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Theoretical Model for Pyruvoyl-Dependent Enzymatic Decarboxylation of α-Amino Acids

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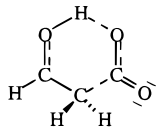
Abstract: The active site of histidine decarboxylase (HDC) has been modeled with both *ab initio* (MP2/6-31G(d)) and DFT (BH&HLYP/6-311G(d,p)) calculations. The results clearly point out the role of zwitterionic transition structures and the importance of hydrogen bonding interactions in enzymatic decarboxylation. A comparison between the gas-phase decarboxylation of aminoformylacetic acid ($H(C{=}O)CH(NH_2)COOH$) and the corresponding process in solution according to the supermolecule model approach with six water molecules is provided. This study analyzes the role of the proton distribution in lowering the reaction barrier in an intermediate Schiff base ($H_2C{=}NCH_2$-COOH) and its transition structure for decarboxylation ($\Delta E^{\ddagger} = 29.8$ kcal mol^{-1} at the MP2/6-31G(d) level of theory). Electronic features displayed by the intermediate imine are analyzed by making use of models of increased complexity. The iminium ion functionality has been established to be the dominant factor in lowering the barrier for the decarboxylation of the α-amino acids through Coulombic stabilization of the developing negative charge on the α-carbon and delocalization of the positive charge induced by proton transfer to the imine nitrogen along the reaction coordinate. Further extension of the model imine by an amide group ($H_2N(C{=}O)CH{=}NCH_2COOH$) lowers the barrier height by an additional 6.7 kcal mol^{-1}. A net transfer of electron density to the amide functionality in the transition state is not in evidence. The stabilizing influence on the barrier height of a hydrogen bonding network with formic acid and a model peptide residue ($H(C{=}O)NHCH_2CHO$) is estimated to be 3.1 kcal mol^{-1} at the BH&HLYP/6-311G(d,p) level.

Introduction

Subtle details of the transition structure for even the simplest enzymatic decarboxylation processes involving α-amino acids remain obscure. In the first theoretical study[1] on the decarboxylation process, where a variety of β-keto acid systems were used as models for enzymatic reactions, evidence was provided that the loss of CO_2 from the simplest β-keto acid, formylacetic acid ($H(C{=}O)CH_2COOH$), proceeds through a cyclic transition structure with essentially complete proton transfer from the carboxylic group to the β-carbonyl oxygen (TS-**A**).



TS-**A**

A classical activation barrier of 28.6 kcal mol^{-1} was found for formylacetic acid, while loss of CO_2 from the corresponding carboxylate anion exhibited a barrier of only 20.6 kcal mol^{-1} (MP4SDTQ/6-31+G(d)//MP2/6-31+G(d)). Since solvent effects typically play only a minor role in determining the rate for decarboxylation,[2] we observed excellent agreement between experiment and theory.[1]

α-Amino acid decarboxylation is a key step in the synthesis of neurotransmitter amino compounds.[3] Mechanistic studies on the enzymatic decarboxylation of α-amino acids have been aimed at the identification of the intermediates. There are essentially two strategies for this decarboxylation process, and

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(1) Bach, R. D.; Canepa, C. *J. Org. Chem.* **1996**, *61*, *6346.*

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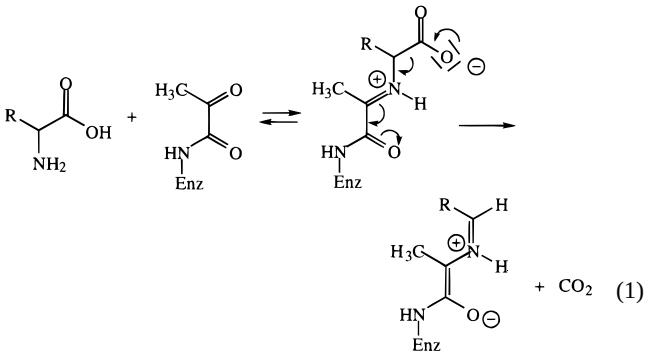
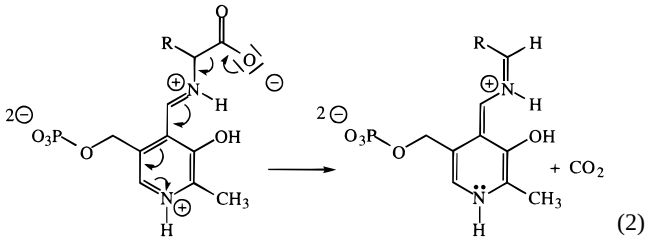
both are thought to take advantage of π-electron delocalization. Enzymes are known to form intermediate imino compounds by reaction of the amino group of the α-amino acid with the carbonyl functionality of the enzyme prosthetic group.[3] Reactions 1 and 2 summarize these concepts for pyruvoyl-dependent



(1)



(2)

and pyridoxal 5′-phosphate (PLP)-dependent enzymatic decarboxylations, respectively. It is thought that the pyruvamide and PLP functionalities serve as an "electron sink" that stabilizes the developing carbanion in the transition structure attending the loss of CO_2. In both cases it has been suggested that the developing negative charge on the α-carbon of the amino acid is dispersed via the π-system of the coenzyme bound to the substrate.[3] In this work we want to assess the effectiveness of this electrophilic assistance to enzyme catalysis, the nature and role of the proton distribution in the imine intermediate (eq 1), and the charge distribution in the transition structure for pyruvoyl-dependent decarboxylation.

Method of Calculation

Theoretical calculations were carried out using the Gaussian94 program system[4a] utilizing gradient geometry optimization.[4b] All structures were fully optimized using second-order Møller−Plesset perturbation theory (MP2), the three-parameter of Becke[5a−b] (B3LYP), or Becke half-and-half[5c] (BH&HLYP) hybrid functionals. The exchange functional is a combination of local spin density, Hartree−Fock, and Becke88. The correlation functional is a combination of the local correlation functional of Vosko−Wilk−Nusair[6] and the gradient corrected correlation functional of Lee−Yang−Parr.[7] These hybrid functionals used in the DFT calculations are defined as follows:

$$E_{XC}^{B3LYP} = 0.20E_X^{HF} + 0.80E_X^{Slater} + 0.72\Delta E_X^{B88} + 0.19E_C^{VWN} + 0.81E_C^{LYP}$$

$$E_{XC}^{BH\&HLYP} = 0.50E_X^{HF} + 0.50E_X^{Slater} + 0.50\Delta E_X^{B88} + E_C^{VWN} + E_C^{LYP}$$

Density functional theory (DFT) with nonlocal exchange-correlation functionals has been shown by Salahub[8] to satisfactorily reproduce the experimental binding energy of water dimer. Truong[9] and Durant[10] have reported the half-and-half hybrid method of Becke (BH&HLYP) to reproduce high-level ab initio structural and energetic information for minima and transition structures. In general, we have found that the effect of MP4SDTQ corrections on MP2 activation barriers for decarboxylation of β-keto acids and inclusion of diffuse functions (6-31+G(d)) is very minimal.[1] The 6-31G(d), 6-31G(d,p), and 6-311G-(d,p) basis sets have been used throughout the study. Vibrational frequency calculations were used to characterize all stationary points as either minima (zero imaginary frequencies) or first-order saddle points (a single imaginary frequency) except for the computationally demanding structures **14**, **15**, and TS-**16** (378 basis functions). The analytical second derivative calculations on structures **3**·$(H_2O)_6$, **3a**·$(H_2O)_6$, and TS-**4**·$(H_2O)_6$ have been carried out on geometries optimized at the RHF/6-31G(d,p) level.

Results and Discussion

Glycine. We initiate this study with the decarboxylation of the simplest α-amino acid, glycine (**1**), that serves as a model system. Glycine is known to exist in the neutral form in the gas phase[11] and in the zwitterionic form in water solution at physiological pH.[12] As a reference point we estimated the energy requirement for decarboxylation of isolated glycine. The loss of CO_2 from **1** appears to proceed without a discernible transition structure and with an exceptionally high energy profile.

