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Reviews

Decarboxylation mechanisms in biological system

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ABSTRACT

This review examines the mechanisms propelling cofactor-independent, organic cofactor-dependent and metal-dependent decarboxylase chemistry. Decarboxylation, the removal of carbon dioxide from organic acids, is a fundamentally important reaction in biology. Numerous decarboxylase enzymes serve as key components of aerobic and anaerobic carbohydrate metabolism and amino acid conversion. In the past decade, our knowledge of the mechanisms enabling these crucial decarboxylase reactions has continued to expand and inspire. This review focuses on the organic cofactors biotin, flavin, NAD, pyridoxal 5′-phosphate, pyruvoyl, and thiamin pyrophosphate as catalytic centers. Significant attention is also placed on the metal-dependent decarboxylase mechanisms.

Contents

1. Introduction

Decarboxylation is one of the most common processes in nature and one of the most fundamentally important reactions in biological systems. Essentially all of the carbon dioxide evolved in fermentation and respiration is generated by the decarboxylation of organic acids [1]. Decarboxylases are known for their roles in a wide variety of catabolic and anabolic pathways including decarboxylation of α- and β-keto acids, amino acid conversions, and carbohydrate synthesis [2]. Within the IUPAC classification scheme, decarboxylases are currently divided into at least 90 different classes [3]. Substantial efforts have been applied to the study of the origin and the mechanisms of production of metabolic carbon dioxide, and considerable knowledge has been accumulated regarding the decarboxylation mechanisms in biological systems. Enzymatic decarboxylation usually utilizes either an organic cofactor such as pyridoxal 5′-phosphate and biotin, or an inorganic cofactor, such as an iron or zinc complex, in the catalytic reaction (Table 1). Only

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Table 1
Catalytic strategy for biological decarboxylation.

Catalytic cofactor/coenzyme			Representative enzyme
None			Orotidine monophosphate decarboxylase
Organic		Biotin	Oxaloacetate decarboxylase, glutaconyl-CoA decarboxylase
		Flavin	Lantibiotic-biosynthesizing enzyme EpiD, 4-phosphopantethenoyl cysteine decarboxylase
		Glycyl radical	4-Hydroxyphenylacetate decarboxylase
		$NAD^+/NADP^+$	Methylmalonyl CoA decarboxylase
		Pyridoxal 5′-phosphate	Glycine decarboxylase; ornithine decarboxylase; DOPA decarboxylase
		Pyruvoyl	Arginine/aspartate/histidine decarboxylase; *S*-adenosylmethionine decarboxylase
		Thiamin diphosphate	Pyruvate dehydrogenase multienzyme complex; phosphonopyruvate decarboxylase
Inorganic	Alkaline earth metal	Mg^{2+}	3-Keto-L-gulonate 6-phosphate decarboxylase
	d-Block metal	Fe^{2+}/O_2 (oxidative)	Gallic acid decarboxylase; CloR decarboxylase; α-ketoglutarate-dependent dioxygenases
		Mn^{2+}/O_2	Oxalate decarboxylase
		M^{2+} (oxidant-independent) (M=Zn, Fe, Co, Cd, or Mn)	α-Amino-β-carboxymuconate-ε-semialdehyde decarboxylase

a handful of decarboxylases, e.g. orotidine monophosphate decarboxylase (OMPDC) [4,5] and methylmalonyl CoA decarboxylase from *Escherichia coli* [6], do not utilize any cofactors. In this review, representative decarboxylation mechanisms are outlined.

2. Enzymatic decarboxylation without an exogenous cofactor

The active sites of most decarboxylases bind organic or metal cofactors, which activate decarboxylation and/or stabilize the carbanion upon the elimination of carbon dioxide from the substrate [7]. Orotidine 5′-monophosphate decarboxylase (OMPDC) [5,8–10], 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline decarboxylase (OHCUD) [11,12], methylmalonyl CoA decarboxylase (MMCD) from *E. coli* [6], and malonate semialdehyde decarboxylase (MSAD) from *Pseudomonas pavonaceae* [13,14] are among a few decarboxlylases that do not contain any cofactors. These enzymes catalyze non-oxidative decarboxylation reactions.

OMPDC catalyzes the critical final step in the pyrimidine biosynthetic pathway [5,8]. This enzyme has drawn much attention because its rate enhancement is one of the highest of any known enzymes [15]. The crystal structures of OMPDC from *Pyrococcus furiosus* with and without substrate have recently been determined at high resolution. These structures have helped to establish a well-defined substrate binding pocket [16]. An extremely hydrophobic pocket is believed to be a strong factor in the rate enhancement compared to rates in the absence of a catalyst [17]. The presence of a carbanionic intermediate is supported through mutagenic [18] and kinetic experiments [18,19], and a carbanion-like intermediate has been trapped in crystals using substrate analogs [20]. However, whether the protonated site occurs at C5 or C6 remains to be determined [9,21–23].

A stepwise mechanism has been proposed for the OMPDC catalysis mechanism: a conserved lysine protonates either the C5 or C6 carbon bound to the leaving carboxylate of the pyrimidine, then an aspartate destabilizes the substrate to prepare it for decarboxylation [10,18]. A concerted and more widely accepted mechanism has also been proposed for OMPDC based on high resolution crystal structures (Fig. 1A) [5,7,11,24]. In this mechanism, the negative charge region of the strictly conserved Asp positions the anionic carboxylate of OMP, which helps to destabilize the ground state of the substrate; meanwhile, the positively charged ammonium group of a strictly conserved lysine is positioned close to the carbon, connected to the leaving carboxylate of the pyrimidine, stabilizing the developing negative charge in the transition state. Thus the OMPDC catalyzed reaction proceeds by a bimolecular electrophilic substitution mechanism (*i.e.*, decarboxylation and protonation are concerted), which will avoid the development of a high energy carbanion intermediate (Fig. 1A). However, recent product deuterium isotope effect studies [25], and quantum mechanical and molecular mechanical simulations [26] bring this mechanism into question. Efforts have also been made to target this active site lysine residue with covalently binding inhibitors [27].

Unlike the mechanism proposed for OMPDC, in which decarboxylation and protonation occur simultaneously at the C5 or C6 carbon of orotidine, decarboxylation and protonation take place stepwise in the proposed mechanism for three other enzymes [11,12]. In the mechanism proposed for OHCUD, the decarboxylation reaction was postulated to occur directly by using the double bond between C5 and N1 as an electron sink to stabilize the negative charge carbanion. A Glu, analogous to the Asp in OMPDC, in the active site may function to destabilize the ground state of the substrate by electrostatic repulsion to facilitate the exit of the carboxylate group. A conserved His in the active site is believed to be involved in the subsequent deprotonation of the hydroxyl group at C4 and the protonation of C5 of the substrate, generating a stereospecific product (Fig. 1B). A similar mechanism in which the protonated imine is believed to be the electron acceptor during decarboxylation has also been proposed [28]. Structures of both the enzyme, and enzyme bound with allopurinol, a recently discovered inhibitor, were published in 2010 [28]. Notably, these structures show a reorganization of the active site upon substrate binding [28].

In the proposed mechanism of MMCD [6], a conserved Tyr forms a hydrogen bond to the leaving carboxyl group of the substrate and orients the substrate in a plane with the thioester carbonyl group. Besides this conserved Tyr, only hydrophobic residues reside close to the negative charge of the leaving carboxyl group. This configuration destabilizes the ground state of the substrate and thus facilitates decarboxylation to leave a neutral carbon dioxide molecule which is more favorable in this hydrophobic environment. Two backbone amide groups from two strictly conserved residues, a His and a Gly, form hydrogen bonds with the thioester carbonyl group of the substrate to produce the required polarization of this bond and to stabilize the proposedanionic intermediate and transition state (Fig. 1C).

A similar mechanism has been proposed for malonate semialdehyde decarboxylase (MSAD) from *P. pavonaceae*, which also catalyzes the decarboxylation of a β-keto acid. In the proposed mechanism based on crystal structure, mutagenesis and inactivation studies [13,14], conserved Pro and Asp residues create a hydrogen bonding network to polarize the β-keto group of the substrate and stabilize the enolate anionic intermediate by donating a proton to the enolate anion. Two Arg residues position the leaving carboxyl group of the substrate such that the scissile bond is in the plane

of the β-keto group and further assist the Pro residue in stabilizing the developing charge anionic intermediate.

Arylmalonate decarboxylase (AMDase) catalyzes the reaction of α-aryl-α-methylmalonates to α-arylpropionates and was firstly purified from soil bacteria [29]. While cofactor independent, it is inhibited by sulfhydryl agents [29]. This enzyme was believed to proceed through a thiol ester intermediate that binds to the enzyme through a Cys residue [30]. This intermediate was trapped using substrate analogs and has been observed using mass spectrometry [30] and FT-IR [31], and the enzyme was reactivated using β-mercaptoethanol [30]. After further consideration the authors later reported that the active site Cys may be used as a proton donor [32]. The most recently proposed model suggests that the reaction occurs in a two-base mechanism through an enolate transition state (Fig. 1D) [32]. AMDase has also been re-engineered as a racemase [33] by a single point mutation and through a dual mutant, leading to its enantiomeric selectivity inverted [34].

Acetoacitate decarboxylase (AADase) is a member of a ketogenic pathway of enzymes that convert excess acetyl CoA into ketone bodies for energy [35]. In 1995, Westheimer suggested that two active site Lys residues situated closely enough in space would

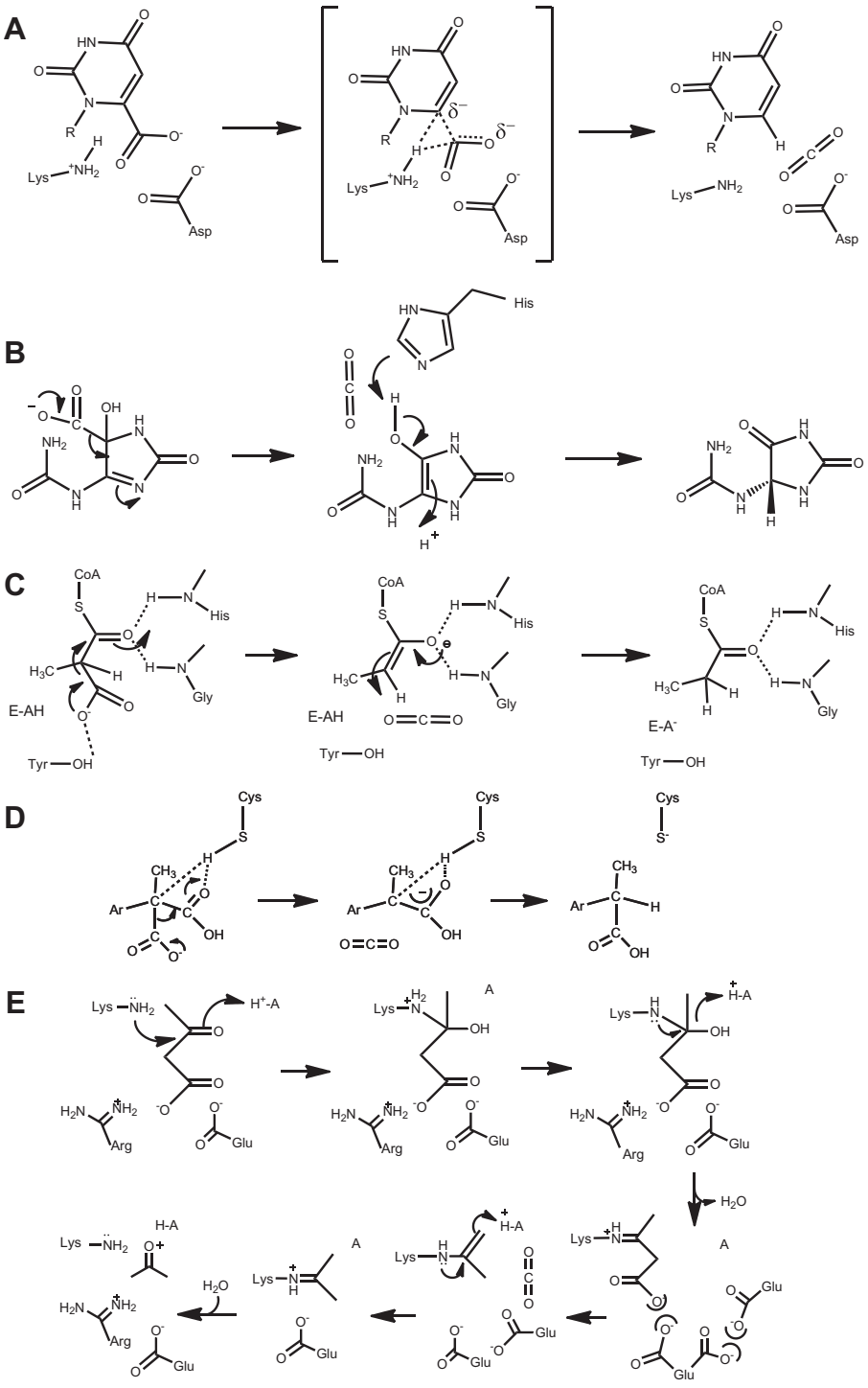

Fig. 1. Representive mechanistic models of decarboxylases without an exogenous cofactor: (A) OMPDC, (B) OHCUD, (C) MMCD, (D) AMD and (E) AAD.

