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Enzymology of Bacterial Lysine Biosynthesis

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1. Introduction

Lysine is an essential amino acid in the mammalian diet, but can be synthesised *de novo* in bacteria, plants and some fungi (Dogovski et al., 2009; Hutton et al., 2007). In bacteria, the lysine biosynthesis pathway, also known as the diaminopimelate (DAP) pathway (Fig. 1), yields the important metabolites *meso*-2,6-diaminopimelate (*meso*-DAP) and lysine. Lysine is utilised for protein synthesis in bacteria and forms part of the peptidoglycan cross-link structure in the cell wall of most Gram-positive species; whilst *meso*-DAP is the peptidoglycan cross-linking moiety in the cell wall of Gram-negative bacteria and also Gram-positive *Bacillus* species (Burgess et al., 2008; Mitsakos et al., 2008; Voss et al., 2010) (Fig. 1).

The synthesis of *meso*-DAP and lysine begins with the condensation of pyruvate (PYR) and *L*-aspartate-semialdehyde (ASA) by the enzyme *dihydrodipicolinate synthase* (DHDPS, EC 4.2.1.52) (Blickling et al., 1997a; Mirwaldt et al., 1995; Voss et al., 2010; Yugari & Gilvarg, 1965). The product of the DHDPS-catalysed reaction is an unstable heterocycle, 4-hydroxy-2,3,4,5-tetrahydro-*L,L*-dipicolinic acid (HTPA) (Fig. 1). HTPA is non-enzymatically dehydrated to produce dihydrodipicolinate (DHDP), which is subsequently reduced by the NAD(P)H-dependent enzyme, *dihydrodipicolinate reductase* (DHDPR, EC 1.3.1.26), to form *L*-2,3,4,5,-tetrahydrodipicolinate (THDP) (Dommaraju et al., 2011; Girish et al., 2011; Reddy et al., 1995, 1996) (Fig. 1). The metabolic pathway then diverges into four sub-pathways depending on the species, namely the succinylase, acetylase, dehydrogenase and aminotransferase pathways (Dogovski et al., 2009; Hutton et al., 2007) (Fig. 1).

The most common of the alternative metabolic routes is the succinylase pathway, which is inherent to many bacterial species including *Escherichia coli*. This sub-pathway begins with the conversion of THDP to N-succinyl-*L*-2-amino-6-ketopimelate (NSAKP) catalysed by *2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase* (THPC-NST, EC 2.3.1.117).

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NSAKP is then converted to N-succinyl-*L,L*-2,6,-diaminopimelate (NSDAP) by *N-succinyldiaminopimelate aminotransferase* (NSDAP-AT, EC 2.6.1.17), which is subsequently desuccinylated by *succinyldiaminopimelate desuccinylase* (SDAP-DS, EC 3.5.1.18) to form *L,L*-2,6-diaminopimelate (*LL*-DAP) (Kindler & Gilvarg., 1960; Ledwidge & Blanchard., 1999; Simms et al., 1984) (Fig. 1). *LL*-DAP is then converted to *meso*-DAP by the enzyme diaminopimelate epimerase (DAPE, EC 5.1.1.7) (Wiseman, & Nichols, 1984) (Fig. 1).

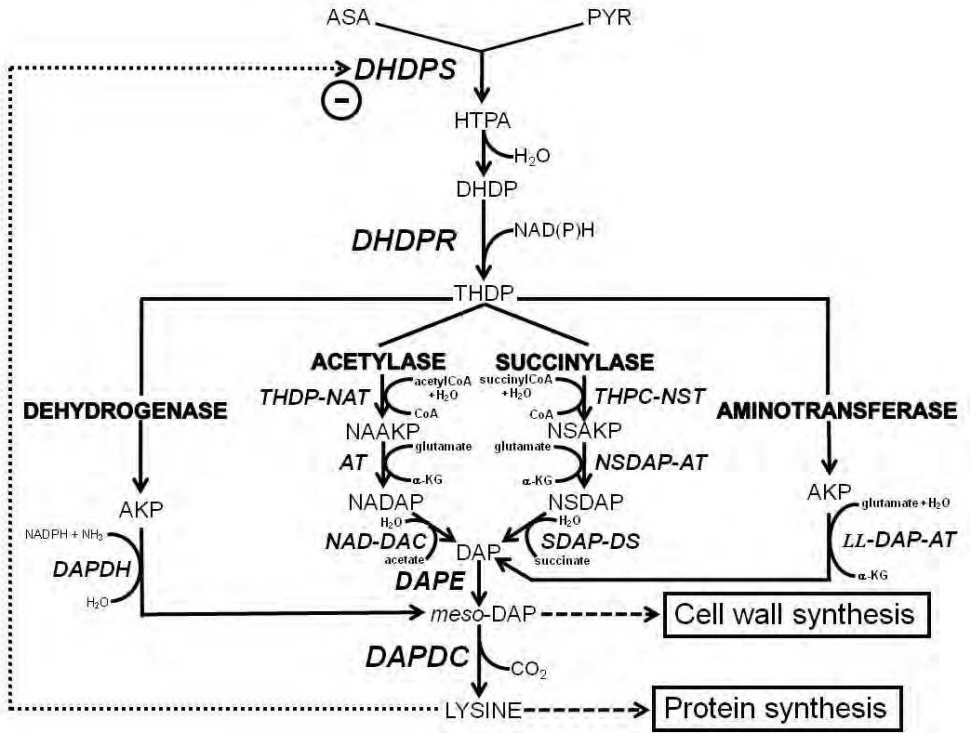


Fig. 1. Diaminopimelate pathway in bacteria.

As for the succinylase pathway, the acetylase pathway involves four enzymatic steps, but incorporates N-acetyl groups rather than N-succinyl moieties. This pathway is common to several *Bacillus* species, including *B. subtilis* and the anthrax-causing pathogen *B. anthracis* (Chatterjee & White., 1982; Peterkofsky & Gilvarg., 1961; Sundharadas & Gilvarg., 1967). The sub-pathway begins with the conversion of THDP to N-acetyl-(*S*)-2-amino-6-ketopimelate (NAAKP) catalysed by *tetrahydrodipicolinate N-acetyltransferase* (THDP-NAT, EC 2.3.1.89), followed by conversion to N-acetyl-(2*S*)-2,6,-diaminopimelate (NADAP) by *aminotransferase A* (ATA, EC 2.6.1). NADAP is subsequently deacetylated to form DAP by the enzyme *N-acetyldiaminopimelate deacetylase* (NAD-DAC, EC 3.5.1.47) (Fig. 1). As in the succinylase pathway, *LL*-DAP is then converted to *meso*-DAP by DAPE (Fig. 1).

There are also two additional sub-pathways that are less common to bacteria. The aminotransferase pathway, catalysed by the enzyme *diaminopimelate aminotransferase* (*LL*-DAP-AT, EC 2.6.1.83), is found in plant, eubacterial and archaeal species (Hudson et al., 2006). This sub-pathway involves the conversion of the acyclic form of THDP, 1,2-amino-6-ketopimelate (AKP), to *meso*-DAP in a single step. *LL*-DAP is then converted in the second step of the sub-pathway to *meso*-DAP by DAPE, as for the acetylase and succinylase pathways (Fig. 1). The dehydrogenase pathway, which is common to *Corynebacterium* and some *Bacillus* species, converts THDP to *meso*-DAP, also in a single step (Misono et al., 1976). This sub-pathway employs the NADPH-dependent enzyme, *diaminopimelate dehydrogenase* (DAPDH, EC 1.4.1.16), which also employs AKP as the substrate (Fig. 1).

All four alternative pathways then converge to utilise the same enzyme for the final step of lysine biosynthesis, namely diaminopimelate decarboxylase (DAPDC, EC 4.1.1.20) (Ray et al., 2002). DAPDC catalyses the decarboxylation of *meso*-DAP to yield lysine and carbon dioxide. This step is important for the overall regulation of the lysine biosynthesis pathway since the downstream product, lysine, has been shown to allosterically inhibit DHDPS from plants and Gram-negative bacteria (Section 2.1.1, Fig. 1). DHDPS is therefore considered the rate-limiting enzyme of the pathway.

This book chapter will describe the function, structure, and regulation of the key enzymes functioning in the lysine biosynthesis pathway. Furthermore, given that several of these enzymes are the products of essential bacterial genes that are not expressed in humans, the pathway is of interest to antibiotic discovery research (Dogovski et al., 2009; Hutton et al., 2007). Accordingly, the chapter will also review the current status of rational drug design initiatives targeting essential enzymes of the lysine biosynthesis pathway in pathogenic bacteria.

2. Dihydrodipicolinate synthase

2.1 Function of DHDPS

Dihydrodipicolinate synthase (DHDPS, EC 4.2.1.52) was first purified in 1965 from *E. coli* extracts (Yugari & Gilvarg, 1965). The enzyme is the product of the *dap*A gene, which has been shown to be essential in several bacterial species (Dogovski et al., 2009; Hutton et al., 2007). The *dap*A product, DHDPS, catalyses the condensation of pyruvate (PYR) and aspartate semialdehyde (ASA) to form 4-hydroxy-2,3,4,5-tetrahydro-*L,L*-dipicolinic acid (HTPA) (Fig. 1). It was first suggested that the product released by DHDPS was dihydrodipicolinate (DHDP), but studies using ^{13}C-labelled pyruvate support the view that the product is the unstable heterocycle HTPA (Blickling et al. 1997a). Rapid decomposition of the ^{13}C-NMR signals of HTPA following its production indicate that formation of DHDP occurs via a nonenzymatic step.

In all cases examined, the DHDPS-catalysed reaction proceeds via a ping-pong kinetic mechanism in which pyruvate binds the active site, resulting in the release of a protonated water molecule. ASA then binds and is condensed with pyruvate to form the heterocyclic product, HTPA (Blickling et al. 1997a).

In the first step of the mechanism, the active site lysine, (Lys161 in *E. coli* DHDPS) forms a Schiff base with pyruvate (Laber et al., 1992) (Fig 2). Formation of the Schiff base proceeds

through a tetrahedral intermediate. It is proposed that a catalytic triad of three residues - Tyr133, Thr44 and Tyr107 (*E. coli* numbering) - act as a proton relay to transfer protons to and from the active site via a water-filled channel leading to bulk solvent (Dobson et al., 2004a). The Schiff base (imine) is converted to its enamine form, which then adds to the aldehyde group of ASA (Blickling et al., 1997a; Dobson et al., 2008). In aqueous solution, ASA is known to exist in the hydrated form rather than the aldehyde, but the biologically-relevant form of the substrate remains to be determined. HTPA is then formed by nucleophilic attack of the amino group of ASA onto the intermediate imine, leading to cyclisation and detachment of the product from the enzyme, with release of the active site lysine residue (Fig 2).