Although a first-order saddle point for glycine decarboxylation has not been found, the isolated products of eq 3 lie 64.5 kcal

$$\underset{\mathbf{1}}{H_2NCH_2COOH} \longrightarrow \underset{\mathbf{2}}{H_3\overset{\oplus}{N}\text{-}CH_2^{\ominus}} + CO_2 \quad \Delta E = 64.5 \text{ kcal mol}^{-1} \qquad (3)$$

mol^{-1} above neutral glycine (**1**) at the MP2/6-31G(d) level, while a product-like complex of the products is 54 kcal mol^{-1} higher in energy than **1**, providing an indication of the difficulty of the uncatalyzed decarboxylation process of an α-amino acid. Unlike the decarboxylation of a β-keto acid,[1] whose intermediate product is a stable enol (or enolate), the decarboxylation of glycine yields a high-energy zwitterionic tautomer of methylamine[13] **2** (eq 3). In the gas phase the energy difference between the neutral form of glycine (**1**) and its geometry-constrained zwitterionic structure is 19.9 kcal mol^{-1} (MP2/6-31G(d)). However, this zwitterionic form of glycine can be stabilized sufficiently by an ammonium cation to lower the energy

(2) Carboxylic acids with potential anionic leaving groups such as benisoxazole-3-carboxylate exhibit pronounced solvent effects. See, for example: (a) Ferris, D. C.; Drago, R. S. *J. Am. Chem. Soc.* **1994**, *116*, *7509*. (b) Gao, J. *J. Am. Chem. Soc*. **1995**, *117*, *8600* and references therein.

(3) (a) Lehninger, A. L.; Nelson, D. L.; Cox, M. M. *Principles of Biochemistry*; Worth Publishers: New York, 1993. (b) Walsh, C. *Enzymatic Reaction Mechanism*; W. H. Freeman and Co.: New York, 1979. (c) van Poelje, P. D.; Snell, E. E. *Annu. Rev. Biochem.* **1990**, *59*, 29.

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(6) Vosko, S. H.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, *58*, 1200.

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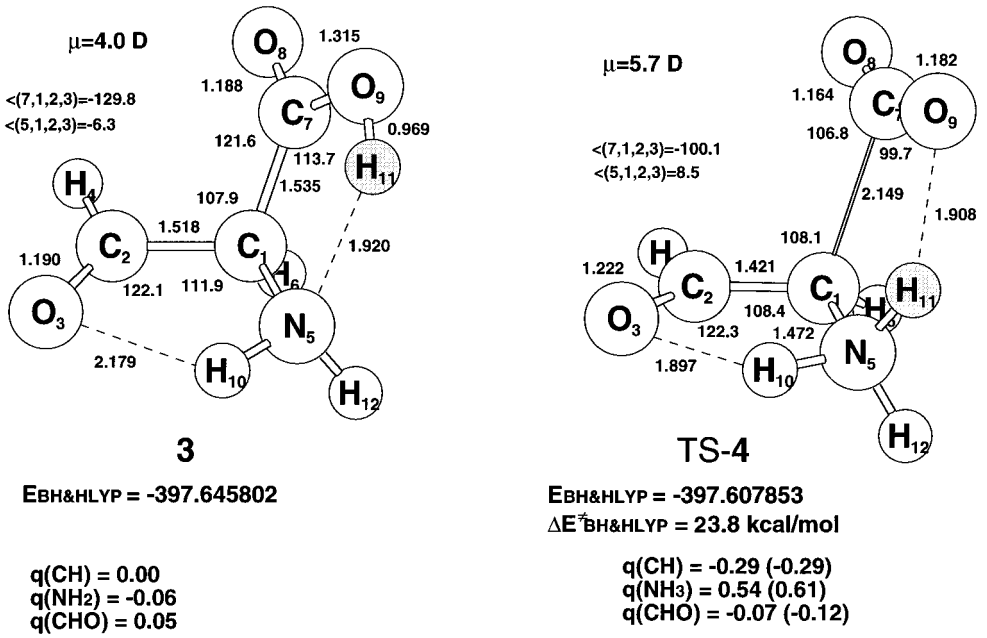
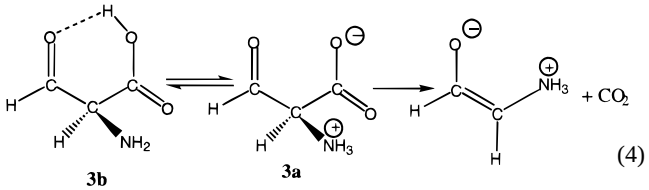


Figure 1. 2-Aminoformylacetic acid (**3**) and its transition structure for decarboxylation (TS-**4**). Geometries are fully optimized at BH&HLYP/6-311G(d,p). Energies are given in hartrees, and the activation barrier is given in kcal/mol. Distances are given in angstroms and angles in degrees. Total dipole moment (μ) is in debye. Group Mulliken charges are given as q, and the net changes are in parentheses.

difference between the two tautomeric forms to 0.7 kcal mol^{-1}. If polar amino acid residues are in their zwitterionic form at the active site of enzymes then even a modest Coulombic interaction with an ammonium cation could markedly alter the position of the equilibrium between the neutral and zwitterionic forms of an α-amino acid.

Effect of Coulombic Stabilization on the Barrier Height. We investigated initially the mechanism of decarboxylation of a series of organic acids including 2-aminoformylacetic acid (**3**) H(C═O)CH(NH_2)COOH, as an isolated gas-phase molecule at the MP2/6-31G(d) level.[1] When the carboxyl proton was shifted in the TS to the more basic α-amino group as in **3a**, the Coulombic stabilization of the adjacent positively charged NH_3 group resulted in a barrier height of $\Delta E^{\ddagger} = 19.0$ kcal mol^{-1}. However, when the proton shift was to the β- carbonyl group as indicated in **3b** and above in TS-**A**, the barrier was significantly increased to 39.4 kcal/mol (eq 4). Part of the



(4)

increase in the barrier is a consequence of the increase in the ground-state energy due to the loss of the strong hydrogen bond to nitrogen in global minimum **3** (Figure 1). An intrinsic reaction coordinate analysis[1] connecting TS-**4** to minimum **3** showed that proton transfer from the carboxylic acid to the adjacent nitrogen (N_5) was complete before the barrier was crossed to maximize the stabilization in the TS. The zwitterionic form of this α-amino acid, **3a**, where the N−H bond distances are necessarily constrained, was estimated to lie about 13 kcal mol^{-1} higher in energy than minimum **3** (Figure 1). Thus a considerable fraction of the gas-phase decarboxylation activation barrier is due to the effective charge separation in zwitterion **3a**. The observation of a pH-dependent decarboxylation rate constant for 2-ammonio-3-oxobutyrate (CH_3(C═O)CH(NH_3^+)CO_2^-) was interpreted as involving a cationic intermediate arising from protonation of the carboxylate group of the zwitterionic form of the α-amino acid.[14a] This type of cyclic transition state involving a protonated carbonyl was first suggested by Westheimer in his classic decarboxylation studies (TS-**A**).[14b,c] The half-life of 2-ammonio-3-oxobutyrate, where the α-amino group was shown to have a pK_a of 8.15, varied from 8.6 s at pH 5.9 to 140 s at pH 11.1, prompting the suggestion that the positively charged carbonyl group accelerated the loss of CO_2. However, this is a formal charge on the oxygen of the protonated carbonyl group that remains negative throughout the reaction coordinate for decarboxylation.[1,15] We attribute the rate acceleration to the Coulombic stabilization of the developing carbanion in the TS by the positive charge at nitrogen (i.e., NH_3^+). We have previously suggested that adjacent positively charged ammonium ions can also Coulombically influence the barriers for oxygen atom transfer from 4α-flavin hydroperoxides.[16]

Since we now include larger more biochemically relevant systems in these decarboxylation studies, we have evaluated the reliability of density functional methods (DFT) for this purpose. The effect of the level of theory and the basis set upon the barriers for decarboxylation of **3** (Figure 1) are given in Table 1. As anticipated, we found the RHF/6-31G(d) level to exaggerate the barrier ($\Delta E^{\ddagger} = 31.8$ kcal mol^{-1}) for decarboxylation of **3** but we have found good agreement between MP2 barriers calculated on either RHF or MP2 geometries. A comparison of MP2 and MP4//MP2 activation barriers for a series of decarboxylation reactions (Table 2) suggests that the MP4SDTQ correlation correction is not needed. Although at

(13) The zwitterionic methylamine tautomer (−)CH_2NH_3(+) is 74.2 kcal mol^{-1} higher in energy than methylamine at the MP2/6-31G(d) level of theory.