cause an electrostatic effect strong enough to deprotonate one Lys residue [36]. He proposed that this effect was responsible for the perturbation of this Lys pK_a from 10.5 to 6. This phenomenon has been regarded as a classic example for dramatic pK_a perturbations in enzymes [37]. Recently, the Allen laboratory determined the crystal structure of AADase, which revealed a new protein fold. The perturbation of the Lys residue was determined to be caused by a highly hydrophobic environment while the second lysine in question is responsible for fasterning the perturbed lysine in the active site [38]. From the structural analysis of both substrate-free and substrate (analog)-bound states of the enzyme, Allen and colleagues proposed a mechanism in which the deprotonated Lys residue catalyzes a nucleophilic attack of an active site acid to the C3 carbonyl reducing it to a secondary alcohol. The lysine bond is further oxidized to form a Schiff base while the C3 alcohol is reduced to water. The formation of this Schiff base and the repulsive force between the substrate's carboxylate group and a nearby glutamate residue force a planar geometry to facilitate the decarboxylation reaction. Carbon dioxide escapes the active site and a water molecule hydrolyzes the Schiff base to a deprotonated lysine and a pyruvate (Fig. 1E) [38].

3. Biotin-dependent decarboxylases

Biotin is a covalently bound cofactor and acts as a mobile carboxyl group carrier. Biotin-dependent decarboxylases exist in all free-living organisms [39]. As members of the sodium ion transport decarboxylase family, the category includes methylmalonyl-CoA decarboxylases, malonate decarboxylases, oxaloacetate decarboxylases, and glutaconyl-CoA decarboxylases from certain anaerobic prokaryotes [40,41]. Biotin-dependent enzymes catalyze carboxyl transfer reactions in numerous metabolic pathways. Specifically, they catalyze the decarboxylation of α-keto acids and thioesters coupled with sodium transport from the cytoplasm into the periplasm under anaerobic conditions.

In general, these enzymes are large molecules comprising three or more subunits: a biotin carboxylase subunit (α-subunit) where the enzymes pick up the carboxyl group from substrates; a decarboxylase subunit (β-subunit) where the decarboxylation takes place and the sodium ion pump is located; and a biotinyl subunit (γ-subunit) containing the specific lysine residue to which biotin covalently binds [39,40,42,43]. A fourth subunit (δ-subunit) has been reported in all glutaconyl-CoA decarboxylases to anchor the α-subunit to the membrane [44], while two additional subunits, δ and ε, are found in malonate decarboxylases and methylmalonyl-CoA decarboxylases [45,46]. A zinc ion is present in the γ-subunit of the oxaloacetate decarboxylase sodium pump. The role of the metal ion is to position the oxaloacetate and to polarize the carbonyl oxygen bond of the substrate and to enhance the carboxyl transferring to biotin [47,48].

Mechanisms of biotin-dependent reactions involve two distinct major steps. Fig. 2 illustrates an example of biotin-dependent decarboxylation described for glutaconyl-CoA decarboxylase [39,40,49]. In this model, the first step of the reaction is a sodium ion-independent transfer of the carboxyl group of the primary substrate to biotin. This transfer takes place in the α-subunit. The carbonyl group of the primary substrate is polarized by hydrogen bonds to the enzyme, forming an oxyanion hole and inducing a transient decarboxylation of the substrate. The hydrogen bonds between the carbonyl oxygen of biotin and the protein residues generate another oxyanion hole, which increases the acidity of the N1 proton of biotin to promote the transfer of this proton to the dienolate anion derived from the substrate. Fixation of carbon dioxide by the enzyme-bound biotin produces carboxybiotin. Next, the carboxybiotin is brought to the decarboxylation site at the β-subunit by the "swing arm" of the γ-subunit, to which the biotin is attached. The second step is a sodium ion transfer-coupled decarboxylation of carboxybiotin, which takes place at the β-subunit. The driving force of such a reaction is the protonation of the 2-carbonyl oxygen of carboxybiotin.

An alternate mechanism for the biotin-dependent oxaloacetate decarboxylase sodium ion pump has been proposed by Dimroth et al. [47,48]. The role of the zinc ion at the γ-subunit is to coordinate and extract electrons from the carbonyl oxygen atom of the oxaloacetate, which in turn facilitates the C—C bond cleavage of the substrate and promotes the formation of carboxybiotin at the α-subunit. The carboxybiotin is then brought to the decarboxylation site at the β-subunit, where the sodium ion binding site is exposed to the cytoplasm. The binding of the first sodium ion to the conserved aspartate-asparagine pair and a second sodium ion to the conserved serine causes the release of a proton, which is relayed to the carboxybiotin by several conserved polar residues in the β-subunit. This process induces a conformational change at the β-subunit and the sodium ion binding site becomes exposed to the periplasm. The carboxybiotin is protonated and then followed by decarboxylation. The sodium ions are released to the periplasm while a proton enters and binds to the conserved Ser residue.

Malonate decarboxylases are reported to utilize CoA or an acyl-carrier protein [43,50]. Although the mechanistic models of malonate decarboxylases and methylmalonyl-CoA decarboxylases are reported to be similar to those of glutaconyl-CoA decarboxylase and oxaloacetate decarboxylase, in terms of the reaction site and the transfer of the carboxybiotin, the chemical mechanisms of these enzymes remain unclear.

4. Flavin-dependent decarboxylases

Flavin-dependent decarboxylases consist of a large family of the homo-oligomeric flavin-containing cysteine decarboxylases (HFCD) and phosphopantothenoylcysteine decarboxylases (PPCDC) from both prokaryotes and eukaryotes [51,52]. The characterized members of HFCD include the lantibiotic-biosynthesizing enzymes EpiD [53,54] and MrsD [55]. These enzymes catalyze the oxidative decarboxylation of the C-terminal peptidyl-cysteines to peptidyl-aminoenthils/aminoenthiolates during the biosynthesis of antibiotics [53,55]. Members of PPCDC catalyze the so-called "simple" decarboxylation of the cysteine moiety of 4′-phosphopantothenoylcysteine to produce 4′-phosphopantetheine during the biosynthesis of coenzyme A (CoA), thus the enzyme is also known as CoaC [56,57]. Although the entire reaction looks like a "simple decarboxylation", it is actually not as simple as it appears [51,58]. A large body of circumstantial evidence suggests that the decarboxylation mechanism of PPCDC is initiated in an oxidative step, followed by the reduction of the intermediate. Thus, the key element of this conversion is an oxidation–reduction reaction. The reaction is far more complex than a simple decarboxylation to CO_2.

Bioinformatics and mass spectroscopic studies have resulted in the assignment of a new member to the HFCD family, Undec1A [59]. This enzyme catalyzes the decarboxylation of L-cysteine to form cysteamine. Besides the HFCD protein family, enzymes with new function and structure have been added recently into this class of decarboxylases, such as Pad1, a flavin mononucleotide (FMN)-dependent UbiX-like decarboxylase, from *E. coli* O157:H7, which does not exhibit overall sequence similarity, the substrate-binding motif, nor the quaternary structure common to the known members of the HFCD protein family [60].

The reaction mechanism of the HFCD protein family has been widely studied. The flavin cofactor bound in PPCDC and EpiD is a FMN molecule [53,58]. In contrast, MrsD binds a flavin adenine dinucleotide (FAD) [55,61]. It seems that all three enzymes catalyze the decarboxylation of cysteine residues with the same biochemical principle. The —CH_2—SH side chain of the C-terminal

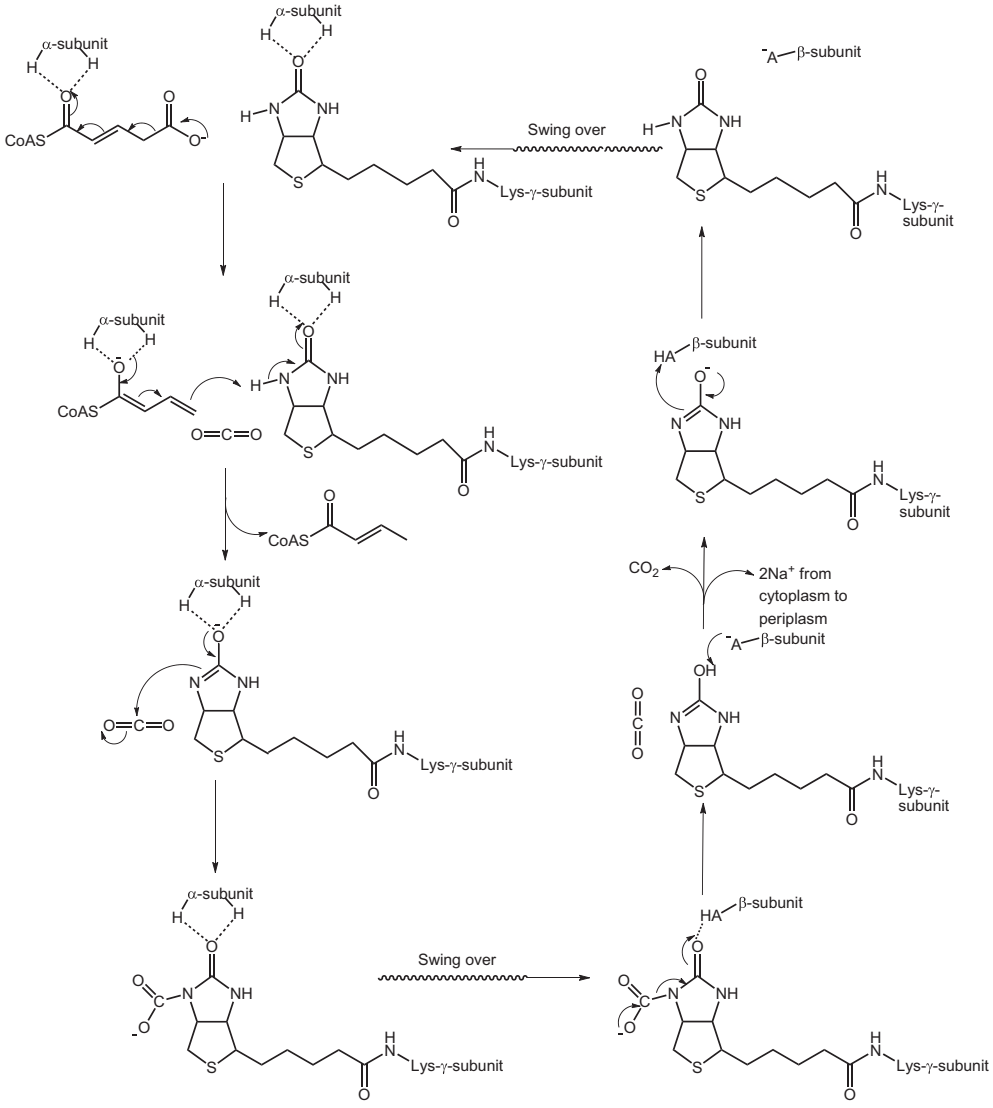

Fig. 2. Proposed mechanism of glutaconyl-CoA decarboxylase, a biotin-dependent decarboxylase [39,40,49].

cysteine residue is oxidized to a thioaldehyde or to a tautomeric enethiol with the concomitant reduction of the flavin cofactor. Decarboxylation of the thioaldehyde/enethiolate intermediate occurs spontaneously, because this step is favored by the delocalization of the negative charge of the adjacent thioaldehyde group in the manner similar to the decarboxylation of β-keto acids. The resulting enethiol is released as the final product of the reaction in EpiD and MrsD [53,55]. Fig. 3 depicts a mechanistic model for the HFCD protein family proposed by Begley et al. [7,51,62]. The observation of a substrate-induced thiolate-flavin charge transfer suggests that a flavin–thiol adduct intermediate (A) is formed and that electron transfer from the thiolate to FMN is mediated by a flavin C4a-thiol adduct [63]. However, a consensus on the electron transfer mechanism has not been reached. The Kupke group argues against the mechanism via the flavin–thiol complex based on the crystal structure of EpiD and PPCDC, proposing instead that the transfer takes place through a single electron transfer mechanism [53,58]. The β-thiolketo acid intermediate (C) has been characterized by a crystallographic study [58] and trapped with a mechanism-based inactivating agent [51].