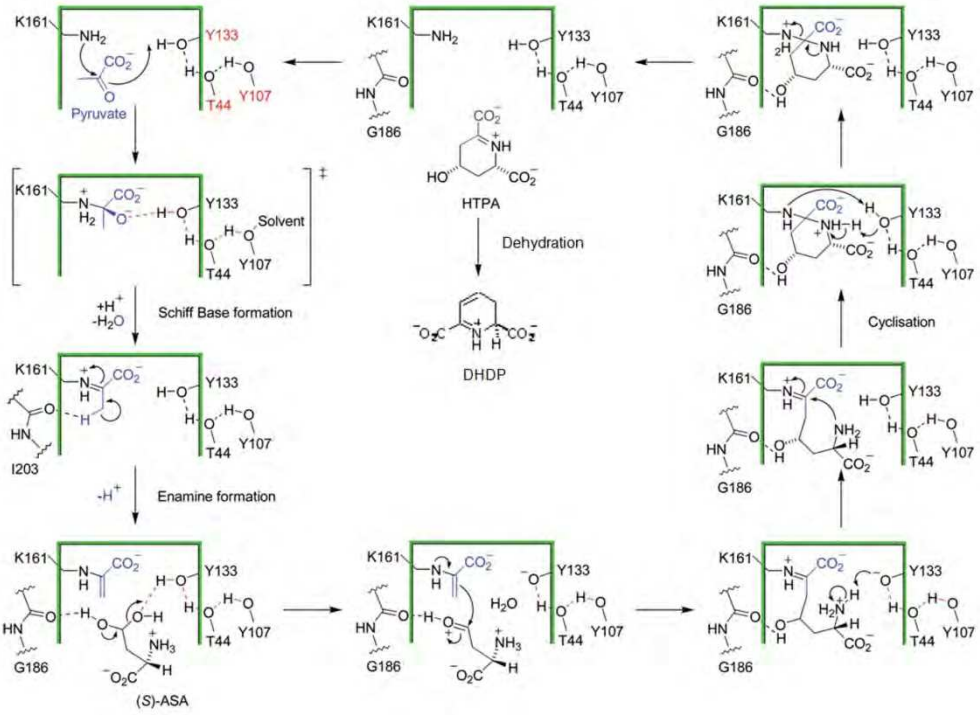


Fig. 2. The catalytic mechanism of DHDPS.

2.1.1 Regulation of DHDPS activity

In some organisms, the activity of DHDPS is regulated allosterically by lysine via a classical feedback inhibition process. Lysine feedback inhibition of DHDPS has been investigated in several plant, Gram-negative and Gram-positive bacterial species to date. Studies involving *Daucus carota sativa* (Matthews et al., 1979), *Pivus sativum* (Dereppe et al., 1992), *Spinacia aloeracea* (Wallsgrove et al., 1980), *Triticum aestivium* (Kumpaisal et al., 1987), and *Zea mays* (Frisch et al., 1991) show that DHDPS from plant species are generally strongly inhibited by

lysine (IC_{50} = 0.01-0.05 mM). In contrast, DHDPS from bacteria are significantly less sensitive to lysine inhibition than their plant counterparts. For example, DHDPS from Gram-negative bacteria, such as *E. coli* (Dobson et al., 2005a; Yugari and Gilvarg, 1965), *Niesseria meningitidis* (Devenish et al., 2009), and *Sinorhizobium. meliloti* (Phenix & Palmer, 2008), display IC_{50} values that range from 0.25 mM to 1.0 mM. Whereas, the enzyme from Gram-positive bacteria such as *Bacillus anthracis* (Domigan et al., 2009), *Bacillus cereus* (Hoganson & Stahly, 1975), *Corynebacterium glutamicum* (Cremer et al., 1988), *Lactobacillus plantarum* (Cahyanto et al., 2006) and *Staphylococcus aureus* (Burgess et al., 2008) show little or no inhibition by lysine.

The crystal structure of DHDPS in complex with lysine from *E. coli* shows that the lysine allosteric binding site is situated in a crevice at the interface of the tight dimer, distal from the active site, but connected to the active site via a water channel (Blickling et al., 1997a). Two inhibitory lysine molecules are bound in close proximity within van der Waals contact to each other. Seven residues located within the allosteric site bind lysine, namely Ala49, His53, His56, Gly78, Asp80, Glu84, and Tyr106 (Blickling et al., 1997a).

Studies show that lysine inhibition is cooperative with the second lysine molecule binding 10^5 times more tightly than the first (Blickling et al., 1997a). The mechanism by which lysine exerts regulatory control over bacterial DHDPS is not well understood, although kinetic and structural studies suggest that it is an allosteric inhibitor, causing partial inhibition (approximately 90%) at saturating concentrations (Blickling et al., 1997a). It has recently been suggested that lysine exerts some effect on the first half reaction by attenuating proton-relay and also the function of Arg138, thought to be crucial for ASA binding (Dobson et al., 2004b). The crystal structure of the *E. coli* DHDPS-lysine complex was solved in the absence of substrate; however, thermodynamic studies have illustrated that the substrate pyruvate has a substantial effect on the nature of enzyme-inhibitor association (Blickling et al., 1997a).

2.2 Structure of DHDPS

2.2.1 Subunit and quaternary structure of DHDPS

DHDPS from *B. anthracis* (Blagova et al., 2006; Voss et al., 2010), *E. coli* (Mirwaldt et al., 1995), *Mycobacterium tuberculosis* (Kefala et al., 2008), *Thermoanaerobacter tengcongensis* (Wolterink-van Loo et al., 2008), *Thermotoga maritima* (Pearce et al., 2006), and several other species is a homotetramer in both crystal structure and solution (Fig. 3). In *E. coli*, the monomer is 292 amino acids in length and is composed of two domains (Mirwaldt et al., 1995). The N-terminal domain is a $(\beta/\alpha)_8$ TIM-barrel (residues 1-224) with the active site located within the centre of the barrel (Fig. 3). The C-terminal domain (residues 225-292) consists of three α-helices and contains several key residues that mediate tetramerisation (Dobson et al., 2005a). The association of the four monomers leaves a large water-filled cavity in the centre of the tetramer, such that each monomer has contacts with two neighbouring monomers only. The tetramer can also be described as a dimer of dimers, with strong interactions between the monomers A & B and C & D at the so-called tight dimer interface, and weaker interactions between the dimers A-B and C-D at the weak dimer interface (Dobson et al., 2005a) (Fig. 3).

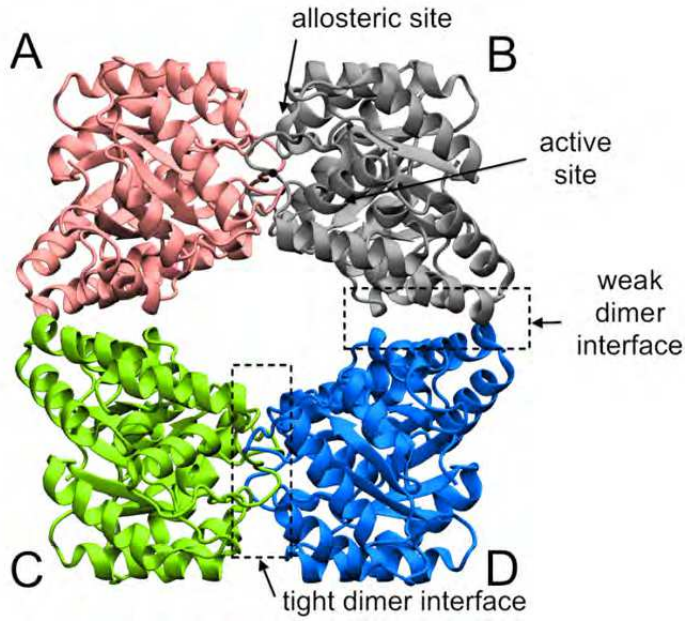


Fig. 3. *E. coli* DHDPS structure . The active sites, allosteric sites, dimerisation interface (tight dimer interface) and tetramerisation interface (weak dimer interface) are shown (PDB: 1YXC).

2.2.2 Active site

The active site is located in cavities formed by the two monomers of the dimer. A long solvent-accessible catalytic crevice with a depth of 10 Å is formed between β-strands 4 and 5 of the barrel (Mirwaldt et al., 1995). Lys161, involved in Schiff-base formation is situated in the β-barrel near the catalytic triad of three residues, namely Tyr133, Thr44 and Tyr107, which act as a proton shuttle (Blickling et al., 1997a) (Fig. 4). Thr44 is hydrogen bonded to both Tyr133 and Tyr107 and its position in the hydrogen-bonding network may play a role in Schiff base formation and cyclisation (Dobson et al., 2005a). The dihedral angles of Tyr107 fall in the disallowed region of the Ramachandran plot, suggesting an important role in the enzyme's function (Mirwaldt et al., 1995). It is believed to be involved in shuttling protons between the active site and solvent (Dobson et al., 2005a). In contrast, Tyr133 plays an important role in substrate binding, donating a proton to the Schiff base hydroxyl. It is also thought to coordinate the attacking amino group of ASA, which requires the loss of a proton subsequent to cyclisation (Fig. 2). A marked reduction in activity is observed in single substitution mutants, highlighting the importance of this catalytic triad (Dobson et al., 2004a).

Situated at the entrance to the active site, Arg138 is essential for ASA binding (Dobson et al., 2005b). In the *E. coli* DHDPS structure, a hydrogen bond is formed between Arg138 and Tyr107 (Dobson et al., 2004a) and a water mediated hydrogen bond is formed between Arg138 and Tyr133 (Dobson et al., 2005a). Arg138 is thus also important for stabilisation of the catalytic triad, both of which are highly conserved in all DHDPS enzymes (Dobson et al., 2005a).

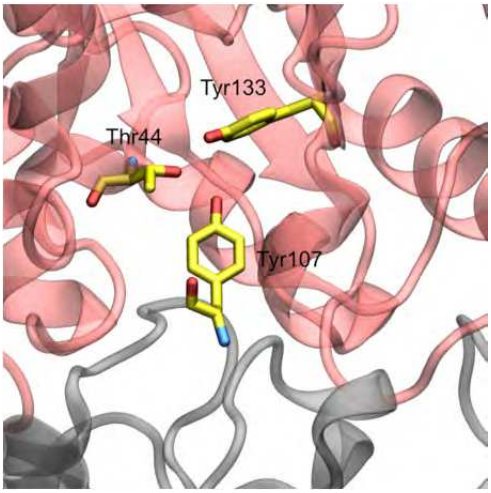


Fig. 4. *E. coli* DHDPS active site, illustrating the catalytic triad Thr44, Tyr133 and Tyr107 interdigitating from the opposing monomer (PDB: 1YXC).

2.2.3 Tight dimer interface

In *E. coli* DHDPS, 1400 Å^2 of surface area from one monomer in each dimer is buried at the tight dimer interface (Dobson et al., 2005a) (Fig. 3). This interface is made up of 25 residues from each monomer, with hydrogen bonds formed between Ser111 and Cys141, and hydrophobic interactions between Leu51 and Ala81, among others. In addition, Tyr107 of one monomer is coordinated with Tyr106 from the adjacent subunit, interdigitating across the monomer-monomer interface and thus forming a stabilising hydrophobic, sandwich-like stacking of aromatic rings.

