loops. The catalytic zinc ion is coordinated to the coformycin 5'-phosphate, an aspartic acid (Asp-736) and three histidine (His-391, 393, and 659) residues. The phosphate group of the inhibitor is located in a polar environment. The transmembrane domain and disordered linker domain of both subunits tether the dimeric globular catalytic domain to the lipid bilayer. The basic residue-rich surface spanning the dimer interface can become quite flat in the region of positive charge and facilitate interaction with negative patches on the surface of the membrane. However, the mechanistic bases for lethality associated with dramatic reductions in plant AMPD activity remain to be elucidated.

Inhibition of the enzymes of amino acid biosynthesis is an important target for several classes of herbicides as detailed earlier.

A new target, tryptophan synthase (TRPS) catalyzes the final two steps in the biosynthesis of tryptophan (Metha and Christen, 2000). It is typically found as an α 2 β 2 tetramer (Raboni et al., 2009). The α subunits catalyze the reversible formation of indole and glyceraldehyde-3-phosphate (G3P) from indole-3-glycerol phosphate (IGP). The β subunits catalyze the irreversible condensation of indole and serine to form tryptophan in a pyridoxal phosphate (PLP) dependent reaction. Each α active site is connected to a β active site by a 25 Å long

hydrophobic channel contained within the enzyme. This facilitates the diffusion of indole formed at α active sites directly to β active sites in a process known as substrate channeling. A rational design of TRPS inhibitors as herbicides based on the structure of the inhibitory indole-3-phosphate (Rhee et al., 1998) in complex with the enzyme has been described (Sachpatzidis et al., 1999). Series of 4-aryl-thiobutylphosphonates were designed in which sulfur mimics the sp^3 -hybridized intermediate of the natural reaction intermediate and opening the heteroaromatic ring of the indole resulted in an increased rotational freedom. Amino group placed in *ortho*-position provided a potent inhibitory compound (IC_{50} = 178 nM) which was shown to bind to the α -site of the enzyme. Later it was shown that the indole-3-acetyl amino acids such as indole-3-acetyl-glycine and indole-3-acetyl-L-aspartic acid are both α -subunit inhibitors and β -subunit allosteric effectors, whereas indole-3-acetyl-L-valine is only an α -subunit inhibitor (Marabotti et al., 2000). The crystal structures of tryptophan synthase complexed with indole-3-acetyl-glycine and indole-3-acetyl-L-aspartic acid revealed that both ligands bind to the active site such that the carboxylate moiety is positioned similarly as the phosphate group of the natural substrates (Weyand et al., 2002).

Since biotin a cofactor for enzymes involved in carboxylation, trans-carboxylation and decarboxylation reactions dethiobiotin synthase (DTBS) can also be a promising target to develop new herbicides. DTBS is a penultimate enzyme in the biotin biosynthesis catalyzing the formation of a cyclic urea precursor of biotin from diaminopelargonic acid, CO_2 and ATP (Marquet et al., 2001). Bacterial enzyme was used as a model for the design and synthesis of DTBS inhibitors as herbicides. (Rendina et al., 1999). In order to mimic the carbamate intermediate of the DTBS various carboxylates and phosphonates were prepared but poor level of inhibition thus weak *in vivo* activities were detected. Co-crystallisation of a diphosphonate derivative with the enzyme revealed a relatively close binding to surface of the enzyme in a solvent exposed region which may explain the weak levels of inhibition. However no other reports were found on the synthesis of more potent inhibitors.

Pyruvate phosphate dikinase (PPDK) is an enzyme that catalyzes the inter-conversion of adenosine triphosphate (ATP), phosphate (Pi), and pyruvate with adenine monophosphate (AMP), pyrophosphate (PPi), and phosphoenolpyruvate (PEP) in the presence of magnesium and potassium/sodium ions (Mg^{2+} and K^+/Na^+) (Evans and Wood, 1968). The three-step reversible reaction proceeds via phosphoenzyme and pyrophosphoenzyme intermediates with a histidine residue serving as the phosphocarrier. The enzyme has been found in bacteria, in C_4 and Crassulacean acid metabolism plants, and in parasites, but not in higher animal forms. Inhibition of PPDK significantly hinders C_4 plant growth (Maroco et al., 1998). A total of 2,245 extracts from 449 marine fungi were screened against C_4 plant PPDKs as potential herbicide target (Motti et al., 2007). Extracts from several fungal isolates selectively inhibited PPDK. Bioassay-guided fractionation of one isolate led to the isolation of the known compound unguinol, which inhibited PPDK with a 50% inhibitory concentration of 42.3 μM . Unguinol had deleterious effects on a model C_4 plant but no effect on a model C_3 plant. The results indicated that unguinol inhibits PPDK via a novel mechanism of action which also translates to a herbicidal effect on whole plants.

