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# Catalysis of decarboxylation by an adjacent negative charge: a theoretical approach

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**Abstract**

We have calculated the rate acceleration in decarboxylation reactions that can be accomplished by charge–charge repulsion between the substrate carboxylate and an adjacent negative charge in media of various dielectric constants. It has been shown that a full negative charge or surrounding partial negative charges will have the same effect. It is concluded that the rate of decarboxylation could be greatly accelerated by the presence of a negative charge nearby. For example, in media with dielectric constants from 5.62 to 20.7, a $10^8$-fold rate acceleration could be achieved by a negative charge placed 3.77 Å away from the substrate carboxylate group. However, p$K_a$ perturbation on two carboxylate groups at close proximity may limit the extent of catalysis. It should also be noted that the extent of catalysis does not change much when the dielectric constant varies from 5.62 to 20.7.


## 1. Introduction

Catalysis of biochemical decarboxylations is accomplished in several ways. Many decarboxylases contain electron sinks such as pyridoxal phosphate or thiamine

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pyrophosphate as cofactor [1]. These cofactors stabilize the carbanion intermediate, formed from decarboxylation, by delocalizing the charge into an electrophilic π-system. There are examples of enzyme-catalyzed decarboxylation that do not require cofactors, such as orotidine-5′-monophosphate (OMP)[2] decarboxylase and acetoacetate decarboxylase [2,3]. However, acetoacetate decarboxylase and similar enzymes are involved in the decarboxylation of β-ketoacids in which the carbanion intermediates are stabilized by delocalization into the neighboring carbonyl or imine group [3].

Decarboxylation could also be accelerated by destabilization of the substrate carboxylate group in the active site of enzymes. Two possible strategies can be envisioned in accomplishing this, a hydrophobic environment or negative charges nearby.

The effect of a hydrophobic medium on the rate of decarboxylation has been well documented in model studies [4–8]. The acceleration in reaction rate is likely due to the desolvation of the carboxylate ion in a less polar environment [7]. It has also been observed that medium effects and desolvation might play a role in enzymatic catalysis [9,10].

The other strategy to destabilize the carboxylate group is through charge–charge repulsion with an adjacent negative charge at the active site. This approach has been alluded to in the catalytic mechanism of histidine decarboxylase [11] and has recently been a target of significant research effort and discussion, especially on the mechanism of OMP decarboxylase [12–17]. Crystallographic studies have revealed that the active site is fairly hydrophobic and the carboxylate groups of the substrate and an active site carboxylate (glutamate or aspartate) are in close proximity [13–15,18].

The proposal that decarboxylation reactions could be catalyzed through charge–charge repulsion is conceptually very simple and attractive. Elegant computational studies have been carried out to assess the effect of the environment of the entire active site (including the negative charge) on the catalysis of the decarboxylation of OMP [14,16]. However, in this study we have focused on the more general question of catalysis of decarboxylation reactions by placing a negative charge at different distances from the carboxylate. The reactions are catalyzed since the external negative charge is farther away from the product carbanion (and thus the transition state) than the carboxylate anion. It is important to estimate the reduction in activation energy by having a negative charge near the carboxylate group. In this report, we have calculated the magnitude of rate acceleration that can be accomplished by charge–charge repulsion between the substrate carboxylate and a full negative charge or surrounding partial negative charges in media of various dielectric constants [19].

## 2. Materials and methods

Acetic acid/acetate and $CO_2$ were optimized using density functional theory (DFT) with the Becke 3-parameter hybrid exchange functional and Lee–Yang–Parr gradient corrected electron correlation functional (B3LYP) with the 6-31++G** ba-

[2] *Abbreviations:* OMP, orotidine 5′-monophosphate; DFT, density functional theory; COSMO, conductor-like screening solvation model.

sis set. To model enzyme active sites, several partially negatively charged residues as well as a single residue with a full negative charge were employed to destabilize the carboxylate group. We have calculated two arrangements for the charge–charge repulsion: one in which the negative charge is surrounded by five partial charges of −0.2 each and the other in which the repulsion is represented by a full negative charge, $F^-$ (Fig. 1).

The energies for both arrangements were determined from single point calculations at the B3LYP/6-31++G** level on the previously optimized substrates while varying the distance between the sites up to 100 Å. Since the degrees of solvation in enzyme active sites are unknown, the single point calculations for these complexes were also performed in simulated polar environments over a range of dielectrics: 1.00, 5.62, 10.36, 20.7, and 78.5. The condensed phase calculations were performed using DFT combined with a conductor-like screening solvation model (COSMO) that treats the solvent as a polarizable, continuous medium. All calculations were done using Gaussian 98 (Gaussian, Pittsburg, PA, (http://www.gaussian.com) on the Livermore Computing Center's DEC Alpha Cluster.

