Investigation of the Uncatalyzed Hydration of CO$_2$ and First Approximations to the Active Site of Carbonic Anhydrase – A Combined *Ab initio* and DFT Study –

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Introduction

Carbonic anhydrase (CA) is found in both plants and animals and the only known biological function of this ubiquitous enzyme is to catalyze the interconversion of CO$_2$ and HCO$_3^-$. In aqueous solution in the absence of CA the reactions

$$H_2O \quad + \quad CO_2 \quad \rightarrow \quad H_2CO_3 \qquad (1)$$

$$OH^- \quad + \quad CO_2 \quad \rightarrow \quad HCO_3^- \quad + \quad H^+ \qquad (2)$$
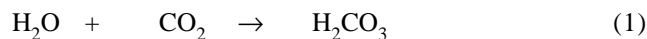
have activation energies of 17.7 kcal/mol (forward) and 14.6 kcal/mol (reverse) for reaction 1 and 13.1 kcal/mol (forward) for reaction 2 [1]. The rate constants for the forward reactions of reaction 1 and 2 are 0.035 s^{-1} and 8500 s^{-1}M^{-1}, respectively. In comparison to the uncatalyzed reaction human CA enhances the hydration of CO$_2$ by a factor of about 10^7. This however implies that the enzyme reduces the energy of activation by about 10 kcal/mol. Although the gas-phase reaction of carbondioxide with water and with hydroxide ion have been studied earlier by others [1-6] we found it necessary to apply density functional theory to the nonenzymatic hydration of CO2 and to compare these results with our ab initio calculations. The active site environment of CA shows that the zinc atom is bound to three histidine imidazole groups with the fourth coordination site occupied by either water or hydroxide ion. In order to understand the central role of the zinc ion in the catalytic mechanism of CA, the next step in our investigation involves the approximation of the active site of this enzyme. Starting with the simplest model, namely the [ZnOH$_2$]$^{2+}$ / [ZnOH]$^+$ system we also considered the analogous triammine and trisimidazole complexes [Zn(NH$_3$)$_3$OH$_2$]$^{2+}$/[Zn(NH$_3$)$_3$OH]$^+$,[Zn(Im)$_3$OH$_2$]$^{2+}$/ [Zn(Im)$_3$OH]$^+$.The optimized structures and energies for the latter two systems and the first steps in the reaction of [ZnOH]$^+$ with CO$_2$ are presented in this poster.