Classical photosystem-II (PSII) inhibitors, such as urea, triazine and triazinone herbicides, bind to the D1 protein in stoichiometric fashion. Due to herbicide binding, the electron flow from PSII is disrupted and carbon dioxide fixation ceases. Since the electron acceptor is not able to accept electrons from photo-excited chlorophyll, free radicals are generated and

chlorosis develops (Ahrens and Krieger-Liszkay, 2001). Stoichiometric inhibition of D1 as an herbicide mode of action has a major disadvantage since high application rates of the herbicide required for the activity. Much lower rates of herbicide would be necessary if the inhibition of the biosynthesis of mature D1 protein were targeted. A carboxyterminal processing protease (CptA) was chosen as a target and tested in a high throughput screen for CptA inhibitors (Duff et al., 2007). CptA, a low abundance enzyme located in the thylakoids catalyzes the conversion of the nascent pre-D1 protein into the active form of D1 by cleaving the 9 C-terminal residues. Under light conditions D1 protein is continuously being damaged by light and is turned over. Thus the active CptA is constantly required under light conditions to maintain functional PSII complexes. The herbicidal effects by the inhibition of CptA protease were reported using novel CptA protease inhibitors. Although K_i values of CptA inhibitors were in micromolar range the *in planta* results suggested that good CptA inhibitors exhibited effective herbicidal activity while compounds with poor inhibitory activity possessed with only observable phytotoxicity.

3. Conclusions

Increasing problems associated with herbicide resistance as well as growing demand for herbicides in the developing world will expectedly spur the research and development for new herbicides. In the last decade, strategies for discovery of new herbicides have shifted from the testing of molecules in whole plant studies towards the use of *in-vitro* assays against molecular targets (Lein et al., 2004). 3D structures produced by X-ray crystallography have become an integral part of the current agrochemical and pharmaceutical discovery process. As genomic and proteomic data becomes increasingly available, a large numbers of validated targets will provide a basis for the structure-based inhibitor design (Walter, 2002) as a routine approach to obtain lead compounds.

4. References

- Ahrens W.H. & Krieger-Liszkay A. (2001). Herbicide-induced oxidative stress in photosystem II. *Trends Biochem Sci.*, 26, 648-653.
- Amrhein N, Deus B, Gehrke P, & Steinrücken HC (1980). The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. *Plant Physiol.* 66, 830-834.
- Ashigh J. & Tardif F. J. (2007). An Ala205Val substitution in acetohydroxyacid synthase of eastern black nightshade (*Solanum Ptychanthum*) reduces sensitivity to herbicides and feedback inhibition. *Weed Sci.*, 55, 558-565.
- Barry G. F. & Kishore G. A. (1995). Glyphosate tolerant plants. US Patent 5,463,175.
- Bernasconi P., Woodworth A. R., Rosen B. A., Subramanian M. V., & Siehl D. L. (1995). A naturally occurring point mutation confers broad range tolerance to herbicides that target acetolactate synthase. *J. Biol. Chem.*, 270, 17381-17385.
- Brownlee J., Johnson-Winters K., Harrison D. H. T., & Moran G. R. (2004). Structure of the ferrous form of (4-hydroxyphenyl)pyruvate dioxygenase from *Streptomyces avermitilis* in complex with the therapeutic herbicide, NTBC. *Biochemistry*, 43, 6370-6377.
- Burton J. D. (1997). Acetyl-coenzyme A carboxylase inhibitors. In: *Herbicide activity: Toxicology, biochemistry and molecular biology*, Roe R. M., Burton J. D., Kuhr R. J., pp 187-205, IOS Press, Washington, DC, USA.