2.2.4 Weak dimer interface

The tight dimer units of the *E. coli* DHDPS tetramer associate via two isologous interfaces formed between corresponding monomers (Fig. 3). This interface buries approximately 538 Å^2 of surface area. Nine residues from each monomer are involved in contacts at the weak dimer interface (Mirwaldt et al., 1995), situated within the α6, α7 and α9-helices. The interface is stabilised by hydrophobic contacts between Leu167, Thr168 and Leu197 (Dobson et al., 2004a). The importance of Leu197 at the interface has been demonstrated with mutations resulting in a dimeric species, unable to form a tetramer (Griffin et al., 2008, 2010). This interface is not conserved in other DHDPS structures. A greater number of contacts are observed at the weak dimer interface in DHDPS from *B. anthracis* (Blagova et al., 2006; Voss et al, 2010), *M. tuberculosis* (Kefala et al., 2008), and most strikingly, *T. maritima* (Pearce et al., 2006) with 20 residues involved in many interactions.

2.2.5 Allosteric site

As described in Section 2.1.1, lysine is an allosteric modulator of DHDPS function, partially inhibiting DHDPS activity. The lysine binding site is situated in a crevice at the interface of

the tight dimer, distal from the active site, but connected via a water filled channel (Fig. 3). The crystal structure of lysine-bound *E. coli* DHDPS shows two lysine molecules bound per dimer (four per tetramer) with each molecule interacting with both monomers and the adjacent lysine molecule (Blickling et al., 1997c) (Fig. 5).

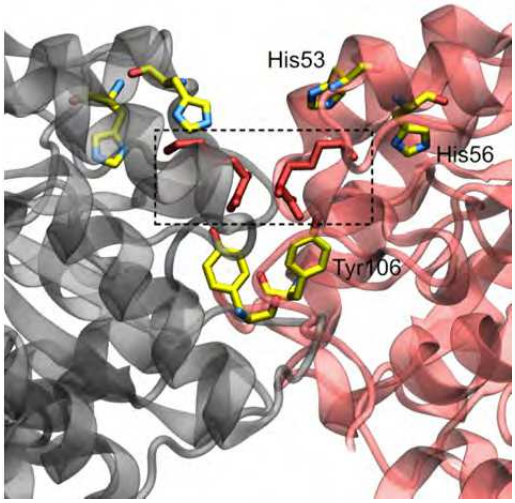


Fig. 5. *E. coli* DHDPS allosteric site with two lysine (boxed - red) molecules bound (PDB: 2ATS).

As stated earlier, seven residues are believed to be involved in binding lysine; Ser48, Ala49, His53, His56, Asn80, Glu84 and Tyr106. All these residues show slightly altered conformations in the presence of lysine, moving to accommodate the molecule (Blickling et al., 1997a). Importantly, Tyr106 moves towards the carboxyl group of lysine, which alters the aromatic stacking of Tyr106 and Tyr107. Otherwise, very few changes are observed upon lysine binding, with no significant secondary structure or quaternary structure change occurring (Dobson et al., 2005a). Most of the residues identified as important in the lysine allosteric binding site are not conserved in those DHDPS enzymes that are not inhibited by lysine (Burgess et al., 2008; Kefala et al., 2008; Voss et al., 2010; Wolterink-van Loo et al., 2008).

2.2.6 Alternative quaternary architecture

Whilst the DHDPS monomer from most bacteria has a molecular mass of approximately 31 kDa, the plant enzymes are larger. For example, DHDPS from *Nicotiana sylvestris* (Blickling et al., 1997b) has a relative molecular mass of 36 kDa, whilst DHDPS from *Pivus sativum* (Dereppe et al., 1992) has been reported to be a homotrimer of 43 kDa monomers based on gel filtration liquid chromatography studies, although this result is uncorroborated. The only plant DHDPS structure solved to date is from *N. sylvestris* (Blickling et al., 1997b). As for the bacterial enzymes, it is a homotetramer, described as a dimer of dimers. The contact areas within the tight dimer are similar within the plant and bacterial enzyme, with 13 of the 19 residues contributing to the interface conserved in both bacteria and plants. However, as Figure 6 shows, the plant dimer of dimers has an alternative architecture, namely the residues involved at the weak dimer interface are located on the opposite face of the monomer. The plant enzyme can thus be described as a "back-to-back" arrangement of dimers

(Fig. 6) compared to the "head-to-head" arrangement observed for bacterial DHDPS (Fig. 3). Compared to the bacterial interface, the weak dimer interface of *N. sylvestris* DHDPS is larger than its bacterial counterpart, burying 810 Å^2 surface area, which is reflected in the greater number of residues contributing to inter-subunit contacts. The additional residues of the C-terminus, as well as the novel quaternary structure of *N. sylvestris* DHDPS, reduces the central water filled cavity and results in a tetramer where all subunits are in contact with each other (Fig. 6). Despite the significant structural differences between the plant and bacterial enzymes, the position and orientation of all active site residues are conserved. Most strikingly, considering the rearrangement of dimers, lysine binds at an equivalent binding pocket at the interfaces of the two monomers of a dimer in both the *E. coli* (Blickling et al., 1997a) and *N. sylvestris* (Blickling et al., 1997b) enzymes. The lysine molecules also bind in the same orientation, with coordination of the α-amino and α-carboxyl groups almost identical.

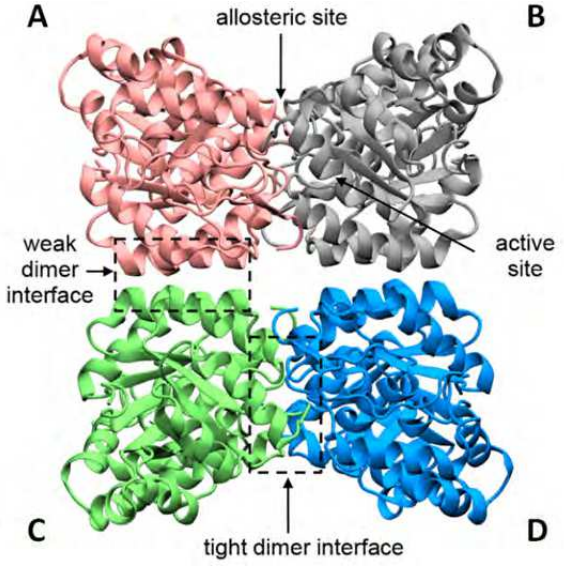


Fig. 6. *N. sylvestris* DHDPS, showing an alternate architecture ("back-to-back" arrangement of dimers) to that observed for bacterial DHDPS enzymes.

In addition, other quaternary structures of bacterial DHDPS enzymes have been reported. For example, DHDPS from methicillin-resistant *S. aureus* (MR*SA*) has recently been shown to be a dimer in solution (Burgess et al., 2008), with enzymatic activity similar to that of the wild-type *E. coli* tetramer. Several DHDPS enzymes have also been identified in *Agrobacterium tumefaciens*, with two forms crystallising as dimers (3B4U, 2R8W) and one as a hexamer (2HMC), although the function of this enzyme has yet to be confirmed.

2.3 Inhibition of DHDPS

A number of potential DHDPS inhibitors have been synthesised and characterised. A variety of heterocyclic analogues of DHDP and HTPA have been shown to act as moderate inhibitors of DHDPS (Hutton et al., 2007). Analogues of the cyclic lactol form of ASA, including homoserine lactone and 2-aminocyclopentanone, show non-competitive moderate

inhibition with K_i = 12-24 mM (Hutton et al., 2007). Analogues of the straight chain hydrate form of ASA have also been investigated, with aspartic acid showing mixed type inhibition with K_i = 90-140 μM (Hutton et al., 2007). Product analogues have also been investigated, exhibiting moderate DHDPS inhibition. More success was achieved with inhibitors based on the acyclic enzyme-bound DHDPS intermediates, such as diethyl (*E,E*)-4-oxo-2,5-heptadienedioate (Turner et al., 2005) and a bis-oxime ester (Boughton et al., 2008), which irreversibly inhibit DHDPS. Interestingly, several of these compounds have displayed clear differentiation in inhibition of DHDPS enzymes from different species (Mitsakos et al., 2008), suggesting the potential for targeting compounds to specific pathogens.

3. Dihydrodipicolinate reductase

3.1 Function of DHDPR

Dihydrodipicolinate reductase (DHDPR, EC 1.3.1.26) was first isolated from *E. coli* in 1965 (Farkas & Gilvarg, 1965). Since then, the enzyme has been characterised from several species including *B. cereus* (Kimura & Goto, 1977), *Bacillus megaterium* (Kimura & Goto, 1977), *Bacillus subtilis* (Kimura, 1975), *C. glutamicum* (Cremer et al., 1988), *Methylophilus methylotrophus* (Gunji et al., 2004), *M. tuberculosis* (Cirilli et al., 2003), *S. aureus* (Dommaraju et al., 2011; Girish et al., 2011), and *T. maritima* (Pearce et al., 2008). DHDPR catalyses the second step in the lysine biosynthesis pathway (Fig. 1), the pyridine nucleotide-dependent reduction of dihydrodipicolinate (DHDP) to form *L*-2,3,4,5,-tetrahydrodipicolinate (THDP) (Dogovski et al., 2009; Hutton et al., 2007).

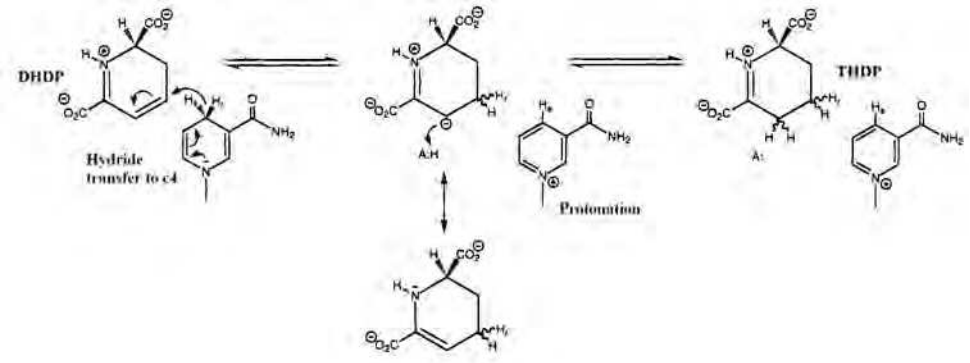


Fig. 7. Schematic representation of the catalytic mechanism of DHDPR.

In *E. coli*, DHDPR is encoded by the *dap*B gene, which is also an essential bacterial gene (Dogovski et al., 2009; Hutton et al., 2007). The open reading frame encodes a 273 amino acid polypeptide with a monomeric molecular weight of 28,758 Da. The enzyme functions by utilising either phosphorylated or non-phosphorylated pyridine nucleotides, NAD(P)H, as hydrogen donors to carry out its reaction. The kinetic mechanism of *E. coli* DHDPR is ordered and sequential (Reddy et al., 1995), involving binding of NAD(P)H followed by DHDP. The reaction is initiated by hydride transfer from the 4-pro-*R* position of NAD(P)H to the C4-position of DHDP, with the resultant enamine then undergoing tautomerisation to form THDP. Upon completion of the reaction, the release of the product THDP is followed by $NAD(P)^+$ release (Reddy et al., 1995) (Fig. 7).