(14) (a) Marcus, J. P.; Dekker, E. E. *Biochem. Biophys. Res. Commun.* **1993**, *190*, 1066. (b) Westheimer, F. H.; Jones, W. A. *J. Am. Chem. Soc.* **1941**, *63*, 3283. (c) Steinberger, R.; Westheimer, F. H. *J. Am. Chem. Soc.* **1951**, *73*, 429.

(15) (a) The computed Mulliken or ChelpG charge[15b] on these atoms bears no resemblance to the formal charge, and as such, formal charges should not be used to make mechanistic predictions. For example, the ChelpG charge on the carbonyl carbon and oxygen of acetone and protonated acetone are 0.53, −0.54 and 0.60, −0.03, respectively. (b) Breneman, C. M. and Wiberg, K. B. *J. Comput. Chem.* **1990**, *11*, 361.

(16) Bach, R. D.; Su, M.-D. *J. Am. Chem. Soc.* **1994**, *116*, 5392.

Table 1. Activation Barriers ($\Delta E^{\ddagger}$) for Decarboxylation of H(C═O)CH(NH_2)COOH (2-Aminoformylacetic acid (**3**)) in the Gas Phase (TS-**4**) and in Water (TS-**4**·$(H_2O)_6$) and the Relative Energies (ΔE) for the Neutral **3** Complexed to Six Water Molecules (**3**·$(H_2O)_6$) and the Zwitterionic Form Complexed to Six Water Molecules (**3a**·$(H_2O)_6$)

computational level	$\Delta E^{\ddagger}$ (hartrees) for TS-**4**[a]	ΔE (kcal mol^{-1}) for **3**·$(H_2O)_6$	$\Delta E^{\ddagger}$ (hartrees) for TS-**4**·$(H_2O)_6$
RHF/6-31G(d,p)//RHF/6-31G(d,p)	31.8	2.6	22.6
MP2/6-31G(d,p)//RHF/6-31G(d,p)	22.3	12.6[c] [−63.5][d]	19.0[e] [−79.4][f]
B3LYP/6-311G(d,p)//RHF/6-31G(d,p)	17.0	11.2 [−68.1]	13.3 [−83.8]
B3LYP/6-311+G(d,p)//RHF/6-31G(d,p)	17.6	6.5 [−49.5]	12.3 [−61.3]
MP2/6-31G(d)//MP2/6-31G(d)[b]	19.0		
B3LYP/6-311G(d,p)//B3LYP/6-311G(d,p)	16.5		
B3LYP/6-311+G(d,p)//B3LYP/6-311+G(d,p)	17.1		
BH&HLYP/6-311G(d,p)//BH&HLYP/6-311G(d,p)	23.8	8.7 [−71.0]	18.9 [−84.7]

[a] $\Delta E^{\ddagger}$ for TS-**4** in the gas phase. [b] For the MP2/6-31G(d) geometry see ref 1. [c] ΔE = relative energy of **3**·$(H_2O)_6$ with respect to **3a**·$(H_2O)_6$. [d] Stabilization energy of gas-phase **3** complexed to six water molecules. [e] $\Delta E^{\ddagger}$ for TS-**4**·$(H_2O)_6$. [f] Stabilization energy of gas-phase TS-**4** complexed to six water molecules.

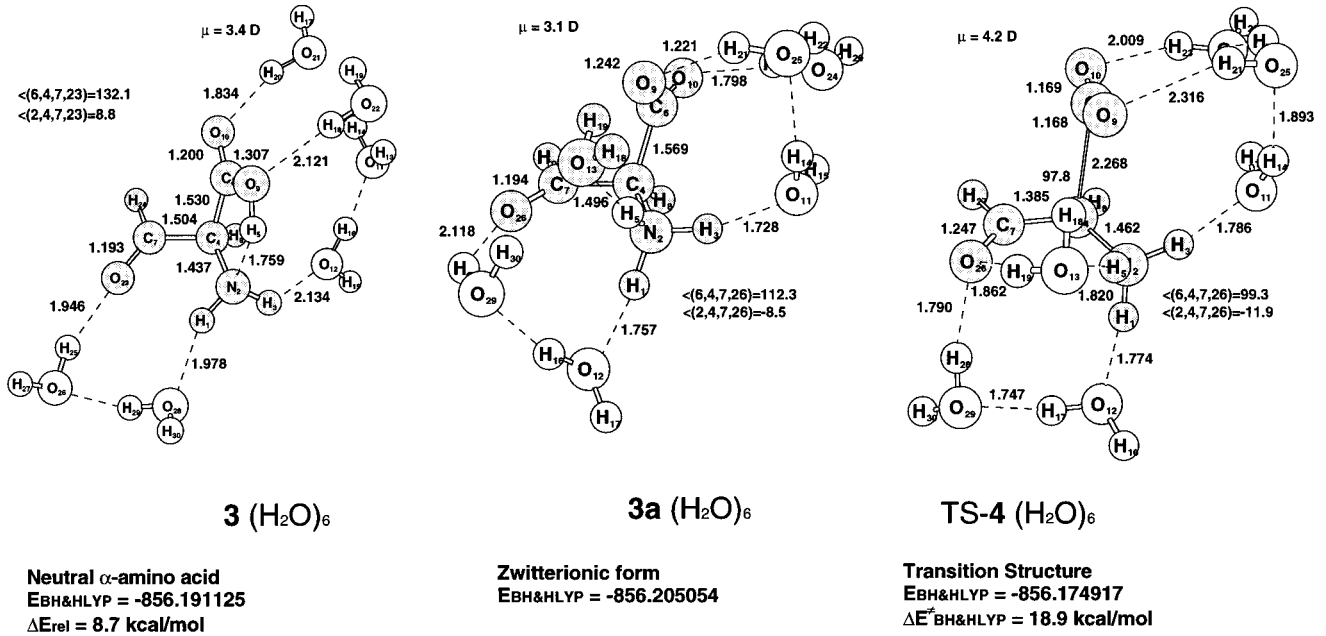


Figure 2. 2-Aminoformylacetic acid cluster with six water molecules: neutral carboxylic acid form (**3**·$(H_2O)_6$), zwitterionic form (**3a**·$(H_2O)_6$), and the transition sttructure for decarboxylation (TS-**4**·$(H_2O)_6$). Geometries are fully optimized at BH&HLYP/6-311G(d,p). Energies are given in hartrees, distances in angstroms, and angles in degrees. Stabilization energies (ΔE_{rel}) represent the interaction of the neutral and zwitterionic forms of **3** and TS-**4** of 2-aminoformylacetic acid (**3**) with six water molecules. Total dipole moments (μ) are in debye.

Table 2. Activation Barriers for the Decarboxylation of Acetic Acid Derivatives X-CH_2COOH (kcal mol^{-1})

X	MP2/6-31G(d)	MP4//MP2	DFT[a]	DFT[b]
H_2N (**1**)	54−64[c]			
CHO[d]	28.5	28.6		
COOH[d]	33.1	33.2		
H_2C═N[e]	29.8	29.2		23.8
H(C═O)CH═N (**5**)	25.1	23.9	29.1	21.4
H_2N(C═O)CH═N (**10**)	23.1			

[a] BH&HLYP/6-311G(d,p). [b] B3LYP/6-311G(d,p). [c] Energy requirement for the loss of CO_2 from **1** (eq 3). [d] Data from ref 1. [e] Unpublished results.

the BH&HLYP/6-311G(d,p) level the activation barrier is somewhat higher relative to the MP2/6-31G(d) barrier (TS-**4**, $\Delta E^{\ddagger}$ = 23.8 kcal mol^{-1}), this agreement is sufficient to permit us to calculate larger systems at the DFT level. In the present case the B3LYP/6-311G(d,p) barrier of 16.5 kcal mol^{-1} is 2.5 kcal mol^{-1} lower than the MP2/6-31G(d) barrier.