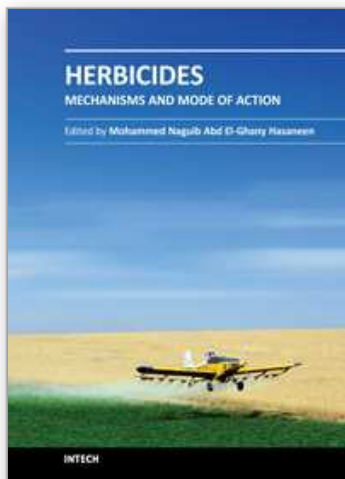
- Bush B. D., Fitchett G. V., Gates D. A., & Langley D. (1993). Carbocyclic nucleosides from a species of *Saccharothrix*. *Phytochemistry*, 32, 737-739.
- Castle L. A., Siehl D. L., Gorton R., Patten P. A., Chen Y. H., Bertain S., Cho H. J., Duck N., Wong J., Liu D., & Lassner M. W. (2004). Discovery and directed evolution of a glyphosate tolerance gene. *Science*, 304, 1151-1154.
- Dancer J. E., Hughes R. G., & Lindell S. D. (1997). Adenosine-5'-phosphate deaminase. A novel herbicide target. *Plant. Physiol.*, 114, 119-129.
- Delye C., Zhang X.-Q., Chalopin C., Michel S., & Powles S. (2003). An isoleucine residue within the carboxyl-transferase domain of multidomain acetyl-coenzyme A carboxylase is a major determinant of sensitivity to aryloxyphenoxypropionate but not to cyclohexanedione inhibitors *Plant Physiol.*, 132, 1716-1723.
- Dayan F. E., Duke S. O., Sauldubois A., Singh N., McCurdy C., Cantrell C. (2007). p-Hydroxyphenylpyruvate dioxygenase is a herbicidal target site for β -triketones from *Leptospermum scoparium*. *Phytochemistry*, 68, 2004-2014.
- Duff S. M. G., Chen Y.-C. S., Fabbri B. J., Yalamanchili G., Hamper B. C., Walker D. M., Brookfield F. A., Boyd E. A., Ashton M. R., Yarnold C. J., & CaJacob C. A. (2007). The carboxyterminal processing protease of D1 protein: Herbicidal activity of novel inhibitors of the recombinant and native spinach enzymes. *Pest. Biochem. Physiol.*, 88, 1-13.
- Duggleby R. G. & Pang S. S. (2000). Acetohydroxyacid synthase. *J. Biochem. Mol. Biol.*, 33, 1-36.
- Evans H. J. & Wood H. G. (1968). The mechanism of the pyruvate, phosphate dikinase reaction. *Proc. Natl. Acad. Sci. USA*. 61, 1448-1453.
- Fonné-Pfister R., Chemla P., Ward E., Girardet M., Kreuz K. E., Honzatko R. B., Fromm H. J., Schär H-P., Grütter M. G., & Cowan-Jacob S. W. (1996). The mode of action and the structure of a herbicide in complex with its target: Binding of activated hydantocidin to the feedback regulation site of adenylosuccinate synthetase. *Proc. Natl. Acad. Sci. USA*, 93, 9431-9436.
- Franz J. E., Mao M. K., & Sikorski, J. A. (1997). *Glyphosate: A Unique Global Herbicide*, ACS Monograph Series, No. 189, ACS, Washington, DC.
- Fritze I. M., Linden L., Freigang J., Auerbach G., Huber R., & Steinbacher S. (2004). The crystal structures of *Zea mays* and *Arabidopsis* 4-Hydroxyphenylpyruvate Dioxygenase. *Plant Physiol.*, 134, 1388-1400.
- Funke T., Han H., Healy-Fried M. L., Fischer M., & Schönbrunn E. (2006). Molecular basis for the resistance of Roundup Ready crops. *Proc. Natl. Acad. Sci. USA*, 103, 13010-13015.
- Funke T., Yang Y., Han H., Healy-Fried M., Olensen S., Becker A., & Schönbrunn E. (2009). Structural basis of glyphosate resistance resulting from the double mutation Thr⁹⁷→Ile and Pro¹⁰¹→Ser in 5-enolpyruvylshikimate-3-phosphate synthase from *Escherichia coli*. *J. Biol. Chem.*, 284, 9854-9860.
- Gerwick B. C., Subramanian M. V., Loney-Gallant V., & Chandler D. P. (1990). Mechanism of action of the 1,2,4-triazolo[1,5- α]pyrimidine. *Pestic. Sci.*, 29, 357-364.
- Han B. W., Bingman C. A., Mahnke D. K., Bannen R. M., Bednarek S. Y., Sabina R. L., Phillips, G. N. Jr. (2006). Membrane association, mechanism of action, and structure of *Arabidopsis* embryonic factor 1 (FAC1). *J. Biol. Chem.*, 281, 14939-14947.

