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The Catalytic Mechanism of Acetoacetate Decarboxylase: A Detailed Study of Schiff Base Formation, Protonation States, and Their Impact on Catalysis

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Abstract

The enzyme Acetoacetate Decarboxylase (AAD) has a crucial function in the process of decarboxylating the substrate acetoacetate (AA). It has been extensively studied over the years, but its exact catalytic mechanism has remained partly unsolved due to the difficulty in assessing reaction intermediates. In this study, we combine molecular dynamics and electronic structure calculations to rediscover its catalytic mechanism. Our results show that the presence of the substrate, the acetoacetate, significantly influences the electrostatic potential of the active site. Furthermore, our simulations show that the decarboxylation reaction can take place by means of a direct proton transfer instead of via an enamine intermediate, which is thought to be strictly necessary. This work provides new insights into the role of the electrostatic interactions on the catalytic activity of AAD and for the first time connects it to the catalytic mechanism of other decarboxylases.

Introduction

Acetoacetate decarboxylase (AAD) is an enzyme which performs the decarboxylation of acetoacetate (AA) rendering acetone as a product (see Figure **??**).[1] The underlying molecular mechanism for the reaction has long been reported to occur through an enamine (Schiff base) intermediate.[2–8] The formation of such an intermediate requires K115 to be deprotonated at the optimal pH of the enzyme (6), indicating a significant shift on its natural pK_a value.[9,10] Initially, it was proposed that K116 caused a pK_a shift of K115; however, subsequent studies have demonstrated that the amine moieties of K115 and K116 are separated by a distance of 14.8 Å and cannot cause such a shift.[11] Recent X-ray crystallography studies have revealed the structure of the enamine intermediate in the presence of the substrate analogue 2,4-pentanedione (PTD), and have attributed the pK_a shift to desolvation effects.[11,12] Such a large pK_a shift would not be unique to this system, with well documented examples in other type of enzymes.[13,14]

However, it has recently been shown that the electrostatic interactions with the neighbouring E76 play a central role in the proposed activation mechanism. In order to promote a pK_a shift at K115, E76 needs also to be protonated.[15] In this sense, the pK_a value of E76 has already been observed to be shifted upwards.[12] The pK_a value of K115 shifts due to the fact that E76 is in the neutral state. This would mean that both E76 and K115 exhibit a pK_a shift from their standard values, mutually influencing each other.[15] The neutral state

of E76 seems to be corroborated by a mutagenesis study where E76 was substituted by Q, with a 250-fold decrease in k_{cat} and no apparent influence on the K_m value.[11] This would correspond to a rise in the activation barrier by approximately 3 kcal/mol (based on the Eyring equation), potentially indicating a shift in the acid/base intermediates utilized during the catalytic process.

It is therefore clear that the local electrostatics within the active site play a significant role in determining the pK_a values of residues. However, it is important to acknowledge the limitations of previously published works. Theoretical calculations did not account for the presence of a negatively charged substrate in the pocket,[12,15] while the experimental determination of pK_a shift on residue K115,[9] used a neutral ligand.

On the proposed reaction mechanism itself, it is said that AAD performs the decarboxylation reaction after the formation of the enamine intermediate.[11] In this sense, the enamine acts as an electron sink facilitating the reaction.[16] The enamine formation has previously been shown to take place within the AAD-like superfamily.[17,18] However, it is worth mentioning that the computed rate limiting energy barrier for the enamine formation is reported to be higher than the effective barrier which can be derived from k_{cat} (14 kcal/mol).[19] Moreover, in the case of AAD this intermediate was characterized upon the presence of a substrate analogue, PTD; albeit similar to the AA substrate, this analogue lacks the carboxylic group which is cleaved by the enzymatic action (see Figure **??**).

On the other hand, in the seminal studies by Westheimer and coworkers,[20] the formation of the Schiff base in presence of AA was confirmed by reduction with sodium borohydride. However, the isopropyllysine which is thereby formed could be a result of either the substrate AA or the product acetone. Studies were conducted at the time, adding separately substrate or product (acetone) to the system and comparing the inhibition rates. The addition of AA resulted in much larger inhibitions than acetone for most assays, about 90% compared to 10% depending on the conditions. This would seem to confirm that the enamine is formed before decarboxylation. However, a few open questions remained, noted by the authors themselves. First of all, some assays did show larger inhibitions for acetone, without any direct explanation. The difference in binding affinities between the molecules could also be a reason behind the inhibition percentage differences, factoring in the instability of sodium borohydride. Secondly, residual activity was consistently observed even after the formation of isopropyllysine. There could be two possible explanations for this. On one hand, it could be due to an incomplete reaction, where only some of the lysines undergo reduction. The other explanation could be that the reaction still proceeds with the presence of a chemically inert lysine derivative, which would go against the Schiff base mechanism.