### 2.1. Method validation

To determine the effect of aqueous solvation on the energy and structure, the interaction energies of the compounds were also determined using DFT combined with COSMO. COSMO models the surrounding solvent by means of polarization charges distributed on the solvent exposed surface of the molecule. Since the system being studied undergoes molecular separation out to 100 Å the solvent accessible surface will be broken into two separate closed surfaces. Since it is not readily obvious that an implicit solvent model would work under such circumstances, we tested this model for the simple test system of two bare anions, represented by fluoride ions in these test calculations.

The potential energy of ionic interactions separated by a distance, $r$, is

$$E = 332.07\frac{q_1 q_2}{\varepsilon r_{12}},$$

where $q$ is the charge of the ion and $\varepsilon$ the dielectric constant of the surrounding medium and $r$ is in angstroms and $E$ in kcal/mol. Because a gas-phase medium has a dielectric constant of 1, a plot of the potential energy versus $1/r$ should result in a

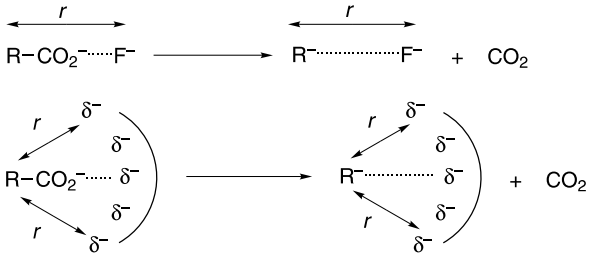


Fig. 1. Decarboxylations in the presence of adjacent negative charge(s).

straight line with slope equal to 332.07 kcal/mol/Å. Similarly, this plot in a medium of water (dielectric constant = 78.5) should give a slope of 4.23. We have plotted the potential energy as predicted from DFT quantum chemical simulations performed in the gas-phase and in water (using the COSMO model) for two fluorine anions separated at a range of different distances. The slope for the gas-phase curve is 332.06 with a regression coefficient, $R^2 = 1.0000$, agreeing nearly perfectly with Coulomb's law. While the slope for the aqueous phase curve is 4.36 with an $R^2 = 0.9992$, deviation from linearity is only observed at close nuclei–nuclei distances ($r \leqslant 7$ Å). These results validate the use of the COSMO model for calculating the condensed phase interactions of widely separated ions.

## 3. Results and discussion

The decarboxylations of acetate and benzoate were calculated according the equations shown in Fig. 1. A fluoride anion ($F^-$) is used to model the adjacent negative charge. It has been demonstrated that decarboxylation is a barrierless process in the gas phase and therefore, the enthalpies of the reactions in Fig. 1 are equivalent to the activation enthalpies ($\Delta H^{\ddagger}$) of the reactions [12].

It is likely that a few partially negatively charged residues, instead of one residue with a full negative charge, might be employed to destabilize the carboxylate group in the active site of enzymes. Therefore, we have also calculated the situation in which the negative charge is provided by five surrounding partial charges ($\delta^- = -0.2$) with a total charge of $-1$. We have found that there is little difference between the effects caused by the two arrangements. In Fig. 2, the reaction enthalpies of the two arrangements, calculated at the B3LYP/6-31++G** level of theory, are plotted against the distance between the carbanion and the negative charge(s). Because both approaches give similar results, the simpler one from Fig. 1 (i.e., charge = $F^-$) was been employed in all subsequent calculations.

The energies of charge–charge interactions are expected to be heavily dependent on the dielectric constant of the media. The dielectric constant of the active site of enzymes has been estimated from 4 to 15. We have therefore carried out the

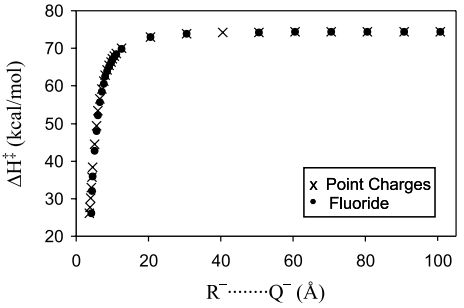


Fig. 2. Plot of decarboxylation energy as a function of charge–charge distance (B3LYP/6-31++G** level).

calculations on the decarboxylation of acetate in different polar environments with dielectric constants ranging from 1 (gas phase) to 78.5 (water). To do this, we have employed the conductor-like screening solvation model (COSMO) in conjuction with the B3LYP/6-31++G** level to determine the energies. Using a series of simulated dielectrics with this model, the decarboxylation enthalpies of acetate were plotted against the $R^- \cdots F^-$ distance (Fig. 3). The decarboxylation of benzoate has also been modeled and the results are very similar to those from acetate.