- Haruyama H., Takayama T., Kinoshita T., Kondo M., Nakajima M., & Haneishi T. (1991). Structural elucidation and solution conformation of the novel herbicide hydantocidin. *J. Chem. Soc. Perkin Trans. 1*, 1637-1640.
- Hattory J., Rutledge R., Labbe H., Brown D., Sunohara G., & Miki B. (1992). Multiple resistance to sulfonylureas and imidazolinones conferred by an acetohydroxyacid synthase gene with separate mutations for selective resistance. *Mol. Gen. Genet.*, 232, 167-173.
- Isaac B. G., Ayer S. W., Letendre L. J., & Stonard R. J. (1991). Herbicidal nucleosides from microbial sources. *J. Antibiotics*, 44, 729-732.
- Job V., Molla G., Pilone M. S., & Pollegioni L. (2002). Overexpression of a recombinant wild-type and His-tagged *Bacillus subtilis* glycine oxidase in *Escherichia coli*. *Eur J. Biochem.*, 269, 1456-1463.
- Kavana M. & Moran G. R. (2003). Interaction of (4-hydroxyphenyl)pyruvate dioxygenase with specific inhibitor 2-[2-nitro-4-(trifluoromethyl)benzoyl]-1,3-cyclohexadione. *Biochemistry*, 42, 10238-10245.
- Klausener A., Raming K., & Stenzel K. (2007) Modern tools for drug discovery in agricultural research, In: *Pesticide Chemistry. Crop Protection Public Health, Environmental safety*. Ohkawa H., Miyagawa H., Lee P. W., pp 55-63, Wiley-VCH, Weinheim.
- LaRossa R. A. & Schloss J. V. (1984). The sulfonylurea herbicide sulfometuron is an extremely potent and selective inhibitor of acetolactate synthase in *Salmonella typhimurium*. *J. Biol. Chem.*, 259, 8753-8757.
- Le D. T., Yoon M-Y., Kim Y. T., Choi J.-D. (2005). Roles of three well-conserved arginine residues in mediating the catalytic activity of tobacco acetohydroxy acid synthase. *J. Biochem.*, 138, 35-40.
- Lee H., Rustgi S., Kumar N., Burke I., Yenish J. P., Kulvinder S. G., von Wettstein D., & Ullrich S. E. (2011). Single nucleotide mutation in the barley acetohydroxyacid synthase (AHAS) confers resistance to imidazolinone herbicides. *Proc. Natl. Acad. Sci. USA*, 108, 8909-8913.
- Lein W. F., Börnke F., Reindl A., Erhardt T., Stitt M., & Sonnewald U. (2004). Target-based discovery of novel herbicides. *Curr. Opin. Plant Biol.*, 7, 219-225.
- Lydon J. & Duke S. O. (1999). Inhibitors of glutamine biosynthesis, In *Plant amino acids: biochemistry and biotechnology*, Singh B. K., pp 445-464, Marcel Dekker, New York, USA.
- Marabotti A., Cozzini P., & Mozzarelli A. (2000). Novel allosteric ejectors of the tryptophan synthase K2L2 complex identified by computer-assisted molecular modeling. *Biochim. Biophys. Acta*, 1476, 287-299.
- Maroco J. P., Ku M. S. B., Lea P. J., Dever L. V., Leegood R. C., Furbank R. T., & Edwards G. E. (1998). Oxygen requirement and inhibition of C4 photosynthesis – an analysis of C4 plants deficient in the C3 and C4 cycles. *Plant Physiol.* 116, 823-832.
- Marquet A., Bui B. T., & Florentin D. (2001). Biosynthesis of biotin and lipoic acid. *Vitam. Horm.*, 61, 51-101.
- McCourt J. A., Pang S. S., King-Scott J., Guddat L.W., & Duggleby R. G. (2006). Herbicide-binding sites revealed in the structure of plant acetohydroxyacid synthase. *Proc. Natl. Acad. Sci. USA*, 103, 569-573.

