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Transition structures for carbon dioxide and formaldehyde hydroxylation reactions in the coordinate sphere of zinc*

An *ab initio* RHF SCF MO analytical gradient study

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Summary. The transition structure and transition vector for carbon dioxide hydroxylation in presence of zinc coordinated with three ammonia molecules have been characterized at a high basis set level. Following the directions of the transition vector the reactants appear to make a rotatory-like motion: the zinc-oxygen bonds with carbon dioxide and hydroxyl ion change antisymmetrically, while the linearity of carbon dioxide is lost and the hydroxide attacks the electrophilic center; as a result, the hydroxide moiety in bicarbonate is not directly bound to zinc in the product.

The hypothesis that zinc catalyzes hydroxylation of carbonyl containing compounds with a mechanism similar to the one used for carbon dioxide hydroxylation is explored. The reaction mechanism of formaldehyde hydroxylation in the coordination sphere of a bare-zinc cation has been studied at the same high basis set level. The results give support to our initial hypothesis and give clear evidence that the intramolecular proton transfer mechanism can be discarded.

Key words: CO_2 – Formaldehyde – Hydroxylation – Transition structure – Zinc

1 Introduction

The pioneering *ab initio* MO studies carried out by Mme Pullman and coworkers [1] showed, among other things, the ability zinc has to reduce the deprotonation enthalpy of water bound to the metal thereby giving an early quantum chemical support to the now commonly accepted zinc-hydroxide mechanism of carbonic anhydrases [2–6].

Although for the enzyme catalyzed reaction the rate limiting step does not correspond with the chemical interconversion one [2, 6], the fundamental chemi-

* This paper is respectfully dedicated to Madame Alberte Pullman who has pioneered quantum chemical studies of carbonic anhydrase molecular mechanism

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cal process catalyzed by the enzyme takes place at the coordination sphere of the metal. It consists in a fairly simple chemical reaction between the hydroxyl ion reacting with carbon dioxide to form bicarbonate; the enzyme catalyzes the decomposition of bicarbonate at the same active site. Along both reaction directions there are finite activation energies.

The strategy in the present and previous study of this system [7] consists in the theoretical characterization of a transition structure for interconvertion by using the simplest molecular model that could sustain the chemical transformation. In this manner, a high level of basis set can be used; thereafter, further complexities modeling protein features can be added on at a later stage. In the present case, as zinc cation is essential for catalysis the minimal molecular model is given by $[Zn(OH)(CO_2)]^{+1}$. Thus, a transition structure was identified as a first order saddle point, with the reaction vector actually describing the reaction step in the catalytic mechanism [7]. The energy hypersurface associated to this model represents the essential features of the zinc-hydroxide mechanism in the sense that there is a first order saddle point that connects the valley of reactant (CO_2 and hydroxide ion) to the valley of product (bicarbonate bound to zinc) [7] with finite activation barriers from both sides. Even this simple model elicits the importance of the metal since, for the free reactants in vacuum, bicarbonate dehydroxylation reaction requires about 90 kcal/mol [7] and no activation barrier is found in the pathway between reactants ($OH^- + CO_2$) and product (HCO_3^-).

The search of saddle points is not trivial. Early studies with the minimal and more complex models [8] had failed in finding a transition structure for this simple reaction. More recently, semiempirical AM_1 studies [9] were successful in finding out a maximum along a reaction coordinate although no Hessian diagonalization was reported. In our previous study of the minimal system in vacuum, the transition structure found at a high level of basis set suggested an alternative mechanism where direct nucleophilic attack was not necessary to accomplish interconversion [7]. This may be a significant result if the saddle point structure remains when structural features found in the real enzyme are added to the molecular model. The coordination shell in the enzyme can be expected to be the source of strong interactions. In the first part of this paper a transition structure is identified with three ammonia molecules that provide, together with the reactants, a full complement of valence electrons to the zinc cation.

In carbonic anhydrases, zinc not only can participate in the reversible hydration of carbon dioxide and bicarbonate dehydration but also the enzyme catalyzes hydroxylation of carbonyl containing molecules such as formaldehyde [4, 5]. Following our simple strategy, we numerically identify the transition structure and the transition vector for the hydroxylation of formaldehyde at the coordination sphere of a bare-zinc cation in order to identify the simplest molecular model capable of describing the interconversion process. One important objective we have in mind is to explore the possibility that the transition structure and transition vector for hydroxylation of carbonyl bond containing compounds catalyzed by zinc cation share geometrical features as well as parts of the atom displacements engaged in the transition vector [10].

In Sect. 2 are described the method of calculation (RHF-SCF-MO scheme), the basis sets and the molecular models. The results and discussion are gathered in Sect. 3.

2 Methods and models

Particular efforts are made to characterize first order saddle points from the outset; such structures describe well defined molecular rearrangements when the two directions of the transition vector (TV) are followed in the internal space with the help of energy minimization procedures. Those saddle points describing the chemical interconversion process correspond with the standard transition state structures (TS) whose changes are well defined and may be related with experimentally based molecular mechanism [11–15]. While our studies address mechanistic issues, the energetics that should be relevant for the actual enzyme process is not rendered in these in vacuum calculations; enzyme's particular groups and global solvent and protein effects are not included. Implicit in our treatment is the hypothesis that such intermolecular effects will not change the nature (geometry and reaction vector) of the transition structure. If such situation really happens, the structure in vacuum may still be used to gauge the differences provided the molecular model is at least partially relevant. Furthermore, the geometric information obtained can be useful in analyzing statistical mechanical simulations using for instance Warshel's empirical valence bond approach [16] which is designed to get a thermodynamic level of description or even using it as an input to parametrize such procedures.