The Role of Hydrogen Bonding in Stabilizing Zwitterionic Structures. A number of key polar interactions at the active site of pyruvoyl-dependent histidine decarboxylase (HDC) have been suggested by Hackert and co-workers.[17] The amino acid residues implicated in the secondary bonding are Ser-81, Asp-63, and the imidazole ring of the substrate itself. Additional bonding stabilization was attributed to hydrophobic interactions between residues Ile-59 and Phe-83 with the imidazole group. A key specific interaction between a hydrogen bond of the peptide carbonyl of Phe-195 and the iminium nitrogen of the zwitterionic form of histidine was also identified. We chose water molecules as models for interacting amino acid residues to see if secondary bonding interactions of this nature can significantly affect the barrier height for enzymatic decarboxylation reactions. Gordon[18a] has established that two water molecules represent the minimal interaction required to stabilize the zwitterionic form of glycine at the HF/6-31G(d) level. The effect of one and two water molecules on the decarboxylation of benzisoxazole-3-carboxylic acid has also been studied by

(17) (a) Gallagher, T.; Snell, E. E.; Hackert, M. L. *J. Biol. Chem.* **1989**, *264*, 12737. (b) Gallagher, T.; Rozwarski, D.A.; Ernst, S. R.; Hackert, M. L. *J. Mol. Biol.* **1993**, *230*, 516. (c) Parks, E. H; Ernst, S. R.; Hamlin, R.; Xuong, Ng. H.; Hackert, M. L. *J. Mol. Biol.* **1985**, *182*, 455. (d) Hackert, M. L.; Meador, W. E.; Oliver, R. M.; Salmon, J. B.; Recsei, P. A.; Snell, E. E. *J. Biol. Chem.* **1981**, *256*, 687.

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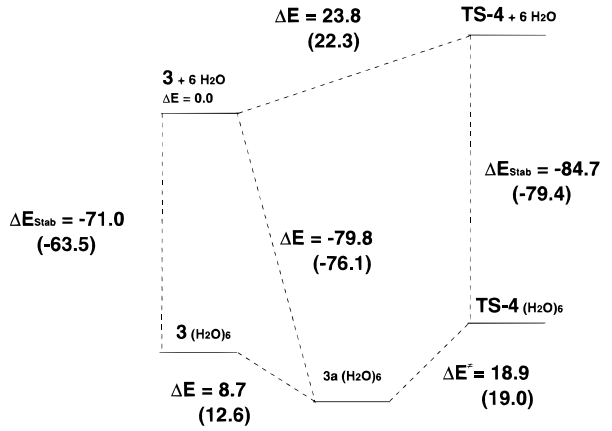


Figure 3. Energy diagram showing the relative energies of gas-phase 2-aminoformylacetic acid (**3**) and its transition structure for decarboxylation (TS-**4**). The relative energies of the cluster of **3** with six water molecules (**3**·$(H_2O)_6$), its zwitterionic form (**3a**·$(H_2O)_6$), and the transition state for decarboxylation (TS-**4**·$(H_2O)_6$) are also given. The energy differences are in kcal/mol at the BH&HLYP/6-311G(d,p)//BH&HLYP/6-311G(d,p) level of theory. The MP2/6-31G(d,p)//RHF/6-31G(d,p) values are given in parentheses. The interaction energies are defined in eqs 5a,b.

Houk.[18b] We allowed 2-aminoformylacetic acid (**3**) to interact with six water molecules in a supermolecule approach (Figure 2).[18c] A water molecule was placed initially at each of the three oxygen and acidic hydrogen atoms. Upon geometry optimization, a critical point corresponding to both a neutral **3**·$(H_2O)_6$ and zwitterionic form **3a**·$(H_2O)_6$ could be located at both RHF and BH&HLYP levels of theory (Figure 2). With this degree of solvation the zwitterionic form **3a**·$(H_2O)_6$ is 8.7 kcal/mol *lower* in energy than **3**·$(H_2O)_6$ at the BH&HLYP/6-311G(d,p) level. The stabilization of neutral **3** with six water molecules (**3**·$(H_2O)_6$), as defined in eqs 5a,b,

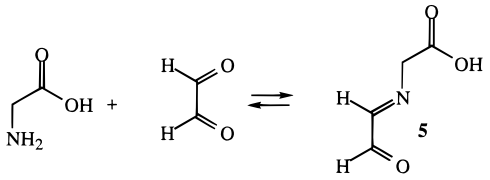
$$\mathbf{3} + 6H_2O \rightarrow \mathbf{3}\cdot(H_2O)_6 \quad \Delta E_{Stab} = -71.0 \text{ kcal mol}^{-1} \quad (5a)$$

$$\text{TS-}\mathbf{4} + 6H_2O \rightarrow \text{TS-}\mathbf{4}\cdot(H_2O)_6 \quad \Delta E_{Stab} = -84.7 \text{ kcal mol}^{-1} \quad (5b)$$

is predicted to be 71.0 kcal mol^{-1} at the same level (Figure 3). The reaction barrier for the loss of CO_2 from zwitterionic cluster **3a**·$(H_2O)_6$ is 18.9 kcal mol^{-1} (Figure 2). The transition structure (TS-**4**·$(H_2O)_6$) more closely resembles zwitterion **3a**·$(H_2O)_6$ than its neutral tautomer **3**·$(H_2O)_6$. This barrier is 4.9 kcal mol^{-1} lower than the gas-phase decarboxylation of **3** (TS-**4**) in the absence of hydrogen bonding (Figure 1). Assuming comparable entropies of activation this corresponds to an increase in the rate for decarboxylation of 4×10^3. These data strongly suggest that the relative stabilization of neutral versus zwitterionic forms by the local environment is mainly responsible for determining the magnitude of the barrier for enzymatic decarboxylation. In fact cluster **3a**·$(H_2O)_6$ is lower in energy than **3**·$(H_2O)_6$ despite the fact that in the absence of water the geometry-constrained gas-phase zwitterionic structure **3a** is about 13 kcal mol^{-1} higher in energy than neutral (**3**) (eq 4). The dipole moments of gas-phase **3** (μ = 4.0 D) suggests that its zwitterionic transition structure TS-**4** (μ = 5.7 D) would be more highly solvated by hydrogen bonding. Indeed, the dipole moment of zwitterion **3a**·$(H_2O)_6$ (μ = 3.1 D) is slightly lower than the neutral complex **3**·$(H_2O)_6$, where μ = 3.4 D. The topology of the potential energy surface for these two clusters differs significantly from the corresponding one for isolated **3** in that the absolute minimum is now zwitterionic in nature and the barrier for decarboxylation of **3a**·$(H_2O)_6$ is reduced.

To understand these changes on the PES upon interaction with the water molecules we computed the interaction energies of gas-phase 2-aminoformylacetic acid (**3**) and its corresponding decarboxylation transition structure TS-**4** with six water molecules (eqs 5, Table 1). Since the transition structure for the decarboxylation of **3** resembles a zwitterion, it is more highly stabilized by water. The relative energies of the species involved in eqs 4 and 5 are shown in Figure 3. The greater stabilization energy of **3a** (ΔE_{Stab} = 79.8 kcal mol^{-1}) relative to **3** and six water molecules at an infinite distance reflects the more acidic ammonium ion of zwitterion **3a** that hydrogen bonds more strongly to water than the neutral NH_2 group in **3** (ΔE_{Stab} = 71.0 kcal mol^{-1}). This results in a decarboxylation barrier that is decreased with respect to the gas-phase process TS-**4** ($\Delta\Delta E^{\ddagger}$ = −4.9 kcal mol^{-1} at the BH&HLYP/6-311G(d,p) level). A more realistic model for enzymatic decarboxylation would stabilize the zwitterion to the extent where it exists as a stationary point with an energy comparable to that of neutral **3**. This would provide the through-bond Coulombic stabilization noted in TS-**4** without the typical barrier increase associated with a lowering of the ground-state energy. This premise is of course contingent upon the fact that the transition structure also resembles a zwitterion and therefore does not realize any additional stabilization relative to reactant **3a**. The above results with the 2-aminoformylacetic acid (**3**) cluster with water clearly show how zwitterion **3a** can be stabilized by the solvent (or interactions at the active site) until the zwitterionic form exists at an energy minimum. These data suggest that the higher efficiency of the active site of HDC is due to more specific stronger hydrogen bonding interactions.

