On Transition Structures for Hydride Transfer Step in Enzyme Catalysis. A Comparative Study on Models of Glutathione Reductase Derived from Semiempirical, HF, and DFT Methods

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As a model of the chemical reactions that take place in the active site of gluthatione reductase, the nature of the molecular mechanism for the hydride transfer step has been characterized by means of accurate quantum chemical characterizations of transition structures. The calculations have been carried out with analytical gradients at AM1 and PM3 semiempirical procedures, *ab initio* at HF level with 3-21G, 4-31G, 6-31G, and 6-31G* basis sets and BP86 and BLYP as density functional methods. The results of this study suggest that the endo relative orientation on the substrate imposed by the active site is optimal in polarizing the C4−Ht bond and situating the system in the neighborhood of the quadratic region of the transition structure associated to the hydride transfer step on potential energy surface. The endo arrangement of the transition structure results in optimal frontier HOMO orbital interaction between NADH and FAD partners. The geometries of the transition structures and the corresponding transition vectors, that contain the fundamental information relating reactive fluctuation patterns, are model independent and weakly dependent on the level of theory used to determine them. A comparison between simple and complex molecular models shows that there is a minimal set of coordinates describing the essentials of hydride transfer step. The analysis of transition vector components suggests that the primary and secondary kinetic isotope effects can be strongly coupled, and this prompted the calculation of deuterium and tritium primary, secondary, and primary and secondary kinetic isotope effects. The results obtained agree well with experimental data and demonstrate this coupling.

1. Introduction

Redox reactions associated to hydride transfer (HT) processes are known to play a leading role in many enzymatic systems.[1] The HT step is catalyzed by enzymes of the dehydrogenase and flavoprotein reductase families;[2] these enzymes are implicated in important biological reactions,[3] with the hydrogen transferred from the 4-position of the nicotinamide adenine dinucleotide or a phosphate derivative ($NAD(P)^+$/NAD(P)H) moiety toward a particular substrate or vice versa.

In this paper, we report a theoretical study on the structural, electronic and mechanistic aspects of the HT in glutathione reductase (GR) enzyme, belonging to the flavoprotein reductase family, which serves an important function in intracellular redox processes by making available free thiols in the form of reduced glutathione.[4] GR (EC 1.6.4.2) catalyzes the reversible pyridine nucleotide dependent reduction of oxidized glutathione (GSSG) to form 2 mol of reduced glutathione (GSH):

$$NAD(P)H + H^+ + GSSG \rightarrow NAD(P)^+ + 2GSH$$

The catalytic process of this enzyme is a two step or ping pong mechanism, and the overall reaction has been demonstrated to be kinetically described as the sum of two half reactions:[4]

$$E + NAD(P)H + H^+ \rightarrow EH_2 + NAD(P)^+$$

$$EH_2 + GSSG \rightarrow E + 2GSH$$

where E is the enzyme and EH_2 is the reduced enzyme.

In the first step studied here a HT process takes place with formation of the reduced enzyme as a stable intermediate. In the second step, glutathione disulfide is reduced.[5] The first step is the rate-limiting step for the spinach, yeast, and *Escherichia coli* GR when NADPH is replaced by NADH as reductant[6] or 2,4,6-trinitrobenzenesulfonate is used as substrate.[7] However, the second step becomes rate limiting when NADPH is used as reductant with the yeast enzyme.[8] Although the surroundings of the active site and the structures of the binary complexes of the enzyme and numerous nucleotides have been determined by X-ray structure analysis

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at high resolution,[5,9] not only the mechanistic aspects of this reaction but also the electronic nature of the formally termed hydride being transferred are still a matter of controversy.[10−13]