(i) Methods: The calculations have been performed with the programs MONSTERGAUSS [17] for the optimizations and GAUSSIAN-86 [18] for the analytic force constant matrix estimations. Stationary points on the energy hypersurfaces have been located with the VA05 subroutine of Powel [19] and descents along the transition vectors have been made with the conjugate gradient method of Davidon [20].

The zinc basis set consisted of the (14*s*, 9*p*, 5*d*) primitives gaussian functions reported by Wachter [21] and contracted to (6, 2, 2, 2, 1, 1/6, 2, 1/3, 2) by Clementi and coworker [22]. For O, N and C, the (9*s*, 5*p*) basis sets of Van Duijneveldt [23] contracted to (5, 2, 1, 1/3, 2) by Clementi [22] have been used. For hydrogen, a 4*s* basis set with a (3, 1) contraction has been used [24].

Bond orders (BOs) and net atomic charges have been obtained from standard population analysis techniques [25, 26]. A good correlation between BOs and calculated force constants has been found. Mulliken effective charges are given because differences among them over molecular fragments sense fairly well charge transfers [27]. Correlation energies are estimated with the standard MP2 scheme [28].

(ii) Searches on the energy hypersurfaces: the search after saddle points is difficult due to the inverted shape of the energy hypersurfaces, namely, the carbon dioxide plus zinc-hydroxide dissociation energy is much above the energy of the transition structure for interconversion. It is then quite common when minimum energy like profiles are used as searching device to end up directly in the products, namely bicarbonate with the hydroxyl moiety bound to zinc. The techniques used here to bracket first order saddle points have been already described by us [14, 15]. Here we use the TS described previously as a seed to search for a new saddle point. The idea is that for hydroxylation reactions on the carbonyl function at the coordination sphere of zinc, the topology of the TS is invariant.

Stationary points are characterized by diagonalization of the Hessian matrix (force constant matrix in internal coordinates). For a minimum, all eigenvalues

are positive; first, second and higher order saddle points have one, two, and higher numbers of negative eigenvalues, respectively. A transition structure is a first order point calculated over the whole hypersurface. To find out a reaction path, descents from the corresponding saddle point along the directions defined by the transition vectors are made; this permits connecting related minima on the hypersurface [29, 30]. The study of alternative pathways requires a characterization of all (if possible) saddle points related to the corresponding mechanistic proposals.

Due to the extreme computer resources demanded by the ammonia liganded system, the TS and product have been geometry optimized to an average gradient length of about 0.04 mdynes. It is our experience that with these systems, geometry optimization does not change the qualitative picture and gains in energy are not large.

(iii) Mechanistic issues. In the first stage of the molecular mechanism of carbonic anhydrase, zinc water bound molecule is deprotonated. Carbon dioxide, or any other carbonyl compound entering the active site, may be attacked by the zinc-hydroxide center in two different manners as indicated in the schemes of Fig. 1. A direct nucleophilic attack would avoid substrate binding to zinc. In the second case binding to the metal is required in order to activate the carbonyl carbon center. These are the mechanistic issues at the interconversion step which are explored in the hydroxylation of methanal.

3 Results and discussion

3.1 Carbon dioxide hydroxylation-bicarbonate dehydroxylation

The transition structure for the carbon dioxide hydroxylation and bicarbonate dehydroxylation calculated with the ammonia ligands is schematically shown in Fig. 2.

To analyze the role of the stereochemistry around zinc, a pyramidal arrangement was chosen to start the study; this is schematically depicted in the lower inset of Fig. 2. The internal coordinates related to the actual transition vector

