D. DECARBOXYLASES

Background

Because of the stability of carbon dioxide, decarboxylation reactions are generally spontaneous processes. For example the decarboxylation of acetic acid (Figure D.1) takes place with a ΔG° of -6 kcal/mol. However, the reaction is exceptionally slow thanks to a very high $\Delta G^{\neq}$. The intermediate in the decarboxylation leads to negative charge build up on the methyl group that has no obvious means of stabilization. Methyl anions just aren't going to form as intermediates at room temperature

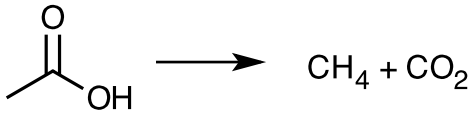


Figure D.1. The decarboxylation of acetic acid.

On the other hand some decarboxylations proceed through stable carbanion intermediates and proceed relatively rapidly at room temperature. Those reactions are also readily catalyzed by enzymes, which can provide further stabilization. A couple of examples discussed below are **malonyl-CoA decarboxylase, oxaloacetate decarboxylase** and **acetoacetate decarboxylase**. Then there are the more difficult cases that still must be processed by enzymes. Once case, **orotidine decarboxylase**, is a miracle, but others proceed through the use of **enzyme cofactors** or **coenzymes** that provide unique chemical functionalities to the enzyme, providing a mechanistic path through a stabilized carbanion. **Histidine decarboxylase** uses an intrinisic **pyruvoyl** cofactor generated in situ, while **L-DOPA decarboxylase**, performing similar chemistry, uses a **pyridoxal** cofactor. **Pyruvate decarboxylase**, uses **thiamine**, which is essential in the decarboxylation of α-ketoacids. These are their stories.

Decarboxylations of β-Keto Acids

Malonyl-CoA Decarboxylase[1]

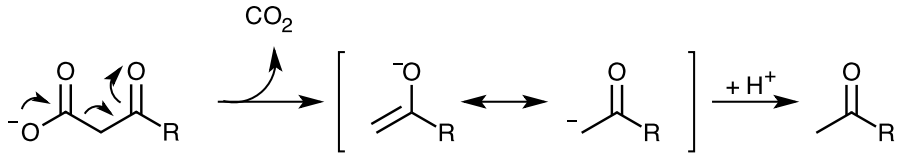


Figure D.2. Decarboxylation of a β-keto carboxylate. Note the resonance stabilization of the intermediate.

[1] Actually, this is an activity contained by an enzyme that uses the product of decarboxylation, acetyl-CoA, in a subsequent step. But we're just interested in decarboxylation today.

As a rule, the decarboxylations of β-keto acids are unchallenging chemical transformations. The CO_2 leaves behind a carbanion β to a carbonyl group that is stabilized through resonance. Figure D.2. Malonyl-CoA decarboxylase (MCDCase) decarboxylates malonyl-CoA to produce acetyl-CoA, a key intermediate in metabolism. Coenzyme A is the prototypical "big moosey creature" but can be simply considered in this case to be a thiol that condenses with malonic acid to form a thioester (Figure D.3).

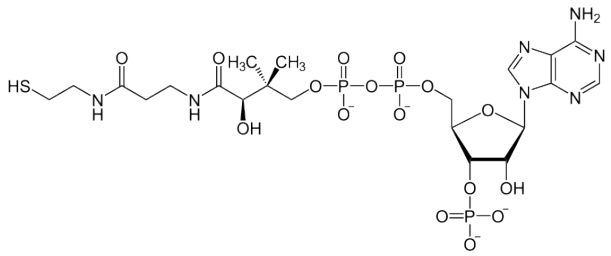


Figure D.3. Structure of coenzyme A (CoA). Note thiol functionality.