In the case of PPCDC, the enethiolate intermediate is then reduced to a thiol, giving the net decarboxylation of cysteine accompanied by the re-oxidation of the cofactor $FMNH_2$ back to FMN. A conserved active site cysteine residue plays an important role in catalysis by transferring a proton to the C=C of the enethiolate intermediate, although whether the protonation site is at C_α or at C_β is under debate [51,58]. The rationale for the enethiolate becoming reduced in PPCDC but not in EpiD and MrsD is attributed to a conserved active site cysteine residue in PPCDC, which is replaced by a serine or threonine residue in EpiD and MrsD. The reduced enethiolate in PPCD, but not in EpiD or MrsD is attributed to a conserved active site cysteine which is replaced by a serine or threonine in the latter enzymes. Neither serine nor threonine is strong enough to induce protonation of the C=C double bond necessary for the reduction to occur [51]. Despite the agreement on the important role of the active site cysteine residue, the detailed reduction reaction mechanism remains unsettled. Similar to that of the oxidative decarboxylation reaction, some evidence suggests electron transfer occurs via a flavin–thiol adduct while other evidence supports a direct hydride transfer from $FMNH_2$ to the thiolate intermediate [51,58,62].

5. Glycyl radical-dependent decarboxylases

Glycyl radical is generated by removing a hydrogen atom from a glycine. Enzymes have the glycyl radical localized on protein main

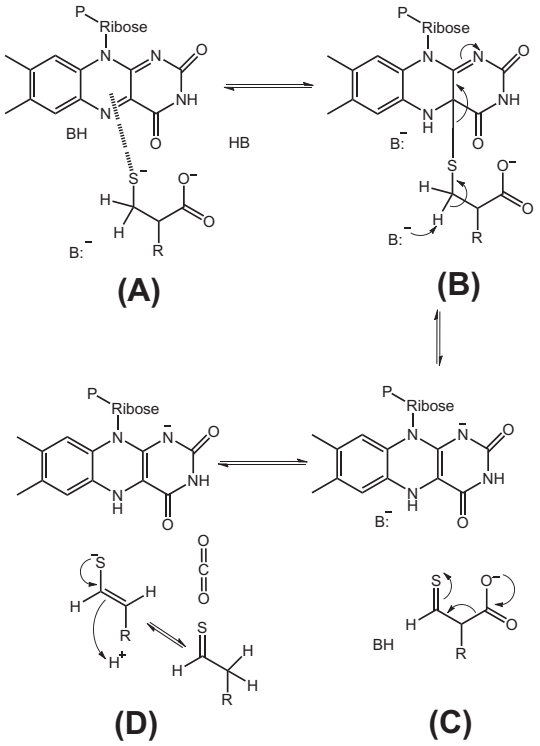

Fig. 3. A proposed model for the flavin-dependent decarboxylase reactions.

chain and use it for catalysis belong to the glycyl radical enzyme (GRE) family. Members in this family are involved in a large variety of functions. Pyruvate formate lyase, benzylsuccinate synthase and anaerobic ribonucleotide reductase are among the best characterized members. 4-Hydroxyphenylacetate decarboxylase (HPAD) is a new member of GRE family [64]. This enzyme catalyzes a Kolbe-type decarboxylation [65] by using a protein-based glycyl/thiyl radical to catalyze the last step of tyrosine fermentation in clostridia. The decarboxylation product *p*-cresol is a virulence factor of the human pathogen *Clostridium difficile*. HPAD is initially synthesised in inactive proenzyme forms and require posttranslational activation by dedicated iron–sulfur clusters for catalytic activity. These activating proteins are members of the *S*-adenosylmethionine (SAM) radical enzyme superfamily and catalyze the reductive cleavage of SAM to yield methionine and a 5′-deoxyadenosyl radical [66–69]. The radical abstracts the *pro-S*-hydrogen atom from the α-carbon of a specific glycyl residue in the proenzyme of HPAD, and forms a protein-bound thiyl radical that is essential for catalytic activity. As seen in other glycyl radical enzymes, the activated form of HPAD is extremely sensitive towards oxygen inactivation like other members in this enzyme family [70].

The crystal structures of the enzyme from *C. scatologenes* in both substrate-free and substrate-bound (obtained by crystal soaking) are available [65]. The substrate carboxyl group is shown interacting with the active site cysteine residue, and the substrate hydroxyl group is anchored by a histidine and a glutamic acid residue. In the catalytic cycle, the HPAD thiyl radical first abstracts a hydrogen atom from the substrate, then the anion radical substrate decarboxylates spontaneously. A neutral radical substrate is further formed by obtaining a hydrogen atom. The enzyme bound thiyl radical is regenerated by donating a hydrogen atom to the neutral radical intermediate and leads to the formation of the final product, *p*-cresol (Fig. 4).

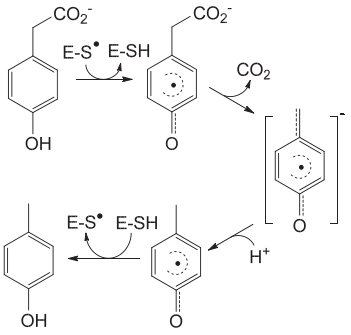

Fig. 4. A mechanistic model for 4-hydroxyphenylacetate decarboxylase.

6. NAD$^+$/NADP$^+$-dependent decarboxylases

Both NAD$^+$ and NADP$^+$ are common cellular oxidizing agents. It is thus not surprising that NAD$^+$/NADP$^+$-dependent decarboxylase reactions always proceed in an oxidative fashion. Enzymes known to utilize NAD$^+$ and NADP$^+$ for decarboxylation include malic enzymes, which catalyze the reversible oxidative decarboxylation of L-malate to pyruvate [71–74]; isocitrate dehydrogenase, which generates α-ketoglutarate in the citric acid cycle [75]; 6-phosphogluconate dehydrogenase, which catalyzes the reversible oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate [76]; tartrate dehydrogenase, which in addition to oxidizing tartrate to oxaloglyconate, catalyzes the oxidative decarboxylation of D-malate to pyruvate and mesotartate to D-glycerate [77]; UDP-glucuronate decarboxylase, which converts UDP-glucuronate to UDP-xylose [78,79]; ArnA, which oxidizes UDP-glucuronic acid to produce UDP-4-keto-arabinose [80,81]; 4α-carboxysterol-C_3-dehydrogenase and C_4-decarboxylase, which removes CO_2 from several substrates in higher plants [82].

Several NAD(P)$^+$-dependent decarboxylases, such as malic enzymes and isocitrate dehydrogenase, require a divalent metal ion as activator. The most common metal ions employed are Mg^{2+} and Mn^{2+}. Tartrate dehydrogenase is unique because it requires both a divalent metal ion and a monovalent metal ion for catalysis. In contrast, 6-phosphogluconate dehydrogenase/decarboxylase and prephenate dehydrogenase do not require any metal ion for their catalytic activity [79,83].

The substrates of these enzymes generally contain a β-hydroxyl group linked to a carboxyl group. Among this class of enzymes, malic enzymes are the most extensively studied. Fig. 5 illustrates a proposed mechanism for malic enzymes. Besides its function in maintaining the integrity of the enzyme's oligomeric structure, the divalent metal ion is suggested to be important in catalysis [84]. The metal ion keeps malate in place by binding to the C4-carboxyl group and the β-hydroxyl group. The catalysis by malic enzymes generally proceeds in a stepwise acid–base mechanism. First, a general base deprotonates the β-hydroxyl group and facilitates a hydride transfer from malate to the NAD(P)$^+$ cofactor, leading to the dehydrogenation of malate, production of oxaloacetate, and reduction of the nicotinamide cofactor [83,85]. This general base, which becomes a general acid, then promotes the decarboxylation of oxaloacetate to generate enolpyruvate by giving the proton back to the resulting enolate group [84]. The coordination of the metal ion to the keto group helps to polarize this group and facilitate the decarboxylation reaction [83]. The leaving carboxyl group is out of the plane created by the other three carbon atoms of malate [73], which facilitates the decarboxylation reaction. Tautomerization of enolpyruvate to form pyruvate is assisted by a pair of general acid and general base residues [84]. Some malic enzymes can directly use oxaloacetate as a substrate [86]. Other

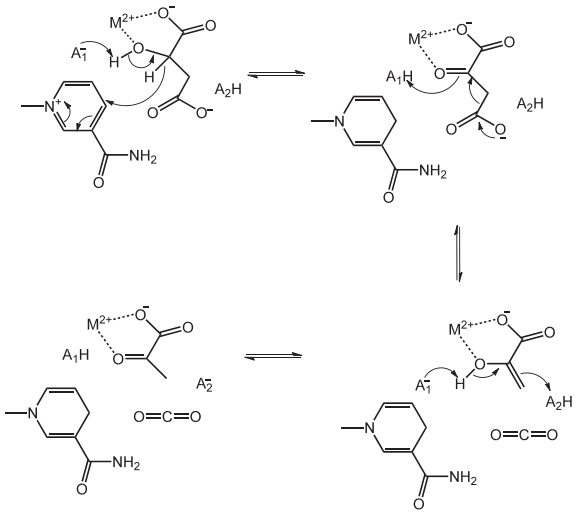

Fig. 5. A proposal for the mechanism of NAD(P) $^{+}$-dependent decarboxylases.

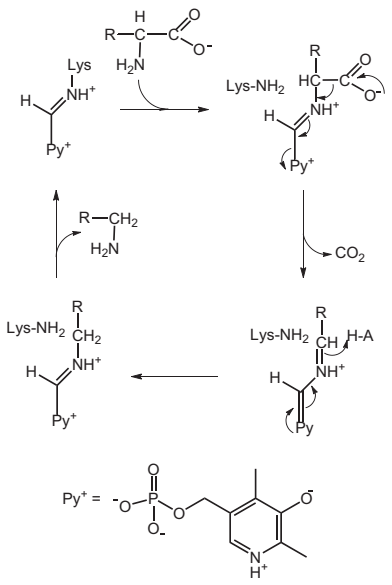

Fig. 6. PLP-dependent decarboxylase reactions.

enzymes, such as 6-phosphogluconate dehydrogenase and PA4872 from the PEP mutase/isocitrate lyase superfamily, utilize a mechanism similar to that illustrated for malic enzymes [87,88].