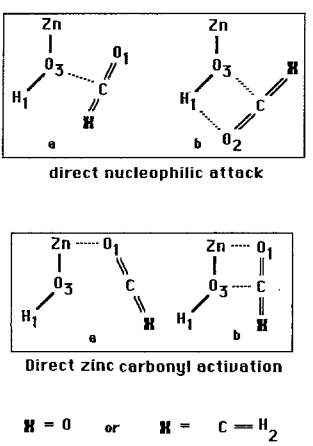

Fig. 1. Schematic display of the mechanistic issues discussed in this paper

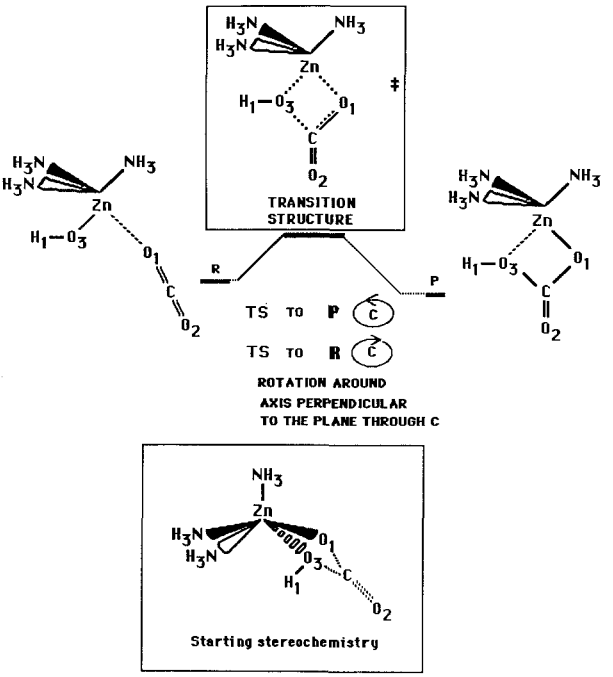

Fig. 2. Transition state structure for the interconversion reaction between carbon dioxide and an hydroxide bound to zinc. The coordination shell is completed with three ammonia molecules. Also indicated are the putative reactant (**R**) and the product (**P**); this latter is fully characterized as a minimum. The initial spatial arrangement of the reactants at the coordination shell of zinc is given as an inset (*lower part*)

were frozen and the coordinates defining the stereochemistry of ligands optimized. The system evolves toward a *deformed tetrahedral arrangement.* In this configuration only a first order saddle point obtains. Its geometry is depicted in Fig. 3.

The structure of this stationary point corresponds fairly well with the one calculated using only a bare-zinc cation. The degree of charge transfer from the hydroxyl and carbon dioxide moieties towards zinc is different, as well as the quantitative values of the force constants are smaller when the coordination

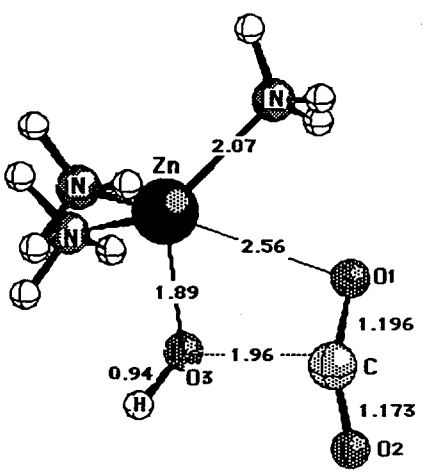

Fig. 3. Geometry of the first order saddle point which represents the transition structure for the interconversion reaction. This can be considered as fairly good portrait of the TS for the interconversion step in carbonic anhydrases. $<O3CO1 = 95.8°$; $<O1CO2 = 156.9°$; $<HO3CO1 = 179.9°$; $<HO3CO2 = -0.1°$

sphere is present. Interestingly, the zinc-oxygen distances, both of the outgoing and ingoing atoms, are larger in the ammonia complex than in the bare-zinc calculation. The hydroxyl oxygen moves from a distance of 1.76 Å in the complex $(NH_3)_3ZnOH^+$ to 1.89 Å at the saddle point.

Viewed from the saddle point perspective, the interconversion reaction can be pictorially described as a rotation-like motion of the reactants around an axis perpendicular to the molecular plane made by them and passing through the carbonyl carbon (see Fig. 2). In the anti-clockwise direction bicarbonate is formed as carbon dioxide approaches zinc and the hydroxyl ion moves away from zinc; in the opposite direction, hydroxyl ion moves towards zinc while carbon dioxide leaves it. We use a properly defined Z-matrix to perform such displacements. Along both directions the energy decreases as it should for a system having negative curvature in its hypersurface. In Fig. 2 the schematic structures are given; full characterization of the bicarbonate complex was achieved; the reactants were not optimized. We anticipate, however, that carbon dioxide is bound to the complex $(NH_3)_3ZnOH^+$ with an angle of about 90° between O–Zn–O atoms.

The model so described will be referred to as the rotary model for the interconversion process. It clearly corresponds to an alternative mechanism for the interconversion process. As shown in our previous paper [7], bicarbonate can move to a monodentate complex; this one is a saddle point of first order that opens the way to a bidentate bicarbonate complex. It is at this stage that the walls of carbonic anhydrase active sites plays a fundamental role. A thorough structural discussion in the active site of the enzyme was already done [7] and will not be repeated here.

The activation energy for bicarbonate dehydroxylation in the rotary model is about 17 kcal/mol. This value is larger than the one found for the bare-zinc case. The reason for this difference is found in the fact that the reactants bonding to zinc is weaker for the ammonia liganded than bare-zinc systems. The energy required to overcome the barrier is larger when the ammonia ligands are present than in absence of them. This result can be due to the gain in strength of the carbon-oxygen bond that is to be broken. Of course, compared with free reactants, where the barrier required to decompose bicarbonate is equal to about 90 kcal/mol [7], the activation energy is now reasonably small. The role of zinc in changing the energetics is therefore enormous.