3.1.2 Nucleotide preference of bacterial DHDPR

Pyridine nucleotide-dependent dehydrogenases typically have a strong preference for either NADPH or NADH as co-factors (Cirilli et al., 2003; Pearce et al., 2008; Reddy et al., 1996). In most cases dual-cofactor enzymes preferentially utilise NADPH over NADH. In light of this observation, there has been significant interest in studying the molecular basis of nucleotide preference. All NAD-dependent dehydrogenases contain the consensus sequence GXGXXG or GXXGXXG and conserved acidic amino acids 20-30 residues downstream of this glycine rich region (Dommaraju et al., 2011). The main chain nitrogen of the second residue (X) in the consensus sequence interacts with this conserved acidic residue. *E. coli* DHDPR has an unusual pyridine nucleotide specificity, exhibiting only a modest selectivity for its nucleotides. Kinetic studies show that *E. coli* DHDPR utilises NADH only slightly more efficiently than NADPH (Reddy et al., 1996). This is consistent with the observation that the binding affinity of *E. coli* DHDPR to NADH (K_D = 0.26 μM) is stronger than that of NADPH (K_D = 1.8 μM) (Reddy et al., 1996). Structural studies of *E. coli* DHDPR show the existence of hydrogen bonds between the side-chain of the acidic residue Glu38 and that of the O3′ of the adenine ribose of NADH. It is hypothesised that the basic residue Arg39, also found in the nucleotide binding pocket, can interact with the negatively charged 2′ phosphate of NADPH, thus enabling the enzyme to utilise both NADH and NADPH. Kinetic analysis of DHDPR from *M. tuberculosis* also shows that the enzyme exhibits only a moderate preference for NADH. The crystal structures of *M. tuberculosis* DHDPR in two ternary complexes (DHDPR-2,6-PDC-NADH and DHDPR-2,6-PDC-NADPH) demonstrate that the number of hydrogen bonds between DHDPR and the nucleotides NADH and NADPH are very similar (Cirilli et al., 2003; Reddy et al., 1996; Scapin et al., 1997).

3.2 Structure of DHDPR

3.2.1 Subunit and quaternary structure of DHDPR

The three-dimensional structure of DHDPR has been elucidated by X-ray crystallography from five diverse bacterial species, namely, *Bartonella henselae*, (PDB: 3IJP), *E. coli* (Scapin et al., 1995, 1997), *M. tuberculosis* (Cirilli et al., 2003), *S. aureus* (Girish et al., 2011), and *T. maritima* (Pearce et al., 2008). DHDPR from *E. coli* (Fig. 8) was the first DHDPR enzyme to be extensively studied in terms of structure and function (Farkas & Gilvarg, 1965; Reddy et al., 1995; Scapin et al., 1995, 1997).

DHDPR is a tetrameric enzyme consisting of four identical monomers (Fig. 8). Each monomer is comprised of an N-terminal nucleotide binding domain and a C-terminal substrate binding domain (Fig. 9). In *E. coli* DHDPR, the nucleotide binding domain is formed by the first 130 and last 36 residues of the polypeptide chain, whereas the substrate binding domain is formed by residues 130-240. The nucleotide binding domain consists of four α-helices and seven β-strands, which are arranged to form a Rossmann (dinucleotide binding) fold. The substrate binding domain contains two α-helices and four β-strands, which form an open mixed β-sandwich (Scapin et al., 1995). Interactions between the four subunits of the tetramer occur exclusively between residues of the substrate binding domain. A long loop (Leu182 to Gly204) also extends from the substrate binding domain and plays an important role in maintaining the quaternary structure of the enzyme. The four monomers interact by pairing the four β-strands on the substrate binding domain to form a 16-stranded, mixed, flattened β-barrel (Fig. 8). This central barrel is anchored by the four long loops (Leu182 to Gly204) that extend from

the body of the substrate binding domain of each monomer and wrap around the mixed β-sheet of the neighboring monomer. Residues 65-74 and 127-130 form flexible hinge regions between the nucleotide and substrate binding domains (Scapin et al., 1995).

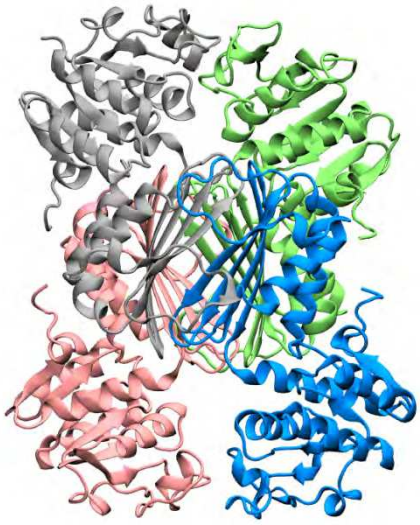


Fig. 8. Structure of *E. coli* DHDPR (PDB: 1ARZ).

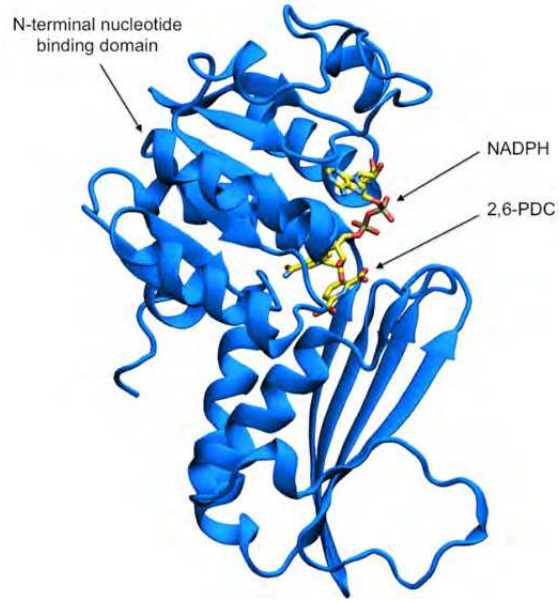


Fig. 9. Structure of the *E. coli* DHDPR monomer bound to NADH and the substrate analogue, 2,6-PDC (PDB: 1ARZ).

3.2.2 Substrate binding site

The consensus sequence, E(L/A)HHXXKXDAPSGTA is found in the substrate binding domain of all known bacterial DHDPR enzymes (Pavelka et al., 1997). This sequence is thought to contain residues involved in binding of substrate and/or catalysis. Molecular modelling studies, using the apo form (enzyme in the absence of substrate) of *E. coli* DHDPR as a structural template, suggest a cluster of five basic residues are the key catalytic site residues (Scapin et al., 1997), namely His159, His160, Arg161, His162 and Lys163 (all contained within the consensus sequence). These residues are located in the loop connecting β-strand B7 to α-helix A5. Structural studies of *E. coli* DHDPR in complex with NADH and the substrate analogue and inhibitor, 2,6-pyridinedicarboxylate (2,6-PDC), show that 2,6-PDC is bound to the substrate binding domain of DHDPR, in a spherical cavity bordered by residues from both the nucleotide binding (Gly102-Phe106 and Ala126-Ser130) and substrate binding domains (Ile155-Gly175 and Val217-His220) (Scapin et al., 1997). The bound inhibitor makes several hydrogen bonding interactions with the atoms of the conserved E(L/A)HHXXKXDAPSGTA motif. Similar interactions are observed between 2,6-PDC and DHDPR from *M. tuberculosis* (Cirilli et al., 2003).

3.2.3 Nucleotide binding site

The nucleotide binding domain of DHDPR adopts a Rossmann fold, which is typical of nucleotide-dependent dehydrogenases (Fig. 9). The consensus sequence (V/I)(A/G)(V/I)-XGXXGXXG located within this domain, is conserved in all NAD(P)H-dependent dehydrogenases, including DHDPR (Pavelka et al., 1997). Structural analyses of *E. coli* DHDPR show that this motif extends from the C-terminal end of β-strand B1 to the loop that connects B1 to α-helix A1. An acidic residue (Glu38 in *E. coli* DHDPR) is located approximately 20 amino acids downstream of the conserved consensus sequence. The two hydroxyl groups from the adenine ribose are known to interact with the side-chain of Glu38 and also the backbone atoms of the glycine rich motif GXXGXXG. Several hydrophobic interactions exist between the adenine ring of NADH and the residues Arg39, Gly84 and His88. The pyrophosphate group of NADH is located over the α-helix A1 and interacts with residues contained within the loop connecting β-strand B1 and α-helix A1 (Reddy et al., 1996; Scapin et al., 1997).

3.3 Inhibition of DHDPR

The substrate analogue, 2,6-PDC, is a competitive inhibitor (K_i = 26 μM) of DHDPR (Scapin et al., 1995) (Fig. 10A). Other substrate analogues such as picolinic acid (Fig. 10B), isopthalic acid (Fig. 10C), pipecolic acid (Fig. 10D) and dimethyl chelidamate (Fig. 10E), are much weaker inhibitors, each displaying an IC_{50} > 10 mM (Hutton et al., 2003). A vinylogous amide that acts as a competitive inhibitor of DHDPR (K_i = 32 μM) has been described and is one of the most potent inhibitors of DHDPR reported to date (Caplan et al., 2000). Molecular modeling in tandem with conventional drug screening strategies has identified novel inhibitors, including sulfones and sulfonamides, with K_i values ranging from 7-90 μM (Caplan et al., 2000). However, a sub-micromolar inhibitor of DHDPR has not been discovered to date.

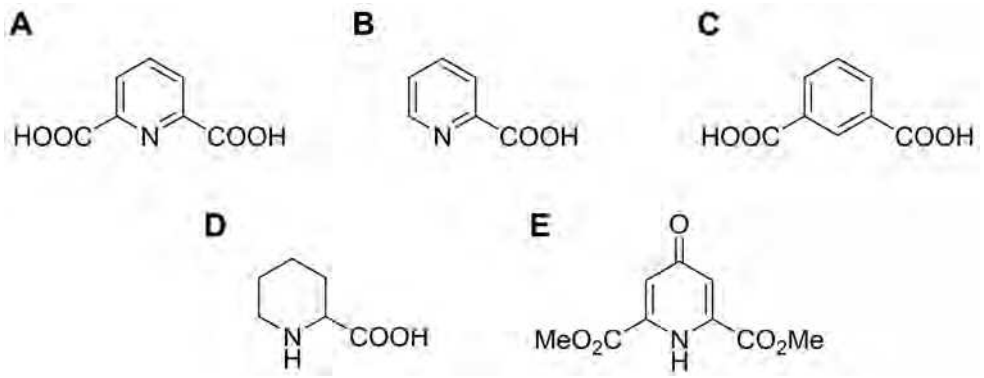


Fig. 10. Inhibitors of DHDPR.

4. Succinylase pathway

4.1 Tetrahydrodipicolinate N-succinyltransferase

Tetrahydrodipicolinate N-succinyltransferase (THPC-NST, EC 2.3.1.117) is a succinyl-coenzyme A (SCoA) dependant enzyme that catalyses the conversion of cyclic *L*-2,3,4,5,-tetrahydrodipicolinate (THDP) to acyclic N-succinyl-*L*-2-amino-6-ketopimelate (NSAKP) (Simms et al., 1984) (Fig. 1). The reaction occurs via a *L*-2-amino-6-ketopimelate (AKP) intermediate. The transfer of an acyl group functions to maintain a linear conformation of the product of the reaction (NSKAP) and exposes the 6-keto group for subsequent transamination (Beaman et al., 2002). Substrate and cofactor kinetic parameters for *E. coli* THPC-NST have been determined. Studies show that the K_M^{app} for THDP and succinyl-CoA are 20 μM and 15 μM, respectively (Berges et al., 1986b; Simms et al., 1984).