In summary, we believe that there is a reasonable degree of uncertainty on the proposed intermediate. As some of the conclusions reached from the cited studies may not be entirely relevant upon the presence of the AA substrate in the pocket. Additionally, the formation of the enamine could be disconnected from the catalytic process and instead, be a secondary reaction with ketone-containing compounds that exhibit favorable binding to the site.

In this study, we present a thorough examination on AAD, an enzyme whose catalytic activity is primarily linked to a neutral K115 residue. Previous investigations showed that the surrounding environment plays a critical role in inducing a pK_a shift, either through desolvation effects or electrostatic interactions. We investigate the pK_a shifts that take place within the active pocket without any ligand, as well as in the presence of AA and PTD, comparing with both theoretical and experimental reported values. We include detailed descriptions for the formation of the Schiff base intermediate and the catalysed decarboxylation reaction. Finally, we provide a simple alternative for the decarboxylation reaction excluding the formation of the widely accepted enamine intermediate.

Methods

Replica Exchange constant pH simulations

All replica exchange constant pH simulations were carried out with the AMBER 20 software package,[21,22] using sander or pmemd, employing the FF10 force field[23,24] for the protein and the GAFF force field for the ligand,[25,26] whose point charges were estimated with RESP.[27] The protein and the protein-ligand complexes were based on the previously published structure (PDB code 3BH2) which were set in a periodic cuboid box of 8 Å, between the protein and the periodic box wall, of TIP3P water molecules and neutralized with Na^+ and Cl^- counterions.[28]

Parametrization. The AA substrate and PTD were parameterised on the basis of Gaussian09 software package calculations.[29] Geometry and frequency calculations were performed at the B3LYP/def2-SVP level using the D3 dispersion correction from Grimme and coworkers.[30–34] The point charges were computed with the RESP procedure at the HF/6-31G* level, as established in the standard protocol.[27]

System preparation. In order to prepare the monomeric protein-ligand complex system, the ligand was placed in the active site of the AAD enzyme manually. Then, the hydrogen atoms of the system were first minimized for 5000 cycles (1000 with steepest descendent and 4000 with conjugate gradient), by restraining the rest of the atoms with a 10.0 kcal/mol/Å^2 force constant. Following another 5000 cycle minimization (1000 cycles with steepest descendent and 4000 with conjugate gradient), constraining everything except the ligand with a 5.0 kcal/mol/Å^2 force constant. Later on, another 5000 cycles of minimization were performed (1000 cycles with steepest descendent and 4000 with conjugate gradient), constraining everything except the ligand and the water molecules with a 5.0 kcal/mol/Å^2 force constant. Then, the system was minimized for 5000 cycles (2000 with steepest descendent and 3000 with conjugate gradient), restraining the backbone atoms of the protein with a 5.0 kcal/mol/Å^2 force constant. Finally, the system was minimized for 5000 cycles (1000 with steepest descendent and 4000 with conjugate gradient) without restraints.

Simulation of the trajectories. The systems were heated up from 10 to 300 K for 300 ps using a NVT ensemble using Langevin dynamics with a collision frequency of 5.0 ps^{-1}. Followed by another 100 ps at 300 K in the same ensemble. The system was equilibrated for 4 ns in NPT ensemble at 300 K, with isotropic position scaling (relaxation time of 1.0 ps and a collision frequency of 1.0 ps^{-1}). The production phase was then performed using the NVT ensemble, with a collision frequency of 5.0 ps^{-1} for 16 replicas, each of them being 32 ns, which leads to a total of 512 ns long trajectory. E76 and K33 were set to be the titratable residues, the protonation state change was attempted every 200 fs and a replica exchange every 4 ps. The heating and production phase were performed using GPUs.[35,36] The cut off for non-bonded interactions was set to 8 Å, employing particle-mesh Ewald summation with a fourth-order B-spline interpolation and a tolerance of 10^{-5}. The non-bonded list was updated every 50 fs and the MD time step was set to 2 fs, employing the SHAKE algorithm[37] to constrain bonds involving hydrogen atoms.

