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New Insights into the Mechanistic Details of the Carbonic Anhydrase Cycle as Derived from the Model System $[(NH_3)_3Zn(OH)]^+/CO_2$: How does the H_2O/HCO_3^- Replacement Step Occur?

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Dedicated to Professor Dr. Heinrich Vahrenkamp on the occasion of his 60th birthday

*The full reaction path for the conversion of carbon dioxide to hydrogencarbonate has been computed at the B3LYP/6-311 + G** level, employing a $[(NH_3)_3Zn(OH)]^+$ model catalyst to mimic the active center of the enzyme. We paid special attention to the question of how the catalytic cycle might be closed by retrieval of the catalyst. The nucleophilic attack of the catalyst on CO_2 has a barrier of 5.7 kcal mol^{-1} with inclusion of thermodynamic corrections and solvent effects and is probably the rate-determining step. This barrier corresponds well with prior experiments. The intermediate result is a Lindskog-type structure that prefers to stabilize itself via a rotation-like transition state to give a Lipscomb-type product, which is a monodentate hydrogencarbonate complex. By addition of a water molecule, a pentacoordinated adduct with pseudo-trigonal-bipyramidal geometry is formed. The water molecule occupies an equatorial position, whereas the hydrogencarbonate ion is axial. In this complex, proton transfer from the Zn-bound water molecule to the hydrogencarbonate ion is extremely facile (barrier 0.8 kcal mol^{-1}), and yields the* trans,trans-*conformer of carbonic acid rather than hydrogencarbonate as the leaving group. The carbonic acid molecule is bound by a short O···H–O hydrogen bond to the catalyst $[(NH_3)_3Zn(OH)]^+$, in which the OH group is already replaced by that of an entering water molecule. After deprotonation of the carbonic acid through a proton relay to histidine 64, modeled here by ammonia, hydrogencarbonate might undergo an ion pair return to the catalyst prior to its final dissociation from the complex into the surrounding medium.*

KEYWORDS:

bioinorganic chemistry · carbonic anhydrase · density functional calculations · enzyme models · lyases

Introduction

Carbonic anhydrases (CAs) are a class of zinc enzymes[1] which are of eminent biological importance in photosynthesis, respiration, and physiological buffers in organisms.[2] They accelerate the hydration of carbon dioxide by a factor of 10^7,[3] thus resulting in reaction rates typical for diffusion-controlled processes. Ever since the seminal paper by Liang and Lipscomb on the theoretical study of the uncatalyzed hydration of carbon dioxide in the gas phase to yield hydrogencarbonate,[3] suggestions[4, 5] have been raised about the mechanism of the catalytic hydration performed by carbonic anhydrases.

The activation barrier for the enzymatic reaction is, as deduced from the experimental rate constant,[4] about 3 kcal mol^{-1} assuming the same frequency factor as for the uncatalyzed reaction, for which the activation energy in solution has been found to be 17.7 kcal mol^{-1}.[6] The total free energy of the reaction can be inferred either from the difference in barriers for the forward and reverse reaction (+3.1 kcal mol^{-1})[3] or from the equilibrium constant which gives a comparable value of +4.1 kcal mol^{-1}.[6]

The active center of the enzyme is known to contain a single Zn^{2+} cation[4] which is bound to three imidazole ligands from histidine amino acid residues. Merz, Hoffmann, and Dewar proposed[7] a catalytic cycle, following earlier suggestions,[8, 9] that involved first the deprotonation of Zn-bound water molecule, followed by the nucleophilic attack by the Zn-OH unit on the carbon atom of free CO_2 (i.e. without the involvement of an encounter complex or an inner-sphere mechanism). The barrier for the deprotonation step (13.1 kcal mol^{-1}) was estimated at the semiempirical AM1 level of theory with respect to the separated reactants. The zinc-bound hydrogencarbonate ion thus generated stabilizes itself by a rearrangement for which two

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alternatives are again discussed: the Lindskog[5] and the Lipscomb[11] mechanism. The product hydrogencarbonate is then replaced by an external water molecule that is finally deprotonated to restore the catalyst.[7]

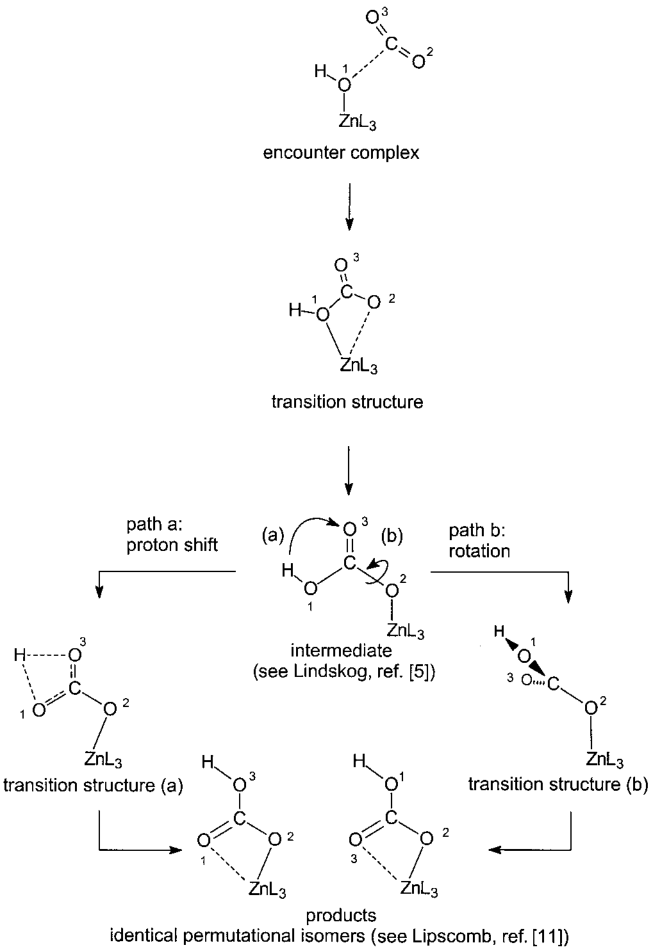
Remarkably, an alternative way to close the catalytic cycle has also been found by Merz et al.:[7] The leaving group, rather than hydrogencarbonate, is proposed to be carbonic acid, which is generated by an intramolecular proton transfer in a transition structure that involves a pentacoordinated zinc ion (barrier 18.1 kcal mol^{-1} at the AM1 level). After formation of H_2CO_3, this molecule is immediately deprotonated through a proton bridge to the His 64 residue. This accounts for the fact that carbonic acid has not been detected experimentally.[12] The species that enters the dehydration step (the reverse reaction) is still the hydrogencarbonate ion, thereby conserving the ping-pong kinetics experimentally observed.[12]