- Metha P. K. & Christen P. (2000). The molecular evolution of pyridoxal-5'-phosphate-dependent enzymes. *Adv. Enzymol. Relat. Areas Mol. Biol.*, 74, 129-184.
- Mohamed Naseer Ali M., Kaliannan P., & Venuvanalingam P. (2005). *Ab initio* computational modeling of glyphosate analogs: Conformational perspective. *Struct. Chem.*, 16, 491-506.
- Moran G. R. (2005). 4-Hydroxyphenylpyruvate dioxygenase. *Arch. Biochem. Biophys.*, 433, 117-128.
- Motti C. A., Bourne D. G., Burnell J. N., Doyle J. R., Haines D. S., Liptrot C. H., Llewellyn L. E., Ludke S., Muirhead A., & Tapiolas D. M. (2007). *Appl. Environ. Microbiol.*, 1921-1927.
- Padgett S. R., Kolacz K. H., Delannay X., Re D. B., LaVallee B. J., Tinius C. N., Rhodes W. K., Otero Y. I., Barry G. F., Eichholz D. A., Peschke V. M., Nida D. L., Taylor N. B., & Kishore G. M. (1995). Development, identification, and characterization of a glyphosate-tolerant soybean line. *Crop Sci.*, 35, 1451-1461.
- Pallett K. E., Little J. P., Sheekey M., Veerasesaran P. (1998). The mode of action of isoxaflutol. I. Physiological effects, metabolism, and selectivity. *Pestic. Biochem. Physiol.*, 62, 113-124
- Pang S.S., Guddat L. W., & Duggleby R. G. (2004). Crystallization of *Arabidopsis thaliana* acetohydroxyacid synthase in complex with the sulfonylurea herbicide chlorimuron ethyl. *Acta Cryst. D*, 60, 153-155.
- Pascal R. A., Oliver M. A., & Chen, Y.-C. J. (1985). Alternate substrates and inhibitors of bacterial 4-hydroxyphenylpyruvate dioxygenase. *Biochemistry*, 24, 3158-3165.
- Perez-Jones A., Park K.-W., Polge N., Colquhoun J., Mallory-Smith C. A. (2007). Investigating the mechanisms of glyphosate resistance in *Lolium multiflorum*. *Planta*, 226, 395-404.
- Pedotti M., Rosini E., Molla G., Moschetti T., Savino C., Vallone B., & Pollegioni L. (2009). Glyphosate resistance by engineering the flavoenzyme glycine oxidase. *J. Biol. Chem.*, 284, 36415-36423.
- Pollegioni L., Schönbrunn E., & Siehl D., (2011). Molecular basis of glyphosate resistance – different approaches through protein engineering. *FEBS J.*, 278, 2753-2766.
- Prisbylla M. P., Onisko B. C., Shribbs J. M., Adams D. O., Liu Y., Ellis M. K., Hawkes T. R., & Mutter L. C. (1993). The novel mechanism of action of herbicidal triketones, *Proc. British Crop Prot. Conf. – Weeds*, vol. 2, 731-738.
- Raboni S., Bettati S., & Mozzarelli A. (2009). Tryptophan synthase: a mine for enzymologists. *Cell. Mol. Life Sci.*, 66, 2391-2403.
- Raspail C., Graindorge M., Moreau Y., Crouzy S., Lefevre B., Robin A. Y., Dumas R., & Matringe M. (2011). 4-Hydroxyphenylpyruvate dioxygenase catalysis: Identification of catalytic residues and production of a hydroxylated intermediate shared with a structurally unrelated enzyme. *J. Biol. Chem.*, <http://www.jbc.org/cgi/doi/10.1074/jbc.M111.227595>.
- Rendina A. R., Taylor W. S., Gibson K., Lorimer G., Rayner D., Lockett B., Kranis K., Wexler B., Marcovici-Mizrahi D., Nudelman A., Nudelman A., Marsilii E., Chi H., Wawrzak Z., Calabrese J., Huang W., Jia J., Schneider G., Lindqvist Y., & Yang G. (1999). The design and synthesis of inhibitors of dethiobiotin synthetase as potential herbicid. *Pestic. Sci.*, 55, 236-247.