From the mechanistic view point, the calculations presented here, and those reported previously [7], indicate that a zinc activation of the carbonyl carbon makes part of the reaction coordinate; using the schemes of Fig. 1 it can be said that there is a simultaneous interaction of the oxygen with zinc, and carbon center with the hydroxyl oxygen (option *b* of the direct zinc carbonyl activation). Thus, the high nucleophilic power of free hydroxyl ion is substantially reduced by its coordination to zinc. This effect is understandable if zinc is to change the pK_a of water making it more acidic as it is required by its enzymatic mechanism. Thus, in order to recover its nucleophilicity, the hydroxyl ion must move away and the carbonyl oxygen moves towards the zinc. This is the type of atom displacement described in the transition vector reported in [7] and with the one obtained in the present study (not reported here to avoid redundancies). The view obtained with lower levels of basis sets that carbon dioxide attacks directly the hydroxide ion is not granted by this more rigorous calculation.

3.2 Formaldehyde hydroxylation reaction mechanism

Energy entries, geometrical parameters and force constants for the reactants with and without zinc are consigned in Table 1. Both the direct nucleophilic attack and zinc activation mechanisms have been studied (cf. Fig. 1).

Direct nucleophilic model. The transition structure for the direct nucleophilic attack of zinc bound hydroxide onto carbon dioxide is displayed in Fig. 4. The stationary structures related by the transition vector are also depicted. Quantitative data are given in Table 2.

At the reactive saddle point a careful analysis of the methanal-OH framework shows that the geometric arrangement for the intramolecular proton transfer looks like the one described for the same reaction in vacuum [31–39]. This intramolecular proton transfer (IPT) involves the concerted displacement of

Table 1. Total electronic energies at the SCF and SCF + MP2 levels of theory for the moieties intervening in the interconversion step of methanal hydroxylation

Structure	SCF Energy (a.u.) (MP2 Energy)	Gradient Length (10-4 mdyne)	Optimized Geometry (Å) (Force Constant (mdyne/Å))
OH—	−75.300912 (−75.403867)	0.17	OH = 0.999 (5.72)
CH2O	−113.802796	0.40	CO = 1.216(14.08); CH = 1.087 (5.16); OCH = 121.8 (1.78)
	(−114.017945)		HCH = 116.4 (1.31)
HCOHOH	−189.185514	5.13	CO1 = 1.249 (11.42); CH1 = 1.973 (5.90); CH2 = 1.182 (2.53)
	−189.506298)		CO2 = 2.949 (6.83); O2H3 = 0.953 (8.68); O1CH1 = 128.1 (8.33) H1CH2 = 121.5 (1.62); O1CO2 = 124.8 (6.40); CO2H3 = 99.9 (0.70) H2CH1O1 = 180.4 (0.06); O2CO1H1 = −0.2 (1.22); H3O2CO1 = −3.1 (0.01)
Zn++	−1776.717508 (−1776.906301)		
ZnOH+	−1852.690103	2.79	ZnO = 1.777 (3.85); OH = 0.953 (8.89); ZnOH = 124.6 (0.43)
	−(1853.006338)		
ZnOCH2++	−1890.680508	1.69	ZnO = 1.82 (3.03); CO = 1.246 (12.59); OH = 1.083 (5.38)
	(−1891.322145)		ZnOC = 179.9 (0.16); OCH = 119.7 (1.74); HOH = 120.6 (1.23)

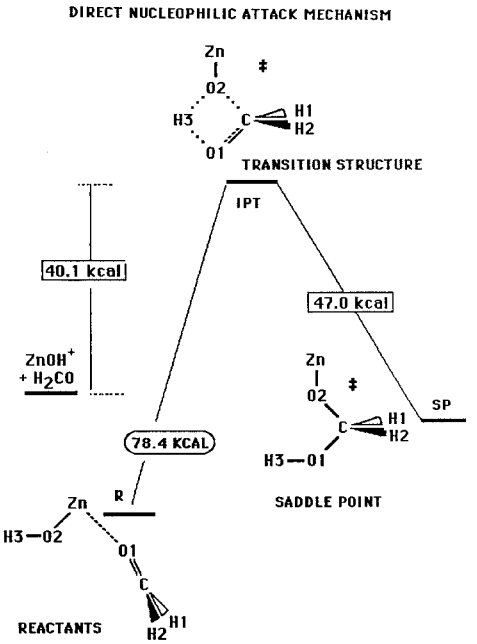

Fig. 4. Transition structure for the intramolecular proton transfer mechanism of methanal hydroxylation. The mechanistic pathway represents the direct nucleophilic attack mentioned in Fig. 1. Note that the curvature at **SP** when coming from the **IPT** structure is positive, thus it is a minimum; however, in an orthogonal direction it is a first order saddle point whose "reactant and product" structures correspond to the actual product **P**

Table 2. Geometrical parameters, diagonal force constants (K) and coefficients (C) of the eigenvector associated with the smallest eigenvalue obtained after diagonalization of the Hessian in the control space for the structures depicted in Fig. 4. **IPT** represents the intramolecular proton transfer; **SP** is the stationary point connected to the **IPT**, along this direction it is a minimum, while it is a saddle point on the global hypersurface