Indeed, an ab initio study (at the SCF level) of the HCO_3^-/H_2O exchange in the $[(NH_3)_3Zn(OH)]^+$ complex has shown that the formation of a pentacoordinated intermediate during the exchange process is highly probable.[13] The authors also suggested a mechanism that implied an intramolecular proton transfer from the water ligand to the hydrogencarbonate ion, prior to the release of carbonic acid. However, a detailed analysis is not presented, and it is doubtfully concluded that "the mechanism of this exchange process has not been fully elucidated".[13]

The rate-determining step had been predicted earlier, based on solvent isotope effects[14], to be the proton transfer step from the zinc-bound water molecule to an available proton acceptor at the active site of the enzyme; this acceptor is probably a nonligated imidazole ring of another histidine residue.[15] Kinetic studies of the dehydration step (the reverse of the final replacement step) indicate that hydrogencarbonate rather than carbonic acid must be the leaving group in the actual CA mechanism and that the rate-determining step is a proton transfer.[16]

Zheng and Merz studied the $[(NH_3)_3Zn(OH)]^+$ CA model system and were the first to suggest that the Lipscomb intermediate is formed from the Lindskog product by a C-O bond rotation with a barrier of 4.1 kcal mol^{-1} at the MP2 level (see ref. [17a] for details on the basis set). Their comprehensive study included thermodynamic corrections and calculations on the free energy of solvation.[17a] They found that the maximum on the energy profile, an encounter complex, is 9.8 kcal mol^{-1} less stable than the separated reactants, whereas the Lindskog product is found to be 9.3 kcal mol^{-1} lower in energy than the Lipscomb form.[17a] As a consequence, the Lindskog product would become a "sink" in the whole mechanism and the backward reaction (dehydration of HCO_3^-) would have to overcome a dauntingly high barrier of about 25 kcal mol^{-1}!

In a more recent paper, Muguruma employed the same model system to investigate all the pertinent features of more realistic imidazole ligands,[17b] while saving computational time. This replacement of NH_3 for imidazole is justified by the finding that NH_3 and imidazole transfer a similar amount of charge to the zinc ion.[18] Muguruma focused on the alternative mechanisms for the formation of the proposed product—a bidentate Lipscomb-type complex (Scheme 1) in which two oxygen atoms have nearly the same distance to the Zn ion.[17b] An initially formed (outer-sphere) encounter complex yields a Lindskog-type intermediate via a transition structure that combines the mode of nucleophilic attack on the CO_2 carbon atom and the rotation-like feature of oxygen ligand exchange (in accord with Lindskog's mechanism[5]). This concertedness of hydration and oxygen exchange conflicts with some earlier work in which a similar model was studied.[19–22]



Scheme 1. *Illustration of the alternative pathways a and b for the formation of the Lipscomb product (L = NH_3).*

The Lindskog intermediate species was described by Muguruma to stabilize itself either by a rotation around the C-O2(Zn) bond (barrier 3.5 kcal mol^{-1}),[17b] producing a bidentate Lipscomb-type intermediate, or alternatively but less preferred, by a proton shift according to the Lipscomb mechanism (Scheme 1).[11] As reported earlier by Zheng and Merz, Muguruma found all species on the reaction path to be lower in energy than the reactants (both at the HF and the MP2/HF levels of theory, applying nonstandard basis sets, see ref. [17b] for details). There was no consideration given to either the initial deprotonation or to the final replacement step of hydrogencarbonate (or carbonic acid). However, it appears to be widely accepted that the initial deprotonation step of a Zn-bound water molecule in the CA mechanism is rate-determining.[16a, 23, 24] Such a deprotonation step might involve a proton relay through at least two water molecules.[24]

Other authors have proposed different sets of model complexes.[25] The question of the binding mode of hydrogencarbonate (bidentate versus monodentate) is of some interest. Zhang and van Eldik[16a] conducted kinetic studies on the complexes of hydrogencarbonate with tri- and tetradentate macrocyclic N-donor ligands. They claimed that a bidentate (rather than monodentate) binding mode that occurs with the 12-membered macrocyclic triamine 1,5,9-triazacyclododecane ([12]aneN_3)[25] causes a decrease in the catalytic activity of their model with respect to the activity of [12]aneN_4, which shows about $^1/_3$ of the CA catalytic activity and binds the hydrogencarbonate ion as a monodentate ligand.[16a, 23] A crystallographic analysis on human type-II carbonic anhydrase (HCA II) shows that hydrogencarbonate is a pseudo-bidentate ligand.[26]

Detailed AM1 computations by Hartmann et al. on the $[Im_3Zn(OH)]^+/CO_2$ system (Im = imidazole) show a high barrier (36.8 kcal mol^{-1}) for the Lipscomb process.[19] In contrast to the semiempirical work of Merz et al.,[7] an encounter complex was proposed and, as in Muguruma's work,[17b] a distinction made between eclipsed and staggered conformations of the Zn-OH moiety with respect to the N ligands. However, an assisting external water molecule was needed to make the location of a Lindskog-type transition state (TS) for oxygen exchange feasible.[19]

Interestingly, these authors have already distinguished between oxygen exchange and C-O2 bond rotation in the formation of the Lipscomb (monodentate) intermediate (Scheme 1).[19] Strikingly, Hartmann et al.[19] predicted the existence of a species with a Zn-O(H)-C unit as a result of nucleophilic attack on CO_2 (i.e. immediately following the encounter complex on the reaction pathway), whereas the results of Zheng and Merz[17a] as well as those of Muguruma[17b] indicate that the modes of oxygen exchange and nucleophilic attack are concerted.[17]

The present work is designed to unravel the details of the elusive and intricate mechanism of catalytic CO_2 hydration. We report here for the first time a complete description of the carbon dioxide hydration cycle by means of the $[(NH_3)_3Zn(OH)]^+$ CA model catalyst, which includes the replacement of the product (hydrogencarbonate or carbonic acid) from the catalyst by water. We pay special attention to the question of how the catalytic cycle might be closed by retrieval of the catalyst since we feel that this particular part of the reaction sequence has not yet been described in sufficient detail.