The nature of the molecular mechanism for the HT step on different models representing this catalytic process has been theoretically investigated.[14−23] In particular, we have studied model systems ranging from liver alcohol dehydrogenase,[16] to lactate dehydrogenase,[19] to formate dehydrogenase,[20] to dihydrofolate reductase.[23] On the basis of this body of information, a quantum description based on transition structure (TS) characterization of chemical reaction of enzyme catalysis has been presented by us.[24] The key hypothesis of our work is that the in vacuo calculated TS informs us on the geometry of the activated complex at the active site of the given enzyme and on the reactive fluctuation pattern there. These findings may be transposed to the HT associated to the GR enzyme, and the present study has three main purposes: (i) the determination of the TS and possible intermediates to describe the mechanism, (ii) the characterization of the electronic nature of the different steps leading to the final result described as the transport of two electrons and one proton in the form of an hydride ion, and (iii) a comparison among the theoretical results obtained with different methods and models and experimental results. This could give useful indications concerning the reliability of the procedures employed.

In section 2, the computing models and methods are presented. In section 3, we analyze and discuss the relevance of the results to the catalytic mechanism of GR. A section of final remarks closes this paper.

2. Computing Models and Methods

Computational chemical models are playing an ever increasing role in chemical research. During the last few years, quantum mechanical calculations have been gradually recognized as a useful tool in research related to the elucidation of molecular reaction mechanisms,[25] and the X-ray crystallographic data are essential to posit the theoretical study in a functional perspective to understanding the enzymatic process. In constructing the geometry of a model system, knowledge of the enzyme structure is required and, in particular, the shape of its active center where the chemical reaction takes place. Human glutathione reductase is the only enzyme belonging to flavoprotein reductase family that has been solved to high resolution[5,9] (1.5 Å). Common aspects (spectral properties, high active site sequence homology, etc.) and structural and mechanistic similarities between this enzyme and others like lipoamide dehydrogenase,[26] mercuric reductase,[27] and trypanathione reductase[28] have been found. GR represents the most studied flavoprotein due to its favorable spectroscopic properties, its stability, and the catalytic reactivity in the crystalline state. This is the reason why the GR has been usually taken as reference for the rest of the family.

The X-ray studies on GR enzyme[5,9] show that the nicotinamide moiety is tightly bound to the enzyme; therefore it has a definite orientation and distance with respect to the flavin ring. The complex formed between NADPH and the flavine system is in an endo configuration where the hydride donor (C4) and acceptor (N6) centers are nearly on top of each other.[5,9] In the GR enzyme, each active site contains a pair of cysteines forming the redox active disulfide that is in close proximity to the flavin isoalloxazine ring to form a charge transfer complex.[4] This complex is formed immediately after the HT has occurred, involving the rupture of the disulfide bridge together with an electronic release to the nearby sulfur atom.[13] Since the main purpose of this study is to analyze the HT process, the presence of the disulfide was not considered.

A model emerges as a possibility for the HT step imposed by stereochemical considerations.[29] In Figure 1, a schematic view of the arrangement of NAD(P)H and FAD partners obtained from X-ray data is depicted. The relative orientation of both partners corresponds to the

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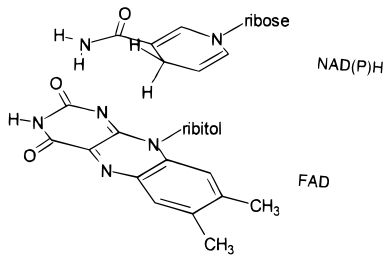

Figure 1. Schematic representation of the arrangements of NAD(P)H and FAD partners obtained from X-ray structure data.

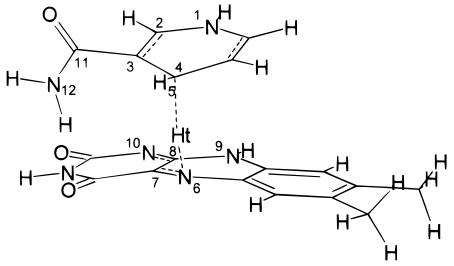

Figure 2. TS geometry and numbering of the atoms for the the hydride transfer reaction of glutathione reductase, model **I**.