	IPT			**SP**		
	Geom.	K	C	Geom.	K	C
ZnO2	1.804	3.27	0.01	1.784	3.68	−0.00
CO1	1.330	6.85	−0.21	1.414	5.97	−0.00
CO2	1.764	0.73	0.48	1.449	4.90	0.00
CH1	1.077	5.50	0.01	1.085	5.17	0.01
CH2	1.077	5.50	0.01	1.085	5.17	0.01
H3O2	1.193	−0.66	−0.75	2.261		
H3O1	1.257			0.954	8.85	−0.00
ZnO2C	147.7	0.40	0.11	128.4	0.46	0.00
O1CO2	84.7	3.62	0.18	107.0	1.84	0.00
CO2H3	70.4	2.08	0.31			
CO1H3				112.6	0.79	0.00
O2CH1	104.1	0.84	−0.09	111.3	1.26	−0.06
O2CH2	104.1	0.84	−0.09	111.3	1.26	0.06
ZnO2CO1	180.0	0.05	−0.00	180.0	0.03	−0.28
O1CO2H3	0.0	0.68	0.00			
H1CO2O1	119.2	1.07	0.04	118.9	1.07	0.05
H2CO2O1	−119.2	1.07	−0.04	−118.9	1.07	0.05
H3O1CO2				0.1	−0.04	0.95

the hydroxide proton H3 to the carbonyl oxygen O1 concomitant with the hydroxide aldehyde C–O2 bond making and with the carbonyl C–O1 π bond breaking. A TS named **IPT** accounting for this process has been located 78 kcal/mol above the reactants. The TS **IPT** and its related stationary structures are reported in Fig. 4 and Tables 2 and 3. The TV amplitudes for **IPT** are mainly directed by the distances O2H3 (amplitude −0.75) and C–O2 (0.48) and by the angle C–O2–H3 (0.31) and describes the intramolecular proton transfer of H3 between O2 and O1. The coordinates involving zinc do not appear in the TV.

Descent along one direction of the TV of **IPT** goes towards reactants formaldehyde and zinc bound hydroxide ion by breaking the hydroxide aldehyde C–O2 bond. Descent along the opposite direction goes towards the stationary structure **SP** by making the C–O2 and O1–H3 bonds and by breaking the O2–H3 σ and CO1 π bonds. The structure **SP** is located 47.0 kcal/mol below **IPT** and 33 kcal/mol above the product **P**. This stationary point accounts for the zinc bound $HOCH_2O^-$ ion. Along the reaction path connecting **SP** to **IPT**, the zinc binding does not change as expected since the metal does not appear in the TV, but the CO2 bond is made: BO and force constant increase from 0.54 and 0.7 mdyne/Å in **IPT** to 0.83 and 4.9 mdyne/Å in **SP**, which indicates a large change in bond strength. Simultaneously, these values for the carbonyl CO1 bond decrease from 1.18 and 6.9 mdyne/Å in **IPT** to 0.89 and 6.0 mdyne/Å in **SP** (see Table 2) signaling a single bond type.

Structure **SP** is a first order saddle point along a direction orthogonal to the one connecting **IPT** with **SP**. The eigenvector corresponding to the unique negative eigenvalue of the Hessian is controlled by the dihedral angles Zn–O2–CO1 (amplitude −0.28) and O2–C–O1–H3 (0.95) and describes the motion of the hydroxide moiety out of the heavy atoms plane. By symmetry reasons, both directions of this eigenvector go towards **P**, the product of the interconversion mechanistic step.

Zinc-activation mechanism. For the alternative mechanism with zinc activation of the carbonyl group, the nucleophilic attack of the hydroxide ion is performed with an extremely low activation barrier. This result can be seen in Fig. 5 where the transition structure for the zinc-activated carbonyl carbon is displayed.

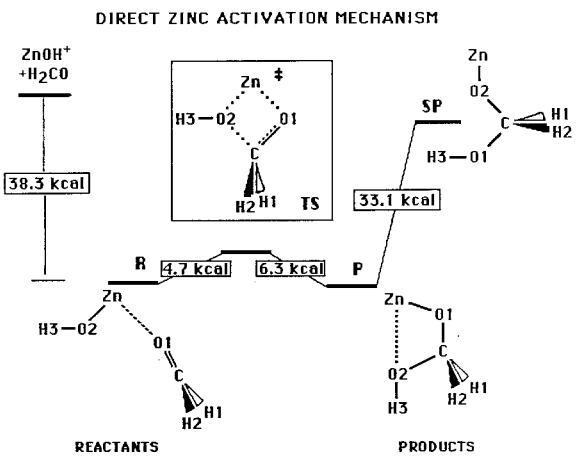

Fig. 5. Reaction profile for the direct activation mechanism in the hydroxylation of methanal

Table 3. Geometrical data and diagonal force constant for the reactant (**R**), transition structure (**TS**) and product (**P**) in the interconversion step of the zinc hydroxide mechanism in the hydroxylation of methanal

	R		**TS**			**P**	
	Geom.	*K*	Geom.	*K*	*C*	Geom.	*K*
ZnO1	2.013	1.11	1.935			1.843	
ZnO2	1.776	4.07	1.817	3.40	0.12	1.997	1.81
CO1	1.224	13.99	1.817	9.20	0.10	1.392	5.71
CO2			2.100	1.56	−0.83	1.548	5.40
CH1	1.083	5.36	1.077	5.52	−0.01	1.080	5.40
O2H3	0.946	9.19	0.945	9.36	0.00	0.949	9.22
ZnO1C	154.5	0.12					
ZnO2H3	133.1	0.34	144.7			154.4	
ZnO2C			82.1	6.01	0.16	87.8	11.4
O1CO2	90.0	0.48	90.5	4.14	0.31	100.8	7.85
CO2H3			133.2	0.41	−0.38	117.8	0.75
O2CH1	120.8*	1.27	95.8	0.66	0.10	106.9	1.16
ZnO2CO1			0.0	0.47	0.00	− 0.0	0.49
H3O2CO1			180.0	0.07	0.00	179.9	0.11
H1CO2O1			121.1	1.22	0.01	119.9	1.06
H2CO2O1			−121.1	1.22	−0.01	−119.9	1.06

In Table 3 the geometry of the saddle point, diagonal force constants and the eigenvector (C-column) of the unique negative eigenvalue are reported.