7. Pyridoxal 5′-phosphate (PLP)-dependent decarboxylases

Pyridoxal 5′-phosphate (PLP) dependent enzymes are ubiquitous and central to a wide variety of anabolic and catabolic pathways in the metabolism of nitrogen-containing compounds. They catalyze a wide range of reactions at the α-, β-, and γ-carbons of amine and amino acid substrates in animals, plants, and microorganisms [89]. The decarboxylation of amine and amino acids is a key step in the synthesis of neurotransmitter amino compounds and other physiologically important compounds. The chemical mechanism of the PLP-dependent decarboxylases, such as dialkylglycine decarboxylase, glutamate decarboxylase, aromatic L-amino acid decarboxylases, and ornithine decarboxylase is well established [90–94]. A representative mechanism of α-decarboxylation catalyzed by PLP-dependent decarboxylases is shown in Fig. 6.

In the resting state of the enzyme, PLP forms an internal aldimine with the ε-amino group of a specific Lys residue [94]. The obligatory first chemical step in all PLP-dependent enzymatic reactions is the formation of a Schiff base intermediate, named the 'external aldimine', between the coenzyme aldehyde and the substrate amino group. The general utility of PLP is derived from its ability to stabilize the carbanions generated adjacent to the Schiff base in the external aldimine intermediate by delocalization of the developed negative charge into the extended π bonded system, *i.e.*, Schiff base and pyridine ring [95]. The PLP cofactor serves as an "electron sink" that stabilizes the developing carbanion in the transition structure which leads to the loss of CO_2. The cleavage of the C—C bond is also aided by a hydrophobic or negatively charged environment, which destabilizes the charged carboxylate and favors the more electrostatically neutral transition state [93,96]. A general acid facilitates the protonation at C_α to form the imine product, which is followed by transaldimination and the release of the corresponding amine product. The protonation can also occur at C_4 which has been proposed for the decarboxylation half reaction of dialkylglycine decarboxylase [94,97,98]. In *P. dacunhae* aspartate β-decarboxylase, an arginine residue has been identified as the primary mediator of the protonation state using rapid scanning stopped-flow kinetics. As such, this residue may be responsible for mediating the reaction cycle [99].

8. Pyruvoyl-dependent decarboxylases

α-Amino acid decarboxylation is a key step in the synthesis of neurotransmitter amino compounds. Two strategies are known for this decarboxylation process, PLP-dependent decarboxylases and pyruvoyl-dependent decarboxylases, and both take advantage of π electron delocalization. These enzymes are known to form intermediate imine compounds through a reaction of the substrate amino group with the carbonyl moiety of the enzyme prosthetic group. It is thought that the pyruvamide and PLP serve as electron sink and stabilize the developing carbanion in the transition structure. The mechanism discussion will be focused on those proposed for pyruvoyl-dependent decarboxylases, including arginine decarboxylase [74], *S*-adenosylmethionine decarboxylase [100], aspartate-1-decarboxylase [101], bacterial histidine decarboxylase [102], and phosphatdylserine decarboxylase [103]. These enzymes produce important biological amines such as decarboxylated *S*-adenosylmethione (the aminopropyl donor for polyamine biosynthesis), histamine (a neurotransmitter), phosphatidylethanolamine (a component of membrane phospholipids), and β-alanine (a precursor of pantothenate and coenzyme A), which are all critical to cellular physiology and thus important targets for drug design. These pyruvoyl-dependent decarboxylases are all synthesised as zymogens. The pyruvoyl cofactor in these decarboxylases is resulting from the self-modification of an internal serine residue of the proenzyme [100,104].

In these decarboxylases, the pyruvoyl group functions through the formation of a Schiff base with the substrate to promote decarboxylation. This mechanism resembles the one postulated for the action of PLP dependent decarboxylases. However, in contrast to PLP-dependent enzymes, where PLP forms an internal aldimine with the ε-amino group of a specific Lys residue in the resting state of the enzyme [94], the carbonyl group of the pyruvoyl enzymes is free [105]. The route of the formation of the intermediate enzyme-substrate imines differs in the two groups of enzymes.

A representative mechanism proposed for pyruvoyl-dependent decarboxylases is illustrated in Fig. 7. The Schiff base intermediate is formed from the pyruvoyl moiety of the enzyme by reacting with the amino group of the amino acid substrate. In this imine intermediate, the acyl-carbonyl group of the pyruvoyl moiety functions as an electron sink, stabilizes the charge developed during the reac-

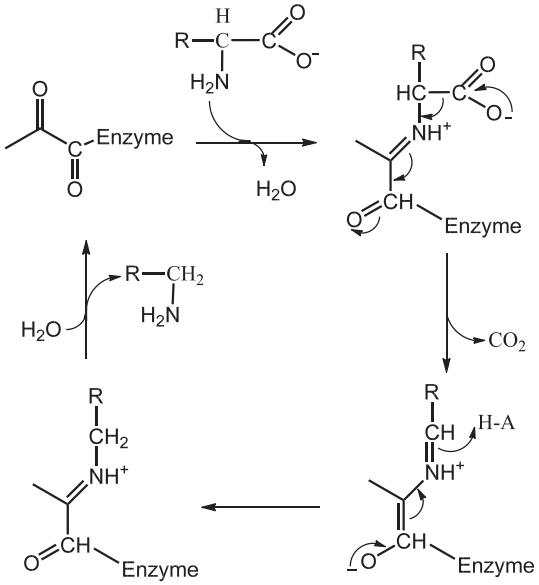

Fig. 7. A proposed mechanism for pyruvoyl-dependent decarboxylase reactions.

tion, and thus assists the decarboxylation. This process is homologous to that of PLP-dependent decarboxylases. Another similarity between the two mechanisms is that the substrate carboxylate in pyruvoyl-dependent decarboxylases is in an environment predominated with hydrophobic and negatively charged residues [105]. This property destabilizes the ground state of the reactant and promotes the leaving of the carboxyl group. The α-carbon is then protonated to form the product imine intermediate, which has been experimentally characterized [102]. Hydrolysis of this imine causes the release of amine product and the regeneration of the pyruvoyl-enzyme.

9. Thiamine diphosphate (ThDP)-dependent decarboxylases

Thiamine diphosphate (ThDP), the biologically active derivative of vitamin B1, is used by various enzymes as a cofactor to perform a wide range of catalytic functions, including the decarboxylation of α-keto acids and transketolation. The ThDP-dependent decarboxylases, such as pyruvate decarboxylase, benzoylformate decarboxylase, oxalyl-CoA decarboxylase, and phenylpyruvate decarboxylase, contain ThDP and a divalent cation (Ca^{2+}, Mg^{2+}, or Mn^{2+}) as cofactors and activators to catalyze the non-oxidative decarboxylation of α-keto acids to corresponding aldehydes and a carboligation reaction forming hydroxyl ketones [106–110]. ThDP binds to the enzyme through a conserved hydrogen-bond network and the coordination of its diphosphate moiety to the Mg^{2+} or other alternative divalent ions [107,111]. The metal cofactor is coordinated to a conserved motif of the enzymes [107,112,113].

In the case of pyruvate decarboxylase, the C-terminal helix and two additional loops close the substrate channel and may create a more hydrophobic active site to facilitate the deprotonation of the C2 carbon of ThDP to stabilize hydrophobic intermediates [114]. The active site of pyruvate decarboxylase contains several hydrophilic functional groups that could otherwise negatively affect the formation of hydrophobic species.

The ability of the thiazolium ring of ThDP to form a nucleophilic anion, to bind to the α-carbonyl group of an α-keto acid, and to stabilize the negative charge created upon cleavage of CO_2 makes the decarboxylation of an α-keto acid to generate an aldehyde possible [108]. The catalytic cycle of ThDP-dependent decarboxylases can be divided into a number of steps (Fig. 8) [106,111,112,115,116]. In the first step, the thiazolium C2 atom is deprotonatated to a highly nucleophilic ylide (ylide-ThDP) using the N4′ nitrogen atom as the general base with the facilitation of the interaction of a conserved acidic residue and the N1′ atom of the pyrimidine ring in the proximity of the coenzyme [116–118]. It has been recently reported that the pyrimidine moiety exists in several tautomeric forms simultaneously and the 1′,4′-iminopyrimidine tautomer is believed to be the intramolecular trigger to produce the reactive ylide-ThDP [106]. Upon the entry of the substrate the ylide-ThDP attacks the substrate carbonyl forming the first covalent tetrahedral intermediate [119]. Decarboxylation of this intermediate leads to a C2-α-carbanion which is resonance-stabilized by its enamine form. The thiazolium ring of ThDP acts as an electron sink after decarboxylation [120,121]. C-protonation of this enamine intermediate yields a second tetrahedral intermediate. Deprotonation of this intermediate with concomitant cleavage of the C—C bond between C2 of ThDP and Cα of the intermediate finally releases the product. The nucleophilic attack of the C2-α-carbanion intermediate on another aldehyde allows for carboligation and the production of a hydroxyl ketone [122,123]. This mechanism is further supported by the irreversible phosphorylation of the enzyme by mechanism-based inhibitors, such as keto acid phosphanates. These inhibitors have also been proven to be effective for benzoylformate decarboxylase [124].

In addition to the nonoxidative decarboxylation conversion of α-keto acids to the respective aldehydes and the carboligation reaction to form hydroxyl ketones, the ThDP-dependent enzymes can also catalyze several other reactions. Such reactions involve the decarboxylation of an α-keto acid, as in the preceding steps, using the thiazolium ring as an electron sink and the oxidation of the enamine intermediate by various oxidative agents through different mechanism. These enzymes include the entire family of α-keto acid dehydrogenase multienzyme complexes which use lipoic acid as the oxidative agent [125], pyruvate-ferredoxin oxidoreductases which employ Fe_4S_4 cluster as the redox agent [116,126], and the pyruvate oxidases which utilize flavin as the oxidant [127]. All decarboxylation reactions in these enzymes are oxidative.

10. Metal- and O_2-dependent decarboxylases

Metal ions are employed as cofactors in many decarboxylases, some of which do not function as catalytic centers while others do. As described above, the Zn^{2+} ion in the biotin-dependent decarboxylases helps to properly orient the substrate while the Mg^{2+} ion in the ThDP-dependent decarboxylases assists in binding the thiamine cofactor. In $NAD(P)^+$-dependent decarboxylases, such as malic enzymes, a divalent metal ion (Mn^{2+} or Mg^{2+}) plays an important role in both catalysis and structure stability. In this section only those decarboxylases containing metal cofactors and not organic cofactors, with the metal ions playing a catalytic role, are discussed.

10.1. Mechanism of ferrous-dependent oxidative decarboxylases

Ferrous ion-dependent decarboxylases include gallic acid decarboxylase [128], bifunction CloR decarboxylase [129], and α-ketoglutarate (αKG)- and nonheme ferrous-dependent dioxygenases [130,131]. Until recently, little was known about gallic acid decarboxylase. CloR decarboxylase is proposed to be a unique member of the α-ketoglutarate (αKG)- and nonheme ferrous-dependent dioxygenase family. This enzyme catalyzes two consecutive oxidative decarboxylations within a single biosynthetic pathway.