Methods

Calculations were carried out on a CONVEX SPP-1000 computer and on HP-735 PA-RISC workstations at the Computer-Chemie-Centrum Erlangen, using GAUSSIAN 94 [7] and the B3LYP hybrid functional [8,9]. The optimized split valence basis set of Ahlrichs et al. [10], augmented with p- and d-type polarization functions, was used for geometry optimizations and frequency analyses. The latter procedure verified that all structures presented in this work are indeed local minima with respect to their potential energy surface. The final adopted zinc-basis set was of the form [14s9p5d/5s3p3d], whereas the oxygen contraction pattern was [7s4p1d/3s2p1d]. The hydrogen [4s/2s] set remained unchanged. For convenience, this type of model chemistry will be further referred to as B3LYP/SHA1. The Moeller-Plesset correlation energy corrected calculations cover geometry optimizations as well as frequency calculations on the MP2(FC) level of theory in conjunction with
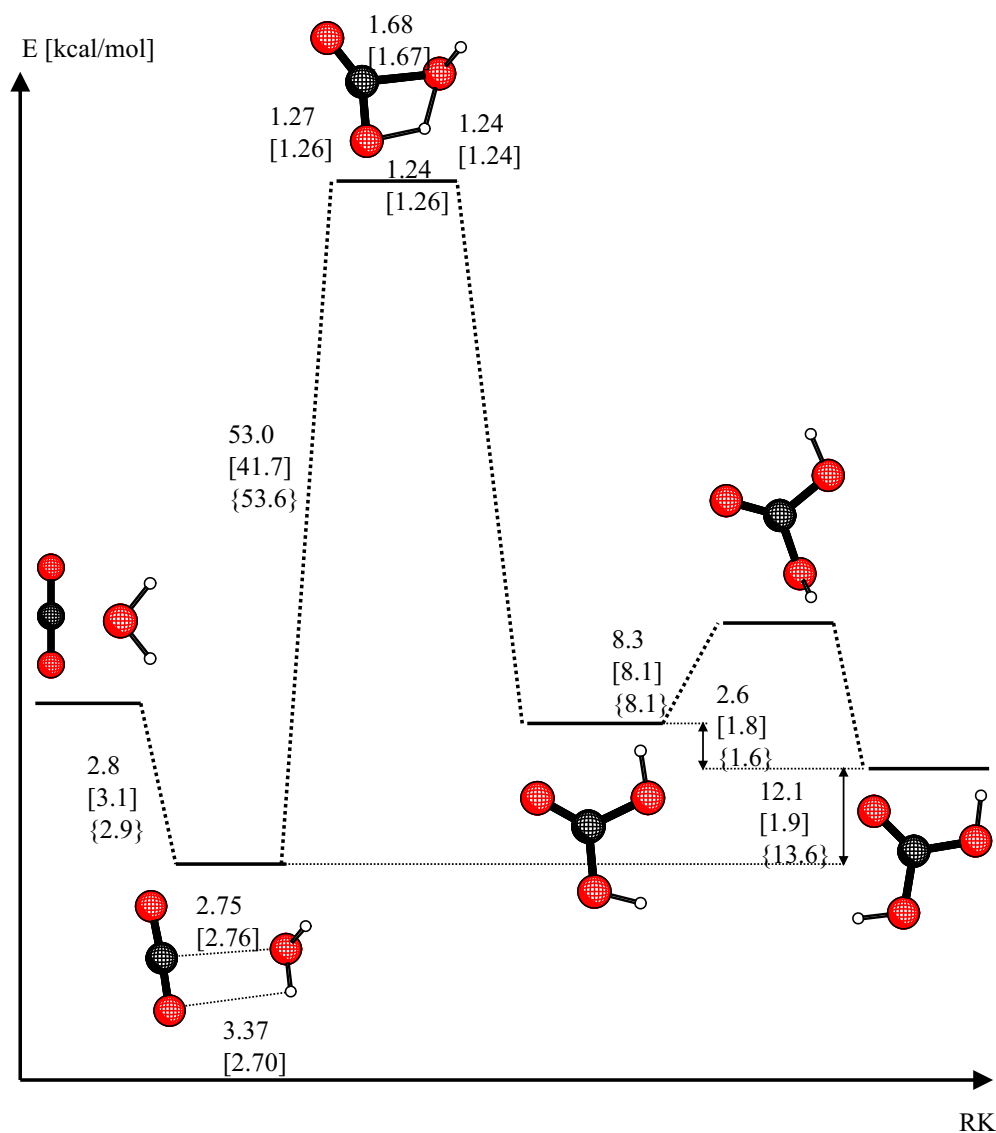
**Figure 1.** *Energy profile of the reaction of carbondioxide and water. The levels of theory are signed with: MP2(FC)/6-31+G(d,p)// MP2(FC)/6-31+G(d,p), {MP4(SDTQ)/6-31+G(d,p)// MP2(FC)/6-31+G(d,p)} and [B3LYP/SHA1//B3LYP/SHA1].*
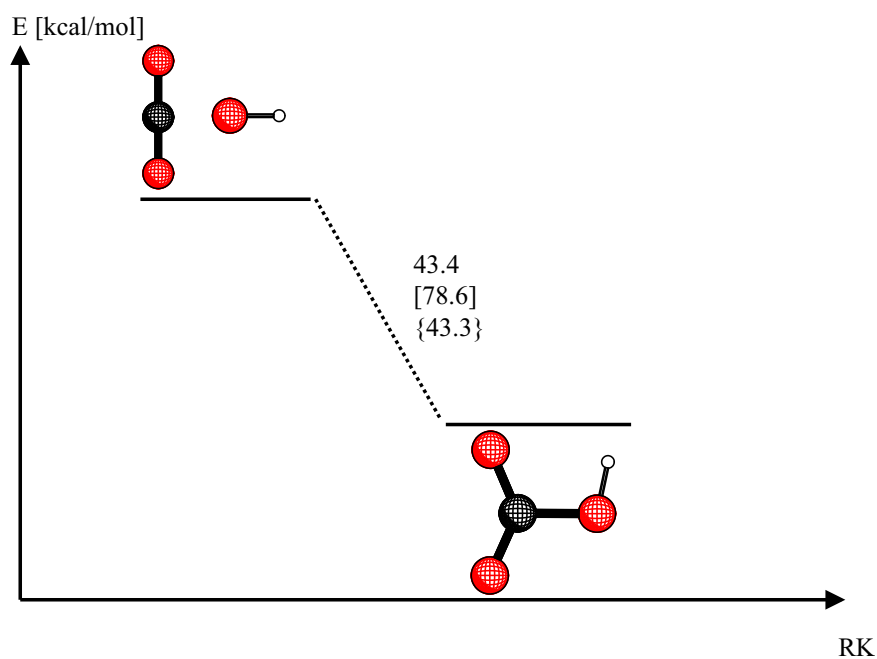
Figure 2. *Energy profile of the reaction of carbondioxide and hydroxide ion. The levels of theory are signed with: MP2(FC)/6-31+G(d,p)// MP2(FC)/6-31+G(d,p), {MP4(SDTQ)/6-31+G(d,p)// MP2(FC)/6-31+G(d,p)} and [B3LYP/SHA1//B3LYP/SHA1].*