- Rhee S., Miles E. W., & Davies D. R. (1998). Cryo-crystallography of a true substrate, indole-3-glycerol phosphate, bound to a mutant (d60n) tryptophan synthase 22 complex reveals the correct orientation of active site Glu49. *J. Biol. Chem.*, 263, 8611-8614.
- Rost T. L. (1984). The comparative cell cycle and metabolic effects of chemical treatments on root tip meristems. *J. Plant Growth Regul.*, 3, 51-63.
- Sachpatzidis A., Dealwis C., Lubetsky J. B., Liang P.-H., Anderson K. S., & Lolis E. (1999). *Biochemistry*, 38, 12665-12674. Crystallographic studies of phosphonate-based alpha-reaction transition-state analogues complexed to tryptophan synthase.
- Schönbrunn E., Eschenburg S., Shuttleworth W. A., Schloss J. V., Amrhein N., Evans J. N. S., & Kabsch W. (2001) Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. *Proc. Natl. Acad. Sci. USA*, 98, 1376-1380.
- Serre L., Sailland D. S., Boudec P., Rolland A., Pebay-Peyroula E., & Cohen-Addad C. (1999). Crystal structure of *Pseudomonas fluorescens* 4-hydroxyphenylpyruvate dioxygenase: an enzyme involved in the tyrosine degradation pathway. *Structure*, 7, 977-988.
- Shah D. D., Conrad J. A., Heinz B., Brownlee J. M., & Moran G. R. (2011). Evidence for the mechanism of hydroxylation by 4-hydroxyphenylpyruvate dioxygenase and hydroxymandelate synthase from intermediate partitionin in active site variants. *Biochemistry*, dx.doi.org/10.1021/bi2009344.
- Shaner D. L., Anderson P. C., Stidham M. A. (1984). Imidazolinones: potent inhibitors of acetohydroxy acid synthase. *Plant Physiol.*, 76, 545-546.
- Shaner D. L. (2003). Herbicide safety relative to common targets in plants and mammals. *Pest. Manag. Sci.* 60, 17-24.
- Shaner D. L. & Singh B. J. (1993). Phytotoxicity of acetohydroxyacid synthase inhibitors is not due to accumulation of 2-ketobutyrate and/or 2-aminobutyrate. *Plant Physiol.*, 103, 1221-1226.
- Shuttleworth W. A., Pohl M. E., Helms G. L., Jakeman D. L., & Evans J. N. S. (1999). Site-directed mutagenesis of putative active site residues of 5-enolpyruvylshikimate-3-phosphate synthase. *Biochemistry*, 38, 296-302.
- Sibony M., Michael A., Haas H. U., Rubin B., & Hurle K. (2001). Sulfometuron-resistant *Amaranthus retroflexus*: Cross-resistance and molecular basis for resistance to acetolactate synthase (ALS) inhibiting herbicides. *Weed Res.*, 41, 509-522.
- Siehl D. L., Subramanian M. V., Walters E. W., Lee S. F., Anderson R. J., & Toschi A. G. (1996). Adenylosuccinate synthetase: Site of action of hydantocidin, a microbial phytotoxin. *Plant Physiol.*, 110, 753-758.
- Siehl D. L., Castle L. A., Gorton R., & Keenan R. J. (2007). The molecular basis of glyphosate resistance by an optimized microbial acetyltransferase. *J. Biol. Chem.*, 282, 11446-11455.
- Sikorski J. A. & Gruys K. J. (1997). Understanding glyphosate's mode of action with EPSP synthase: Evidence favoring an allosteric inhibitor model. *Acc. Chem. Res.*, 30, 2-8.
- Sost D. & Amrhein N. (1990). Substitution of Gly-96 to Ala in the 5-enolpyruvylshikimate-3-phosphate synthase of *Klebsiella pneumoniae* results in a greatly reduced affinity for the herbicide glyphosate. *Arch. Biochem. Biophys.*, 282, 433-436.
- Stallings W. C., Abdel-Meguid S. S., Lim L. W., Shieh H. S., Dayringer H. E., Leimgruber N. K., Stegemann R. A., Anderson K. S., Sikorski J. A., Padgett S. R., & Kishore G. M.