The computational determination of pK_a values may require long simulation times. To confirm that the protonation ratios were converged, extended molecular dynamics were carried out. One can observe the convergence pattern for the ratios in Figs. S1-S3.

Molecular Dynamic simulations

Molecular dynamic simulations were carried with the AMBER 18 software package, using sander or pmemd, employing the FF14SB force field[38] for the protein and the GAFF force field for the ligand, whose point charges were estimated with RESP. The protein-ligand complex was set in a periodic cuboid box of 8 Å, between the protein and the periodic box wall, of TIP3P

water molecules and neutralized with Na^+ and Cl^- counterions.

Simulation of the trajectories. The system preparation followed the same protocol used for the replica exchange calculations. The systems were then, first heated from 0 to 300 K for 300 ps using a NVT ensemble using Langevin dynamics with a collision frequency of 2.0 ps^{-1}. Followed by another 100 ps at 300 K in the same ensemble. We have then equilibrated the system for 4 ns in NPT ensemble at 300 K and with isotropic position scaling and a relaxation time of 1.0 ps with a collision frequency of 1.0 ps^{-1}. The production phase was then performed for 20 ns, using the NPT ensemble, with the same values as set for equilibration phase. The heating and production phase were performed using GPUs. The cut off for non-bonded interactions was set to 8 Å, employing particle-mesh Ewald summation with a fourth-order B-spline interpolation and a tolerance of 10^{-5}. The non-bonded list was updated every 50 fs and the MD time step was set to 2 fs, employing the SHAKE algorithm to constrain bonds involving hydrogen atoms.

QM cluster calculations

In order to perform these calculations we modeled the reaction by including the ligands, i.e. AA and PTD, methylamine and two water molecules. Geometry optimizations and frequency calculations were performed using the Gaussian16 software package.[39] We used Density Functional Theory (DFT), namely the B3LYP functional together with the def2-SVPD basis set. All calculations included the D3 correction and the SMD solvation model with the permittivity of water.[40] The final energies were refined with single point calculations applying the larger def2-TZVPD basis set.

QM/MM Potential of Mean Force simulations

The hybrid quantum mechanics/molecular mechanics (QM/MM) simulations were performed with the AMBER 18 software package, using sander.

Set up. The FF14SB force field was used for the protein and the GAFF force field for the ligand. The QM region was defined by the AA substrate, K115, E61 and E76. K115 was truncated at C_ϵ whereas the glutamate residues were truncated at C_γ. We used the semiempirical DFTB3 Hamiltonian for the QM region.[41,42]

The PMF simulations of the substrate-enzyme complex were conducted in conjunction with the umbrella sampling method along the C-N that is formed in the case of the enamine and the C-C bond that is cleaved in the case of the decarboxylation. We performed simulations setting E76 as protonated and deprotonated, based on the replica exchange simulation results (see the Results and discussion) with the aim of studying its role along the reaction coordinate. MD simulations were performed using equally spaced windows with an increment of 0.05 Å and a bond force constant of 350.0 kcal/mol/$Å^2$. We first conducted the simulations at certain distances, which are then employed as the starting structures for further simulations. The analysis of the results is performed applying the weighted histogram analysis method (WHAM).[43]

Simulation of the trajectories. The initial structure was taken from the minimized structure obtained from MD simulations (see Tables S1 and S2). This system was minimized for 2000 cycles with steepest descent and 3000 steps with conjugate gradient. Then it was heated for 200 ps increasing the temperature at equal intervals from 0 to 300 K at NVT ensemble. Langevin dynamics were used with a collision frequency of 2 ps^{-1}. The system was then equilibrated for another 200 ps at NPT ensemble, using Langevin dynamics with the same collision frequency and Berendsen's barostat with isotropic position scaling, with a pressure relaxation time of 1 ps. The production run was then set to 500 ps for each window, using the same ensemble and parameters as for the equilibration. All the MD simulations were

carried out without SHAKE constraints and with a time step of 0.5 fs.

The obtained last structure of each production phase was employed as a starting point of the following distances that were required for the PMF (see Tables S1-S5 for further details). The procedure as described above (including minimisation and equilibration) was then repeated.