Figure 3. *Energies of deprotonation [kcal/mol] for selected model complexes of the active site of carbonic anhydrase based on B3LYP/SHA1//B3LYP/SHA1 calculations*

$E_{dep} = +422.9$ kcal/mol

$E_{dep} = +55.8$ kcal/mol

$E_{dep} = +150.4$ kcal/mol

$E_{dep} = +180.3$ kcal/mol
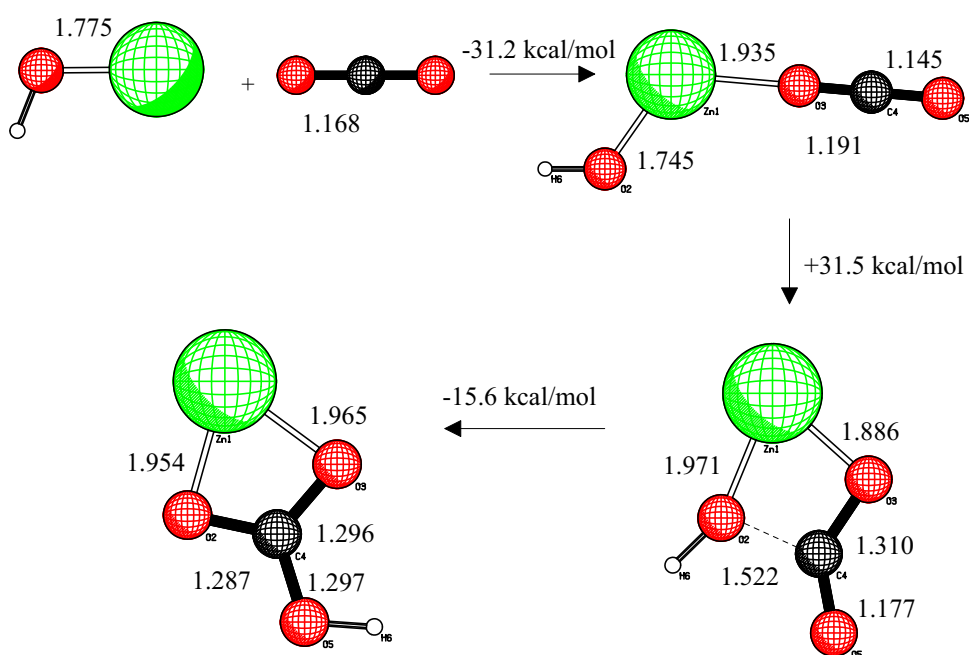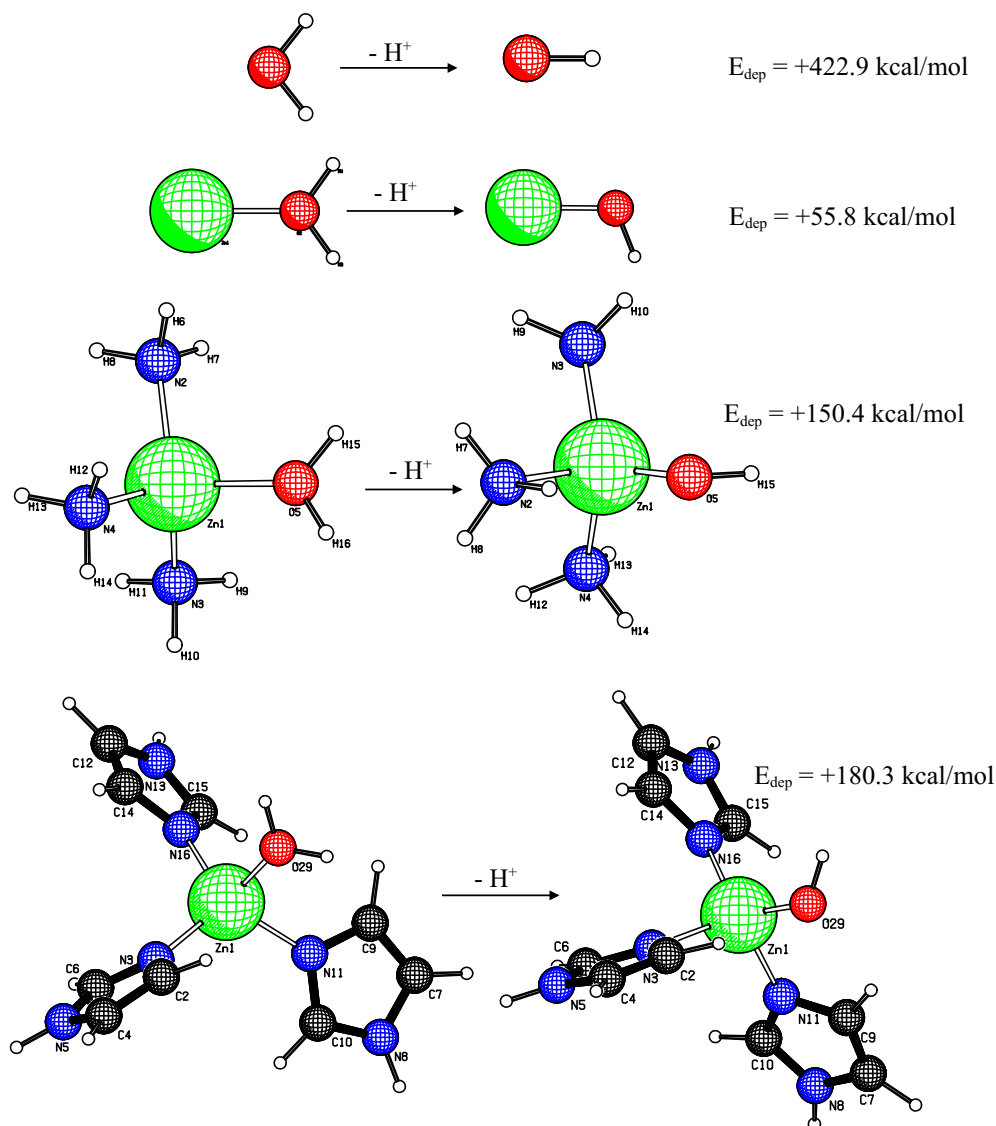
Figure 4. *Suggestion for the first steps in the reaction of [ZnOH]⁻ and CO2 calculated on the B3LYP/SHA1//B3LYP/SHA1 level of theory. Bond lenghts are given in Angstrom*