The binding mode of the hydrogencarbonate ion, the possible preference for the Lindskog mechanism, and the role of a pentacoordinated Zn complex[27] as intermediates are other aspects discussed in this work. Furthermore, we show that the rate-determining step, at least for the model system under consideration, is the nucleophilic attack of the Zn-OH moiety on the CO_2 carbon atom, rather than the initial deprotonation of a Zn-bound water molecule. We thus favor a deprotonation step as the last step in the mechanism via a proton relay involving carbonic acid.

In order to obtain activation barrier predictions that are more relevant than those from gas-phase computations alone, we have included calculations of the free energy of solvation for the evaluation of the activation barrier of CO_2 hydration in a polar aprotic solvent (acetonitrile, $\varepsilon = 35.9$).

Computational Methods

Full geometry optimizations (i.e. without symmetry constraints) were carried out with the GAUSSIAN98 program package[28] at the hybrid B3LYP/6-311 + G** level.[29] The density functional employed contains a term that accounts for the effects of dynamic electron correlation (Coulomb hole).[30] The necessity of using a basis set that contains diffuse functions is caused by the consideration of weakly bound (i.e. van der Waals) complexes and H-bridged species. Because of its d^{10} electron configuration, Zn is especially amenable to quantum chemical computations and is characterized by a low stereorigidity and a low preference for a certain coordination number.

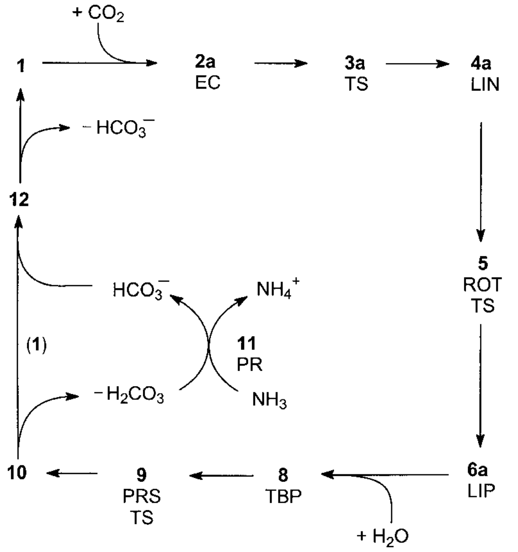
Stationary points were rigorously characterized as minima or transition states according to the number of imaginary modes by applying a second-order derivative calculation (vibrational analysis).[31] Visualization of the reactive mode in the transition structures was used to support the assignments of the pertaining minimum structures. Zero-point energy (ZPE) corrections as well as thermal (ΔH) and entropic ($T\Delta S$) corrections have been made for both activation barriers and reaction energies simulating standard ambient temperature and pressure conditions.

The natural bond orbital (NBO) analysis of Reed and Weinhold[32] has been applied to quantify the transfer of atomic charges in the course of the reactions. The static isodensity surface-polarized continuum model (IPCM)[33] has been used to compute the effect of the free energy of solvation (acetonitrile, $\varepsilon = 35.9$) on the barrier height for the nucleophilic attack.

Results and Discussion

The whole sequence of reaction steps comprising the CA catalytic reaction cycle is displayed in Scheme 2. Absolute and relative energies of the different species can be found in the Supporting Information (Table S-1). CO_2 interacts with the catalyst $[(NH_3)_3Zn(OH)]^+$ (**1**) to form two encounter complexes, **2a** and **2b** (Figure 1), that are almost isoenergetic and differ only in the orientation of the bent Zn-OH moiety (Zn-O-H angle in **2a** is 124.1°) with respect to the ZnL_3 (L = NH_3) ligand sphere: either staggered (**2a**) or eclipsed (**2b**). The distance of the nucleophilic oxygen atom (bound to Zn) to the CO_2 carbon atom is slightly longer in **2a** than in **2b** (Figure 1), which indicates a higher partial charge on oxygen atom O1.[34] In the following discussions, only the energetically preferred staggered forms (denoted by an "**a**") will be discussed in detail. The slight energetical preference for the "**a**" form is probably due to the presence of two intramolecular N-H contacts as compared to one in the "**b**" form.

The subsequent stationary point on the reaction path, **3a** (Figure 1), is a transition structure for the nucleophilic attack on the substrate and represents a maximum of the potential energy in the considered mechanism. The activation barrier (5.7 kcal mol^{-1}) of this complex reaction is thus given by the