The *dap*D gene encoding THPC-NST is found in a large number of bacterial species including *E. coli* and *Mycobacterium* species (Beaman et al., 1997; Richaud et al., 1984; Schuldt et al., 2009). Expression of this gene in *E. coli* is weakly inhibited by lysine (Ou et al., 2008; Richaud et al., 1984). THPC-NST enzymes characterised to date are comprised of approximately 290 residues and show greater than 18% sequence identity (Beaman et al., 1997; Richaud et al., 1984; Schuldt et al., 2009).

The crystal structure of THPC-NST from *Mycobacterium bovis* (Fig. 11) shows that the enzyme forms a homotrimer. The monomer consists of three domains, namely, the (i) N-terminal, (ii) left handed parallel β-helix (LβH), and (iii) C-terminal domains (Beaman et al., 1997). The N-terminal domain is comprised of four α-helices and two hairpin loops. The LβH domain, comprising 50% of the subunit, contains the hexapeptide repeat motif ([LIV]-[GAED]-X_2-[STAV]-X) within each turn of the β-helix. The LβH domain is interrupted by two loops, including a flexible loop (residues 166-175) that is involved in binding substrate. The C-terminal domain consists of a β-stranded structure. All three domains contribute to inter-subunit contacts. The structure of THPC-NST from other bacterial species have since been determined and show a high degree of similarity to that of *M. bovis* THPC-NST (Nguyen et al., 2008; Schuldt et al., 2009).

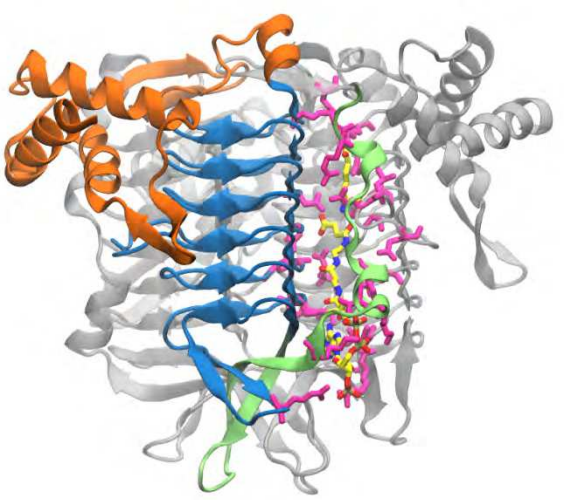


Fig. 11. Structure of trimeric *M. bovis* THPC-NST in complex with *L*-2-aminopimelate and succinamide-CoA. The N-terminal (orange), LβH (blue) and C-terminal (green) domains are indicated. The substrate *L*-2-aminopimelate (yellow) and cofactor succinamide-CoA (yellow) are bound via the THPC-NST active site residues (pink) (PDB: 1KGQ).

Crystal structures of *M. bovis* THPC-NST in complex with substrate analogs and several forms of coenzyme A have resulted in a model describing substrate binding and catalysis (Beaman et al., 1998, 2002). Self-association of the monomer subunit results in a homotrimer complex containing three active sites. The AKP and SCoA binding sites are located at the LβH domain interfaces. Binding of SCoA and possibly AKP is thought to promote a large conformational change that encloses the bound substrate and cofactor within the active site. In this state, the 2-amino group of AKP is placed in close proximity to the SCoA thioester, allowing nucleophilic attack and transfer of the succinyl group (Beaman et al., 2002).

Studies have shown that *L*-2-aminopimelic acid, an analog of AKP, is an inhibitor of THPC-NST, although it does not display antibacterial activity (Berges et al., 1986a). However, peptide derivatives of 2-aminopimelic acid show significant antibacterial activity against a range of Gram-negative bacteria (Berges et al., 1986a).

4.2 N-succinyldiaminopimelate aminotransferase

N-succinyldiaminopimelate aminotransferase (NSDAP-AT, EC 2.6.1.17) catalyses the conversion of NSKAP to N-succinyl-*L,L*-2,6,-diaminopimelate (NSDAP) (Fig. 1). The reaction begins by the formation of a Schiff base linkage between an active site lysine and the cofactor pyridoxal-5′-phosphate (PLP). An amino group, donated by glutamate, is transferred to PLP, to form pyridoxamine phosphate (PMP). The enzyme subsequently transfers the amino group from PMP to NSAKP to yield N-succinyl-*L,L*-2,6,-diaminopimelate (NSDAP) and α-ketoglutarate (Peterkofsky & Gilvarg., 1961; Ledwidge & Blanchard., 1999). Studies of *E. coli* NSDAP-AT report K_M values for the substrates NSKAP and glutamate of 0.5 mM and 0.52 mM, respectively (Peterkofsky & Gilvarg., 1961).

The gene encoding NSDAP-AT (*dapC*), is found in a large number of bacterial species including *Bordetella pertussis* (Fuchs et al., 2000), *C. glutamicum* (Hartmann et al., 2003), *E. coli,* (Peterkofsky & Gilvarg., 1961) and *M. tuberculosis* (Weyand et al., 2006). In *E. coli,* the gene encoding NSDAP-AT is annotated *argD* (Ledwidge & Blanchard., 1999). This enzyme also functions as a N-acetylornithine aminotransferase, a component of the arginine biosynthesis pathway. The *dapC* gene in *B. pertussis* (Fuchs et al., 2000), *C. glutamicum,* (Hartmann et al., 2003), and *E. coli* (Bukari & Taylor., 1971) has been found to map in close proximity to the *dapD* gene on the chromosome. Sequence analyses have shown that NSDAP-AT consists of approximately 400 residues and shares greater than 26% identity across species (Fuchs et al., 2000; Hartmann et al., 2003; Peterkofsky & Gilvarg., 1961; Weyand et al., 2006). The NSDAP-AT sequence is characterised by the presence of the PLP binding sequence motif, SLSKXSNVXGXRAG, that includes an active site lysine residue (underlined) (Fuchs et al., 2000).

Structure studies of *M. tuberculosis* NSDAP-AT in complex with PLP shows that the enzyme forms a homodimer (Fig. 12). The structure is characteristic of the aminotransferase family of class I PLP-binding proteins (Weyand et al., 2007). The monomer subunit is comprised of (i) an α-helical N-terminal extension, (ii) a central domain comprising an 8-stranded β-sheet surrounded by 8 α-helices, and (iii) a C-terminal domain consisting of a four stranded β-sheet flanked by 4 α-helices. The active site of each subunit is located at the dimer interface with residues from both subunits contributing to the architecture of the active sites. PLP is bound to the active site Lys232, presumably via a Schiff base, and makes a number of noncovalent contacts with other residues within the active site via a hydrogen bond network.

A number of hydrazino-dipeptide analogs of NSDAP inhibit NSDAP-AT with K_i values ranging from 22-556 nM and show significant antibacterial activity against *E. coli* (Cox et al., 1998).

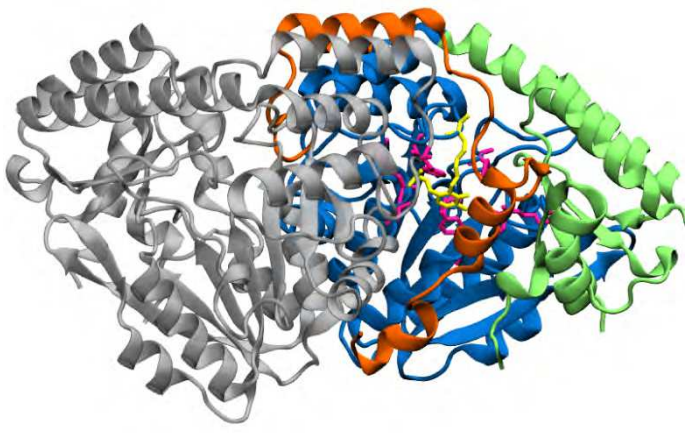


Fig. 12. Structure of dimeric *M. tuberculosis* NSDAP-AT in complex with PLP. The α-helical N-terminal extension (orange), central (blue) and C-terminal (green) domains are indicated. The cofactor PLP (yellow) is bound by the NSDAP-AT active site residues (pink) (PDB: 2O0R).

4.3 Succinyldiaminopimelate desuccinylase

Succinyldiaminopimelate desuccinylase (SDAP-DS, EC 3.5.1.18) catalyses the hydrolysis of N-succinyl-*L,L*-2,6,-diaminopimelate (NSDAP) to yield *L,L*-2,6-diaminopimelate (DAP) and succinate (Kindler & Gilvarg., 1960) (Fig. 1). Kinetic parameters for SDAP-DS from several bacterial species have been reported, with substrate K_M and k_{cat} values ranging from 0.73 - 1.3 mM and 140 - 200 s^{-1}, respectively (Bienvenue et al., 2003; Born et al., 1998; Lin et al., 1988).

The gene encoding SDAP-DS, *dap*E, is present in a large number of bacterial species including, *C. glutamicum* (Wehrmann et al., 1994), *E. coli* (Bouvier et al., 1992), *Haemophilus influenzae,* (Born et al., 1998) and *Salmonella enterica* (Broder & Miller., 2003). In general, SDAP-DS contains approximately 375 residues and shares greater than 22% sequence identity across bacterial species. Alignment of SDAP-DS amino acid sequences show conservation of histidine and glutamate metal binding residues that are characteristic of metal-dependent amidases (Born et al., 1998).

Consistent with the conservation of metal binding residues, the activity of SDAP-DS enzymes are dependent on Zn^{2+} ions (Born et al., 1998; Lin *et al*., 1988). Futhermore, studies involving Zn K-edge extended X-ray absorption fine structure (EXAFS) analyses of *H. influenzae* SDAP-DS indicate that the enzyme contains dinuclear Zn^{2+} active sites (Cosper et al., 2003). Studies of *H. influenzae* SDAP-DS mutants by kinetics, electronic absorption spectroscopy and electron paramagnetic resonance spectroscopy showed that His67 and His349 coordinate Zn^{2+} ions, with His67 functioning in catalysis (Gillner et al., 2009). A similar study showed that residue Glu134 is also involved in catalysis, possibly functioning as an acid/base (Davis et al., 2006).

The crystal structure of zinc bound SDAP-DS has been determined (Fig. 13). Studies have shown that the enzyme forms a homodimer, with each monomer subunit containing a catalytic domain and a dimerisation domain (Nocek et al., 2010). The core of the catalytic domain is composed of an eight-stranded twisted β-sheet that is sandwiched between seven α-helices. The dimerisation domain adopts a two layer α+β sandwich fold and is comprised of a four stranded antiparallel β-sheet and two α-helices.