As a simple enzyme using nothing but amino acid side chains to catalyze the reaction, MCDCase must either succeed in destabilizing the substrate relative to the transition state or stabilizing the transition state relative to the substrate (not quite the same thing). It appears that the enzyme does both things. In a structure of hexanoyl-CoA (a product analog of acetyl-CoA) bound to the enzyme, an "oxyanion" hole for the carbonyl oxygen was observed containing residues Asn336 and His303 (Figure D.4). Each is positioned to form a hydrogen bond with the carbonyl oxygen as it accepts electron density following decarboxylation. Not surprisingly, mutagenesis of these residues leads to sharp decreases in activity. Most mutations to His303 abolish activity, except for the Gln mutation (Table D.1), while the mutation of Asn336 to alanine reduces k_{cat} by 100-fold, indicative of a 2.8 kcal/mol stabilization of the transition state relative to the substrate via that amide-containing side chain.[2]

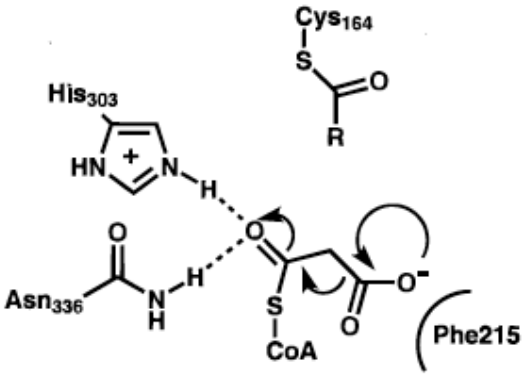


Figure D.4. Arrangement of active site responsible for malonyl-CoA decarboxylation. (Taken without permission from Ref. 2).

[2] Jez et al. (2000) Dissection of Malonyl-CoA Decarboxylation from Polyketide Formation... *Biochemistry* **39**, 890-902.

Substrate destabilization is a little more subtle. Phe215 is positioned roughly where the carboxylate of the malonyl group would be expected to reside. Binding the substrate in that environment is destabilizing relative to solvent. In water, the carboxylate is capable of forming H-bonds and obtaining enthalpic stabilization of its charged state. In the enzyme active site, the carboxylate receives no such stabilizing interactions, while the transition state – which removes negative charge from the carboxylate is expected to be less affected by the presence of phenylalanine at that position. To demonstrate the point, replacement of Phe215 with the polar serine residue lowers k_{cat} fifty-fold.

An alternate interpretation is that Phe215 positions the carboxylate appropriately for reactivity. As decarboxylation proceeds, negative charge is delivered to a p-orbital that develops on the sp^2 hybridized carbon α to the thioester. For maximum stabilization, that p-orbital should be aligned with the p-orbitals of the carbonyl carbon and oxygen to form a three center π molecular orbital. Thus, if the enzyme positions the carboxylate at an angle 90° to the carbonyl group, there will be optimal stabilization of the growing negative charge left behind during loss of CO_2.

Table D.1. Kinetic data from malonyl-CoA decarboxylase.

Enzyme	**k_{cat} (s^{-1})**	**K_m (μM)**	**k_{cat}/K_m ($M^{-1}s^{-1}$)**
WT	70	20	3.6×10^6
Asn336→Ala	0.5	70	7×10^3
His303→Gln	11	25	4.4×10^5
Phe215→Ser	1.3	55	2.4×10^4

Acetoacetate Decarboxylase

This enzyme has two important historical roles. The first is in the formation of Israel. Acetoacetate decarboxylase (AADCase) converts acetoacetate to acetone and CO_2 (Figure D.5) in the bacterium *Clostridium acetobutylicum*. Chaim Weizmann was a biochemist living in England in World War I who developed a fermentation process for the production of acetone, a necessary solvent in the manufacture of cordite. Legend has it that Weizmann traded the recipe for manufacturing cordite for English support of Zionism (see the one-page play *Arthur and the Acetone* in Appendix 1). Its other historical role is in enzyme mechanism. AADCase is one of the early examples of pK_a tuning in an enzyme active site (see below).