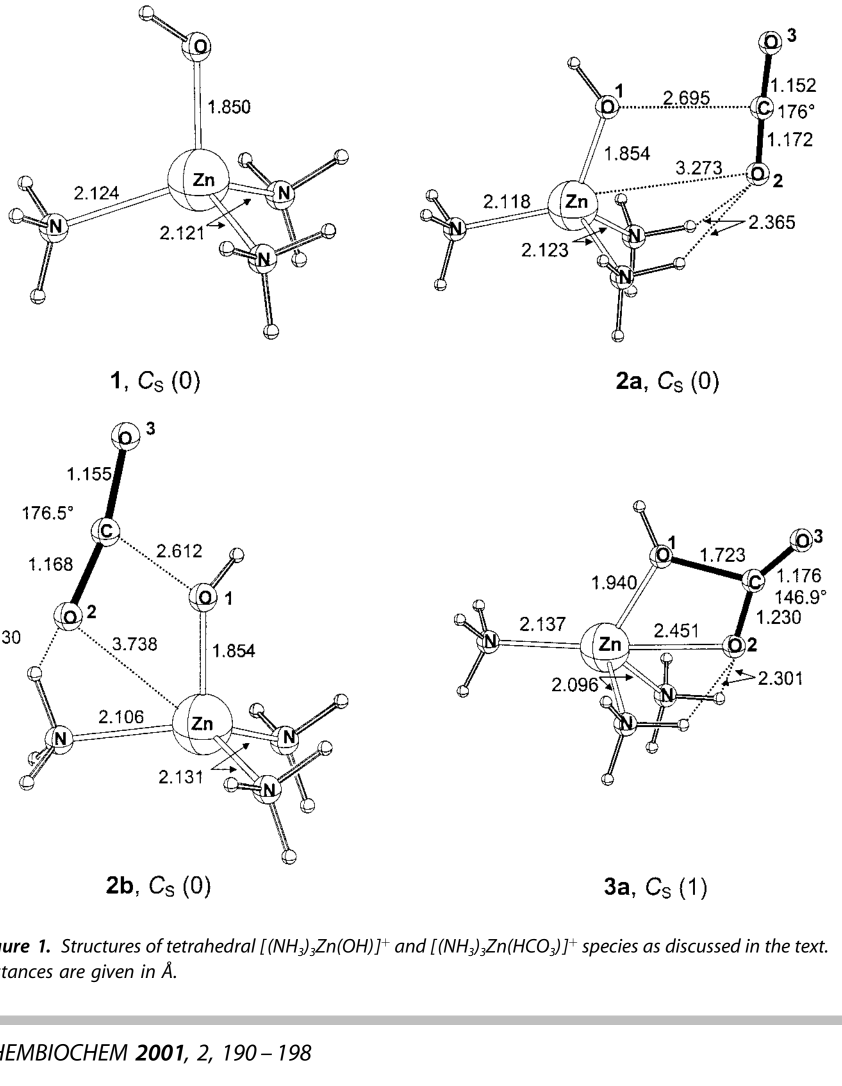


Scheme 2. Catalytic reaction cycle for [$(NH_3)_3Zn(OH)$]$^+$ (1) as a model system for carbonic anhydrase. The net reaction is the conversion of carbon dioxide and water to carbonic acid. For an explanation of the numbering see text (EC = encounter complex, TS = transition state, LIN = Lindskog intermediate, ROT = rotation, LIP = Lipscomb intermediate, TBP = trigonal-bipyramidal, PRS = proton shift, LGC = leaving-group complex, PR = proton relay). Both H_2CO_3 and HCO_3^- are not fully separated from 1. This is denoted by "(1)".

relative energy of **3a** with respect to the energy level of the entry channel (species **1** + CO_2 + H_2O; see Tables S-1 and S-2 in the Supporting Information). This holds as long as the equilibration of the energy stored in the rotational and vibrational degrees of freedom in **3a** with the environment (e.g. the solvent) takes longer than the half-life of **3a**. Note that the CO_2 molecule in TS **3a** is already significantly bent (O-C-O angle 146.8°), and one of the C=O bonds is weakened (1.170 Å in free CO_2 at the same level of theory) in favor of a (short) polarized $C^{(+)}$-$O^{(-)}$ single bond (1.230 Å, partial charge on $q_C = +1.02$ e, partial charge on $q_{O2} = -0.78$ e; as compared to $+0.91$ and -0.45 e in free CO_2, respectively). As a result of the nucleophilic attack, a new, strongly polarized σ bond is formed between O1 (for the numbering see Scheme 1) and C (with 80.1 % contribution from oxygen, $q_{O1} = -1.12$ e, and a Wiberg bond index[28, 32] of 0.442) in which the carbon atom uses an orbital that has the approximate hybridization sp$^{3.41}$ (NBO analysis[31]). Whereas the Zn-O bond lengths in **3a** (Zn-O1: 1.940, Zn-O2: 2.451 Å) seem to imply a bidentate binding mode of the forming hydrogencarbonate ligand, the Wiberg bond indices do not support such an interpretation.

We have also investigated the effect of a polar solvent (acetonitrile, $\varepsilon = 35.9$) on the activation barrier for the hydration reaction. The potential energy difference (without ZPE correction) between the encounter complex **2a** and the transition state for the nucleophilic attack (**3a**) is 10.1 kcal mol^{-1}. After adding the sum of the thermal and entropy corrections (for 298 K, including the zero-point energy), a barrier of 12.4 kcal mol^{-1} is obtained, which would be far too high to explain the facility of the enzymatic hydration. However, taking into account the free energies of solvation, the transition state for the nucleophilic attack on CO_2 is stabilized by 6.0 kcal mol^{-1} with respect to the encounter complex **2a**. The sum of the free energy of solvation and free energy corrections in the gas phase gives an overall barrier height of 5.7 kcal mol^{-1}, which agrees well with predictions based on experimental observations.[6]

The nucleophilic attack (via TS **3a**) of the catalyst on CO_2 yields the Lindskog-type product **4a** (Figure 2) with a planar Zn-O-C(O)-OH unit and a considerably shortened Zn-O2 distance (1.918 Å as compared to 3.273 Å in the encounter complex **2a**). The Zn-O1 distance, on the other hand, increased from 1.854 to 2.822 Å. In the related structure **4b**, the most pronounced difference is the longer Zn-O1 distance (3.062 Å). Proceeding along the sequence **2** → TS **3** → **4** (the rate-determining step), the oxygen atoms O1 and O2 have exchanged their roles: Whereas the calculated distances observe the relation Zn-O1 $\ll$



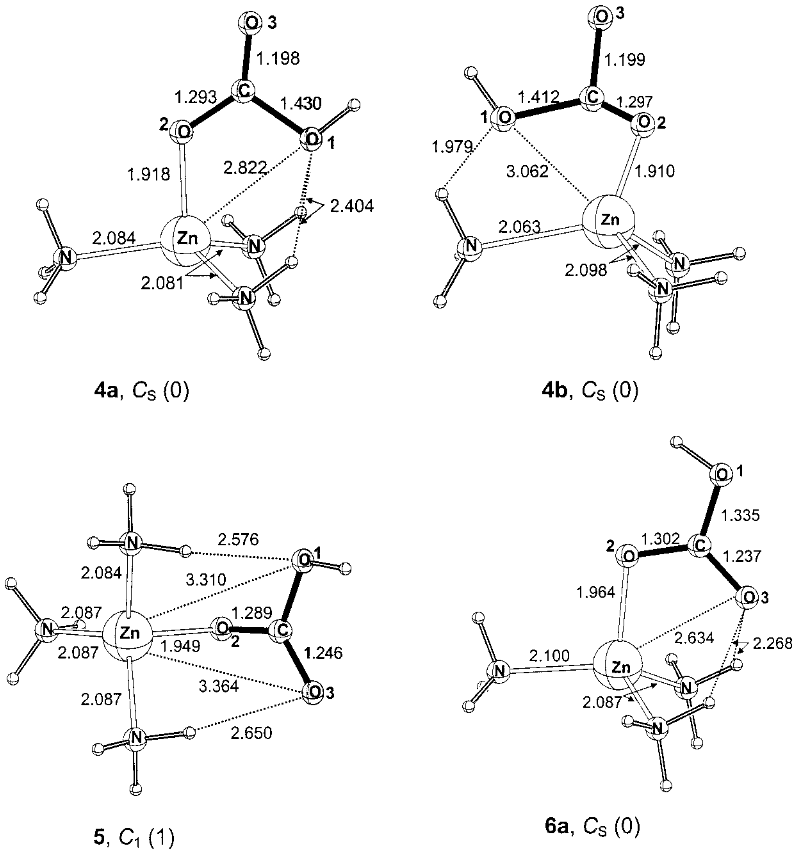
1, C_S (0)

2a, C_S (0)

2b, C_S (0)

3a, C_S (1)

Figure 1. Structures of tetrahedral [$(NH_3)_3Zn(OH)$]$^+$ and [$(NH_3)_3Zn(HCO_3)$]$^+$ species as discussed in the text. Distances are given in Å.