Pyruvoyl Models. To be able to approximate the reactivity of glycine toward enzymatic decarboxylation we need a more realistic model for the amino acid bound at the active site of the enzyme. As a reference point we examined initially the role of electron delocalization of the developing carbanionic center to an adjacent imine on the gas-phase barrier for decarboxylation. As a simplified version of the imine formed in eq 1 we used the condensation of glycine and glyoxal as a model intermediate (**5**, eq 6). An imine of this type provides



(6)

an opportunity to examine the effect of conjugation on the activation barrier since it has been postulated that the "electron sink" provided by the amide carbonyl functionality serves as the driving force to lower the activation energy for decarboxylation.[17] Although most mechanisms for pyruvoyl-dependent enzymes invoke a protonated imine nitrogen in the substrate−Schiff base intermediate at low pH, there seems to have been no explanation offered as to why an iminium cation is an essential feature nor has there been any consideration given to the prospect of a 1,4-proton shift from the neutral carboxyclic acid to the imine nitrogen along the reaction coordinate.

The MP2/6-31G(d) transition structure for decarboxylation of neutral imine **5** (TS-**6**, $\Delta E^{\ddagger}$ = 25.1 kcal mol^{-1}, $\Delta G^{\ddagger}_{298}$ = 25.7 kcal mol^{-1}, $\Delta H^{\ddagger}_{298}$ = 24.2 kcal mol^{-1}, $\Delta S^{\ddagger}_{298}$ = −4.9 cal mol^{-1} K^{-1}) shows that the carboxylic proton is transferred to the imine nitrogen, affording a zwitterionic structure *before the barrier is crossed*. The 1,4-proton transfer occurs along the reaction path to this relatively basic nitrogen. The proton affinity of $H_2C{=}NH$ (−216.1 kcal mol^{-1}) is just 10.8 kcal mol^{-1}

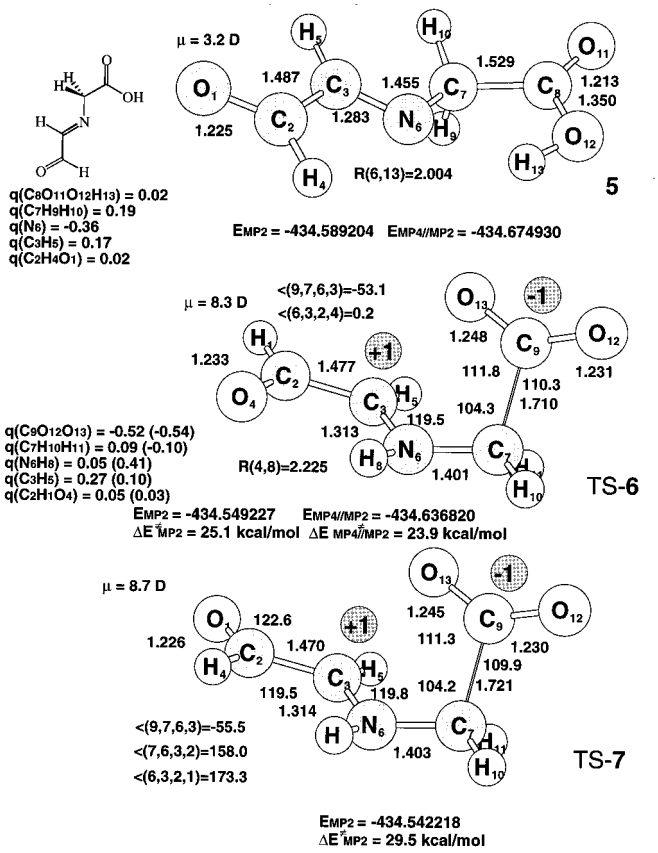


Figure 4. Glycine imine of glyoxal (**5**) and its transition structures for decarboxylation (TS-**6** and TS-**7**). Geometries are fully optimized at MP2/6-31G(d). Energies are given in hartrees, distances in angstroms and angles in degrees. The shaded atoms represent the conjugated π-system. Group Mulliken charges are given as *q*.

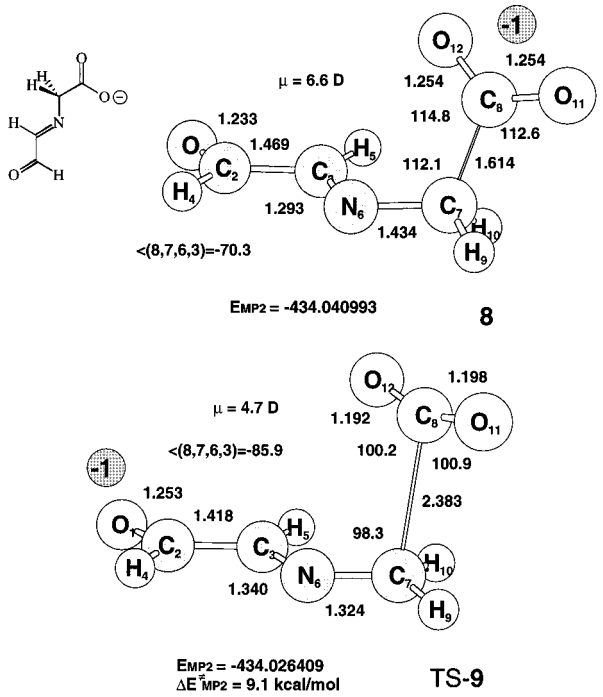


Figure 5. Glycine anionic imine of glyoxal (**8**) and its transition structure for decarboxylation (Ts-**9**). The geometries are fully optimized at MP2/6-31G(d). Energies are given in hartrees, distances in angstroms, and angles in degrees. The shaded atoms represent the conjugated π-system.

lower that the corresponding saturated primary amine H_3C-NH_2 (-226.9 kcal mol^{-1}) at the MP2/6-31G(d) level. The proton shift is accompanied by a rotation of the CO_2 group around the C_7-N_6 bond that aligns the breaking C−C bond with the developing π-system. The transition structure itself lies early on the reaction path, and the formal negative charge on the zwitterionic transition structure is still mostly localized on the carboxylate group. In fact, the major structural change in the formation of TS-**6** is the increased distance of the CO_2 fragment from the α-carbon ($\Delta r(C_7-C_9) = 0.181$ Å) while the C_7-N_6 distance of the developing C═N bond is shortened by only 0.044 Å. The C_2-O_4 distance in the carbonyl group that is thought to serve as an electron sink is increased by only 0.008 Å, and its group charge on going from reactant **5** to the transition structure (TS-**6**) is slightly more positive ($\Delta q = 0.03$) rather than negative. *Thus, the carbonyl group is virtually unaffected by the change in electronic distribution attending decarboxylation*. The developing negative charge on C_7 of the amino acid ($\Delta q = -0.10$, Figure 4) is Coulombically stabilized by the adjacent protonated iminium group that greatly increases its charge upon protonation (N_6-H_8, $\Delta q = 0.41$). Also the group charge on the C−H (C_3-H_5) group in (TS-**6**) increases by 0.10, showing that it is actually the positive charge on the iminium group that is being stabilized. It should be recalled that the positive charge of 1+ on the iminium nitrogen is a formal charge. The calculated Mulliken charge on N_6 in TS-**6** is -0.55, while the N_6-H_8 group charge is 0.05. Breaking the cisoid hydrogen bond between O_4 and H_8 in TS-**6** by rotation around the C_2-C_3 results in TS-**7** and an increase in the barrier of 4.4 kcal mol^{-1}. Triple and quadruple excitations in the perturbative series do not affect greatly the barrier for decarboxylation of **5** ($\Delta E^{\ddagger} = 23.9$ kcal mol^{-1} at the MP4/6-31G(d)//MP2/6-31G(d)). As previously noted for the decarboxylation of 2-aminoformylacetic acid (**3**) the B3LYP method affords a lower activation barrier with respect to MP2 while BH&HLYP gives a slightly higher barrier (Table 1). Since the decrease in barrier height for decarboxylation of **5** is only 4.7 kcal mol^{-1} (Table 2) relative to the simplest model imine (H_2C═$N-CH_2-COOH$) we suggest that the developing positively charged iminium ion in TS-**6** makes the largest contribution to the stability of the transition structure. A similar through-bond Coulombic stabilization has been observed in the decarboxylation of isolated 2-aminoformylacetic acid[1] ($\Delta\Delta E^{\ddagger} = 20.4$ kcal mol^{-1}).