- (1991). Structure and topological symmetry of the glyphosate target 5-enolpyruvylshikimate-3-phosphate synthase: a distinctive protein fold. *Proc. Natl. Acad. Sci. USA*, 88, 5046-5050.
- Tan X., Calderon-Villalobos L. I. A., Sharon M., Zheng C., Robinson C.V., Estelle M., & Zheng, N. (2007). Mechanism of auxin perception by TIR1 ubiquitin ligase. *Nature*, 446, 640-645.
- Tranel P. J., Wright T. R. (2002). Resistance of weeds to ALS-inhibiting herbicides: What have we learned. *Weed Sci.*, 50, 700-712.
- Unno H., Uchida T., Sugawara H., Kurisu G., Sugiyama T., Yamaya T., Sakakibara H., Hase T., & Kusunoki M. (2006). Atomic structure of plant glutamine synthetase. A key enzyme for plant productivity. *J. Biol. Chem.*, 281, 29287-29296.
- Yang C., Pflugrath J. W., Camper D. L., Foster M. L., Pernich D. J., & Walsh T.A. (2004). Structural basis for herbicidal inhibitor selectivity revealed by comparison of crystal structures of plant and mammalian 4-hydroxyphenylpyruvate dioxygenases. *Biochemistry*, 43, 10414-10423.
- Yu Q., Cairns A., & Powles S. (2007). Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. *Planta*, 225, 499-513.
- Walter M. W. (2002). Structure-based design of agrochemicals. *Nat. Prod. Rep.*, 19, 278-291.
- Waltz E. (2010). Glyphosate resistance threatens Roundup hegemony. *Nat. Biotechnol.*, 28, 537-538.
- Weyand M., SCHlichting I., Marabotti A., & Mozzarelli A. (2002). Crystal structures of a new class of allosteric effectors complexed to tryptophan synthase. *J. Biol. Chem.*, 277, 10647-10652.
- Woodward A. W. & Bartel B. (2005). Auxin: regulation, action, and interaction. *Ann. Bot. (Lond.)*. 95, 707-735.
- Zagnitko O., Jelenska J., Tevzadze G., Haselkorn I., & Gornicki P. (2001). An isoleucine/leucine residue in the carboxyltransferase domain of acetyl-CoA carboxylase is critical for interaction with aryloxyphenoxypropionate and cyclohexanedione inhibitors. *Proc. Natl. Acad. Sci. USA*, 98, 6617-6622.
- Zhang H., Tweel B., & Tong L. (2004) Molecular basis for the inhibition of the carboxyltransferase domain of acetyl-coenzyme-A carboxylase by haloxyfop and diclofop. *Proc. Natl. Acad. Sci. USA*, 101, 5910-5915.
- Zhou Q., Liu W., Zhang Y., & Liu K. K. (2007). Action mechanisms of acetolactate synthase-inhibiting herbicides. *Pestic. Biochem. Physiol.*, 89, 89-96.
- Zimdahl R. L. (2002). My view. *Weed Sci.*, 50, 687.



Herbicides - Mechanisms and Mode of Action

Edited by Dr. Mohammed Nagib Hasaneen

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This volume contains two sections: Mechanisms of herbicidal action (chapters 1-4) and Mode of action of selected herbicides on controlling diseased, weed growth and productivity and/or growth and development of field crops (chapters 5-10). Topics by chapters are: molecular mechanism of action, immunosensors, laboratory studies, molecular modeling, weed resistance, community response, use of herbicides in biotech culture, gene flow, herbicides and risk, herbicides persistence. These recurring themes reinforce my view, held over a very long time, that experience with one crop or problem can sometimes be relevant, often to an unexpected extent, to an apparently dissimilar situation in a different crop. I hope that readers interested in herbicides and pesticides will be satisfied with all the chapters in the book as its content might be of interest and value to them in the future.

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