The TV of the structure **TS** accounts for this four bond making/breaking process. Descent along one direction shifts the system towards **R** by breaking the hydroxide aldehyde CO2 bond and by weakening the zinc aldehyde ZnO1 bond. Concomitantly, zinc hydroxide bond Zn–O2 and carbonyl C–O1 bonds are reinforced. The structure **R** is reproted in Fig. 5 and Table 3; it corresponds to a point on the hypersurface located 4.7 kcal/mol below **TS**. The angle O1–Zn–O2 has a value of 90° with a repulsive gradient (−0.19 mdyneÅ/rad), the remaining parameters have been optimized with a gradient liength of 8.3×10^{-5} mdyne. This structure describes a weak interaction of H_2CO with the zinc cation. Comparing with the saddle point figures, the bond order of the zinc aldehyde Zn–O1 decreases from 0.18 in the **TS** structure to 0.06 in **R** although the BO and force constant of the Zn–O2 bond increase from 0.38 and 3.4 mdyne/Å in **P** to 0.55 and 4.1 mdyne/Å in **R** (see Table 3). For the carbonyl C–O1 bond these values increase from 1.38 and 9.2 mdyne/Å in transition structure **TS** to 1.65 and 14.0 mdyne/Å in **R** close to the 1.84 and 14.1 values reported for free formaldehyde (Table 1); this result accounts for a double bond character.

Descent along the other direction in the TV of the **TS** structure goes toward the product structure **P** which is a minimum located 6.3 kcal/mol below the **TS**. This descent corresponds to the making of the hydroxide aldehyde C–O2 zinc aldehyde Zn–O1 bonds and to the breaking of the zinc hydroxide Zn–O2 and carbonyl C–Ol π bonds.

In the product **P**, the zinc aldehyde oxygen bond has a BO three times bigger than the zinc hydroxide bond. Although weak, the hydroxyl oxygen is occupying

Table 4. Effective charges obtained from a Mulliken population analysis

	Zn	OH	H2CO
R	1.609	−0.647	0.038
TS	1.648	−0.580	−0.069
P	1.590	−0.387	−0.203
IPT	1.382	−0.374	−0.008
SP	1.490	−0.369	−0.121

a coordination site. This bonding is important. The monodentate bonding, characteristic of structure **SP** is high above in energy.

The pathway **R-TS-P** represents the hydroxide moiety transfer between the zinc cation and the formaldehyde. This process displays small activation energies. Along this pathway, the effective charge of the zinc cation varies in a very narrow band as it is shown in Table 4. The charge transfer occurs from the hydroxide moiety to the formaldehyde whose effective charge increases from 0.04*e* in structure **R** to 0.20*e* in **P**.

As it was the case for the carbon dioxide substrate (ref. [7] for detailed discussions), the nucleophilicity of the hydroxide ion towards the formaldehyde is inhibited by its binding to the zinc cation. The reaction implies an activation by zinc of the electrophilic carbon center. This appears to be a recurrent aspect for those examples calculated until now with extended basis sets [7, 10].

4 Discussion

We have presented theoretical evidence that the transition structures for hydroxylation of carbon dioxide and formaldehyde are, to within minor geometrical differences, closely related in both reactions. A rotary mechanism where the reactants use two coordination sites of the zinc coordination sphere is the unifying feature. The same microscopic motions were found for direct water attack both onto carbon dioxide and formaldehyde. The intramolecular proton transfer pathway, which is always present as an alternative, appears to be excluded due to their high activation barriers compared to the zinc-sensitive barriers of the rotatable model. Of couse, one can always introduce water molecules that might help lowering such a barrier. However, as the rotary model accomplishes the task in a simpler manner it can be taken as a reasonable alternative mechanism for the interconversion step.

In the rotary model, the roles played by bare zinc in both hydroxylation reaction can be summarized: (i) inhibition of simple hydroxide nucleophilicity strength; (ii) aldehyde carbonyl activation; (iii) well-defined interconversion pathway between formaldehyde and $HOCH_2O^-$ ion with low activation barriers.

For the hydroxylation of methanal, the tetrahedral nature of the carbon center, that is typical for the gas phase reaction of OH^- and H_2CO to yield $HOCH_2O^-$ [33, 37, 40] is not affected by zinc. Note that in vacuum and without the metal, there is no energy barrier to the addition of the negatively charged nucleophile to a neutral carbonyl compound. In the zinc coordination sphere

things go differently, the metal provides the conditions to create a barrier for the hydroxylation reaction.