Results and Discussion

Replica exchange constant pH simulations

Previous theoretical investigations showed that E76 has a higher pK_a value than usual (4.1).[12] Consequently, at the pH optimum of the enzyme the E76 residue can populate the protonated state, which has been shown to lead to a decrease in the pK_a value of K115.[15] At the same time, the experimental results also point to a shift of the K115 pK_a, in the presence of PTD, a substrate analogue.[11] However, it should be noted that the theoretical studies did not include the substrate in the pocket and the experimental results were performed with PTD, which is neutral. Therefore, the presence of AA, a negatively charged substrate, could have a strong impact on the local electrostatic potential.

In order to assert the influence of the substrate charge, we have conducted replica exchange simulations for the protein and also for protein-ligand complexes with AA substrate and the substrate analogue PTD. The obtained pK_a values show an acidic E76, in case of the protein without ligands and in complex with PTD, as shown in Figure **??**. Interestingly, the pK_a value of this residue is shifted up in the presence of the negatively charged AA, by about 3 units from its usual value, i.e. 4.1. Whereas the absence of ligands or the presence of the neutral PTD, leads to a downwards shift of about 1-2 units. At this point, it has to be recalled that the optimal pH of this enzyme is around 6,[10] so based on the obtained pK_a value, E76 can be either protonated or deprotonated. K115 on the other hand, shows a slightly lower pK_a value than usual (10.4) in the case of the empty AAD, but the presence of AA shifts its value upwards, making it a regular lysine residue in terms of its pK_a value.

From the constant pH results we can state that upon the binding of AA, that the protonated and deprotonated states of E76 should be close to equilibrium, whereas K115 would be mainly protonated. In the presence of the neutral PTD, the conclusions are quite different. By eliminating the negative charge of the ligand, E76 is now observed to be acidic and so it is deprotonated. On the other hand, K115 is observed to exhibit a lower pK_a value than a regular lysine residue, indicating that in this case, it can either be protonated or deprotonated. The protonation states are remarkably different comparing the AA substrate and the PTD substrate analogue. Thereby, a pK_a study to extract general conclusions on AAD without an anionic binding molecule could prove deceptive.

The pK_a shift of E76 under the influence of the AA substrate indicates a preference of the binding which can be studied through the use of a thermodynamic cycle (Figure **??**). Here, the difference in the acidity of E76 with and without the substrate is equal to the difference of the binding of AA to the enzyme without and with the proton at E76 respectively, as shown in Equation (1). Thereby, a protonated E76 binds the AA substrate about 6.3 kcal/mol stronger than in the deprotonated case.

$$\Delta G_1 - \Delta G_2 = \Delta G_4 - \Delta G_3 \qquad (1)$$

Molecular dynamic simulations

Motivated by the observed energetic differences in the binding of the AA substrate, upon the different protonation states of E76, we have conducted molecular dynamic simulations with the purpose of characterizing strong noncovalent interactions (in particular hydrogen bonds) between the hydrophilic residues in the active pocket and the AA substrate, considering the protonated and deprotonated states of E76. The results displayed in Figure **??** show

that upon the protonation of E76, this residue forms a stable hydrogen bond with E61, which is then preserved along the trajectory. On the other hand, in the event that E76 is deprotonated, it creates a stable hydrogen bond with K115, which is again preserved along the trajectory. Therefore, two main interactions are possible for E76 and directly determined by its protonation state. A slight difference is observed between the interaction of the carboxylate and the carbonyl moiety of AA with R29. Finally, no difference can be observed in the interaction between the carboxylate and the carbonyl moiety of AA with K115.

QM cluster calculations

At this point, we would like to focus on the formation of the enamine intermediate for both PTD and AA as well as the decarboxylation mechanism for AA. Our cluster calculations involve the aforementioned ligands along with a methylamine, representing the K115 residue. It should be noted that the presented values are not meant to reflect the absolute values of the enzymatic mechanism, as the enzymatic environment is absent. Rather, the calculations are conducted within the dielectric continuum of water, resulting in values that are closer to those of the uncatalyzed reaction. Nonetheless, this approach enables a detailed examination of the entire reaction mechanism, facilitating the comparison of values and comprehension of both the intermediate formation and the catalyzed decarboxylation.

Decarboxylation with and without the enamine. With the previous information at hand, we aimed to assess the impact of the enamine formation to the decarboxylation reaction. In order to do so, we compare the barriers with and without the enamine intermediate step. One should focus on relative variations since important environment effects, which could significantly lower some of the activation energies, are not included in this simplified model.