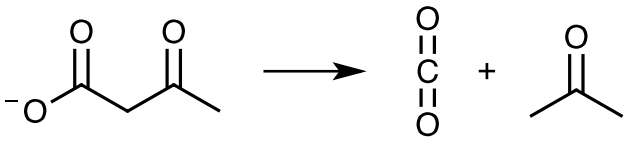


Figure D.5. Decarboxylation of acetoacetate.

The decarboxylation of acetoacetate is not particularly slow. The half-life for the process is about 7 hr, with a k_{uncat} of 3 x 10^{-5} s^{-1}. The enzyme is, however, pretty good at what it does – with a k_{cat} of 1560 s^{-1} and K_m of 8.2 mM. The k_{cat}/K_m value of 2 x 10^5 $M^{-1}s^{-1}$ indicates that the enzyme operates a little ways from the diffusion limit, but the proficiency constant, $k_{uncat}/(k_{cat}/K_m)$ is 10^{-11} M, suggesting a stabilization of the transition state by the enzyme of 15 kcal/mol.

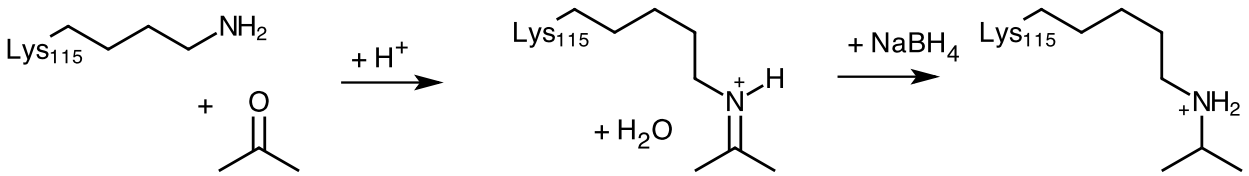


Figure D.6. Formation of a Schiff's base (iminium ion) between Lys115 and acetone. The iminium group can be reduced by borohydride to produce an irreversibly inhibited enzyme.

In the 1960's, the Westheimer group showed that an active site lysine, Lys115, forms a Schiff's base (iminium ion) with the product, acetone, which can be trapped by reduction with $NaBH_4$ (Figure D.6). The value of an Schiff's base is apparent even in a solution study. It has been shown that the use of H_2NCH_2CN to form a Schiff's base with acetoacetate can accelerate the rate of decarboxylation immensely. Because the iminium group is positively charged, there is no build up of positive charge in the transition state for decarboxylation. Instead there is a loss of positive charge on the iminium group, providing an enthalpically more accessible transition state. The decarboxylation of the Schiff's base of acetoacetate proceeds with a rate constant of 0.1 s^1, a 10,000-fold enhancement relative to the β-keto acid (Figure D.7).

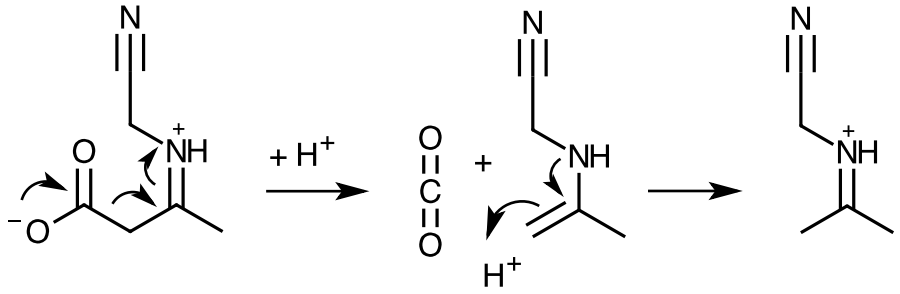


Figure D.7. Formation of a Schiff's base with a simple amine provides access to transition state that *has less charge* than the ground state, creating a lower enthalpic barrier for decarboxylation.