The observed nitrogen isotope effect for HDC indicates that the imine nitrogen in the substrate−Schiff base intermediate complex is ordinarily protonated,[19a] and the pH dependence of the carbon isotope effect indicates that both protonated and unprotonated forms of this intermediate are capable of undergoing decarboxylation.[19b] Consequently, we elected to examine the barrier of deprotonated imine **5**. The barrier for decarboxylation of the free anion of the simplest β-keto acid H(C═O)-$CH_2COO(-)$ is significantly reduced ($\Delta\Delta E^{\ddagger} = -7.9$ kcal mol^{-1} at MP2/6-31G(d)) with respect to its parent carboxylic acid form $H(C=O)CH_2COOH$.[1] The decarboxylation barrier for the anion **8** of imine **5** (TS-**9**, Figure 5, $\Delta E^{\ddagger} = 9.1$ kcal mol^{-1}, $\Delta G^{\ddagger}_{298} = 6.4$ kcal mol^{-1}, $\Delta H^{\ddagger}_{298} = 7.3$ kcal mol^{-1}, $\Delta S^{\ddagger}_{298} = 3.1$ cal mol^{-1} K^{-1}) is also lowered considerably relative to the parent neutral compound ($\Delta\Delta E^{\ddagger} = -16.0$ kcal mol^{-1} at MP2/6-31G(d)). The formation of a carboxylate anion raises the energy of the ground-state reactant system which is typically attended by a lowering of the gas-phase barrier for decarboxylation. In solution the rate of decarboxylation would be slowed significantly by ion

(19) (a) Recsei, P. A.; Snell, E. E. *Annu. Rev. Biochem.* **1984**, *53*, 357. (b) Lynn, M. A.; O′ Leary, M. H. *Biochemistry* **1988**, *27*, 5933. (c) Grate, J. W.; McGill, R. A.; Hilvert, D. J. *J. Am. Chem. Soc.* **1993**, *115*, 1410. (d) Rahil, J.; You, S.; Kluger, R. *J. Am. Chem. Soc.* **1996**, *118*, 12495.

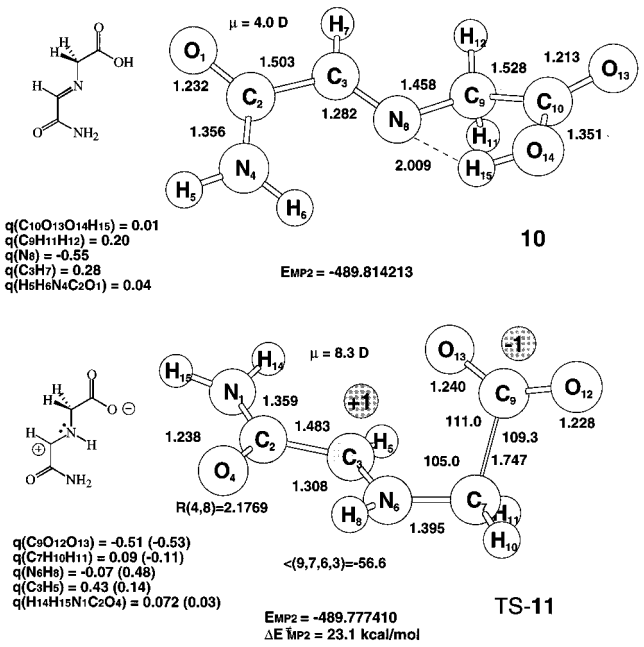
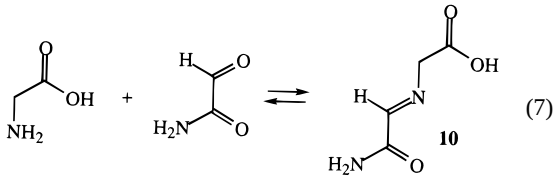


Figure 6. Glycine imine of glyoxylic acid amide (**10**) and its transition structure for decarboxylation (TS-**11**). The geometries are fully optimized at MP2/6-31G(d). Energies are given in hartrees, distances in angstroms, and angles in degrees. The shaded atoms represent the basic π-system. Group Mulliken charges are given as *q*, and the net changes are given in parentheses.

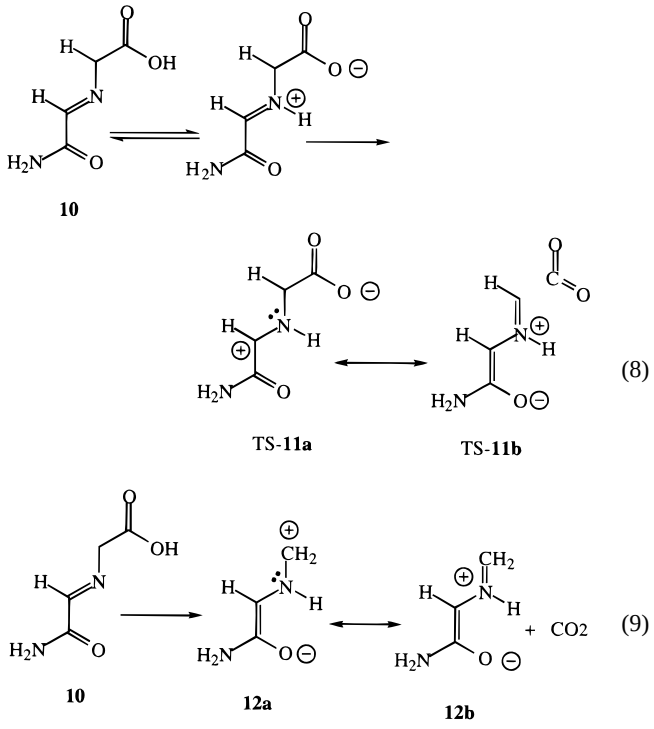
pairing.[19c] It is also noted that the C_7-C_8 bond distance of 1.614 Å in anion **8** compares with the 1.529 Å in parent compound **5**. These data support the general idea[1] that decarboxylation can proceed through the unprotonated form of the carboxylic acid group or that a proton shift to the imine nitrogen can occur in concert with loss of CO_2. However, as noted for model studies on biotin-mediated decarboxylation, the rate of loss of CO_2 from the neutral carboxylic acid is 6×10^3 times faster than that of its anion.[19d]

Since the allylic-type ($CH_2-N{=}CH_2$) conjugation in **5** reduces the barrier height for decarboxylation by almost 30 kcal mol^{-1} relative to glycine (**1**), model compound **5** was extended to include the amide functionality present in the pyruvoyl enzyme (eq 7). As noted above the 1,4-proton shift from the



(7)

carboxylic acid group to the imine nitrogen is complete before the barrier is crossed in TS-**11** (Figure 6). The amidic nitrogen is capable of a further stabilization of the transition structure that results in a lowering of the classical barrier at the MP2 level by only 2.0 kcal mol^{-1} relative to TS-**6** ($\Delta E^{\ddagger} = 23.1$ kcal mol^{-1}, $\Delta G^{\ddagger}_{298} = 23.6$ kcal mol^{-1}, $\Delta H^{\ddagger}_{298} = 22.1$ kcal mol^{-1}, $\Delta S^{\ddagger}_{298} = -5.0$ cal mol^{-1} K^{-1}). Significantly, the $H_2NC{=}O$ amidic functionality in reactant **10** has a group charge of 0.04 and it does not disperse the developing negative charge in TS-**11** *where this group charge is actually slightly more positive* ($q(H_2NCO) = 0.07$). The charge distribution shows that the net charge on the CO_2 fragment of TS-**11** is negative (−0.51) while the charge on the remainder of the pyruvoyl system bears an equal positive charge. Moreover, a resonance structure like TS-**11a** rather than TS-**11b** (eq 8) is responsible for the stability



(8)

(9)

of the transition structure with respect to the energetically prohibitive decarboxylation of isolated glycine. The group charge of 0.43 on the C−H fragment (C_3-H_5) in TS-**11** shows that the positive fractional charge of the zwitterionic structure is delocalized by the π-system of the C=N double bond ($C_3{=}N_6$, Figure 6).