The barrier for decarboxylation is naturally higher than that experimentally observed (20.4 vs 14 kcal/mol), see Figure **??** a). Interestingly, the results demonstrate that indeed, the enamine leads to a slightly lower barrier for the decarboxylation, by about 3 kcal/mol. The model calculations are of interest since one reduces significantly the conformational space of the reaction, making it possible to more clearly look at the difference in reaction steps. But our values need to be ultimately confirmed including the enzyme environment. For this purpose we carried out QM/MM simulations, which are discussed in the following section.

Formation of the enamine in PTD. In the case of the substrate analogue, PTD, the formation of the enamine is observed to be energetically plausible, see Figure **??** b). This aligns with experimental findings from crystal structure characterization, which reveals the presence of an enamine upon the incorporation of PTD.[11] The bottleneck here corresponds to the dehydration step, with a barrier of around 14 kcal/mol, and the formed enamine product is observed to be slightly exergonic (-0.5 kcal/mol). In addition, the deprotonation of K115 is favorable at the optimal pH of the enzyme, as it was already shown from the replica exchange results.

Formation of the enamine in AA. A conservative approach has been adopted in this study. Hence, in this section we consider both the protonated and deprotonated forms of the K115 residue, although the results discussed in the previous section indicated the presence of K115 only in its protonated state.

Our observations, presented in Figure **??** c), indicate that the deprotonated K115 slightly favors the formation of the enamine, with the dehydration step being the bottleneck, with a barrier of approximately 26 kcal/mol. Conversely, the decarboxylation reaction was observed to occur only in the protonated scenario. The enamine intermediate, as a result of its electron-sink nature, acquires a proton, leading to a a significantly lower decarboxylation barrier, from 50.8 down to 20.4 kcal/mol. In this instance, the rate-limiting step is determined to be the transition of the sp^2 hybridized carbon

to an sp^3 hybridization, with a barrier of approximately 26 kcal/mol.

Even if the decarboxylation reaction was found to be energetically more favorable through the intermediate of an enamine, with a reduction of 3 kcal/mol (Figure ?? a)), the added steps might make it less accessible (Figure ?? c)), depending on the substrate and environment. The reaction mechanism for the formation and the dissociation of the enamine involves several chemical transformations, whose barriers are similar in magnitude to that of the decarboxylation without the enamine. As a result and considering the whole picture obtained from this section, the two mechanisms are potentially competitive.

Potential of Mean Force simulations

In this section we present the results of simulations of the Potential Mean Force (PMF), incorporating the enzymatic environment. The objectives of these simulations are to a) elucidate the reaction mechanism governing the formation of the enamine intermediate and the subsequent decarboxylation and b) compare the activation barriers associated with the formation of the enamine intermediate and direct decarboxylation, while also examining the effect of protonation states.

Upon the formation of the enamine, the reaction coordinate is defined along the nitrogen atom that initiates the nucleophilic attack and the carbon atom of the substrate to which the nitrogen atom becomes attached. For the decarboxylation reaction, the reaction coordinate is determined by the carbon-carbon bond of the substrate, which is cleaved resulting in the formation of CO_2. Given that the pK_a value of E76 aligns with the pH when the substrate AA is present in the pocket, we have taken into consideration the possibility of having a protonated and a deprotonated E76.

Formation of the enamine. Our analysis indicates that when the E76 residue is protonated, the formation of the enamine intermediate does not occur. Instead, the formation of the carbinolemaine intermediate is observed. It is important to note that while the proximity of the C and N atoms increases, the reaction profile raises its energy without reaching a minimum energy state.

Conversely, when the E76 residue is deprotonated, it functions as a proton acceptor, leading to the deprotonation of the K115 residue, thereby allowing for the nucleophilic attack of the N atom on the carbonyl moiety of the substrate, see Figure ??. The reaction barrier in this scenario is approximately 23 kcal/mol (22.1 kcal/mol in the case of the QM results), resulting in the formation of the carbinolenamine, which serves as an intermediate in the reaction. It is noteworthy that the carbinolenamine intermediate in this case possesses one less proton than previously. At this point, bringing the C and N atoms closer together, a dehydration reaction takes place, removing the alcohol moiety which forms a water molecule by accepting a proton from the enamine. The reaction has a barrier of roughly 20 kcal/mol (25.6 kcal/mol in the case of the QM results), resulting in the formation of the deprotonated enamine intermediate.