Interestingly, a plot of rate vs. pH for inactivation of the enzyme by 2,4-dinitrophenyl propionate suggests that the pK_a of 6 for the lysine side chain, a significant lowering from the standard value of 10. A neutral lysine is predicted to be a better nucleophile, so the alteration in pK_a serves a distinct biochemical function. In examining potential causes for the depressed pK_a, it was noted

that Lys115 is adjacent in sequence to Lys116. It was suggested that the proximity of two positively charged amines would be mutually destabilizing. If Lys116 is maintained in a cationic state, then Lys115 would have a lower pK_a than the average amine.

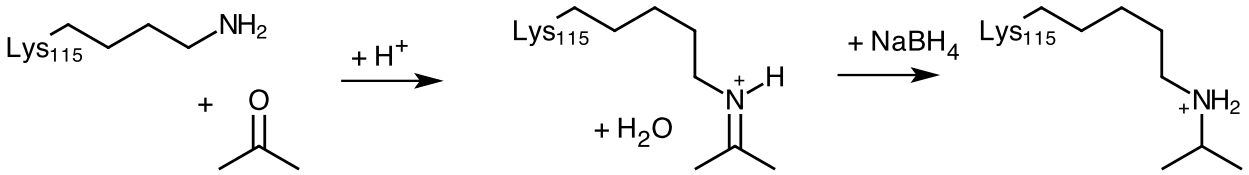
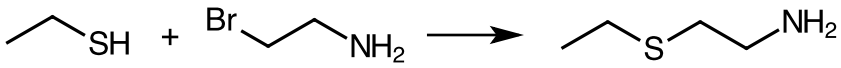


Figure D.6. Formation of a Schiff's base (iminium ion) between Lys115 and acetone. The iminium group can be reduced by borohydride to produce an irreversibly inhibited enzyme.

The Gerlt lab further investigated the pK_a shift of Lys115 by preparing a series of mutations to Lys116.[3] When Lys116 is mutated to a neutral residue, like Gln or Cys, the pK_a of Lys115 rises to over 9 and the enzyme becomes inactive. If, however, Lys116 is mutated to another cationic residdue, Arg, the enzyme retains high activity and a low pK_a for the side chain of Lys115. In a nifty experiment, this paper looks at a covalently modified version of the K116C mutant. When the cysteine is reacted with bromoethylamine, an ammonium ion is reinstated at position 116, and the modified mutant is only three-fold less active than the wild type enzyme.



Scheme D.1

Table D.2. Kinetic data and pK_as of Lys115 in a series of Lys116 mutants.

AADCase Variant	k_{cat} (s^{-1})	K_m(mM)	pK_a of Lys115
WT	1560	8.2	6.40
K116C	0	-	>9.2
K116N	0	-	>9.2
K116R	300	15	6.3
K116C-EA	410	8	5.9

Given this strong support for the modulation of the pK_a of Lys115 by its cationic neighbor, the crystal structure that appeared, finally, in 2009 was a huge surprise.[4] The ammonium group of Lys116 is *nowhere* near Lys115 (Figure D.8A). Instead they point in opposite directions, with the ammonium group of Lys116 fixed in position by two H-bonds 15 Å away from Lys115's –

[3] Highbarger et al. (1997) Mechanism of the Reaction Catalyzed by Acetoacetate Decarboxylase. *Biochemistry* **35**, 41-46.

[4] Ho et al. (2009) The origin of the electrostatic perturbation in acetoacetate decarboxylase. *Nature* **459**, 393-398.

NH_3^+ group, which forms no H-bonds (Figure D.8B). In fact, that is the apparent cause of the low pK_a for Lys115. It is in a non-polar environment and the conjugate acid form is destabilized.