The α-ketoglutarate-dependent dioxygenase reaction proceeds in two half reactions. The first half of the reaction is a ferrous and O_2-dependent oxidative decarboxylation reaction of αKG. The cleavage of the O—O bond results in one oxygen atom inserting into αKG while the other oxygen remaining at the Fe center (Fig. 9). These

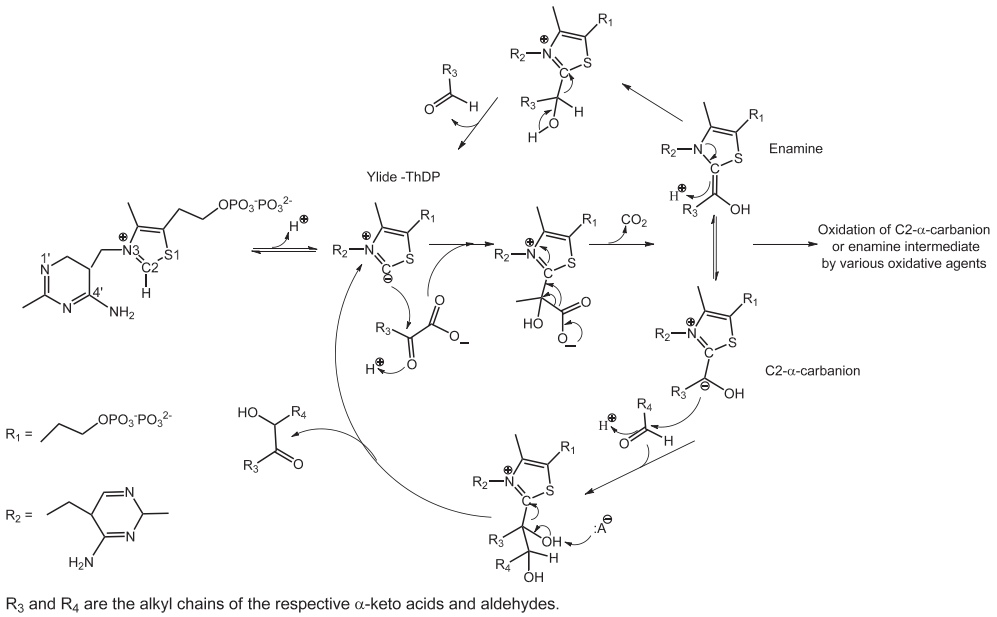

Fig. 8. Reaction mechanism of ThDP-dependent decarboxylases.

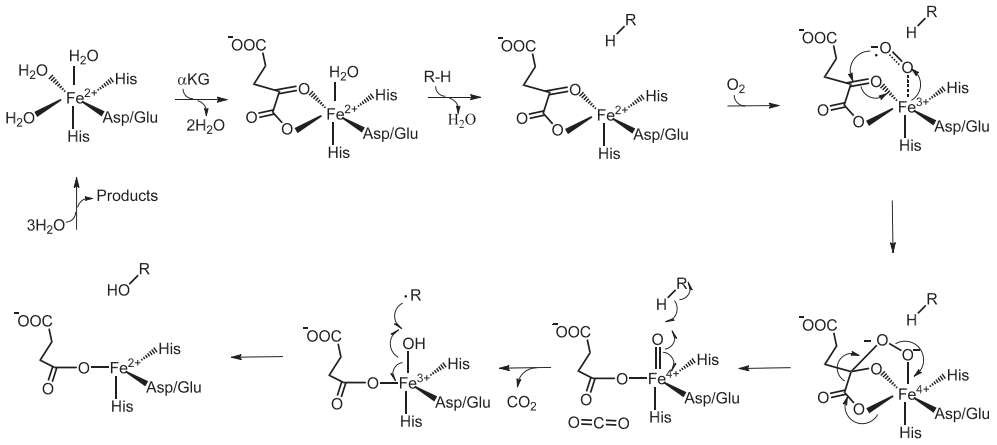

Fig. 9. A proposed mechanism for ferrous-dependent oxidative decarboxylation of αKG.

αKG- and Fe-dependent enzymes are widespread and versatile, catalyzing an exceptionally wide range of energetically demanding biosynthetic and degradative reactions such as the stereo-selective desaturation of unactivated C—C single bonds, ring expansion, oxidative ring closure, and hydroxylation processes [131], which are important in the biosynthesis of collagen [132], antibiotics biosynthesis [133,134], cellular response of hypoxia [135], repair of alkylated DNA [136,137], regulation of gene expression by demethylation of histones [138], and many other biological processes.

The catalytic mechanism of αKG-dependent dioxygenases has been extensively studied. This family of enzymes all require an iron(II) center, which is relatively weakly coordinated by three amino acid side chains, typically in a 2-his-1-carboxylate facial triad [139]. The mechanism for this group of decarboxylases proceeds via an Fe(IV)═O intermediate (Fig. 9), which was first proposed three decades ago and recently confirmed by Bollinger and Krebs using EPR and Mössbauer spectroscopy [140–145] and by Hausinger using continuous-flow resonance Raman spectroscopy [146,147]. In this model, the binding of the enzyme to αKG, the primary substrate, and the binding of molecular oxygen follows an ordered binding mechanism [145,148]. The iron center of the resting enzyme is coordinated with two histidines and one carboxylate (Asp or Glu) residues together with three water molecules. The addition of the co-substrate, αKG, will replace the two water ligands with its 1-carboxyl and 2-keto groups. The primary substrate will then enter and expel the third water molecule, which leads to an empty coordination site ready for a dioxygen molecule to come in and bind to the iron center [145,149]. Addition of dioxygen to the substrate/co-substrate/iron-bound enzyme complex leads to the oxidation and decarboxylation of αKG to produce succinate and form a substrate-hydroxylating Fe(IV)═O species through two non-accumulating complex species in which the Fe(IV)═O intermediate has been trapped and characterized [140,144,146,150–152]. Although the details of the decarboxylating mechanism remain elusive, it has been demonstrated that decarboxylation of αKG occurs prior to or concomitantly with the formation of this substrate-hydroxylating Fe(IV)═O intermediate [151]. Carbon dioxide may remain bound to the metal center in its hydrated or nonhydrated form as observed in the self-hydroxylation reaction study in taurine/αKG dioxygenase [153] and the crystallographic

study in deacetoxycephalosporin C synthase [154]. In the next step, the highly reactive Fe(IV)=O intermediate abstracts a hydrogen atom from the primary substrate and generates a radical species on the substrate. It also causes a reduction of the metal center. The radical abstracts the hydroxyl group from the iron center, resulting in the hydroxylation of the substrate [143,144].

The rate-determining step in the catalytic cycle is the release of products [151]. For some enzymes in this family, the Fe(IV)=O intermediate is believed to perform an electrophilic attack on the aromatic ring of the primary substrate rather than hydrogen abstraction [143,155,156]. In αKG-dependent halogenates, the iron center is coordinated by two histidine residues, αKG, water, and chloride, *i.e.*, the carboxylate of the facial triad is replaced by a chloride ion [157]. It is believed that halogenation activity may have evolved from hydroxylation by iron ligand replacement. A Cl—Fe(IV)=O intermediates purportedly abstracts a hydrogen from the primary substrate while the chlorination occurs by the rebinding of chlorine rather than oxygen to the radical on the substrate [140].

10.2. Mechanism of manganese-dependent decarboxylases

Enzymes that require Mn^{2+} and O_2, but not any organic cofactors, to decarboxylate substrates include oxalate decarboxylase (EC 4.1.1.2) and the closely related oxalate oxidase (EC 1.2.3.4). Moreover, 4-oxalocrotonate decarboxylase (EC 4.1.1.77) forms a complex with vinylpyruvate hydratase and utilizes either Mn^{2+} or Mg^{2+} as a cofactor to convert 2-oxo-3-hexenedioate to 2-oxo-4-hydroxypentanoate [158,159]. Oxalate decarboxylase catalyzes the conversion of oxalate to formate and carbon dioxide. Oxalate decarboxylase utilizes both Mn^{2+} and dioxygen as its cofactors, but the reaction does not involve a net redox change [160]. Oxalate oxidase also requires Mn^{2+} and dioxygen for its catalytic activity. Furthermore, it degrades oxalate to produce carbon dioxide. However, the reaction uses dioxygen as a substrate involves a net redox reaction [161,162]. Both oxalate decarboxylase and oxalate oxidase catalyzing reactions utilize free radicals to cleave a relatively stable carbon–carbon bond. Current proposed mechanisms for oxalate degradation by oxalate decarboxylase and oxalate oxidase suggest several essentially identical steps up to formation of the Mn-bound formyl radical [161,163]. In fact, it has been demonstrated that oxalate decarboxylase can be converted to an efficient oxalate oxidase by mutating amino acids on a flexible active site lid [164]. Fig. 10 illustrates a proposed mechanism for oxalate decarboxylase based on a number of hypotheses [7,161–166].

In 2007, the two metal centers in oxalate decarboxylase were individually characterized using high field, multifrequency EPR spectroscopy [167]. The carbon dioxide anion radical shown in step five in Fig. 10 was trapped in oxalate decarboxylase by Angerhofer and co-workers in 2011 using an EPR spin trapping technique. Through mutagenesis, the authors were able to disrupt a hydrogen bond responsible for closing the flexible loop across the substrate binding channel. This led to a buildup of a carbon dioxide anion radical. A closed conformation is required for decarboxylation, which ensures that the harmful anion radical intermediate remains bound at the active site during catalysis [168]. At the resting state of the enzyme, Mn^{2+} coordinates to three conserved histidine residues, a conserved glutamate or aspartate residue, and two water molecules [162,166]. When malate comes into the active site, it binds to the metal ion monodentately and replaces one of the water molecule ligands. Then dioxygen binds to Mn^{2+} and replaces the other water molecule. One electron transferred from the metal to the binding dioxygen results in a Mn^{3+}-superoxo species, which functions as an electron sink to facilitate the decarboxylation reaction. An electron is drawn from the bound substrate to the metal to generate a radical on the bound oxalate. Cleavage of the C—C bond of this radical intermediate releases CO_2 and gives a manganese ion-bound formyl radical anion intermediate. At this point the mechanisms of oxalate oxidase and oxalate decarboxylase purportedly divert. In the hypothetical mechanism of oxalate decarboxylase, protonation occurs at the carbanionic carbon of the formyl radical intermediate, which is followed by an electron transfer from the manganese ion to the formyl radical forming a product bound Mn^{3+}-superoxo species. Product dissociation from the active site completes the reaction cycle and regenerates the enzyme.

10.3. Mechanism of magnesium-dependent decarboxylases

Divalent metal ions can catalyze a decarboxylation reaction at the β-carbon of α-keto carboxylic acid, while decarboxylases can increase the rate of the reaction dramatically [169]. One of the most extensively studied cases is the decarboxylation of oxaloacetate. The reaction catalyzed by oxaloacetate decarboxylase is dependent on divalent metal ions. This enzyme is active with either Mn^{2+} or Mg^{2+}. Besides the divalent ion, the decarboxylation catalyzed by this enzyme from some organisms also requires biotin, while $NAD(P)^+$ is essential for the enzyme from other species. A proposed mechanism for oxaloacetate decarboxylase from *Pseudomonas putida*, which requires only divalent metal cations, is shown in Fig. 11 (upper reaction scheme) [170]. The decarboxylation catalyzed by 4-oxalocrotonate decarboxylase is believed to proceed in a mechanism similar to that of oxaloacetate decarboxylase [158]. The metal divalent cation is thought to form a complex

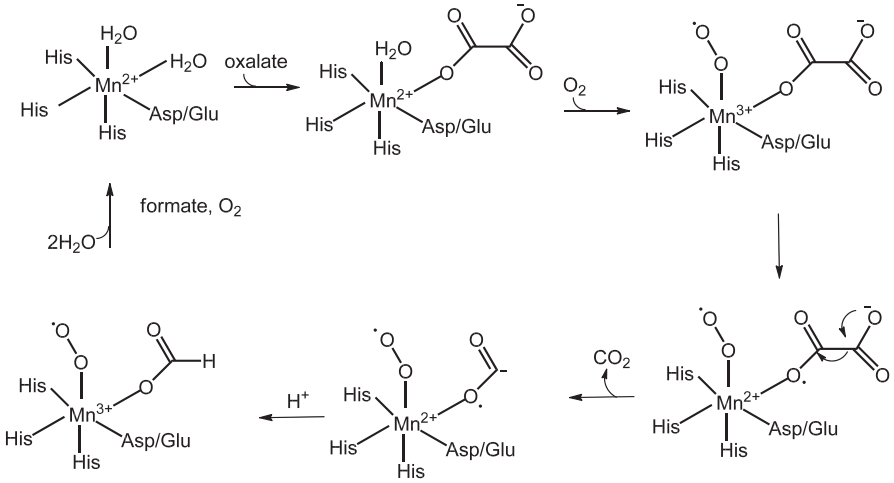

Fig. 10. Proposed mechanism for oxalate decarboxylase.