4a, C_S (0)

4b, C_S (0)

5, C_1 (1)

6a, C_S (0)

Figure 2. *Tetrahedral [Zn(HCO$_3$)(NH$_3$)$_3$]$^+$ species (**4a**–**6a**, see Scheme 2) pertinent to the replacement of hydrogencarbonate by water.*

Zn-O2 in the reactants **2**, the opposite is true for the products **4** (Scheme 1).

Lindskog versus Lipscomb mechanism

In contrast to earlier assumptions,[9, 10, 18] a monodentate Lindskog-type intermediate such as $[L_3Zn\text{-}O^{(+)}(H)\text{-}CO_2^{(-)}]^+$ with a central tricoordinated and a formally positively charged oxygen atom, is not formed. Nucleophilic attack and oxygen exchange take place simultaneously. The Lindskog intermediate **4** preferably stabilizes itself by rotation around the C-O2 bond (path b, Scheme 1) via the transition structure **5** to yield the monodentate Lipscomb-type adduct **6a** (Figure 2), in which the O3 shows just a very small interaction with the Zn cation. The Zn-O2 bond length and bond order (bond length: 1.964 Å, Wiberg bond index:[32] 0.152) contrasts strongly with the values for Zn-O3 (bond length: 2.634 Å, Wiberg bond index: 0.145). The bond orders indicate the predominantly electrostatic nature of these interactions. The two rotational modes as represented by the two conceivable analogous transition structures similar to **5** (for clockwise or anticlockwise rotation) are equivalent because of the chemical identity of the ammonia ligands.

A bidentate Lipscomb intermediate as assumed in ref. [17b] (where a HF level was employed for the geometry optimizations) with comparable Zn-O2 and Zn-O3 bond lengths of 2.003 and 2.280 Å, respectively,[17b] could not be found at the B3LYP level. Hence, the contradicting results reported at uncorrelated levels of theory must be regarded as artificial.

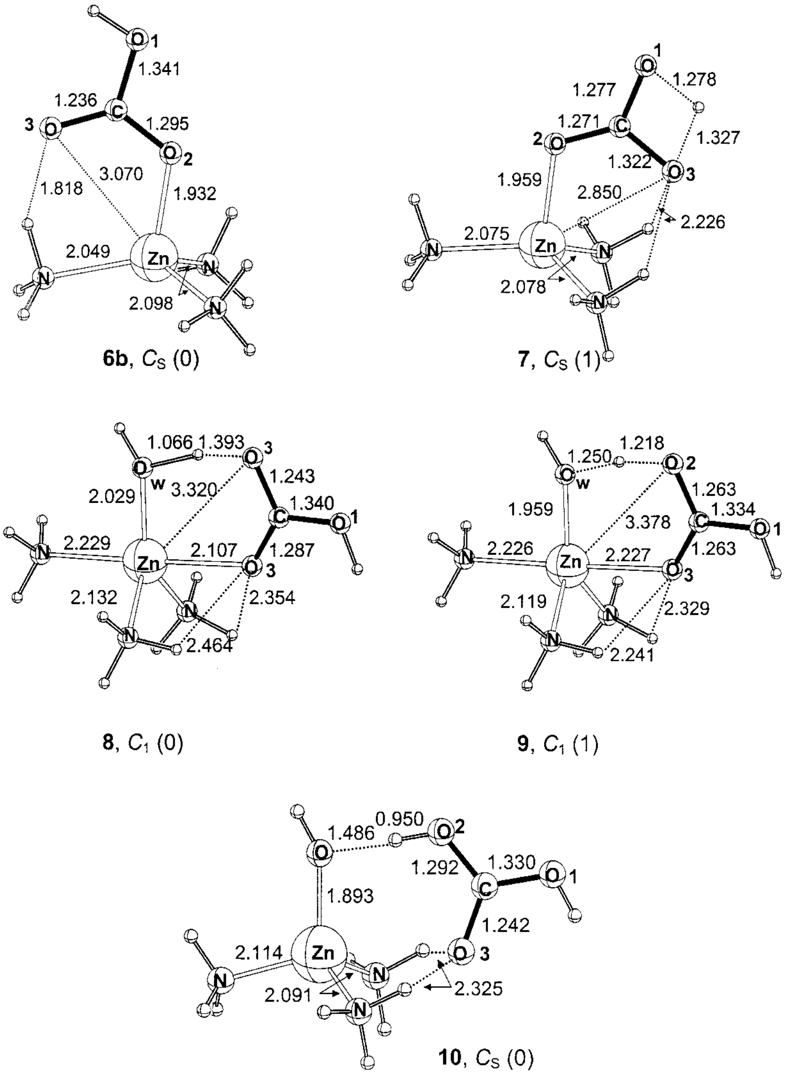
Alternatively, a proton shift (path a, Scheme 1) proceeding over TS **7** (Figure 3) from O1 to O3 can occur, generating a permutational isomer of **6a** (a Lipscomb-type product in which O1 and O3 are permuted). This Lipscomb-type process has, nevertheless, a significantly higher activation barrier (ca. 28 kcal mol^{-1}, see Table S-1 in the Supporting Information) than the C-O2 bond rotation.

In summary, the Lindskog-type mechanism (path b) involves subsequent, rather than concerted, oxygen exchange and C-O2 bond rotation. The reaction pathway might start from a weakly stabilized encounter complex and proceeds via an intermediate with a Zn-O-C(OH) moiety after completed nucleophilic addition, followed by a "rotation-like" reaction step in which the OH group moves to the more remote position as depicted in Figure 2, (structure **6a**). In the alternative Lipscomb-type mechanism (path a), a proton shift from O1 to O3 is preceded by the nucleophilic addition. Although the proton shift might take place in the Lindskog-intermediate before it undergoes internal bond rotation, the difference in the relative activation energies for both processes indicates that such a reaction pathway is rather unlikely.