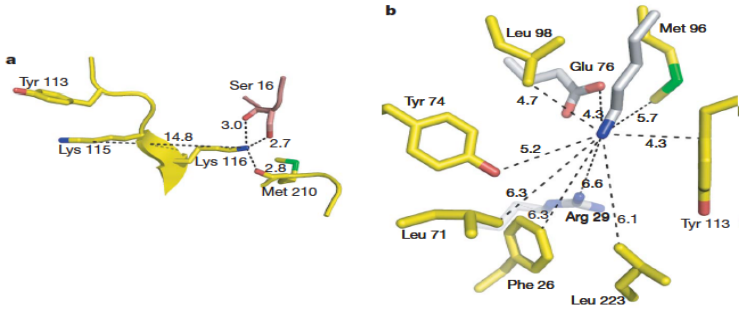


Figure D.8. Structure of the active site a AADCase. (A) Note that the side chains of K115 and K116 are oriented 180° apart. (B) The hydrophobic environment around K115. (Taken without permission from Ref. 4).

While the Schiff's base intermediate is natively more active for decarboxylation than the ketone, additional contributions to activity are made by the active site of AADCase. Glu76 was identified in a liganded complex of AADCase to occupy a position uncomfortably close to the substrate carboxylate, thereby destabilizing the carboxylate relative to solution. As in MCDCase above, this destabilization renders the transition state more enthalpically accessible and contributes to a lower $\Delta G^{\neq}$. Mutation of Glu76 to Gln reduces k_{cat} by 250-fold, indicating a roughly 3 kcal/mol destabilization of the substrate. The authors of the 2009 report present a comprehensive and believable mechanism for catalysis in light of their structure (Figure D.9).

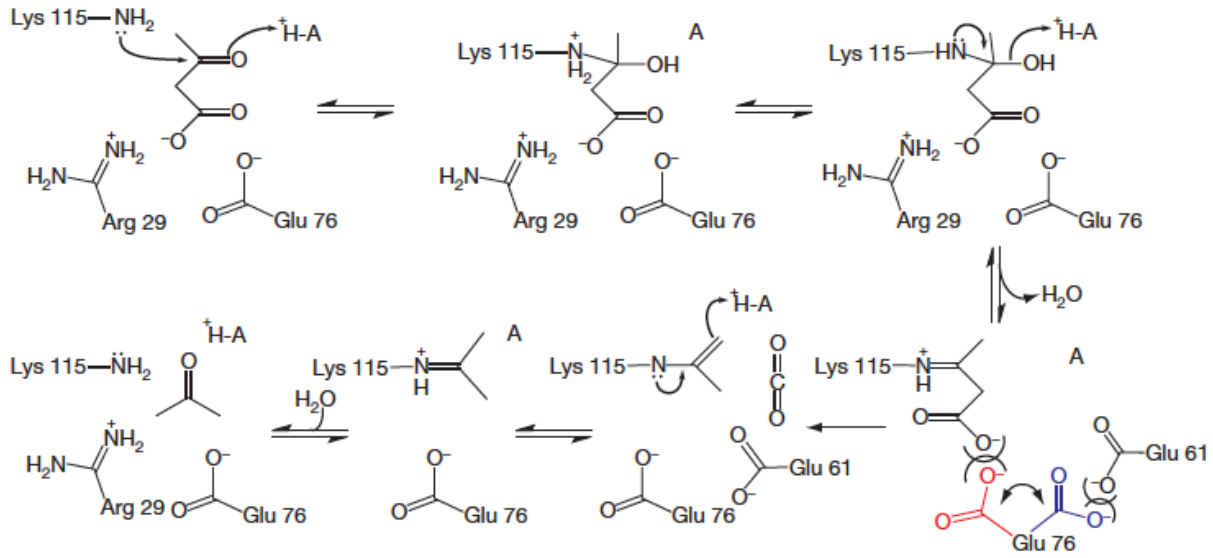


Figure D.9. Mechanism of AADCase (from Ref. 4 without permission).