Decarboxylation of the enamine intermediate. In view of the above discussed results, the analysis of the decarboxylation reaction starting from the enamine, with a deprotonated E76 residue, is presented below. From Figure ??, it can be seen that the reaction mechanism contains a relatively low activation barrier of approximately 5 kcal/mol. However, it is important to remember that the formation of this enamine intermediate is a necessary prerequisite, which also might constitute a rate limiting step.

Direct decarboxylation. Based on the obtained results, our goal is now to analyze the reaction barriers without an enamine intermediate, that is, a direct decarboxylation reaction. Once again, we considered both the deprotonated and protonated states of E76. It can be observed that along the reaction coordinate the two protonation states exhibit quite a different activation barrier. The deprotonated

E76 shows a higher barrier (about 17 kcal/mol) whereas the protonated E76 shows a lower value (about 9 kcal/mol). Given that the chemical reaction is the same, the observed difference is exclusively due to the surrounding environment, and how it potentially stabilises the transition state. It is also interesting to note that the final product stability is also influenced by the protonation of E76. Hence, the deprotonated E76 leads to a slightly endergonic reaction (about 1 kcal/mol) whereas the protonated E76 leads to an exergonic reaction (about -12 kcal/mol).

From the obtained trajectories, one can observe that around the transition states, both the protonated and the deprotonated E76 trajectories, exhibit different protonation state of the substrate, which is probably the reason for the observed energetic difference. The reaction in the presence of the deprotonated E76 features around a distance of 2.55 Å a proton migration from K115 to the substrate forming the keto tautomer of acetone. It is interesting to observe that this same proton has migrated to E76 at C-C distances lower than 2.55 Å. On the other hand, for the reaction when E76 is protonated, an enol form of the tautomer is observed to be formed at distances near the transition state, i.e. from a C-C distance of 1.85 to 2.50 Å, stabilising the transition state. At larger C-C distances, the keto form of acetone is formed as the result of the chemical reaction. Finally, it should be noted that at C-C distances smaller than 1.70 Å, the proton of K115 is present at the substrate, making it a carboxylic acid. This proton is then transferred back to K115 from a C-C distance of 1.75 to 1.90 Å. All the mentioned structures are schematically represented in Figure **??**.

Conclusions

In this study, we investigated the reaction mechanism of AAD enzyme using molecular dynamics and quantum chemical calculations. Our findings present an alternative pathway to previous beliefs about the enamine intermediate and propose the competition of a direct proton transfer mechanism. The protonation state of residue E76 was found to be crucial for the enzymatic regulation and activity, as it enhances the binding of the substrate and stabilizes the transition state and the thermodynamics of the decarboxylation reaction. These findings are supported by the PMF calculations, and give a detailed perspective of the pK_a shift phenomenon.

The direct proton transfer mechanism, which avoids the formation of unnecessary intermediates, is somewhat similar to the one observed in orotidine-5'-monophosphate decarboxylase, where proton transfers through nearby lysine and aspartic acid residues were key components to the stabilisation of the transition state.[44] This may prove to be a widespread mechanism for catalysis of decarboxylation, although one will need to better understand the electrostatic forces behind the latter. Having the two systems as comparison might help in this endeavour as coupled proton transfers are rather difficult to model.[45]

The presented calculations do not challenge the current accepted mechanism of action for acetoacetate decarboxylase. Whatever calculations may be carried out, these need to be supported by further experimental evidence. However, they do highlight an alternative path for reactivity that the system may recourse to upon inhibition or blocking of the catalytic lysine. Our results provide clarity on some open questions in previous studies of AAD and have wide-ranging implications for the field. The continued activity of the enzyme even after the formation of isopropyllysine can be explained by the fact that K115 acts only as a proton donor and is not strictly required for the formation of the enamine and product.

Further experimental studies are needed to validate and extend these findings. These could include investigation of the electrostatic forces underlying the proton transfer mechanism and characterisation of the two different conformations of E76 that we have observed. The results of this study contribute to our understanding of the regulation and activity of enzymes used in decarboxylation reactions.

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Data Availability

The data generated in this study is available at https://doi.org/10.25625/PPWTLP.

Supporting Information Available

The following Supplementary Information is available free of charge. Figures S1-S3, which show the protonation fraction of K115 in the enzyme with and without acetoacetic acid and PTD. Tables S1-S5 detail the initial structures for C-C cleavage and C-N formation simulations generated from the last snapshot for both protonated and deprotonated E76, as well as C-C cleavage from an enamine intermediate.

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