Both the C=O and the O–H bonds can be oriented in a staggered or eclipsed fashion with respect to the ZnL_3 moiety (or *cis* and *trans* relative to each other), giving rise to four different conformational isomers of Lipscomb-intermediates, of which only two have been considered in this work. The C=O bond takes on a staggered conformation in **6a**, whereas it is eclipsed in **6b**. The O–H and C=O bonds are *trans* to each other in **6a** and *cis* in **6b**. The Zn-C contact in **6a** is somewhat shorter (2.634 Å) as compared to that in structure **6b** (3.070 Å) and is accompanied by a small lengthening of the Zn-O2 bond (1.932 to 1.964 Å, see Figures 2 and 3). At this stage in the reaction, about half of the total reaction energy of −25.1 kcal mol^{-1} has already been released (Table S-1 (see Supporting Information) and Figure 4).

The H_2O/HCO_3^- replacement step

A water molecule from the surrounding medium now approaches and complexes with the four-coordinate structure **6a** to give the five-coordinated species **8**. No activation barrier for this process could be found at the B3LYP level of theory. A barrier of 4.5 kcal mol^{-1} for the formation of a similar pentacoordinated complex has been reported at the HF level using a double-zeta basis set (except for STO-3G functions on the hydrogen atoms in the ammonia ligands).[13] This, taken together with the lack of a barrier, as reported in this article, strongly suggests that



6b, C_S (0)

7, C_S (1)

8, C_1 (0)

9, C_1 (1)

10, C_S (0)

Figure 3. Tetrahedral [Zn(HCO$_3$)(NH$_3$)$_3$]$^+$ and trigonal-bipyramidal [Zn(OH$_2$)(HCO$_3$)(NH$_3$)$_3$]$^+$ species pertinent to the replacement of hydrogencarbonate by water.

formation of the five-coordinated species **8** is not the limiting step of the whole HCO_3^-/H_2O exchange process.[13]

When water is attached to the central Zn ion, either a square-pyramidal or a trigonal-bipyramidal pentacoordinated complex could result. Pentacoordinated **8** (Figure 3) is of the trigonal-bipyramidal (TBP) type. There are two possible binding sites (equatorial or axial) for water and the hydrogencarbonate ion. We could, however, find only one complex **8** with the H_2O molecule occupying an equatorial and the hydrogencarbonate an axial binding site. In contrast to the correlated B3LYP results, TBP complexes with a water molecule in both equatorial and axial positions could be located at the HF/3-21G* level.

One O–H bond of the water molecule in **8** is coplanar with the Zn–hydrogencarbonate unit, thus allowing for an optimal stabilization by an O···H–O hydrogen bond as part of a six-membered ring system. This structure differs from the five-coordinated complexes calculated by Solá et al., in which such a bridge is not present.[13] In those structures, preference is given to the formation of hydrogen bonds being part of a four-membered ring system (structures *weba* and *wabe* in ref. [13]). Liang and Lipscomb reported in their theoretical study[6] that, even if some proteinic residues are included, the barrier for direct dissociation of HCO_3^- from a trigonal-bipyramidal complex with water as a fifth ligand is 43 kcal mol^{-1}. Although the deprotonation of the water molecule removes this barrier,[6] the deprotonation itself costs an additional 51.0 kcal mol^{-1}![13]

Comparing **6a** and **8**, it is apparent that the axial Zn-O2 distance (to the eventual leaving group) is already significantly increased in **8** (from 1.964 in **6a** to 2.107 Å in **8**) and the coaxial ammonia ligand becomes much less strongly bound to Zn than the equatorial ones (Figures 2 and 3).

The final replacement step consists of a proton transfer from O_W (from water) to O2 (hydrogencarbonate) to form the *trans,trans*-conformer of carbonic acid, which constitutes a more favorable (i.e. neutral) leaving group than the negatively charged hydrogencarbonate ion, which would have to be separated from a positively charged Zn complex. The transition structure **9** is characterized by an extremely low barrier of 0.8 kcal mol^{-1} (Figure 4a) for the proton shift. The lengthening of the Zn-O2 bond proceeds even further (to 2.226 Å) in **9** as compared to that in **8** (2.107 Å, Figure 2). At the same time, the Zn-O_W distance of 2.029 Å approaches the Zn-O "single-bond" length as found in the catalyst $[L_3Zn(OH)]^+$ (**1**) (1.850 Å). The

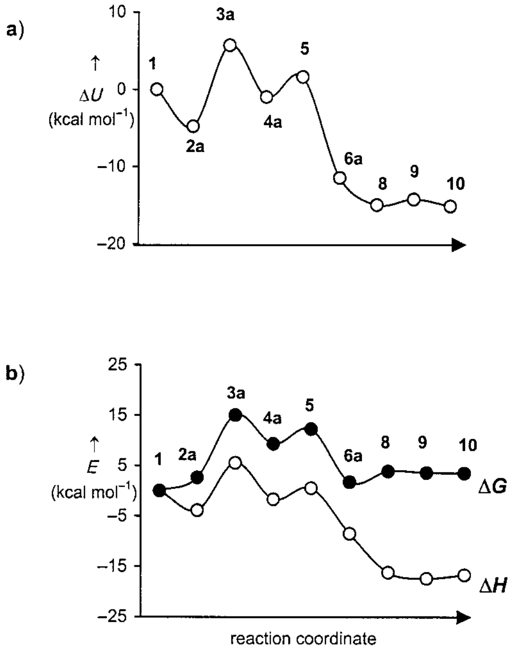


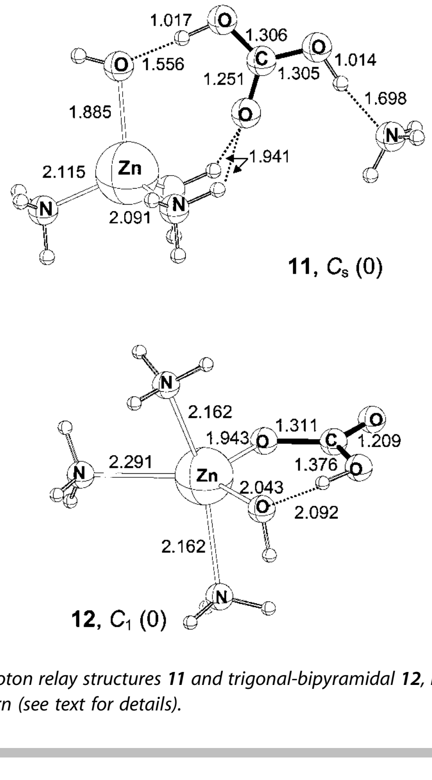
*Figure 4. a) Potential energy as a function of the reaction coordinate along the reaction itinerary **1**→**2a**→**3a**→**4a**→**5**→**6a**→**8**→**9**→**10** (see Table S-1 in the Supporting Information). The hilltop at **3a** gives the position of the TS for the rate-limiting step. b) Depiction of the gas-phase enthalpy and free energy changes in the course of the CO_2 hydration reaction.*

minute variations in the geometry at the reaction center caused by differing orientations of the O1-H bond with respect to the ZnL_3 unit are not discussed here.