Oxaloacetate Decarboxylase

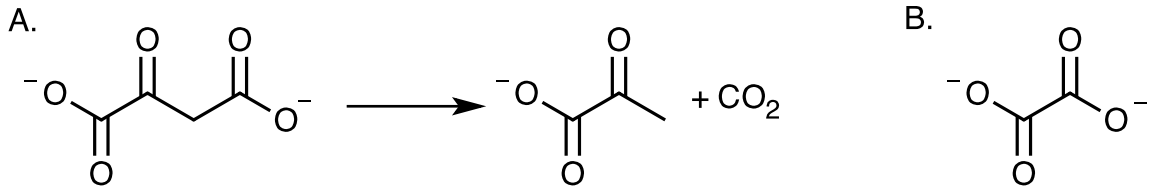


Figure D.10 (A) Reaction catalyzed by oxaloacetate decarboxylate. Oxaloacetate is converted to pyruvate and CO_2. (B) Oxalate, a competitive inhibitor of OADCase.

Although catalysis of the decarboxylation of β-keto acids is facile, and as shown above, can be achieved using only the 20 regularly occurring amino acids, it is possible to achieve acceleration through other routes. Metal ions are routinely used in enzyme catalysis thanks to their properties as Lewis acids and their capacity to perform redox chemistry. Oxaloacetate decarboxylase (Figure D.10) uses an active site Mg^{2+} ion as a Lewis acid to stabilize the growing anionic charge on the β-keto group that appears during decarboxylation. The Mg^{2+} may be viewed as a **co-factor** in this instance. OADCase is a remarkable enzyme, accelerating the uncatalyzed reaction with a k_{uncat} of 2.5 x 10^{-4} s^{-1} to 7500 s^{-1} (among the fastest rate constants for enzymes acting on organic substrates). That represents a 30 million fold stabilization of the transition state vs. the substrate by the enzyme (~10 kcal/mol). With a K_m of 2.2 mM, the efficiency constant is 3 x 10^{-6} M and the proficiency constant, $k_{uncat}/(k_{cat}/K_m)$ of 10^{-10} M shows absolute binding of the transition state by 14 kcal/mol. The enzyme shows little difference in activity between pH 5 and 10, indicating the absence of GA/GB catalysis. C-C bond breaking may be rate-determining step, and subsequent protonation of the enolate is likely rapid (Figure D.11).

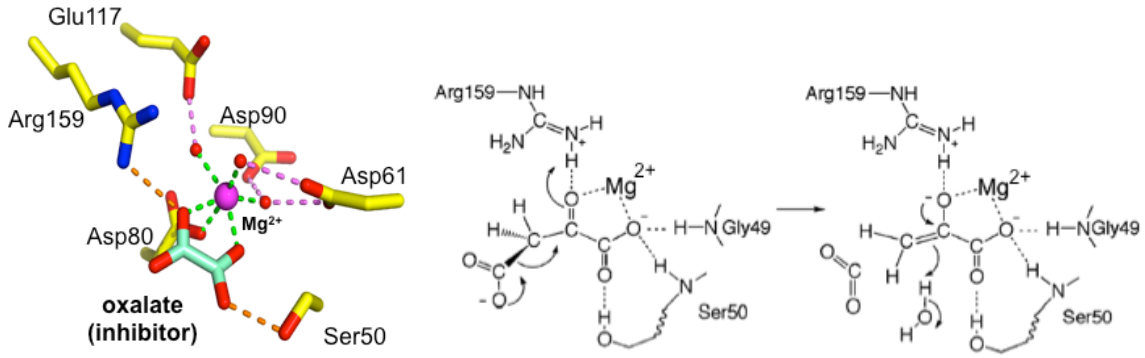


Figure D.11. Structure of OADCase active site in the presence of the inhibitor oxalate. Note that oxalate (in cyan) is a bidentate ligand to Mg^{2+} (magenta sphere). A propose mechanism showing the stabilization of negative charge following decarboxylation by Mg^{2+} is shown at right.