The rotary model enforces a space requirement in the coordination sphere of zinc. A coordination shell with four fixed ligands will have more difficulties than one with three ligands to accommodate the molecular motions required by the transition vector without introducing strong steric effects. Three fixed ligand positions occupy one hemisphere around the metal thereby leaving the space for ease incoming/outgoing of molecules. In carbonic anhydrases, three histidines are coordinating the metal plus one exchangeable water molecule in a deformed tetrahedral arrangement. The stereochemistry appears as optimal in view of the present mechanistic model. The fact that a pyramidal arrangement evolves towards a deformed tetrahedral conformation of reactants is an indication in favour of this idea. Furthermore, as it has been shown by Argos et al. [41], after analyzing entries in the Protein Data Bank for a number of zinc-containing enzymes, one of the characteristics of the coordination sphere of the metal is a deformed tetrahedral arrangement [42]. One can then understand that one extra monodentate ligand would block one position at the coordination sphere of zinc and may actually act as competitive inhibitor.

The preceding arguments are reinforced by the results obtained from the elegant experimental work done by Kimura et al. [43]. There, it is actually shown that the most efficient biomimetic systems have zinc tetracoordinated.

The carbon dioxide/bicarbonate interconversion mechanism herein discussed is an alternative [44] to current models. It must be further tested with experiments. In fact, it predicts that the active site may be inactivated if the rotary motion continues until a bidentate bicarbonate complex is formed. In biomimetic systems, such effects have been detected. In carbonic anhydrases, it can be predicted that a replacement of the residue Thr-199 by a non-hydrogen bonding one, would likely permit the reactants to rotate more freely in the coordination sphere of zinc, thereby opening the possibility for bidentation of bicarbonate and consequent inhibition [7].

The extrapolation of in vacuum *ab initio* MO studies of first order saddle points obtained with analytical gradients and actual calculation of the Hessians to discuss enzyme catalyzed reactions raises important questions: What do the calculations really represent? What do the calculations tell us? are two among other interrogations. Two main hypotheses are behind our way to extrapolate: (1) There exists a minimal molecular model with a first order saddle point which describes the essentials of the chemical interconversion step in a given enzyme mechanism; (2) The corresponding transition structure, that encodes the fundamental information relating reactant and product valleys, is an invariant feature: namely, it is essentially the same in vacuum and in the active site of the enzyme; this ought to be understood in the sense that it encodes the same information on the reaction. *Ab initio* calculations of these type of saddle points carried out at a high level of basis set representation permit the actual determination of high quality geometric structures and corresponding force constants. One constructs, in this manner, not an artificial situation, but a minimal model to produce testable information. For example, starting from such information, and the constraints put upon the relative displacements of reactant moieties, Bigeleisen type calculations can be made to get kinetic isotope effects. This approach has been tested with fairly good success in, for instance, our work on formate dehydrogenase mechanism and alcohol dehydrogenase from liver [45–47]. In the present work we have found a fairly good invariance of the geometry and

transition vector for the interconversion step when strong interactions, represented by the model coordination sphere, are incorporated in the calculational scheme.

If one is talking about the same interconversion chemical step, the transition structure invariance to surrounding medium effects is required; such effects cannot alter the information content embodied in the saddle point. The pathway towards reactants and products, on the contrary, may be fully determined by the environments. Thus, at variance with the TSs behavior, one would expect that the reactant and product configurations and electronic properties to become strongly modulated by the protein environment. Pauling's lemma [11], namely, that the shape of the active site is complementary to the form of the activated complex for the reaction catalyzed by the enzyme, can be extended in the following sense: the geometry of the activated complex corresponds to the geometry predicted by the first order saddle point structure describing the chemical step in vacuum. It follows then that, most likely, the geometries of reactants and/or products are not necessarily those pertaining to their equilibrium conformations, but they will be moulded by the enzyme into conformations resembling the transition structure. Support for this idea is emerging from the study of enzyme model systems [7, 10, 45–48], especially with the finding made for RUBISCO carboxylation-oxygenation reaction [48].

Note that the idea that the enzyme-catalyzed reaction would look like the reaction *in vacuum* has already been discussed in the literature [49]. One may or may not agree with the arguments given there, but the point is that an *in vacuum* system will certainly give insights on the reaction catalyzed by an enzyme if the interconvertion mechanistic step is the same in both situations (the TSs must be closely related). In the present case, the questions raised in the preceding paragraphs should be discussed in the light of the new paradigm, namely: the *in vacuum* calculated TS structures inform us on the potential geometry the activated complex will have at the active of the given enzyme. This does not mean, of course, that protein surrounding effects are not important in enzyme catalysis [50, 51].