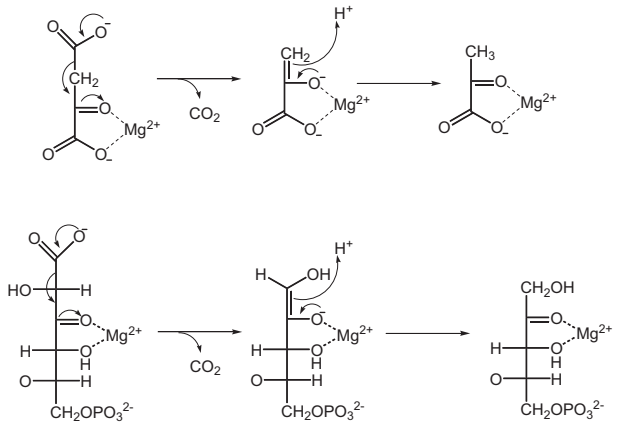

Fig. 11. Hypothetical mechanisms for magnesium-dependent decarboxylases.

with the α-keto acid moiety and one of the oxygen atoms from the carboxyl group of the substrate, functioning as an electron sink to stabilize the enolate intermediate [169,171,172]. The enzyme serves to provide substrate specificity [169] and to lower the energy of the ground state to a much more favorable value [173]. Protonation at the C_β position yields the product pyruvate. The proton incorporated in the product can be in a retained or inverted stereochemical configuration compared to the leaving carboxyl group of the substrate [174].

A recent study reported that the decarboxylation catalyzed by 3-keto-L-gulonate 6-phosphate decarboxylase is also Mg^{2+} ion-dependent. The reaction mechanism of this enzyme has been extensively studied [175–180]. These studies have provided a hypothetical mechanism shown in Fig. 11 (lower reaction scheme). In the proposed mechanism, the departure of CO_2 is facilitated by forming an enediolate intermediate stabilized by the Mg^{2+} ion. However, the required Mg^{2+} ion does not ligate to the oxygen of the carboxyl group. Instead it coordinates to the oxygen of the keto-group at the C_3 position and the oxygen of the hydroxyl-group at the C_4 position of the substrate, forming a *cis*-1,2-enediolate intermediate. Protonation of C_1 of the intermediate is completed through a proton relay system involving two alternative water molecules in the active site and the side chains of a conserved histidine or a conserved arginine as the ultimate proton sources. Protonation can lead to either R or S configurations depending on which of the two conserved residues is used as the ultimate source of the proton.

11. Transient metal-dependent, O_2-independent decarboxylases

α-Amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) was initially thought to be a cofactor-free enzyme [181,182]. It has recently been found to be a metalloenzyme belonging to the amidohydralase superfamily [183,184]. This enzyme is an illustrative example of the ACMSD protein family (also known as the Amidohydrolase_2 subfamily) [185], whose members are decarboxylases or hydrotases rather than hydrolases or deaminases. Members of the ACMSD protein family catalyze a C—C bond cleavage reaction instead of hydrolytic reaction [186,187]. Understanding the mechanism of ACMSD will shed light on the mechanisms of the continually emerging members of the ACMSD protein family. However, it is unknown whether ACMSD adopts the mechanism from the amidohydrolase_1 subfamily or follows a model evolved by other decarboxylases [188]. Fig. 12 is a mechanistic model adopted from the well-characterized amidohydrolase_1 subfamily. In the first step, the substrate binds to the enzyme with its C2 carboxylate close but not to the metal center at a rate of $2.4 \times 10^6\ s^{-1}\ M^{-1}$ [189]. An active site histidine, His228 as numbered in the *Pseudomonas fluorescens* (*Pf*) enzyme [186], assists in the deprotonation of a water ligand to form hydroxide, which then nucleophilically attacks the double bond between C_2 and C_3 of the substrate, resulting in a hydrated tetrahedral intermediate. The subsequent collapse of the tetrahedral intermediate is assisted by the concerted deprotonation of His228 and the rebinding of the hydroxyl group to the metal ion. The carboxylate attached to the C3 position becomes the leaving group. The decarboxylation step is a rapid reaction (>500 s^{-1}) but the release of the product aminomuconate-ε-semialdehyde is slow (9 s^{-1}) [189].

The amidohydrolase-derived mechanism shown in Fig. 12 predicts that the metal-bound hydroxide is the catalytic driving force of the decarboxylation reaction. The p*K*a value of the water ligand is lowered from 15.7 of the unbound state to about 9.6 because of its coordination to zinc [190,191]. This value can be further reduced to nearly the physiological pH with the assistance of an active site base catalyst, presumably His228 in *Pf*ACMSD, and therefore generates a hydroxide anion, which performs a nucleophilic attack on the C2 and C3 double bond of the substrate. As expected, mutation at His228 significantly reduces the enzyme activity and removal of the metal bound water molecule completely deprives the enzyme activity [184].

This hydroxide attack mechanism is believed to be present in the enzyme 4-oxalomesaconate hydratase, which was identified as a part of this family in a recent bioinformatics study [186]. The hydratase enzyme catalyzes the addition of a hydroxide ion and a proton across a C=C bond of its substrate. The added hydroxide is preserved in the product so that the proposed hydroxide attack for the ACMSD enzyme family is visualized in this specific member, as if it were an interruption of an ACMS decarboxylation reaction. The metal-bound hydroxide attack of the substrate is also proposed to occur in another structurally characterized decarboxylase member of the ACMSD family, γ-resorcylate decarboxylase [192]. Therefore, the metal-bound hydroxide assisted C—C/C=C cleavage is likely to be a common mechanism for the ACMSD subfamily of the amidohydrolase superfamily.

Acknowledgments

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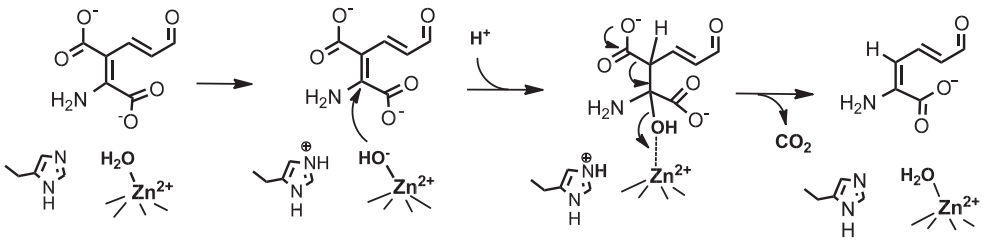

Fig. 12. A working hypothesis for mechanism of the ACMSD reaction.