More Difficult Decarboxylations

Histidine Decarboxylase

Not all decarboxylations are performed on β-keto acids. Not all decarboxylations leave behind a resonance stabilized carboanion. In those instances, where more challenging chemistry is involved, an enzyme may resort to more specialized co-factors than the Mg^{2+} ion used in OADCase. Histidine decarboxylase from bacteria is an interesting case of an enzyme modifying itself so as to produce the necessary functional groups to promote catalysis. Two serine residues within the protein chain self-cleave rearrange to produce a pyruvoyl group that is connected via an amide to the C-terminal fragment of the original protein Figure D.12).

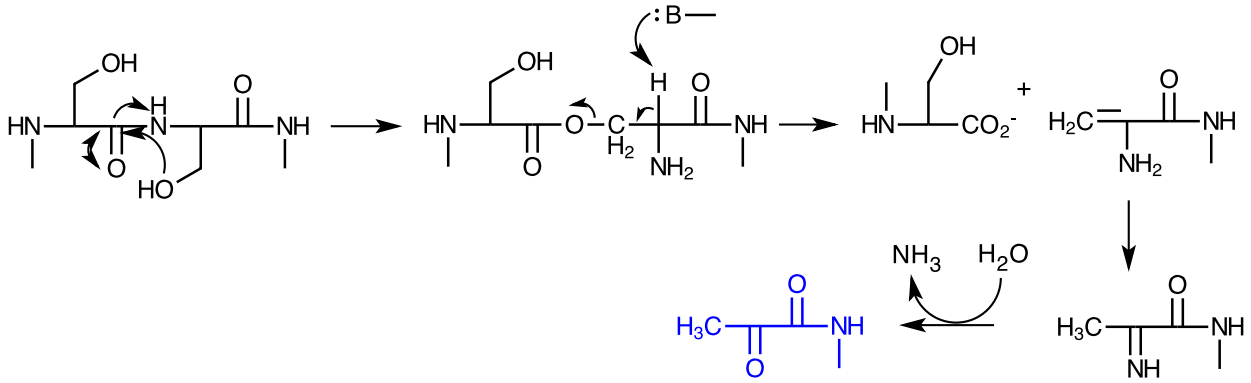


Figure D.12. Formation of a pyruvoyl amide (the blue product). An intramolecular attack leads to formation of an ester, replacing the amide linkage. Base cleavage leads to formation of a dehydroalanine residue (an enamine) which rearranges to an imine and is hydrolyzed to form the pyruvoyl amide.

The virtue of the pyruvoyl group is that it can condense with an amino acid in such a way as to create an electron sink for the pair of electrons that are left behind during decarboxylation (Figure D.13). The amino acid reacts with the α-carbonyl of the pyruvoyl group to create a Schiff's base. Decarboxylation can proceed with a resonance shift of the growing negative charge well away from the α-carbon of the amino acid, where there is no good mechanism for stabilizing an anion. Following reprotonation of the α-carbon, the resulting amine compound is released by hydrolysis. *I will do more with this section in a later draft.*

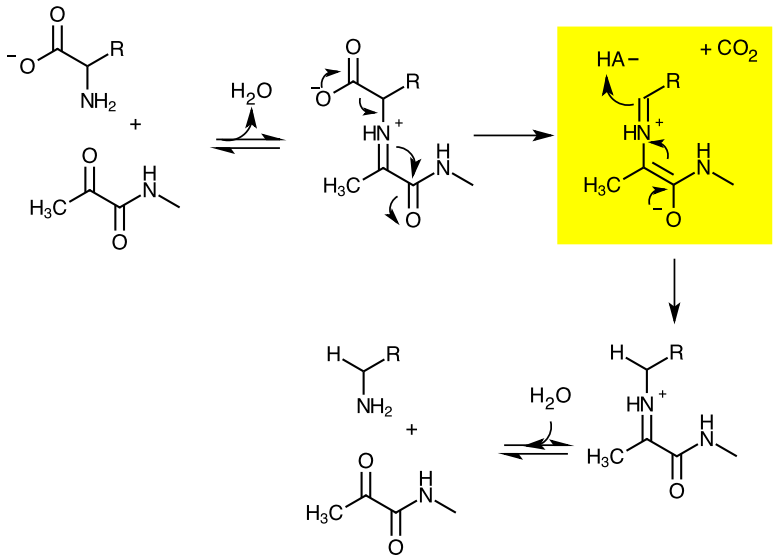


Figure D.13. Condensation of an amino acid with a pyruvoyl group to yield a Schiff's base, followed by decarboxylation to yield a relatively stable anionic intermediate (in yellow).