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References

1. Pullman A (1981) Ann NY Acad Sci 367:340 and references therein
2. Lindskog S (1986) in: Bertini I, Luchinat C, Maret W, Zeppezaur M (eds) 307, Birkhäuser, Boston
3. Vallee BL, Galdes A (1984) Adv Enzymol 56:283
4. Lindskog S (1983) in: Spiro TG (ed) Zinc enzymes. Wiley, NY
5. Chesnovsky D, Navon G (1980) Biochemistry 19:1866
6. Coleman JE (1986) in: Bertini I, Luchinat C, Maret W, Zeppezauer M (eds) 49, Birkhäuser, Boston
7. Jacob O, Cardenas R, Tapia O (1990) J Am Chem Soc 112:8692

8. Liang JY, Lipscomb WN (1987) Biochemistry 26:5293; Liang JY, Lipscomb WN (1989) Int J Quantum Chem 36:299; Liang JY, Lipscomb WN (1986) J Am Chem Soc 108:5051; Jonsson B, Karlstrom G, Wennerstrom H (1978) J Am Chem Soc 100:1658
9. Merz KM, Hoffmann R, Dewar MJS (1989) J Am Chem Soc 111:5636
10. Jacob O, Tapia O (1992) Int J Quantum Chem 41:1271
11. Pauling L (1948) Nature (London) 161:707
12. McIver JW (1974) Accounts of Chemical Research 7:72
13. Truhlar DG, Hase WL, Hynes JT (1983) J Phys Chem 87:2664
14. Tapia O, Andres J (1984) J Chem Phys Lett 109:471
15. Tapia O, Lluch JM, Cardenas R, Andres J (1989) J Am Chem Soc 111:829
16. Åqvist J, Warshel A (1992) J Mol Biol 224:7
17. Program MONSTERGAUSS, Peterson MR, Poirier RA (1980) University of Toronto, Ontario, Canada
18. Program GAUSSIAN-86, Frisch MJ, Binkley JS, Schlegel HB, Raghavachari K, Melius CF, Martin RL, Stewart JJP, Bobrowicz FW, Rohhfing CM, Kahn LR, Defrees DJ, Seeger R, Whiteside RA, Fox DJ, Fleuder EM, Pople JA (1984) Carnegie-Mellon Quantum Publishing Unit, Pittsburgh, PA
19. Powel MJD, Atomic Energy Research Establishment, Harwell, UK
20. Davidson WC (1975) Mathematical Programming 9:1
21. Watchers AJH (1970) J Chem Phys 52:1033
22. Gianolo L, Pavani R, Clementi E (1978) Gazz Chim Italiana 108:181
23. Van Duijneveldt F (1971) IBM Techn Report RJ945, Dec
24. Exponents for the 1*s* contraction are 26.972837, 4.0685679, 0.92177523 with coefficients 0.0033494, 0.23472695, 0.81375733; the exponent for the 2*s* orbital is 0.21328984. Scale factors of 1.2 and 1.15 were used for the 1*s* and 2*s* orbitals, respectively.
25. Mayer I (1983) Chem Phys Lett 97:270
26. Mulliken RS (1955) J Chem Phys 23:1833
27. Otto P, Ladik J (1980) Int J Quantum Chem 18:1143
28. Binkley JS, Pople JA (1975) Int J Quant Chem 9:229
29. Schlegel HB (1987) Adv Chem Phys 67:249
30. Gandour RD, Schowen RL (eds) (1978) Transition states of biochemical procesess. Plenum, NY
31. Burgi HB, Lehn JM, Wipff G (1974) J Am Chem Soc 96:1956
32. Burgi HB, Dunitz JD (1983) Acc Chem Res 16:153
33. Williams IH, Maggiora GM, Schowen RL (1980) J Am Chem Soc 102:7831
34. Scheiner S, Lipscomb WN, Kleier DA (1976) J Am Chem Soc 98:4770
35. Alagona G, Scrocco E, Tomasi J (1975) J Am Chem Soc 97:6976
36. Dewar MJS, Strocch DM (1985) J Chem Soc Chem Commun 94
37. Madura JD, Jorgensen WL (1986) J Am Chem Soc 108:2517
38. Weiner SS, Singh C, Kollman PA (1985) J Am Chem Soc 107:2219
39. Chandrasekhar J, Smith SF, Jorgensen WL (1984) J Am Chem Soc 106:3049
40. Spangler D, Williams IH, Maggiora GM (1983) J Comp Chem 4:524
41. Argos P, Garavito RM, Eventoff W, Rossman MG, Brändén CI (1978) J Mol Biol 126:141
42. We have extended this analysis with basically similar results to those found by Argos et al.
43. Kimura E, Shiota T, Koike T, Shiro M, Kodama M (1990) J Am Chem Soc 112:5805
44. Lipscomb WN (1991) Int J Quantum Chem S25:1
45. Tapia O, Cardenas R, Andres J, Krechl J, Campillo M, Colonna-Cesari F (1991) Int J Quantum Chem 39:767
46. Tapia O, Cardenas R, Andres J (1992) Chem Phys Lett 189:395
47. Tapia O, Cardenas R, Andres J, Colonna-Cesari F (1988) J Am Chem Soc 110:4046
48. Tapia O, Andres J (1992) Mol Eng 1
49. Dewar MJS (1986) Enzymes 36:8
50. Tapia O (1992) J Math Chem 10:139
51. Tapia O (1992) Theoretical Evaluation of Solvent Effects in: Maksic ZB (ed) Theoretical models of chemical bonding, v 4, p 435, Springer-Verlag NY, Berlin, HD; Tapia O, Nilsson O (1992) in: Bentran J (ed) Molecular aspects of biotechnology: computational models and theories, pp 123–152, Kluwer, Dordrecht