The reductions in activation enthalpy ($\Delta\Delta H^{\ddagger}$) of the reaction due to the presence of the negative charge at a certain distance can be calculated by comparing the reaction enthalpy at that distance to that of the uncatalyzed reaction at the corresponding dielectric constant. The decarboxylation enthalpies for the uncatalyzed reactions are 74.5, 69.7, 67.7, 67.5, and 66.1 kcal/mol at dielectric constants of 1, 5.6, 10.36, 20.7, and 78.5, respectively. The first part of Table 1 lists the results when the negative charge is 6.566 Å away from the carbanion. This leads to a distance of 4.71 Å between the negative charge and the carboxylate group. Table 1 shows that at a distance of 4.71 Å between the substrate carboxylate group and an adjacent negative charge, the activation enthalpy of the reaction can be reduced by an amount ranging from 18.7 to 4.6 kcal/mol. For example, if the dielectric constant of the enzyme interior is 5.62, the rate acceleration ($k_{cat}/k_{noncat}$) can be as large as 50,000-fold. It is interesting

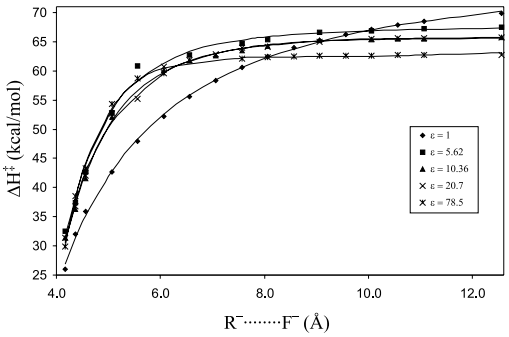


Fig. 3. Plot of decarboxylation energy as a function of $R^- \cdots (F^-)$ distance employing various dielectric constants (COSMO B3LYP/6-31++G** level).

Table 1
Catalysis by an adjacent negative charge at different distance and media

| Dielectric constant | $R^- \cdots F^- = 6.56$ Å and $RCO_2^- \cdots F^- = 4.71$ Å | | $R^- \cdots F^- = 5.56$ Å and $RCO_2^- \cdots F^- = 3.77$ Å | |
|---|---|---|---|---|
| | $\Delta\Delta H^{\ddagger}$ (kcal/mol) | $k_{cat}/k_{noncat}$ | $\Delta\Delta H^{\ddagger}$ (kcal/mol) | $k_{cat}/k_{noncat}$ |
| 1 | 18.7 | $5.2 \times 10^{13}$ | 26.2 | $1.6 \times 10^{19}$ |
| 5.62 | 6.4 | $4.9 \times 10^{4}$ | 11.4 | $2.3 \times 10^{8}$ |
| 10.36 | 6.1 | $3.0 \times 10^{4}$ | 11.3 | $1.9 \times 10^{8}$ |
| 20.70 | 5.8 | $1.8 \times 10^{4}$ | 10.9 | $9.9 \times 10^{7}$ |
| 78.5 | 4.6 | $2.4 \times 10^{3}$ | 7.9 | $6.2 \times 10^{5}$ |

to note that the amount of reduction in activation energy does not change as much as one would expect from varying the dielectric constants from 5.62 to 78.5. However, with such a short distance between two similar charges, the dielectric of the surrounding medium does not seem to be able to provide effective shielding of the electrostatic repulsion. As a result, the active sites of the decarboxylases are not required to be extremely hydrophobic for this type of catalysis to take effect.

Shorter distances between the substrate carboxylate group and the catalytic negative charge such as 3.5 Å or shorter have been reported for enzymes such as histidine decarboxylase and ODCase [11,13–15]. At this distance, tremendous rate acceleration can be realized. For example, when the negative charge is 3.77 A away from the carboxylate group, the reaction can be catalyzed by around $10^8$-fold in media with dielectric constants from 5.6 to 20.7, as shown in Table 1. However, one factor will complicate the situation when there is too short a separation between the substrate carboxylate group and the negatively charged catalytic residue, which is likely to be a side-chain carboxylate group. If the distance between two carboxylate groups is too short, their p$K_a$ values are likely perturbed and one of them should be protonated [17]. In other words, to gain a large amount of electrostatic catalysis in the decarboxylation process, the system would create a highly unstable carboxylate that would likely be protonated. This puts a limit on the amount of catalysis that can be provided by such an electrostatic destabilization of the ground-state carboxylate. One way enzymes might employ to alleviate this problem is to partially reduce the negative charge on the catalytic residue. The partial negative charge will be able to catalyze the reaction, though the $\Delta\Delta H^{\ddagger}$ is reduced. Rate acceleration of more than $10^4$-fold can still be achieved even if the $\Delta\Delta H^{\ddagger}$ values in the later part of Table 1 are halved to about 5–6 kcal/mol.

The results from quantum mechanics calculations have demonstrated that a nearby negative charge can greatly catalyze a decarboxylation reaction. The presence of a destabilizing negative charge combined with the hydrophobicity of the active site as seen in some enzymes may have an even greater catalytic effect, though our results have shown that the requirement for hydrophobicity is not as rigorous as one would expect. Our calculations have provided a quantitative base from which the extent of catalysis through the destabilization of the substrate could be estimated. However, the impact of the electrostatic destabilization on the p$K_a$'s of the species in the active site of the enzyme will limit the catalysis to the difference between the p$K_a$'s the carboxylates and the effective pH of the active site (approximately a factor of $10^3$–$10^5$).

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