The terminal product **10**, which represents the "exit channel" in the absence of a polar solvent, is a hydrogen-bridged weakly bound associate complex of the formed carbonic acid and the retrieved catalyst **1** (Figure 3). Solá et al. were not able to locate such a $[(NH_3)_3Zn(OH)(H_2CO_3)]^+$ species and doubted that it is "a true minimum on the potential hypersurface", although they anticipated a ligated carbonic acid.[13] The carbonyl oxygen atom of the carbonic acid in **10** is no longer ligated with the Zn cation (Zn-O3 distance: 3.171 Å). Thus, the L_3Zn-OH moiety of **10** is, in principle, ready to attack a new CO_2 substrate molecule. Hence, it is possible to close the catalytic cycle without an initial deprotonation step!

To account for the established fact that carbonic acid cannot be detected experimentally,[12] we propose (as Merz, Hoffmann, and Dewar, based on semiempirical data, have done[7] earlier) the occurrence of a proton relay, which facilitates the deprotonation of the initially generated leaving molecule, carbonic acid, to give the hydrogencarbonate ion. In this relay, an imidazole nitrogen atom of the histidine 64 residue in the active site of carbonic anhydrase functions as the proton acceptor. The corresponding structural model **11**, involving the catalyst, carbonic acid, and an ammonia molecule, which represents the imidazole ring in His64, is depicted in Figure 5. Further corroboration for this proposal comes from an analysis that demonstrates the surprising kinetic stability of carbonic acid in the absence of water.[35]

The zinc – nitrogen distance with optimized hydrogen bonds has been computed to be 6.9 Å, while the experimental value is 7.5 Å.[36] Considering the possibly nonoptimal hydrogen-bonding distances in the actual pocket of the enzyme (due to other restraints), this correspondence with the experimentally determined distance appears to be excellent.



11, C_s (0)

12, C_1 (0)

Figure 5. *Proton relay structures **11** and trigonal-bipyramidal **12**, resulting from ion pair return (see text for details).*

The carbonic acid moiety in **10** might be either deprotonated prior or after its release from the Zn complex. As a rough estimate, the energy required for a proton transfer from the complexed carbonic acid to ammonia in the gas phase is $+156.5\,kcal\,mol^{-1}$ due to the charge separation involved. In acetonitrile this value decreases to $+13.3\,kcal\,mol^{-1}$ [Eq. (1 a) and Figure 6].

$$[(NH_3)_3Zn(OH)(H_2CO_3)]^+ (\mathbf{10}) + NH_3 \longrightarrow [(NH_3)_3Zn(OH)]^+ (\mathbf{1}) + HCO_3^- + NH_4^+ \tag{1 a}$$

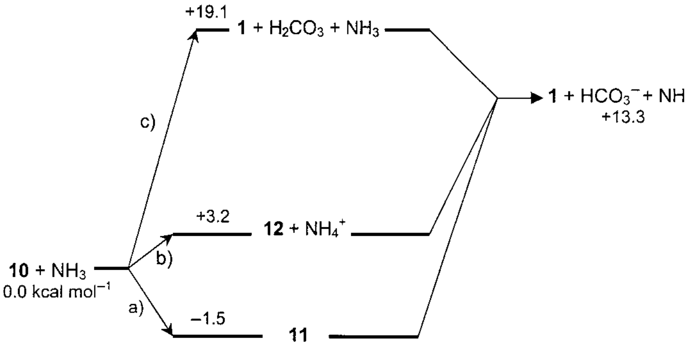


Figure 6. *Illustration of the three possible alternative reaction pathways to achieve both the proton transfer to ammonia and the release of hydrogencarbonate. All energies are given in* $kcal\,mol^{-1}$.

The three alternative pathways (Figure 6) differ in the distribution of energy on two subsequent equilibrium steps. In the case of pathway a), a proton relay via the H-bridged species **11** occurs. The formation of **11** is *exothermic* both in the gas phase and in solution (-12.7 and $-1.5\,kcal\,mol^{-1}$, respectively). The total free energies in solution and the gas-phase total energies of the relevant smaller molecules can be found in Table S-2 of the Supporting Information. The dissociation of **11** into the catalyst, hydrogencarbonate ion, and ammonium ion requires $+169.2$ ($+14.8$ in acetonitrile) $kcal\,mol^{-1}$.

In the remaining alternatives (Figure 6), carbonic acid is either released from **10**, a process that requires 26.2 (19.1) $kcal\,mol^{-1}$, [pathway c)], or is first deprotonated before being released [pathway b)]. The reaction energy involved in transferring a proton from the noncomplexed carbonic acid to ammonia has been obtained as $+130.2$ (-5.8) $kcal\,mol^{-1}$. This again results in a barrier of $+13.3\,kcal\,mol^{-1}$ in the solvent.

Comparing just the pathways b) and c) of Figure 6, it is obviously more likely that carbonic acid is deprotonated before it leaves the catalyst. However, the proton transfer step is still endothermic in the case of *complexed* carbonic acid as part of a proton relay [pathway a)]. The inclusion of specific solution effects drastically reduces the barrier to this process. Nonetheless, we do not rule out the possibility that this deprotonation step might actually become rate-limiting.

Another aspect to be considered is the fate of the hydrogencarbonate ion. Equation (1 b) illustrates that hydrogencarbonate, instead of leaving the active site immediately after the deprotonation of carbonic acid, might undergo an ion pair return to yield the distorted trigonal-bipyramidal complex **12** (Figure 5), a complex that is similar to a $[(NH_3)_3Zn(OH)_{eq}(HCO_3)_{eq}]$ species that had been found computationally by Solá et al.[13]

$$[(NH_3)_3Zn(OH)(H_2CO_3)]^+ \ (\mathbf{10}) + NH_3 \longrightarrow [(NH_3)_3Zn(OH)(HCO_3)_{eq}] \ (\mathbf{12}) + NH_4^+ \quad (1\,b)$$

Ammonia stands here for any external base, thus making this mechanism most likely in the absence of the enzyme. Complex **12** resulted directly from the geometry optimization of deprotonated **10**. Further credibility is given to the involvement of **12** because although the process corresponding to Equation (1 b) is endothermic by 55.9 kcal mol^{-1}, it becomes exothermic by 3.2 kcal mol^{-1} in solution [pathway b), Figure 6]. Nevertheless, such Zn^{2+} complexes with two anions in the coordination sphere are quite unstable.[37] The subsequent dissociation of **12** into the catalyst and hydrogencarbonate requires an additional 100.5 (10.1) kcal mol^{-1}.