L-DOPA Decarboxylase

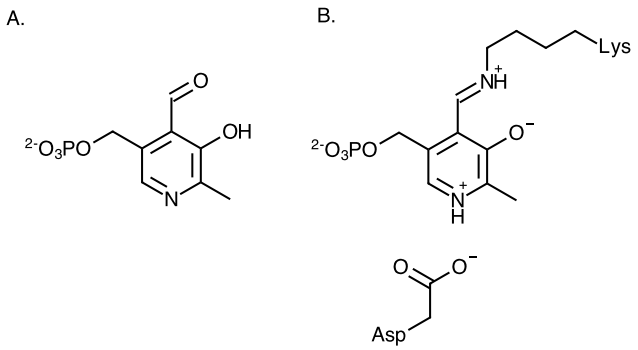


Figure D.14. (A) Structure of pyridoxal phosphate. (B) Structure of pyridoxal phosphate bound to L-DOPA decarboxylase via a Schiff's base. Note the ionization state of the cofactor stabilized by the aspartyl residue and Schiff's base.

Most amino acid decarboxylases do not operate via a self-generated functional group, but rather rely on an enzyme co-factor that possesses chemistry favorable to the decarboxylation of the amino acid. The B vitamins are, broadly speaking, the collection of enzyme cofactors that provide a great deal of the essential chemistry to primary metabolism not already available to amino acids. Two of them are broadly used in decarboxylations, B1 (thiamine) and B6 (pyridoxal). L-DOPA, L-3,4-dihydroxyphenylalanine, is a precursor to dopamine and other neurotransmitters. The conversion to dopamine is a vitamin B6-dependent reaction catalyzed by L-DOP decarboxylase. Like many other B6-dependent enzymes, pyridoxal phosphate (the active form of the co-factor) is covalently bound in the active site via a Schiff's base to a lysine side chain, and its protonated form is stabilized by a strategically placed aspartyl residue.

Wolfenden has explored the thermodynamics of glycine decarboxylation from 170-260°C and obtained an uncatalyzed rate constant of 1 x 10^{-17} s^{-1}, with a $\Delta H^{\neq}$ of 39 kcal/mol and $-T\Delta S^{\neq}$ of 1.1 kcal/mol. Clearly the challenge is enthalpic rather than entropic.[5] Interestingly, a study cited in that work indicates that PLP, on its own, is capable of promoting amino acid decarboxylation with a 10^{11}-fold rate enhancement (k_{PLP} = 1 x 10^{-6} s^{-1}) on its own, in the absence of an enzyme. Thus the cofactor is key to the function of L-DOPA decarboxylase, which has pretty remarkable kinetics (Table D.3).

Table D.3. Kinetic data for L-DOPA decarboxylase

Parameter	Value
k_{cat}	4.3 s^{-1}
K_m	70 μM
k_{cat}/K_m	6 x 10^4 $M^{-1}s^{-1}$
k_{cat}/k_{uncat}*	10^6
$k_{uncat}/(k_{cat}/K_m)$*	10^{-10} M

*vs. PLP reaction without enzyme

Sorry – ran out of time in notes. ...

Pyruvate Decarboxylase

Orotidine Decarboyxlase

[5] Snider MJ, Wolfenden R. (2000) The rate of spontaneous decarboxylation of amino acids. *J. Am. Chem. Soc.* **122**, 11507-8.