References

[1] S. Ochoa, Physiol. Rev. 31 (1951) 56–106.
[2] U. Schorken, G.A. Sprenger, Biochim. Biophys. Acta—Gen. Subj. 1385 (1998) 229–243.
[3] <http://www.chem.qmul.ac.uk/iubmb/enzyme/EC4/1/1/>.
[4] N. Wu, W. Gillon, E.F. Pai, Biochemistry 41 (2002) 4002–4011.
[5] T.C. Appleby, C. Kinsland, T.P. Begley, S.E. Ealick, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 2005–2010.
[6] M.M. Benning, T. Haller, J.A. Gerlt, H.M. Holden, Biochemistry 39 (2000) 4630–4639.
[7] T.P. Begley, S.E. Ealick, Curr. Opin. Chem. Biol. 8 (2004) 508–515.
[8] I. Lieberman, A. Kornberg, E.S. Simms, J. Biol. Chem. 215 (1955) 403–451.
[9] M.A. Rishavy, W.W. Cleland, Biochemistry 39 (2000) 4569–4574.
[10] P. Harris, J.C. Navarro Poulsen, K.F. Jensen, S. Larsen, Biochemistry 39 (2000) 4217–4224.
[11] K. Kim, J. Park, S. Rhee, J. Biol. Chem. 282 (2007) 23457–23464.
[12] L. Cendron, R. Berni, C. Folli, I. Ramazzina, R. Percudani, G. Zanotti, J. Biol. Chem. 282 (2007) 18182–18189.
[13] G.J. Poelarends, H. Serrano, W.H. Johnson Jr., C.P. Whitman, Biochemistry 44 (2005) 9375–9381.
[14] J.J. Almrud, G.J. Poelarends, W.H. Johnson Jr., H. Serrano, M.L. Hackert, C.P. Whitman, Biochemistry 44 (2005) 14818–14827.
[15] A. Radzicka, R. Wolfenden, Science 267 (1995) 90–93.
[16] K. Tokuoka, Y. Kusakari, S.R. Krungkrai, H. Matsumura, Y. Kai, J. Krungkrai, T. Horii, T. Inoue, J. Biochem. 143 (2008) 69–78.
[17] C.A. Lewis, R. Wolfenden, Biochemistry 48 (2009) 8738–8745.
[18] K.K. Chan, B.M. Wood, A.A. Fedorov, E.V. Fedorov, H.J. Imker, T.L. Amyes, J.P. Richard, S.C. Almo, J.A. Gerlt, Biochemistry 48 (2009) 5518–5531.
[19] K. Toth, T.L. Amyes, B.M. Wood, K. Chan, J.A. Gerlt, J.P. Richard, J. Am. Chem. Soc. 129 (2007) 12946–12947.
[20] S.A. Barnett, T.L. Amyes, B.M. Wood, J.A. Gerlt, J.P. Richard, Biochemistry 47 (2008) 7785–7787.
[21] X.B. Wang, J.E. Dacres, X. Yang, L. Lis, V.M. Bedell, L.S. Wang, S.R. Kass, J. Am. Chem. Soc. 125 (2003) 6814–6826.
[22] M. Fujihashi, A.M. Bello, E. Poduch, L.H. Wei, S.C. Annedi, E.F. Pai, L.P. Kotra, J. Am. Chem. Soc. 127 (2005) 15048–15050.
[23] W.Y. Feng, T.J. Austin, F. Chew, S. Gronert, W. Wu, Biochemistry 39 (2000) 1778–1783.
[24] J. Retey, H.W. Liu, Curr. Opin. Chem. Biol. 8 (2004) 451–453.
[25] K. Toth, T.L. Amyes, B.M. Wood, K. Chan, J.A. Gerlt, J.P. Richard, J. Am. Chem. Soc. 132 (2010) 7018–7024.
[26] C.L. Stanton, I.F.W. Kuo, C.J. Mundy, T. Laino, K.N. Houk, J. Phys. Chem. B 111 (2007) 12573–12581.
[27] A.M. Bello, E. Poduch, M. Fujihashi, M. Amani, Y. Li, I. Crandall, R. Hui, P.I. Lee, K.C. Kain, E.F. Pai, L.P. Kotra, J. Med. Chem. 50 (2007) 915–921.
[28] J.B. French, S.E. Ealick, J. Biol. Chem. 285 (2010) 35446–35454.
[29] K. Miyamoto, H. Ohta, Eur. J. Biochem. 210 (1992) 475–481.
[30] T. Kawasaki, M. Watanabe, H. Ohta, Bull. Chem. Soc. Jpn. 68 (1995) 2017–2020.
[31] T. Kawasaki, Y. Fujioka, K. Saito, H. Ohta, Chem. Lett. 3 (1996) 195–196.
[32] K. Matoishi, M. Ueda, K. Miyamoto, H. Ohta, J. Mol. Catal. B—Enzym. 27 (2004) 161–168.
[33] Y. Terao, K. Miyamoto, H. Ohta, Chem. Commun. 34 (2006) 3600–3602.
[34] Y. Ijima, K. Matoishi, Y. Terao, N. Doi, H. Yanagawa, H. Ohta, Chem. Commun. 7 (2005) 877–879.
[35] M.P. Kalapos, Biochim. Biophys. Acta—Gen. Subj. 1621 (2003) 122–139.
[36] F.H. Westheimer, Tetrahedron 51 (1995) 3–20.
[37] T.K. Harris, G.J. Turner, IUBMB Life 53 (2002) 85–98.
[38] M.C. Ho, J.F. Menetret, H. Tsuruta, K.N. Allen, Nature 459 (2009) 393–397.
[39] P.V. Attwood, J.C. Wallace, Acc. Chem. Res. 35 (2002) 113–120.
[40] W. Buckel, Biochim. Biophys. Acta—Gen. Subj. 1505 (2001) 15–27.
[41] P.R. Hall, R. Zheng, L. Antony, M. Pusztai-Carey, P.R. Carey, V.C. Yee, EMBO J. 23 (2004) 3621–3631.
[42] M. Balsera, R.M. Buey, X.D. Li, J. Biol. Chem. 286 (2011) 9457–9467.
[43] S. Chohnah, Y. Takamura, Microbes Environ. 19 (2004) 179–189.
[44] A. Braune, K. Bendrat, S. Rospert, W. Buckel, Mol. Microbiol. 31 (1999) 473–487.
[45] S. Chohnan, T. Fujio, T. Takaki, M. Yonekura, H. Nishihara, Y. Takamura, FEMS Microbiol. Lett. 169 (1998) 37–43.
[46] J.B. Huder, P. Dimroth, J. Biol. Chem. 268 (1993) 24564–24571.
[47] P. Jockel, M. Schmid, J. Steuber, P. Dimroth, Biochemistry 39 (2000) 2307–2315.
[48] P. Dimroth, P. Jockel, M. Schmid, Biochim. Biophys. Acta—Gen. Subj. 1505 (2001) 1–14.
[49] K.S. Wendt, I. Schall, R. Huber, W. Buckel, U. Jacob, EMBO J. 22 (2003) 3493–3502.
[50] H. Hilbi, I. Dehning, B. Schink, P. Dimroth, Eur. J. Biochem. 207 (1992) 117–123.
[51] E. Strauss, H. Zhai, L.A. Brand, F.W. McLafferty, T.P. Begley, Biochemistry 43 (2004) 15520–15533.
[52] P. Hernandez-Acosta, D.G. Schmid, G. Jung, F.A. Culianez-Macia, T. Kupke, J. Biol. Chem. 277 (2002) 20490–20498.
[53] M. Blaesse, T. Kupke, R. Huber, S. Steinbacher, EMBO J. 19 (2000) 6299–6310.
[54] T. Kupke, M. Uebele, D. Schmid, G. Jung, M. Blaesse, S. Steinbacher, J. Biol. Chem. 275 (2000) 31838–31846.
[55] F. Majer, D.G. Schmid, K. Altena, G. Bierbaum, T. Kupke, J. Bacteriol. 184 (2002) 1234–1243.
[56] M. Daugherty, B. Polanuyer, M. Farrell, M. Scholle, A. Lykidis, V. de Crecy-Lagard, A. Osterman, J. Biol. Chem. 277 (2002) 21431–21439.
[57] E. Strauss, C. Kinsland, Y. Ge, F.W. McLafferty, T.P. Begley, J. Biol. Chem. 276 (2001) 13513–13516.
[58] S. Steinbacher, P. Hernandez-Acosta, B. Bieseler, M. Blaesse, R. Huber, F.A. Culianez-Macia, T. Kupke, J. Mol. Biol. 327 (2003) 193–202.
[59] C. Jiang, B. Wu, Biochem. Biophys. Res. Commun. 357 (2007) 421–426.
[60] E.S. Rangarajan, Y. Li, P. Iannuzzi, A. Tocilj, L.W. Hung, A. Matte, M. Cygler, Protein Sci. 13 (2004) 3006–3016.
[61] M. Blaesse, T. Kupke, R. Huber, S. Steinbacher, Acta Crystallogr. D. Biol. Crystallogr. 59 (2003) 1414–1421.
[62] E. Strauss, T.P. Begley, Bioorg. Med. Chem. Lett. 13 (2003) 339–342.
[63] E. Strauss, T.P. Begley, J. Am. Chem. Soc. 123 (2001) 6449–6450.
[64] T. Selmer, P.I. Andrei, Eur. J. Biochem. 268 (2001) 1363–1372.
[65] B.M. Martins, M. Blaser, M. Feliks, G.M. Ullmann, W. Buckel, T. Selmer, J. Am. Chem. Soc. 133 (2011) 14666–14674.
[66] A.F. Wagner, M. Frey, F.A. Neugebauer, W. Schäfer, J. Knappe, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 996–1000.
[67] X. Sun, J. Harder, M. Krook, H. Jörnvall, B.M. Sjöberg, P. Reichard, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 577–581.
[68] E. Torrents, G. Buist, A. Liu, R. Eliasson, J. Kok, I. Gibert, A. Gräslund, P. Reichard, J. Biol. Chem. 275 (2000) 2463–2471.
[69] T.L. Grove, J.S. Benner, M.I. Radle, J.H. Ahlum, B.J. Landgraf, C. Krebs, S.J. Booker, Science 332 (2011) 604–607.
[70] L. Yu, M. Blaser, P.I. Andrei, A.J. Pierik, T. Selmer, Biochemistry 45 (2006) 9584–9592.
[71] R.Y. Hsu, H.A. Lardy, J. Biol. Chem. 242 (1967) 520–526.
[72] G. Loeber, A.A. Infante, I. Maurer-Fogy, E. Krystek, M.B. Dworkin, J. Biol. Chem. 266 (1991) 3016–3021.
[73] X. Tao, Z. Yang, L. Tong, Structure 11 (2003) 1141–1150.
[74] D.E. Graham, H. Xu, R.H. White, J. Biol. Chem. 277 (2002) 23500–23507.
[75] C.B. Grissom, W.W. Cleland, Biochemistry 27 (1988) 2934–2943.
[76] S. Pontremoli, A. De Flora, E. Grazi, G. Mangiarottig, A. Bonsignore, B.L. Horecker, J. Biol. Chem. 236 (1961) 2975–2980.
[77] W.E. Karsten, P.A. Tipton, P.F. Cook, Biochemistry 41 (2002) 12193–12199.
[78] J.L. Moriarity, K.J. Hurt, A.C. Resnick, P.B. Storm, W. Laroy, R.L. Schnaar, S.H. Snyder, J. Biol. Chem. 277 (2002) 16968–16975.
[79] M. Bar-Peled, C.L. Griffith, T.L. Doering, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 12003–12008.
[80] P.Z. Gatzeva-Topalova, A.P. May, M.C. Sousa, Biochemistry 43 (2004) 13370–13379.
[81] G.J. Williams, S.D. Breazeale, C.R. Raetz, J.H. Naismith, J. Biol. Chem. 280 (2005) 23000–23008.
[82] D. Gachotte, R. Barbuch, J. Gaylor, E. Nickel, M. Bard, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 13794–13799.
[83] W.W. Cleland, Acc. Chem. Res. 32 (1999) 862–868.
[84] G.G. Chang, L. Tong, Biochemistry 42 (2003) 12721–12733.
[85] D. Liu, W.E. Karsten, P.F. Cook, Biochemistry 39 (2000) 11955–11960.
[86] P.D. Sender, M.G. Martin, S. Peiru, C. Magni, FEBS Lett. 570 (2004) 217–222.
[87] C.C. Hwang, A.J. Berdis, W.E. Karsten, W.W. Cleland, P.F. Cook, Biochemistry 37 (1998) 12596–12602.
[88] B.C. Narayanan, W. Niu, Y. Han, J. Zou, P.S. Mariano, D. Dunaway-Mariano, O. Herzberg, Biochemistry 47 (2008) 167–182.
[89] F.W. Alexander, E. Sandmeier, P.K. Mehta, P. Christen, Eur. J. Biochem. 219 (1994) 953–960.
[90] H. Hayashi, F. Tsukiyama, S. Ishii, H. Mizuguchi, H. Kagamiyama, Biochemistry 38 (1999) 15615–15622.
[91] D.E. Stevenson, M. Akhtar, D. Gani, Biochemistry 29 (1990) 7631–7647.
[92] G.D. Kalyankar, E.E. Snell, Biochemistry 1 (1962) 594–600.
[93] L.K. Jackson, H.B. Brooks, D.P. Myers, M.A. Phillips, Biochemistry 42 (2003) 2933–2940.
[94] M.D. Toney, Arch. Biochem. Biophys. 433 (2005) 279–287.
[95] C. Walsh, Enzymatic reactions requiring pyridoxal phosphate, in: A.C. Bartlett, L.W. McCombs (Eds.), W.H. Freeman and Co., San Francisco, 1979, pp. 777–827.
[96] M.H. O'Leary, G.J. Piazza, Biochemistry 20 (1981) 2743–2748.
[97] X. Zhou, M.D. Toney, Biochemistry 38 (1999) 311–320.
[98] X. Zhou, X. Jin, R. Medhekar, X. Chen, T. Dieckmann, M.D. Toney, Biochemistry 40 (2001) 1367–1377.
[99] R.S. Phillips, S. Lima, R. Khristoforov, B. Sudararaju, Biochemistry 49 (2010) 5066–5073.
[100] W.D. Tolbert, J.L. Ekstrom, I.I. Mathews, J.A. Secrist III, P. Kapoor, A.E. Pegg, S.E. Ealick, Biochemistry 40 (2001) 9484–9494.
[101] A. Albert, V. Dhanaraj, U. Genschel, G. Khan, M.K. Ramjee, R. Pulido, B.L. Sibanda, F. von Delft, M. Witty, T.L. Blundell, A.G. Smith, C. Abell, Nat. Struct. Biol. 5 (1998) 289–293.
[102] T.A. Alston, R.H. Abeles, Biochemistry 26 (1987) 4082–4085.
[103] W. Dowhan, Methods Enzymol. 280 (1997) 81–88.
[104] J.L. Ekstrom, W.D. Tolbert, H. Xiong, A.E. Pegg, S.E. Ealick, Biochemistry 40 (2001) 9495–9504.