An extension of the C=N π-system has only a minor effect on the barrier for decarboxylation. Table 2 summarizes the effect of progressively extending the degree of electron delocalization on the reaction barrier showing that the imine functionality plays the major role. The impact on the reaction barrier of extending the parent glycine molecule **1** with either a carbonyl group or an imine functionality ($N{=}CH_2$) is to lower it by ca. 35 kcal mol^{-1}. However, the additional carbonyl group as in **5** or an amide as in **10** serves to lower the barrier height relative to $H_2C{=}N-CH_2-COOH$ by 5−7 kcal mol^{-1}. The reduction in the barrier, however, is not accompanied by a transfer of electron density to the amide oxygen as required by conventional wisdom (eq 1). Thus, the so-called "electron sink" is not operative and the amide functionality serves to modify the ground state. We suggest that the Coulombic stabilization induced by proton transfer to the adjacent nitrogen makes the greatest contribution to the stability of the transition structure (TS-**11**). This concept is also supported by the charge distribution in the product **12** of the decarboxylation of **10** (eq 9). The charge distribution in **12** is remarkably different from that in the corresponding TS-**11** and shows an overall neutral polarized structure $q(H_2N-CO) = -0.13$ and $q(CH_2) = 0.09$ with resonance structure **12a** being the most representative. The data on the calculated charge distribution, however qualitative, clearly show that the type of polarization with the negative end on the carbonyl group occurs only at the product stage in the reaction path. The early transition structures exhibit structural and electronic features very close to zwitterionic intermediates.

At this point a comment concerning the origin of the so-called "electron sink effect" is in order. Resonance structures described by eq 1 suggest that the carbonyl oxygen of the amide residue bound to the enzyme has effectively a net formal charge of −1. However it should be recalled that formal charges do not correspond to actual computed charges,[1] as exemplified by

Table 3. Activation Energies (kcal mol^{-1}) for the Decarboxylation of the Glycine Imide of Glyoxal (**5**) H(C═O)CH═NCH$_2$COOH

computational level	TS-**6** (syn)	TS-**7** (anti)
MP2/6-31G(d)//MP2/6-31G(d)	25.1	29.5
MP4/6-31G(d)//MP2/6-31G(d)	23.9	
B3LYP/6-311G(d,p)//B3LYP/6-311G(d,p)	21.4	
BH&HLYP/6-311G(d,p)//BH&HLYP/6-311G(d,p)	29.1	

Table 4. Relative Energies (kcal mol^{-1}) of the Glycine Imide of Glyoxal (**5**) H(C═O)CH═NCH$_2$COOH Hydrogen Bonded to Formic Acid and Model Peptide **13**[a]

computational level	**14**	zwitterion **15**	TS-**16**
BH&HLYP/6-311G(d,p)//-BH&HLYP/6-311G(d,p)	0.0 [−24.0][b]	19.0 [−5.0][b]	26.0 [−27.2][c]

[a] The sum of the energies of isolated fragment **13** and HCOOH is −512.142 953 at the same level of theory. [b] Interaction energy of **5** with isolated fragment **13** and HCOOH. [c] Interaction energy of TS-**7** with isolated fragment **13** and HCOOH.

the group charge on the N_6-H_8 group, that is always close to neutrality ($q = -0.07$). Besides, arguments set forth by Wiberg[20] concerning the C−N rotational barrier in simple amides suggest that the carbonyl group is equally polarized in both the ground-state reactant and transition structure for C−N bond rotation. By analogy, we suggest that the amide group of the prosthetic group is equally polarized in both reactant **10** and its transition structure (TS-**11**) for decarboxylation. Indeed, the calculated charges on the carbon and oxygen of the amide carbonyl group in **10** and TS-**11** are 0.75, −0.60 and 0.75, −0.61, respectively. In addition, we observe no change in the geometry of the carbonyl group; the C−O bond distances in **10** and TS-**11** are 1.232 and 1.238 Å, respectively. Consistent with this suggestion the group charge on the $H_2N-C═O$ amide group ($H_5H_6N_4C_2O_1$) is 0.04 in minimum **10** and, as noted above, is slightly more *positive* (0.07) in TS-**11**. There is little question that the amide functionality lowers the barrier for decarboxylation ($\Delta\Delta E^{\ddagger} = 6.7$ kcal mol^{-1}). However, *we see no evidence consistent with the resonance interaction shifting electron density to the amide carbonyl oxygen as suggested by eq 1*. The primary function of the imine functionality is to provide an electron-deficient carbon at C_3 as a polarizable group adjacent to the protonated nitrogen N_6. The group charge on the C−H fragment (C_3-H_7) is 0.28 in reactant **10** and 0.43 in TS-**11**.

Model of the Active Site. Histidine decarboxylase has been reported to accommodate the carboxylic acid group of the intermediate Schiff base in a hydrophobic pocket containing Glu-197 as the only polar residue.[17a] Consequently, we chose to model the decarboxylation reaction of imine **5** in a more hydrophobic enzyme-like environment. The following structures have been optimized at the BH&HLYP/6-311G(d,p) level of theory. The results in Tables 1−3 show that this method gives results that are consistent with the MP2/6-31G(d) level of theory both in the energetics of the hydrogen bonds and the reaction barrier for decarboxylation. The hydrogen bonding network provided by the enzyme active site is highly specific and directional in contrast to that shown in structures **3**·$(H_2O)_6$ and **3a**·$(H_2O)_6$. The Glu-197 and Phe-195 residues that are present at the active site of the pyruvoyl-dependent histidine decarboxylase[17a] are modeled in structures **14** and **15** (Figure 7) by a formic acid and the model fragment peptide H(C═O)-HNCH$_2$CHO (**13**), respectively. It has been suggested that the amide nitrogen helps pull the required electron from the departing carboxylate group through the conjugated system.[17]

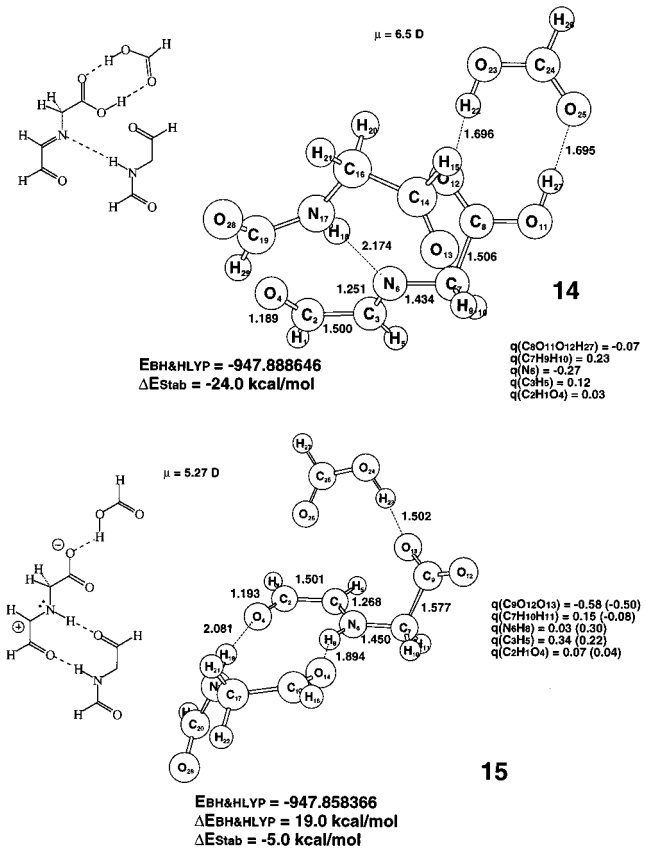
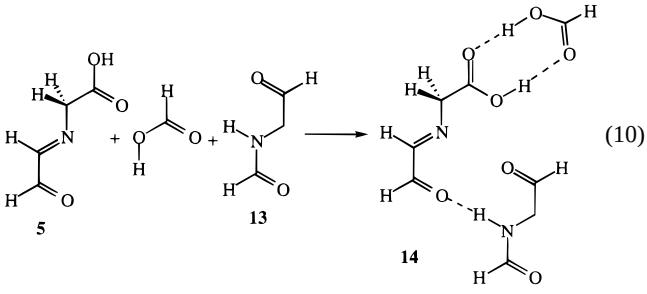


Figure 7. Glycine imine of glyoxal (**5**, shaded structure) hydrogen bonded to formic acid and model peptide **13**. Neutral form (**14**) and zwitterionic form (**15**). Geometries are fully optimized at BH&HLYP/6-311G(d,p). Energies are given iin hartrees, distances in angstroms, and angles in degrees. Group Mulliken charges are given as *q*, and the net changes are in parentheses.