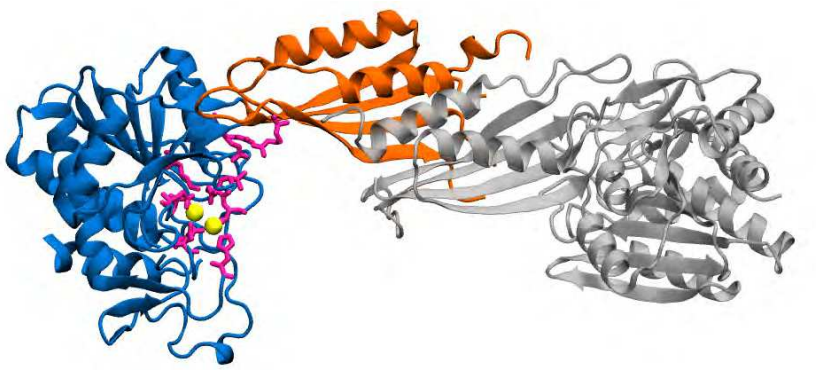


Fig. 13. Structure of dimeric *H. influenzae* SDAP-DS in complex with two zinc ions. the dimerisation (orange) and catalytic (blue) domains are indicated. Zinc ions (yellow) are bound by SDAP-DS active site residues (pink) (PDB: 3IC1).

The catalytic domain incorporates a negatively charged active site cleft, containing two zinc ions. One zinc ion is coordinated by the imidazole group and sidechain oxygens of His67 and Glu163, respectively, whilst another zinc ion is coordinated in a similar manner by His349 and Glu135. The zinc ions are bridged together by interaction with Asp100 and a water/hydroxide.

The availability of a structural model has resulted in a proposed mechanism for hydrolysis of NSDAP by SDAP-DS (Born et al., 1998; Nocek et al., 2010). It is hypothesised that NSDAP adopts an extended conformation when bound to the active site of the enzyme. The NSDAP amide carbonyl coordinates to an active site Zn^{2+} ion and becomes avaliable for nucleophilic attack. This binding event displaces a bridging water molecule, resulting in its hydrolysis by Glu134 and the generation of a zinc bound nucleophilic hydroxide. The hydroxide then attacks the target carbonyl carbon to form a η-1-μ-transition-state complex, which then resolves to release DAP and succinate.

The DAP isomers *L,L*-DAP and *D,L*-DAP are competitive inhibitors of *H. influenzae* SDAP-DS, exhibiting K_i values of 8 and 12 mM, respectively (Born et al., 1998). Studies employing Zn K-edge EXAFS suggest that the *H. influenzae* SDAP-DS inhibitor, 5-mercaptopentanoic acid, may exert its effect through binding to active site Zn^{2+} ions (Cosper et al., 2003).

5. Acetylase pathway

5.1 Tetrahydrodipicolinate N-acetyltransferase

Tetrahydrodipicolinate N-acetyltransferase (THDP-NAT, EC 2.3.1.89) is an acetyl-coenzyme A (ACoA) dependant enzyme that catalyses the conversion of cyclic THDP to acyclic N-acetyl-(*S*)-2-amino-6-ketopimelate (NAAKP) (Chatterjee & White., 1982) (Fig. 1). The transferred acyl group maintains the linear conformation of the product and exposes the 6-keto group for subsequent transamination (Beaman et al., 2002). Crude cell extracts from *B. megaterium* were found to contain active THDP-NAT (Chatterjee & White., 1982). THDP-NAT enzymes are thought to be largely confined to *Bacillus sp.* (Weinberger & Gilvarg., 1970).

5.2 Aminotransferase A

Aminotransferase A (ATA, EC 2.6.1) is a PLP-dependant enzyme that catalyses the conversion of NAAKP and glutamate to N-acetyl-(2*S*)-2,6,-diaminopimelate (NADAP) and α-ketoglutarate (Ledwidge & Blanchard., 1999; Peterkofsky & Gilvarg., 1961) (Fig. 1). It has been speculated that the ATA reaction mechanism resembles that of NSDAP-AT (Section 4.2) (Ledwidge & Blanchard., 1999; Peterkofsky & Gilvarg., 1961). Crude cell extracts from *B. megaterium* were found to contain active ATA (Chatterjee & White., 1982), with ATA activity identified by monitoring enzyme activity in the reverse direction utilising an acid ninhydrin assay (Chatterjee & White., 1982; Sundharadas & Gilvarg., 1967).

5.3 N-acetyldiaminopimelate deacetylase

N-acetyldiaminopimelate deacetylase (NAD-DAC, EC 3.5.1.47) catalyses the hydrolysis of NADAP to form DAP and acetate (Fig. 1). NAD-DAC was first identified from studies involving the isolation of a *B. megaterium* DAP auxotroph (Saleh & White., 1979; Sundharadas & Gilvarg., 1967). The mutant strain possesses a non-functional form of NAD-

DAC and consequently accumulates NADAP. Early studies of this enzyme centred on Gram-positive species, with NAD-DAC activity identified by utilising an acid ninhydrin assay to detect NADAP formation in crude cell extracts (Chatterjee & White., 1982; Weinberger & Gilvarg., 1970). The distribution of NAD-DAC has since been investigated in large number of Gram-negative and Gram-positive bacteria. Interestingly, the enzyme appears to be restricted to *Bacillus sp*. (Weinberger & Gilvarg., 1970).

6. Aminotransferase pathway

6.1 Function of diaminopimelate aminotransferase

Diaminopimelate aminotransferase (*LL*-DAP-AT, EC 2.6.1.83) is a PLP-dependant enzyme that catalyses the conversion of *L*-2,3,4,5,-tetrahydrodipicolinate (THDP) to *L,L*-2,6-diaminopimelate (*LL*-DAP) (Fig. 1). This transamination reaction utilises glutamate as an amino donor to yield α-ketoglutarate. (Hudson et al., 2006, 2008; Liu et al., 2010; McCoy et al., 2006)

The enzyme was first isolated from plant and cyanobacterial species and thus demonstrated a new branch of the lysine biosynthesis pathway existed (Hudson et al., 2006). Although plants are known to synthesise lysine *de novo*, components of the pathway required for conversion of THDP to *meso*-DAP had not been identified previously despite years of investigation. Studies of crude cell extracts had shown that plants do not catalyse reactions specific to the succinylase, acetylase or dehydrogenase branches of the pathway. This was subsequently confirmed with the observation that annotated plant genomes, including that from *Arabidopsis thaliana*, lack some or all genes associated with the three classical branches (Chatterjee et al., 1994; Hudson et al., 2005). The identification and characterisation of *LL*-DAP-AT from *A. thaliana* demonstrated for the first time the means by which plant species catalyse the conversion of THDP to *meso*-DAP via the aminotransferase sub-pathway (Hudson et al., 2006).

More recently *LL*-DAP-AT has been identified in algal, archaeal and bacterial species including, *Chlamydia trachomatis* (McCoy et al., 2006), *Chlamydomonas reihardtii* (Hudson et al., 2011), *Methanocaldococcus jannaschii* (Liu et al., 2010), and *Protochlamydia amoebophila* (McCoy et al., 2006). Comparative genomic analyses shows that *LL*-DAP-AT is restricted to the eubacterial lineages, *Bacteroidetes*, *Chlamydiae*, *Chloroflexi*, *Cyanobacteria*, *Desulfuromonadales*, *Firmicutes*, and *Spirochaeta*; and the archaea, *Archaeoglobaceae* and *Methanobacteriaceae* (Hudson et al., 2008). The phylogeny of *LL*-DAP-AT from these species has established the existence of two classes of *LL*-DAP-AT orthologues, namely, DapL1 and DapL2, which differ significantly in primary amino acid sequence. DapL1 and DapL2 are found predominantly in eubacteria and archaea, respectively (Hudson et al., 2008).

LL-DAP-AT enzymes are classified as members of the PLP-dependant protein superfamily of class I/II aminotransferases (Hudson et al., 2008; Jensen et al., 1996; Sung et al., 1991). Orthologues are in general 410 amino acids in length and can share as little as 29% sequence identity. Kinetic parameters for the *LL*-DAP-AT reaction have been determined for enzymes from a number of species, including *A. thaliana, C. trachomatis, Desulfitobacterium hafniense, Leptospira interrogans, Methanobacterium thermoautotrophicus, Morella thermoacetica,* and *P. amoebophila.* (Hudson et al., 2006, 2008; McCoy et al., 2006). In the human pathogen *C.*

trachomatis, the K_M values for the substrates THDP and glutamate have been reported as 19 μM and 2.1 μM, respectively (Hudson et al., 2008).

6.2 Structure of *LL*-DAP-AT

At present, the PDB reports twelve *LL*-DAP-AT X-ray crystal structures from three species, namely, *A. thaliana, C. trachomatis and C. reihardtii* (Watanabe et al., 2007, 2008, 2011; Dobson et al., 2011). The tertiary and quaternary structure of all three proteins are very similar with *LL*-DAP-AT existing as a homodimer (Fig. 14).

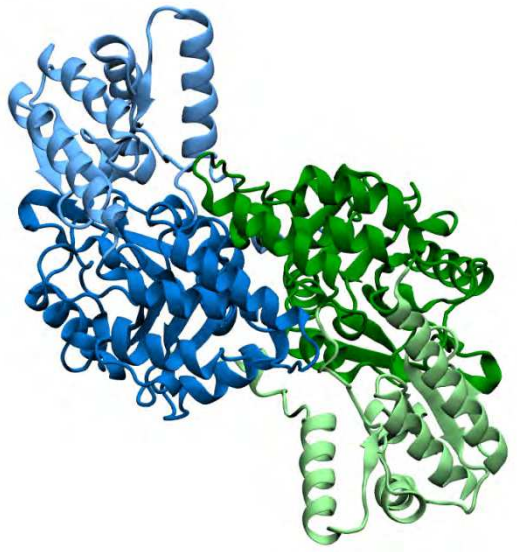


Fig. 14. Structure of dimeric *C. trachomatis LL*-DAP-AT. Monomers, indicated in blue and green, associate to form a functional dimer (PDB: 3ASA).

The subunit structure of *C. trachomatis LL*-DAP-AT is described as containing two domains, a large domain (LD) (residues 48-294) and a small domain (SD) (residues 1-47 and 295-394) (Watanabe et al., 2011; Watanabe & James, 2011). The LD is composed of α-β-α sandwich, whilst the SD assumes an α-β complex (Fig. 14). The LD is involved in binding PLP and also dimer formation, whereas the SD forms an N-terminal arm and also the C-terminal region. The active site is situated in a groove between the two domains of the monomer (Fig. 14). Importantly, the dimer structure is proposed to be essential for function as both subunits participate in substrate binding. Study of the structures of apo and ligand-bound forms of *C. trachomatis LL*-DAP-AT have revealed that the enzyme adopts an open and closed conformation (Watanabe et al., 2011). In the absence of ligand, the enzyme assumes an open state, whereby the active site is exposed to solvent. Upon PLP binding, the enzyme adopts a closed conformation. Within the active site, PLP is covalently linked to Lys236 via a Schiff base and is stabilised through an aromatic stacking interaction with Tyr128. PLP also forms a network of hydrogen bonding interactions with residues within the enzyme active site (Watanabe et al., 2011) (Fig 15).