The gas-phase value is in accord with the value reported by Kraus and Garmer for a similar complex with an axial HCO_3^- ligand, 105 kcal mol^{-1}.[38] Values reported by Solá et al. referred to complexes with equatorial hydrogencarbonate and are significantly higher (at least 114.4 kcal mol^{-1}), but are also drastically reduced by inclusion of solution effects in a medium with $\varepsilon = 78.36$ (water) from, for example, 131.8 to 16.0 kcal mol^{-1}.[13]

In contrast, deprotonation of $[(NH_3)_3Zn(OH_2)]^{2+}$ has been computationally found to require about 40.3 kcal mol^{-1} in a medium with $\varepsilon = 78.36$, whereas the deprotonation of water in a pentacoordinated complex similar to **8** requires, even in solution, 51.0 kcal mol^{-1}.[13] We therefore propose pathway a), as described in Figure 6 needed for the enzymatic mechanism due to its good correspondence with X-ray structural data.[36] However, it is very probable that hydrogencarbonate is not immediately released into the medium, but undergoes an ion pair return with **1** prior to its final dissociation from **12**.

Figure 4 a shows the "mountain ridge" of the potential energy for the complete $[(NH_3)_3Zn(OH)]^+$-catalyzed hydration of carbon dioxide as a cross-section of the potential surface along the minimum energy pathway beginning from the separated reactants, **1** (entry channel), to the H-bridged product complex **10**.

Figure 4 b depicts the changes in enthalpy and free energy along the reaction path. Only the free energy reproduces satisfactorily the experimentally observed endothermicity of the overall reaction (theory: +3.4 kcal mol^{-1}, experiment: +3 – +4 kcal mol^{-1}).[6, 10] From the free energy point of view, an encounter complex intermediate that would form from the separated reactants does not exist: The free energy of **2 a** with respect to the entry channel (separated reactants), including thermodynamic corrections to the gas-phase results, is 2.6 kcal mol^{-1}.

Conclusion

In this work we report for the first time the reaction pathway for a complete cycle of catalytic CO_2 hydration by means of a $[(NH_3)_3Zn(OH)]^+$ model catalyst to mimic the carbonic anhydrase functionality. We also provide a more comprehensive and detailed investigation of the structure – activity relationships in this system than has been reported in earlier work.[7, 13, 17]

In addition, we have carried out for the first time full geometry optimizations on this system which include electron correlation effects. We argue that the most likely scenario for the H_2O/HCO_3^- replacement step involves first the generation of carbonic acid as a leaving group in the coordination sphere of zinc—a reconfirmation of a mechanism proposed by Merz, Hoffmann, and Dewar in 1989.[7] After deprotonation via a proton relay to histidine 64, the resulting hydrogencarbonate ion might undergo an ion pair return with the positively charged Zn complex. Finally, HCO_3^- dissociates into the surrounding medium, thereby regenerating the catalyst. This overall process requires at least 13.3 kcal mol^{-1} as activation energy in acetonitrile. We do not know how the protein environment could influence this value.

Nevertheless, the rate-determining step is not an initial deprotonation of a Zn-bound water molecule, as repeatedly described in previous studies,[5–11] but is rather the nucleophilic attack on carbon dioxide which is accompanied by a rotation-like movement (Lindskog-type mechanism) that exchanges the role of the oxygen atoms which are closest to Zn (see Scheme 1).

Only monodentate Lipscomb-type hydrogencarbonate complexes are produced by internal proton shifts (Lipscomb-type mechanism) or by an energetically preferred C-O2 bond rotation from these Lindskog adducts. This leaves more room for an entering water molecule to form a five-coordinated complex, which appears to be required in order to exchange water for hydrogencarbonate.

The formation of carbonic acid through cleavage of the Zn-O bond to the leaving group is assisted by an intramolecular proton transfer from the water molecule that occupies a fifth (equatorial) coordination site at the zinc center in a distorted trigonal-bipyramidal ligand environment. The barrier for this process is extremely low, 0.8 kcal mol^{-1}. The catalyst is consequently regenerated without the necessity of an initial deprotonation, and moreover, without the adverse effects of undue charge separation in the product complex. The necessary final proton transfer from carbonic acid to His 64 is therefore part of a proton relay from a Zn-bound water molecule to the imidazole N atom in the amino acid residue. This endothermic reaction step might become rate-limiting under certain conditions.

Considering the free energy differences in the gas phase, rather than the potential energies themselves, encounter complexes do not correspond to energy minima. Only by additional inclusion of the free energy of solvation in a sufficiently polar solvent such as acetonitrile were we able to reproduce the experimental activation barrier (in water) satisfactorily. The value of 5.7 kcal mol^{-1} corresponds well with the experimental value of about 3 kcal mol^{-1} in the CA enzyme, and constitutes a rather dramatic decrease with respect to the barrier of the uncatalyzed CO_2 hydration in neutral water, that is, 17.7 kcal mol^{-1}!

While a $^{16}O/^{18}O$ isotopic labeling experiment appears to be suited for distinction among the several mechanistic possibilities, such an experiment might suffer from the conformational lability of the hydrogencarbonate complexes and the possibility of oxygen label scrambling.[39]

To further improve the validity of the model system, imidazole rings should replace the ammonia ligands. Imidazole differs the most from ammonia in the increased nucleophilicity of the Zn-O1H oxygen atom and the reduced acidity of Zn-bound water.[18, 19] In addition, even some simplification of the model system can be expected from the absence of the intricacies engendered by the NH hydrogen bonds.

We have shown that only a complete analysis of all pertinent aspects of conformation and electronic structure can elucidate the reaction mechanism in systems that should serve to model enzymatic catalysis, exemplified by that of carbonic anhydrase. Our contribution should help to pave the way for a detailed understanding of the catalytic mechanisms in related enzymes and of how those mechanisms can be studied and/or employed for synthetic purposes. Hence, extension of this work will include further heterocumulenes, such as CO_2, CS_2, RNCO, etc., to close the gap between the native molecule and synthetical reality.

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