[105] T. Gallagher, E.E. Snell, M.L. Hackert, J. Biol. Chem. 264 (1989) 12737–12743.
[106] F. Jordan, Science 306 (2004) 818–820.
[107] W. Versees, S. Spaepen, J. Vanderleyden, J. Steyaert, FEBS J. 274 (2007) 2363–2375.
[108] D. Kern, G. Kern, H. Neef, K. Tittmann, M. Killenberg-Jabs, C. Wikner, G. Schneider, G. Hubner, Science 275 (1997) 67–70.
[109] A. Schellenberger, Biochim. Biophys. Acta—Gen. Subj. 1385 (1998) 177–186.
[110] F. Jordan, N.S. Nemeria, E. Sergienko, Acc. Chem. Res. 38 (2005) 755–763.
[111] M.A. Lie, L. Celik, K.A. Jorgensen, B. Schiott, Biochemistry 44 (2005) 14792–14806.
[112] F. Jordan, Nat. Prod. Rep. 20 (2003) 184–201.
[113] T.G. Mosbacher, M. Mueller, G.E. Schulz, FEBS J. 272 (2005) 6067–6076.
[114] S. Konig, M. Spinka, S. Kutter, J. Mol. Catal. B—Enzym. 61 (2009) 100–110.
[115] D.J. Kuo, F. Jordan, J. Biol. Chem. 258 (1983) 13415–13417.
[116] S.W. Ragsdale, Chem. Rev. 103 (2003) 2333–2346.
[117] F. Jordan, N.S. Nemeria, S. Zhang, Y. Yan, P. Arjunan, W. Furey, J. Am. Chem. Soc. 125 (2003) 12732–12738.
[118] Y.A. Muller, G.E. Schulz, Science 259 (1993) 965–967.
[119] K. Tittmann, R. Golbik, K. Uhlemann, L. Khailova, G. Schneider, M. Patel, F. Jordan, D.M. Chipman, R.G. Duggleby, G. Hubner, Biochemistry 42 (2003) 7885–7891.
[120] R. Kluger, G. Ikeda, Q. Hu, P. Cao, J. Drewry, J. Am. Chem. Soc. 128 (2006) 15856–15864.
[121] E. Fiedler, S. Thorell, T. Sandalova, R. Golbik, S. Konig, G. Schneider, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 591–595.
[122] O.P. Ward, A. Singh, Curr. Opin. Biotechnol. 11 (2000) 520–526.
[123] H. Iding, P. Siegert, K. Mesch, M. Pohl, Biochim. Biophys. Acta—Gen. Subj. 1385 (1998) 307–322.
[124] A.K. Bera, L.S. Polovnikova, J. Roestamadji, T.S. Widlanski, G.L. Kenyon, M.J. McLeish, M.S. Hasson, J. Am. Chem. Soc. 129 (2007) 4120–4121.
[125] M. Fries, H.I. Jung, R.N. Perham, Biochemistry 42 (2003) 6996–7002.
[126] S.O. Mansoorabadi, J. Seravalli, C. Furdui, V. Krymov, G.J. Gerfen, T.P. Begley, J. Melnick, S.W. Ragsdale, G.H. Reed, Biochemistry 45 (2006) 7122–7131.
[127] G. Wille, D. Meyer, A. Steinmetz, E. Hinze, R. Golbik, K. Tittmann, Nat. Chem. Biol. 2 (2006) 324–328.
[128] M. Zeida, M. Wieser, T. Yoshida, T. Sugio, T. Nagasawa, Appl. Environ. Microbiol. 64 (1998) 4743–4747.
[129] F. Pojer, R. Kahlich, B. Kammerer, S.M. Li, L. Heide, J. Biol. Chem. 278 (2003) 30661–30668.
[130] A.G. Prescott, M.D. Lloyd, Nat. Prod. Rep. 17 (2000) 367–383.
[131] R.P. Hausinger, Crit. Rev. Biochem. Mol. Biol. 39 (2004) 21–68.
[132] K.I. Kivirikko, T. Pihlajaniemi, Adv. Enzymol. Relat. Areas Mol. Biol. 72 (1998) 325–398.
[133] M.D. Lloyd, H.J. Lee, K. Harlos, Z.H. Zhang, J.E. Baldwin, C.J. Schofield, J.M. Charnock, C.D. Garner, T. Hara, A.C. Terwisscha van Scheltinga, K. Valegard, J.A. Viklund, J. Hajdu, I. Andersson, A. Danielsson, R. Bhikhabhai, J. Mol. Biol. 287 (1999) 943–960.
[134] X. Yin, T. O'Hare, S.J. Gould, T.M. Zabriskie, Gene 312 (2003) 215–224.
[135] C.J. Schofield, P.J. Ratcliffe, Nat. Rev. Mol. Cell Biol. 5 (2004) 343–354.
[136] T. Duncan, S.C. Trewick, P. Koivisto, P.A. Bates, T. Lindahl, B. Sedgwick, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 16660–16665.
[137] S.C. Trewick, T.F. Henshaw, R.P. Hausinger, T. Lindahl, B. Sedgwick, Nature 419 (2002) 174–178.
[138] Y. Tsukada, J. Fang, H. Erdjument-Bromage, M.E. Warren, C.H. Borchers, P. Tempst, Y. Zhang, Nature 439 (2006) 811–816.
[139] E.L. Hegg, L. Que, Eur. J. Biochem. 250 (1997) 625–629.
[140] C. Krebs, D. Galonic Fujimori, C.T. Walsh, J.M. Bollinger Jr., Acc. Chem. Res. 40 (2007) 484–492.
[141] J.C. Price, E.W. Barr, T.E. Glass, C. Krebs, J.M. Bollinger Jr., J. Am. Chem. Soc. 125 (2003) 13008–13009.
[142] M. Costas, M.P. Mehn, M.P. Jensen, L. Que Jr., Chem. Rev. 104 (2004) 939–986.
[143] M.L. Neidig, A. Decker, O.W. Choroba, F. Huang, M. Kavana, G.R. Moran, J.B. Spencer, E.I. Solomon, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 12966–12973.
[144] L.M. Hoffart, E.W. Barr, R.B. Guyer, J.M. Bollinger Jr., C. Krebs, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 14738–14743.
[145] E.I. Solomon, T.C. Brunold, M.I. Davis, J.N. Kemsley, S.K. Lee, N. Lehnert, F. Neese, A.J. Skulan, Y.S. Yang, J. Zhou, Chem. Rev. 100 (2000) 235–350.
[146] D.A. Proshlyakov, T.F. Henshaw, G.R. Monterosso, M.J. Ryle, R.P. Hausinger, J. Am. Chem. Soc. 126 (2004) 1022–1023.
[147] P.K. Grzyska, E.H. Appelman, R.P. Hausinger, D.A. Proshlyakov, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 3982–3987.
[148] P.L. Roach, I.J. Clifton, C.M. Hensgens, N. Shibata, C.J. Schofield, J. Hajdu, J.E. Baldwin, Nature 387 (1997) 827–830.
[149] J.M. Elkins, M.J. Ryle, I.J. Clifton, J.C. Dunning Hotopp, J.S. Lloyd, N.I. Burzlaff, J.E. Baldwin, R.P. Hausinger, P.L. Roach, Biochemistry 41 (2002) 5185–5192.
[150] M.J. Ryle, A. Liu, R.B. Muthukumaran, R.Y. Ho, K.D. Koehntop, J. McCracken, L. Que Jr., R.P. Hausinger, Biochemistry 42 (2003) 1854–1862.
[151] J.C. Price, E.W. Barr, L.M. Hoffart, C. Krebs, J.M. Bollinger Jr., Biochemistry 44 (2005) 8138–8147.
[152] J.C. Price, E.W. Barr, B. Tirupati, J.M. Bollinger Jr., C. Krebs, Biochemistry 42 (2003) 7497–7508.
[153] M.J. Ryle, K.D. Koehntop, A. Liu, L. Que Jr., R.P. Hausinger, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 3790–3795.
[154] H.J. Lee, M.D. Lloyd, K. Harlos, I.J. Clifton, J.E. Baldwin, C.J. Schofield, J. Mol. Biol. 308 (2001) 937–948.
[155] P.F. Fitzpatrick, Biochemistry 42 (2003) 14083–14091.
[156] G.R. Moran, Arch. Biochem. Biophys. 433 (2005) 117–128.
[157] L.C. Blasiak, F.H. Vaillancourt, C.T. Walsh, C.L. Drennan, Nature 440 (2006) 368–371.
[158] T.M. Stanley, W.H. Johnson Jr., E.A. Burks, C.P. Whitman, C.C. Hwang, P.F. Cook, Biochemistry 39 (2000) 718–726.
[159] S. Harayama, M. Rekik, K.L. Ngai, L.N. Ornston, J. Bacteriol. 171 (1989) 6251–6258.
[160] A. Tanner, L. Bowater, S.A. Fairhurst, S. Bornemann, J. Biol. Chem. 276 (2001) 43627–43634.
[161] D. Svedruzic, S. Jonsson, C.G. Toyota, L.A. Reinhardt, S. Ricagno, Y. Lindqvist, N.G. Richards, Arch. Biochem. Biophys. 433 (2005) 176–192.
[162] O. Opaleye, R.S. Rose, M.M. Whittaker, E.J. Woo, J.W. Whittaker, R.W. Pickersgill, J. Biol. Chem. 281 (2006) 6428–6433.
[163] V.J. Just, M.R. Burrell, L. Bowater, I. McRobbie, C.E. Stevenson, D.M. Lawson, S. Bornemann, Biochem. J. 407 (2007) 397–406.
[164] M.R. Burrell, V.J. Just, L. Bowater, S.A. Fairhurst, L. Requena, D.M. Lawson, S. Bornemann, Biochemistry 46 (2007) 12327–12336.
[165] L.A. Reinhardt, D. Svedruzic, C.H. Chang, W.W. Cleland, N.G. Richards, J. Am. Chem. Soc. 125 (2003) 1244–1252.
[166] R. Anand, P.C. Dorrestein, C. Kinsland, T.P. Begley, S.E. Ealick, Biochemistry 41 (2002) 7659–7669.
[167] A. Angerhofer, E.W. Moomaw, I. Garcia-Rubio, A. Ozarowski, J. Krzystek, R.T. Weber, N.G.J. Richards, J. Phys. Chem. B 111 (2007) 5043–5046.
[168] W. Imaram, B.T. Saylor, C.P. Centonze, N.G.J. Richards, A. Angerhofer, Free Radical. Biol. Med. 50 (2011) 1009–1015.
[169] R. Steinberger, F.H. Westheimer, J. Am. Chem. Soc. 73 (1951) 429–435.
[170] G.L. Waldrop, B.F. Braxton, J.L. Urbauer, W.W. Cleland, D.M. Kiick, Biochemistry 33 (1994) 5262–5267.
[171] B. Vennesland, M.C. Gollub, J.F. Speck, J. Biol. Chem. 178 (1949) 301–314.
[172] J.F. Speck, J. Biol. Chem. 178 (1949) 315–324.
[173] J.H. Hurley, S.J. Remington, J. Am. Chem. Soc. 114 (1992) 4769–4773.
[174] J.A. Piccirilli, J.D. Rozzell Jr., S.A. Benner, J. Am. Chem. Soc. 109 (1987) 8084–8085.
[175] E. Wise, W.S. Yew, P.C. Babbitt, J.A. Gerlt, I. Rayment, Biochemistry 41 (2002) 3861–3869.
[176] E.L. Wise, W.S. Yew, J.A. Gerlt, I. Rayment, Biochemistry 42 (2003) 12133–12142.
[177] W.S. Yew, E.L. Wise, I. Rayment, J.A. Gerlt, Biochemistry 43 (2004) 6427–6437.
[178] E.L. Wise, W.S. Yew, J.A. Gerlt, I. Rayment, Biochemistry 43 (2004) 6438–6446.
[179] W.S. Yew, J. Akana, E.L. Wise, I. Rayment, J.A. Gerlt, Biochemistry 44 (2005) 1807–1815.
[180] E.L. Wise, W.S. Yew, J. Akana, J.A. Gerlt, I. Rayment, Biochemistry 44 (2005) 1816–1823.
[181] S. Fukuoka, K. Ishiguro, K. Yanagihara, A. Tanabe, Y. Egashira, H. Sanada, K. Shibata, J. Biol. Chem. 277 (2002) 35162–35167.
[182] T. Muraki, M. Taki, Y. Hasegawa, H. Iwaki, P.C. Lau, Appl. Environ. Microbiol. 69 (2003) 1564–1572.
[183] T. Li, A.L. Walker, H. Iwaki, Y. Hasegawa, A. Liu, J. Am. Chem. Soc. 127 (2005) 12282–12290.
[184] T.F. Li, H. Iwaki, R. Fu, Y. Hasegawa, H. Zhang, A.M. Liu, Biochemistry 45 (2006) 6628–6634.
[185] A. Liu, H. Zhang, Biochemistry 45 (2006) 10407–10411.
[186] D. Martynowski, Y. Eyobo, T.F. Li, K. Yang, A.M. Liu, H. Zhang, Biochemistry 45 (2006) 10412–10421.
[187] A. Liu, T. Li, R. Fu, Encyclopedia of Life Sciences, John Wiley & Sons, Ltd., Chichester, 2007. http://dx.doi.org/10.1002/9780470015902.a0020546. <http://www.els.net/>.
[188] M.E. Glasner, J.A. Gerlt, P.C. Babbitt, Curr. Opin. Chem. Biol. 10 (2006) 492–497.
[189] T. Li, J.K. Ma, J.P. Hosler, V.L. Davidson, A. Liu, J. Am. Chem. Soc. 129 (2007) 9278–9279.
[190] J.M. Bergy, J.L. Tymoczko, L. Stryer, Biochemistry, fifth ed., W.H. Freeman and Company, New York, 2002.
[191] F.A. Frey, A.D. Hegeman, Enzymatic Reaction Mechanisms, Oxford University Press, New York, 2007.
[192] M. Goto, H. Hayashi, I. Miyahara, K. Hirotsu, M. Yoshida, T. Oikawa, J. Biol. Chem. 281 (2006) 34365–34373.