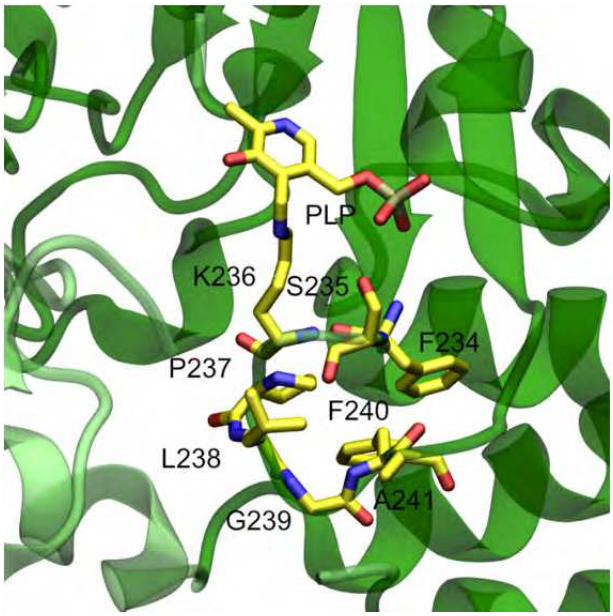


Fig. 15. Catalytic site of *LL*-DAP-AT from *C. trachomatis* (PDB: 3ASA). Ligand binding induces a closed conformation. PLP is covalently linked to Lys236 *via* a Schiff base.

6.3 Inhibition of *LL*-DAP-AT

A number of potential *LL*-DAP-AT inhibitors have been synthesised and characterised. In a screen involving 29,201 molecules, 15 compounds displayed IC_{50} values ranging from 20 μM to 60 μM, with the best hit being an aryl hydrazide showing an IC_{50} of 5 μM (Fan et al., 2010). However, the best hit appears to be an uncompetitive inhibitor and probably reacts irreversibly with PLP. Analogues of this compound have been synthesised and studies show that they fail to effectively inhibit *LL*-DAP-AT. In addition, there are two rhodanine-based molecules reported that show IC_{50} values of 41 μM and 46 μM (Fan et al., 2010).

7. Diaminopimelate epimerase

7.1 Function of DAPE

Diaminopimelate epimerase (DAPE, EC 5.1.1.7) catalyses the penultimate step in the lysine biosynthetic pathway whereby *L,L*-2,6-diaminopimelate (*LL*-DAP) is converted to *meso*-DAP (Fig. 1, Fig. 16). In *E. coli*, the enzyme is encoded by the *dap*F gene and is constitutively expressed (Neidhardt & Curtiss, 1996). DAPE was first characterised in 1957 using enzyme derived from crude extracts of *E. coli* (Work, 1957). The enzyme specifically recognises the *LL*-DAP isomer (Anita et al., 1957), whereas the *DD*-DAP isomer is not a substrate or inhibitor of the enzyme. Early studies noted that DAPE was inhibited by low concentrations of thiol-binding reagents and could be reactivated by reducing agents, suggesting the presence of an essential sulfhydryl group (Work, 1957). This finding was subsequently confirmed upon purification of DAPE to homogeneity (Wiseman, & Nichols, 1984).

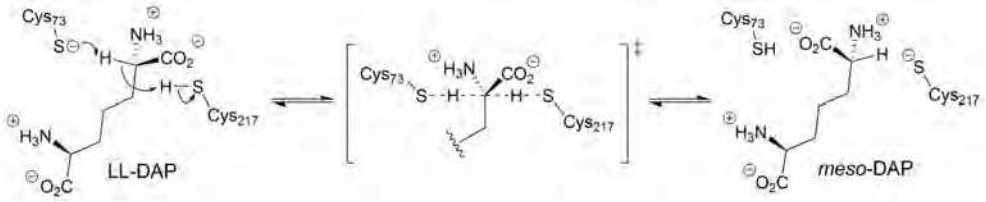


Fig. 16. DAPE catalysed reaction.

DAPE catalyses the conversion of *LL*-DAP to *meso*-DAP by employing a "two-base" mechanism (Wiseman, & Nichols, 1984). The reaction involves two active site Cys residues, where the first Cys residue (73 in *H. influenzae*) acts as base abstracting proton from *LL*-DAP, while the second Cys residue (217 in *H. influenzae*) re-protonates the molecule to generate *meso*-DAP. The enzyme is also capable of catalysing the reverse reaction, with the two Cys residues reversing their roles (Wiseman, & Nichols, 1984).

7.2 Structure of DAPE

The structures of DAPE from four species have been described. These include DAPE from *B. anthracis* (PDB:2OTN), *H. influenza* (Cirilli et al., 1998; Lloyd et al., 2004), and *M. tuberculosis* (Usha et al., 2009); and also the plant species *A. thaliana* (Pillai et al., 2009). The enzyme is a symmetrical monomer comprised of two domains containing eight β-strands and two α-helices (Cirilli et al., 1998) (Fig. 17).

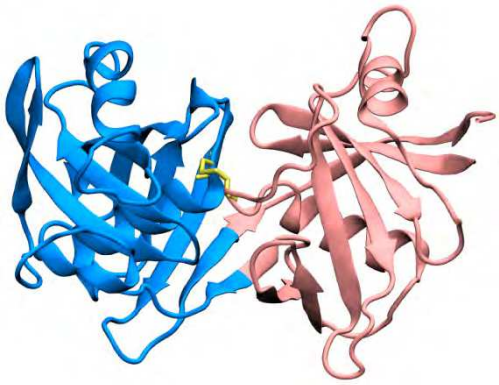


Fig. 17. Structure of DAPE from *H. influenzae*. Domains are coloured pink and blue, active site cysteines (disulfide linked) are shown in yellow (PDB: 1BWZ).

This fold, first observed in *H. influenzae* DAPE, is now referred to as the DAP epimerase-like fold. The structure of DAPE from *H. influenzae* shows that each domain of the enzyme contributes one active site Cys (residues 73 and 217). The distal, non-reacting end of the substrate interacts via a number of hydrogen bonds to residues Asn157, Asp190, Arg209, Asn64, and Glu208 (Fig. 18). The nature of this interaction ensures that only the *LL*-DAP stereoisomer is recognised. Interestingly, DAPE adopts two distinct conformational states. In the absence of substrate, the enzyme exists in an open conformation, and upon binding substrate adopts a closed conformation (Pillai et al., 2007).

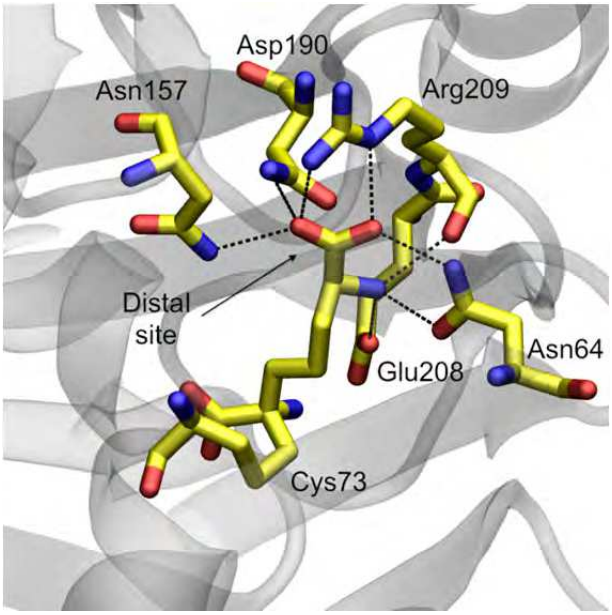


Fig. 18. Catalytic site of DAPE from *H. influenzae*. Hydrogen bond interactions (black dotted lines) at the distal site of the substrate analogue *LL*-AziDAP (arrow indicating position of the analogue) (PDB: 2GKE).

7.3 Inhibition of DAPE

Substrate analogues of DAP have been used as the basis for the generation of inhibitors of DAPE. These inhibitors take advantage of the anionic character at the α-carbon during the reaction or mimic the planar transition state. The most potent inhibitors are shown in Fig. 19 (Williams et al., 1996).

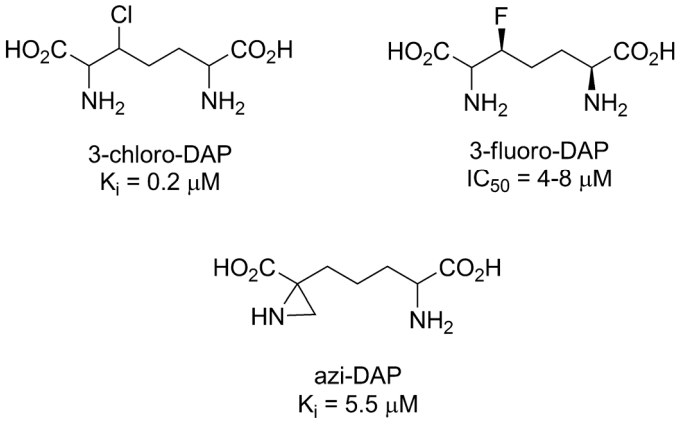


Fig. 19. Inhibitors of DAPE.

8. Dehydrogenase pathway

8.1 *Diaminopimelate dehydrogenase*

Diaminopimelate dehydrogenase (DAPDH EC 1.4.1.16) is a NADPH dependant enzyme that catalyses the reductive amination of *L*-2-amino-6-ketopimelate (AKP), the acyclic form of *L*-2,3,4,5,-tetrahydrodipicolinate (THDP), to produce *meso*-DAP (Misono et al., 1976; Misono & Soda., 1980) (Fig. 1). It is assumed that the reaction occurs via an imine intermediate as a result of amination of *L*-2-amino-6-ketopimelate. Reduction of the imine by hydride transfer from NADPH generates *meso*-DAP (Scapin et al., 1998).

Only a small group of Gram-positive and Gram-negative bacteria posses DAPDH activity. These include *Bacillus sphaericus*, *Brevibacterium sp.*, *C. glutamicum* and *Proteus vulgaris* (Misono et al., 1979). Characterised DAPDH enzymes are comprised of approximately 320 residues and share greater than 27% sequence identity (Ishino et al., 1987; Hudson et al., 2011b). Kinetic studies of DAPDH from *C. glutamicum* has yielded K_M values for NADPH, *L*-2-amino-6-ketopimelate and ammonia of 0.13 mM, 0.28 mM and 36 mM, respectively (Misono *et al*., 1986).

Some bacterial species possessing DAPDH activity use multiple pathways to synthesise lysine. For example, *C. glutamicium* (Schrumpf et al., 1991) can synthesise lysine by either the dehydrogenase or succinylase pathway, whilst *Bacillus macerans* (Hudson et al., 2011b) can employ enzymes of the dehydrogenase or acetylase pathways.

DAPDH from *C. glutamicum* forms a homodimer (Scapin et al., 1996) (Fig. 20). The DAPDH monomer subunit is comprised of (i) a dinucleotide binding domain, that is similar to but not identical to a classical Rossman fold, (ii) a dimerisation domain, and (iii) a C-terminal domain (Fig. 20). Monomer subunits interact via two α-helices and a three-stranded antiparallel β-sheet to form the dimer.