a 6-31+G(d,p) basis set. For the refinement of the total energies, single point calculations were performed using MP4(SDTQ)/6-31+G(d,p) calculations. All of the energy values given in the figures are corrected by means of zero-point energies (ZPE).

Results and Discussion

The energy barrier for the reaction of CO_2 with H_2O (Figure 1) varies with respect to the computational technique from 53.6 kcal/mol (MP4/6-31+G(d,p)//MP2/6-31+G(d,p)) to 41.7 kcal/mol (B3LYP/SHA1//B3LYP/ SHA1). The deviation from the experimental energy of activation measured in solution (17.7 kcal/mol) can be explained by means of solvent interactions, which have an important contribution to the barriers. However, in comparison to semiempirical and *ab initio* results found in the literature [1-4], density functional calculations give better values for the energy barrier. The overall reaction yielding the trans-trans isomer of the carbonic acid is predicted to be endothermic with reaction energies ranging from 13.6 kcal/mol (MP4/6-31+G(d,p)//MP2/6-31+G(d,p)) to 1.9 kcal/mol (B3LYP/SHA1// B3LYP/SHA1). Again density functional theoretical calculations resemble the experimental value of 4.8 kcal/mol better than any other computational technique described in the literature. For the analogous gas-phase hydration of CO_2 by OH^- instead of H_2O (Figure 2), we found, as have other workers [1-4], that this reaction is activationless, while the experimental energy of activation in solution is 13.1 kcal/mol. Almost all of this error is again associated with the underestimation of the solvent contribution. The calculated reaction energies vary from -43.3 kcal/mol mol (MP4/6-31+G(d,p)//MP2/6-31+G(d,p)) to -78.6 kcal/mol (B3LYP/ SHA1//B3LYP/ SHA1). Now, MP4 calculations in conjunction with a diffuse basis set give results remarkably close to the experimental value of -49 kcal/mol, while density functional theory yields energies that are in the range of semiemprical (AM1) ones [2].

　　Theoretical and experimental work have shown that the deprotonation of zinc-bound water is more favorable than either the deprotonation of zinc-bound imidazolium or that of imidazole itself [2]. Hence it seems that the role of zinc is to reduce the pK_a of bound H_2O by electrostatic interaction, thus allowing the hydroxyl group to retain significant nucleophilic character to attack CO_2. This effect can be shown by looking at the deprotonation energies of a selection of model complexes (Figure 3). For the extreme

cases, i.e the totally uncatalyzed and catalyzed deprotonation, respectively, these energies were calculated to be +422.9 kcal/mol and +55.8 kcal/mol (B3LYP/SHA1/ /B3LYP/SHA1). On approaching the active site environment of CA by means of complexation of the zinc with ammonia and imidazole, we obtained energy values between these limits. For the σ-donor ligand we obtained +150.4 kcal/mol while the σ-acceptor ligand yields +180.3 kcal/mol. In contrast to this, the calculations for the first steps in the reaction of [ZnOH]⁻ with CO_2, show that the direct complexation of carbondioxide is more favorable than the nucleophilic attack of the zinc-bound hydroxide ion. However due to the saturation of the coordiantion sphere with other ligands, we expect this behaviour to be changed when dealing with other model complexes like the triammine and trisimidazole complexes.

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