In this model selective hydrogen bonding is provided between the carboxylic acid groups of model imine **5** and formic acid. In neutral **14**, that is 24.0 kcal mol^{-1} lower in energy than its isolated components, the structure is held together by the weak hydrogen bond between N_6 of **5** and the N−H ($N_{17}-H_{18}$) of **13** (eq 10).



(10)

The next step is to transfer the proton from the carboxylic acid group to the imine nitrogen to form stabilized zwitterion **15** that now possesses the activated iminium functionality essential for decarboxylation. In zwitterionic intermediate **15**, the N−H of **13** is hydrogen bonded to the free carbonyl oxygen O_4 of pyruvamide **5** and a new and much stronger hydrogen bond is developed between the more acidic iminium hydrogen (H_8) and O_{14} of **13**. Zwitterion **15** is not as greatly stabilized as **14** with respect to isolated reactants ($\Delta E_{Stab} = -5.0$ kcal

(20) Wiberg, K. B; Breneman, C. M. *J. Am. Chem. Soc.* **1992**, *114*, 831.

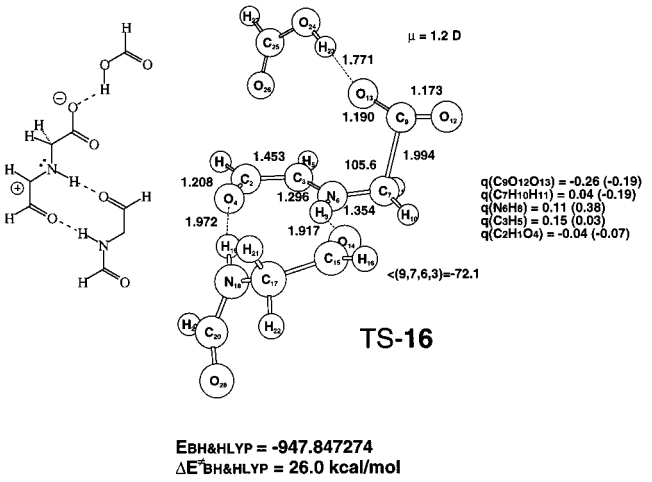


Figure 8. Glycine imine of glyoxal (**5**, shaded structure) hydrogen bonded to formic acid and model peptide **13**; transition structure for decarboxylation (TS-**16**). Geometry is fully optimized at BH&HLYP/6-311G(d,p). The energy is given in hartrees, distances in angstroms, and angles in degrees. The Mulliken group charges are given as *q*, and the net changes are in parentheses.

mol^{-1}) as a consequence of its greater local charge separation despite the fact that its total dipole moment is decreased.

The mechanistic role assigned to the backbone nitrogen amide of residue 195 is to stabilize the oxyanion that purportedly forms in the TS on the amide carbonyl (electron sink) of the pyruvoyl group.[17b] However, this interaction is present in ground-state zwitterion **15** (O_4-H_{19} = 2.081 A) as well as in TS-**16** (O_4-H_{19} = 1.972 Å). The role of the formic acid in TS-**16** (Figure 8) is to stabilize the carboxylate anion (O_{13}) while the carbonyl group (O_{14}) of model peptide **13** interacts strongly with the iminium hydrogen (H_8). The barrier height for decarboxylation of model system **14** is 26.0 kcal mol^{-1}, while that for the "gas-phase" decarboxylation of **5** is 29.1 kcal mol^{-1} at the same level of theory. The activation barrier measured from intermediate zwitterionic structure **15** is only 7.0 kcal mol^{-1}. One of the hydrogen bonds between the two carbonyl groups is lost in TS-**16** but this loss is energetically more than compensated for by the much stronger bonding interactions involving charged groups. As a result of this specific interaction, that provides a different arrangement of the hydrogen bonding for the transition structure relative to the minimum intermediate **14**, TS-**16**, as depicted for **5** in eq 10, is greatly stabilized. In fact, the energy of interaction between the transition structure for the gas-phase decarboxylation (TS-**6**) and the two model amino acid residues in TS-**16** is 27.2 kcal mol^{-1}, a value slightly greater than ΔE_{Stab} = −24.0 kcal mol^{-1} for reactant **14**.[21] It should be recalled that the constrained zwitterion of glycine **1** is 19.9 kcal mol^{-1} higher in energy than glycine, and we estimated that constrained zwitterion **5** would exhibit a comparable increase in energy. The additional stabilizing interactions at the HDC active site may further stabilize zwitterion **15** and TS-**16**, influencing the activation barrier for loss of CO_2. If the energy of **15** could be further reduced by specific hydrogen bonding in an actual enzymatic environment to a point where it is comparable in energy to neutral **14**, then the overall barrier for decarboxylation should be close to 7 kcal mol^{-1}. Thus, we suggest that it is the role of the amino acid residues at the active site to stabilize a zwitterionic structure either as a ground-state intermediate or as a TS in order to effectively reduce the activation energy for decarboxylation. In fact, the optimal situation exists when the molecular architecture of the active site of the enzyme is designed such that the energies of the neutral reactant intermediate and the zwitterion are comparable so that at equilibrium a favorable concentration of zwitterion is present and the ground-state energy of the reactant is not significantly lowered, thereby effecting an increase in the barrier. In addition to the extension of the conjugation of the delocalized ground state π-system, the role of the amide functionality could well be to provide a hydrogen bonding network to maintain the planarity of the pyruvoyl system in the TS for decarboxylation. The primary driving force in these enzymatic decarboxylations is the stabilization of the developing charge by adjacent positively charged heteroatoms. It should also be pointed out that thiamin-diphosphate-dependent enzymes (ThDP)[22] may also utilize couloumbic stabilization to effect decarboxylation. We have reached similar conclusions regarding adjacent iminium ions in studies on PLP-dependent enzymes where the role of the pyridine ring has been examined and also found to have no discernible "electron sink" effect. These studies are in progress.

(21) The interaction energy is computed relative to gas-phase TS-**6**, formic acid, and fragment **13**.

Conclusions

1. In a nonenzymatic environment the transition structures for decarboxylation closely resemble solvated zwitterionic species. The carboxylic acid proton is shifted to an adjacent base along the reaction pathway (carbonyl for β-keto acid or the nitrogen of an α-amino acid). The zwitterionic intermediate is essential to the overall decarboxylation process, and it is formed to provide Coulombic stabilization of the developing charge in the transition structure.

2. A primary function of the HDC is to provide a hydrogen bonding environment that can stabilize the zwitterionic form of the Schiff base of the α-amino acid in either the ground or transition state for decarboxylation.

3. The extended conjugation attending the formation of intermediate imine **5** is capable of lowering the energetic requirement for decarboxylation of glycine from the 64.5 kcal mol^{-1} of the isolated species **1** to 25.1 kcal mol^{-1} due to electron delocalization and Coulombic stabilization in TS-**6**.

4. Zwitterionic structures for glycine (**3**) and pyruvamide model **5** could not be located on the gas-phase surfaces, but they can be stabilized by key interactions with NH_4^+, water (**3a**·$(H_2O)_6$), or amino acid residues at the active site (**15**).

5. The zwitterionic structure **3a**·$(H_2O)_6$ is lower in energy (ΔE = −8.7 kcal mol^{-1}), is more polar, has a higher dipole moment, and is more highly stabilized by water than neutral **3**·$(H_2O)_6$.

6. An increase in the stabilization energy of the decarboxylating zwitterionic intermediate by hydrogen bonds with water leads to a lowering of the decarboxylation barrier ($\Delta\Delta E^{\ddagger}$ = 4.9 kcal mol^{-1}) as exemplified in TS-**4**·$(H_2O)_6$.

7. Hydrogen bonds to more acidic sites such as iminium =N−H(+) ions and carboxylic acids have a greater effect upon the barrier than multiple but weaker hydrogen bonds. The stronger and more specific hydrogen bonds in TS-**16** with respect to TS-**4**·$(H_2O)_6$ appear to be consistent with the suggested mechanism of HDC.

8. The electron sink or net transfer of electron density to the amide group in the transition state plays no role in lowering the activation barrier for decarboxylation.

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