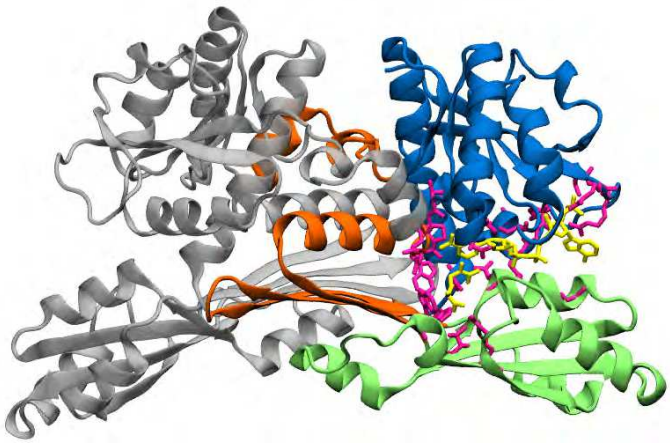


Fig. 20. Structure of dimeric *C. glutamicum* DAPDH in complex with NADPH and *L*-2-amino-6-methylene-pimelate. The dimerisation (orange), dinucleotide binding (blue), and C-terminal (green) domains are indicated. The cofactor NADPH (yellow) and inhibitor *L*-2-amino-6-methylene-pimelate (yellow) are bound by active site residues (pink) (PDB:1F06).

The crystal structure of the *C. glutamicum* DAPDH in complex with ligand shows that the oxidised cofactor, NADP+, is bound within each of the dinucleotide binding domains (Scapin et al., 1996). The domains exhibit open and closed conformations thought to represent the binding and active states of DAPDH, respectively (Scapin et al., 1996). In the closed conformation the $NADP^+$ pyrophosphate forms seven additional noncovalent contacts. Subsequent studies demonstrate the product, *meso*-DAP, binds within an elongated cavity formed at the interface of the dimerisation and dinucleotide binding domains (Scapin et al., 1998).

Crystal structures of *C. glutamicum* complexed with the inhibitors (2*S*,5*S*)-2-amino-3-(3-carboxy-2-isoxazolin-5-yl)-propanoic acid (K_i = 4.2 μM) and *L*-2-amino-6-methylene-pimelate (K_i = 5 μM) show that they form similar interactions with DAPDH as the product *meso*-DAP (Scapin et al., 1998). An additional hydrogen bond between the α-amino group of the *L*-2-amino-6-methylene-pimelate and the indole ring of DAPDH Trp144 is thought to account for the strong competitive inhibition observed (Scapin et al., 1998).

9. Diaminopimelate decarboxylase

9.1 Function of DAPDC

Diaminopimelate decarboxylase (DAPDC, EC 4. 1. 1. 20) is a PLP-dependant enzyme that is responsible for catalysing the final reaction of the lysine biosynthesis pathway (Fig. 1). In this non-reversible reaction, DAPDC converts the substrate *meso*-DAP to lysine and carbon dioxide (Fig. 21). Unlike other PLP-dependant decarboxylases that decarboxylate an *L*-stereocentre, DAPDC specifically cleaves the *D*-stereocentre carboxyl group. Thus, the enzyme possesses a means to differentiate between two stereocentres (Gokulan et al., 2003; Ray et al., 2002). DAPDC is classified as a type III class PLP enzyme, from the alanine racemase family.

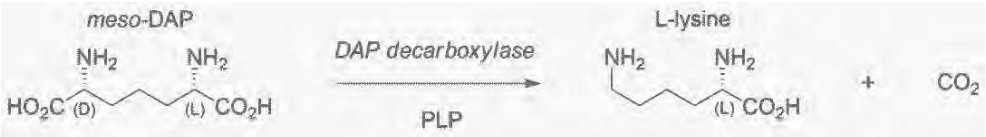


Fig. 21. DAPDC catalysed reaction.

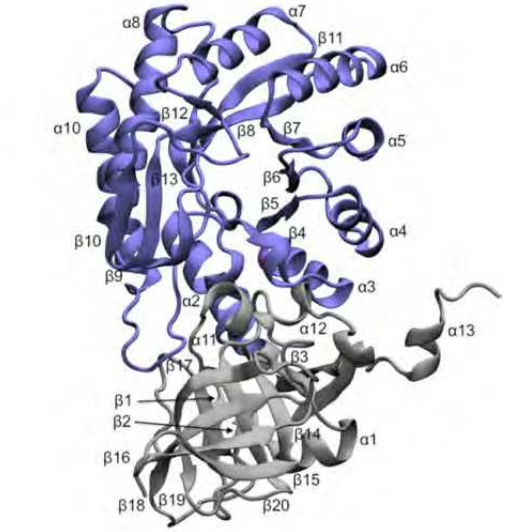
Compared to other enzymes within the lysine biosynthesis pathway, DAPDC has not been studied extensively. Consequently, the catalytic mechanism is poorly defined. However, current understanding of the structure and function of this enzyme is based on work performed on DAPDC from *Helicobacter pylori* (Hu et al., 2008), *M. tubercolosis* (Weyand et al., 2009), and *Methanococcus jannaschii* (Ray et al., 2002).

9.2 Structure of DAPDC

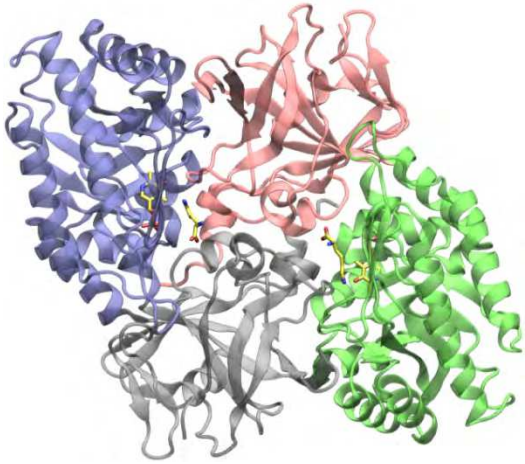
The crystal structures of DAPDC from seven species have been determined. There appears to be no consensus in quaternary structure of the enzyme as monomeric, dimeric, and tetrameric forms of DAPDC have been described. This is unusual, and possibly not a true reflection of what occurs in nature. Studies have shown that the active site of DAPDC is located at the dimer interface (Hu et al., 2008; Ray et al., 2002; Weyand et al., 2009). This implies that the dimer is the minimal catalytic unit. Therefore, monomeric forms of DAPDC

are likely to be non-functional; however, this does not rule out the existence of active tetrameric forms of DAPDC.

In species such as *M. jannaschii* (Ray et al., 2002) and *M. tubercolosis,* (Gokulan et al., 2003; Weyand et al., 2009) DAPDC is composed of a homodimer, whereby subunits associate to form a head-to-tail quaternary architecture (Fig. 22).



(A)



(B)

Fig. 22. Structure of *M. tuberculosis* DAPDC. (A) *M. tuberculosis* DAPDC monomer - The N-terminal (purple) and C-terminal (grey) domains are indicated. (B) *M. tuberculosis* DAPDC dimer – The active site is situated at the homodimer interface. PLP (yellow) and lysine (yellow) are located within the active site cavity (PDB: 1HKV).

The DAPDC monomer is composed of two domains, consisting of an N-terminal 8-fold α/β-barrel domain and a C-terminal β-sheet domain (Gokulan et al., 2003; Ray et al., 2002) (Fig. 22A). In *M. tuberculosis* DAPDC, the N-terminal α/β-barrel domain (residues 48-308) is comprised of β-strands β4–β13 and helices α2-α10 (Fig. 22A). The C-terminal domain (residues 2-47 and 309-446) is comprised of β-strands β1-β3, β14-β21 and helices α1, α11-α13 (Gokulan et al., 2003) (Fig. 22A). The active site is located at the interface between the α/β-barrel domain of one subunit and β-sheet domain of both subunits (Gokulan et al., 2003) (Fig. 22B).

The X-ray structure of *H. pylori* DAPDC has allowed identification of key residues involved in substrate and cofactor recognition. The enzyme was crystallised in the presence of PLP and lysine. The *H. pylori* structure is very similar to that of *M. tuberculosis* DAPDC, forming a homodimer in a head-to-tail conformation. In this enzyme, PLP forms Schiff base linkages with Lys46 and lysine to produce a lysine-PLP external aldimine. This aldimine is believed to mimic the catalytic intermediate formed between *meso*-DAP and PLP (Hu et al., 2008).

9.3 Inhibition of DAPDC

Diaminopimelic acid analogues (Fig. 23) have been synthesised to study the inhibition of DAPDC from *B. sphaericus* (Kelland et al., 1986). Mixtures of isomers of N-hydroxydiaminopimelate and N-aminodiaminopimelate are potent competitive inhibitors of DAPDC, with K_i values of 0.91 mM and 0.1 mM, respectively. Lanthionine sulfoxides (Fig. 23) are good competitive inhibitors, providing about 50% inhibition at 1 mM. Weaker competitive inhibitors include the *meso* and *LL*-isomers of lanthionine sulfone and lanthionine, whereas the *DD*-isomers (Fig. 23) were less effective.

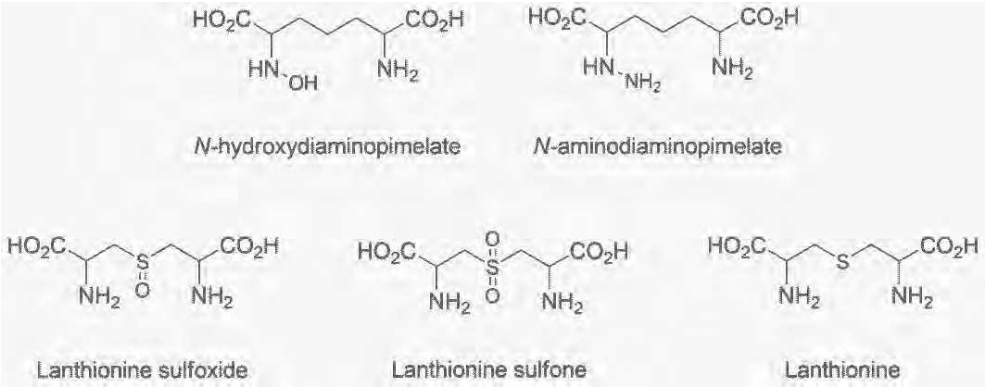


Fig. 23. Inhibitors of DAPDC.

10. Conclusions

Significant advances in our understanding of the enzymes of the lysine biosynthetic pathway have occurred in recent years, particularly through detailed kinetic and structural studies of wild-type and mutant enzymes. While advances in inhibitor design have not been as dramatic, our increased structural knowledge augurs well for the design of potent enzyme inhibitors in the near future, and subtle differences between the structures of the enzymes from different pathogenic species offers great potential of designing pathogen-

specific antibiotics. The improvements in our understanding of the lysine biosynthetic pathway in recent years will no doubt advance our efforts toward the ultimate goal of developing novel antibiotics that target this essential bacterial pathway.

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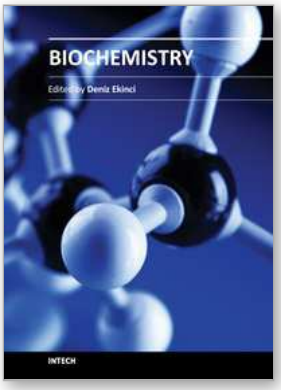

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Over the recent years, biochemistry has become responsible for explaining living processes such that many scientists in the life sciences from agronomy to medicine are engaged in biochemical research. This book contains an overview focusing on the research area of proteins, enzymes, cellular mechanisms and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in biochemistry. Particular emphasis is devoted to both theoretical and experimental aspect of modern biochemistry. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in biochemistry, molecular biology and associated areas. The book is written by international scientists with expertise in protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the book will enhance the knowledge of scientists in the complexities of some biochemical approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of biochemistry.

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