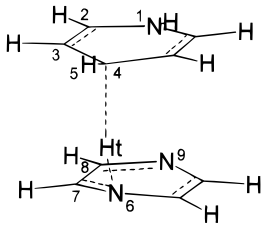

Figure 3. TS geometry and numbering of the atoms for the the hydride transfer reaction of glutathione reductase, model **II**.

model reported by Schulz *et al.*[30] and Tapia *et al.*[31] Two molecular models have been selected: (i) a complex model, **I**, used for semiempirical and *ab initio* calculations at the HF/3-21G level, assembled with the flavine, represented by an isoalloxazine tricyclic ring, and the coenzyme NAD, represented by the 1,4-dihydronicotinamide ring (see Figure 2); and (ii) the reduced model, **II**, used for the ab initio and DFT procedures, formed by pyrazine and 1,4-dihydropyridine rings (see Figure 3).

A complete application of *ab initio* methods is generally prohibitive in terms of computational effort for any but the smallest systems. Semiempirical methods are intended for studying large molecular systems of chemical or biological interest, and they have progressed over the last few years to a surprising level of accuracy and reliability, considering the limitations of the underlying approximations.[32] On the other hand, much interest has been given recently to methods based on density functional theory (DFT)[33−35] as an alternative to *ab initio* schemes.[36,37] Although DFT took relatively long to find a wider acceptance among chemists, there is now rapidly increasing evidence that molecular density functional calculations give remarkably accurate results for molecular structures and electronic properties. Furthermore, since it takes into account most of the dynamical and nondynamical correlation effects, the DFT results are of a quality comparable to the conventional post Hartree−Fock (HF)[38] methods.

Semiempirical calculations, AM1[39] and PM3,[40] were carried out using the MOPAC93[41] program. The *ab initio* calculations have been performed with the GAUSSIAN 92/DFT series of programs[42] at HF/3-21G, HF/4-31G, HF/6-31G and HF/6-31G* levels. For the DFT calculations, we employed the Becke exchange functional,[43] which includes the Slater exchange along with corrections involving the gradient of the density, and the gradient corrections provided by the Perdew 86 expression, along with his 1988 local correlation functional (BP86)[44,45] and also the correlation functional of Lee, Yang, and Parr which includes both local and non-local terms (BLYP).[46,47] These calculations have been carried out in conjunction with the 6-31G* basis set and high accuracy grid, and integrals were used in all DFT calculations.

The molecular geometries were optimized using the Berny analytical gradient optimization routines.[48,49] The strategy followed for obtaining the TS is to start searching directly in the quadratic zone on the energy hypersurface. This method has recently been presented by us.[50] Examination of the TSs has been achieved by the evaluation of the Hessian matrix; the nature of these stationary points was established by calculating analytically and diagonalizing this matrix of energy second derivatives to determine the unique imaginary frequency. Once the stationary points are obtained, the Hessian is recalculated and the zero-order atom dynamics and the

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Table 1. Selected Geometrical Parameters and Net Atomic Charges for the TSs Obtained for the Molecular Model I

	AM1	PM3	HF/3-21G
distances (Å)			
C4−Ht	1.477	1.507	1.413
N6−Ht	1.238	1.257	1.223
C4−N6	2.678	2.716	2.587
C3−C4	1.439	1.444	1.440
N6−C7	1.370	1.375	1.359
C7−C8	1.466	1.440	1.413
bonds angles (deg)			
Ht−C4−N1	108.5	112.6	103.3
H5−C4−N1	158.5	158.3	160.6
C4−Ht−N6	161.1	160.8	158.0
dihedral angles (deg)			
N1−C4−N6−N9	0.0	0.0	5.4
N12−C11−C3−C4	−29.3	−24.7	−25.8
Net atomic charges			
N1	−0.35	0.23	−0.95
C4	−0.12	−0.12	−0.42
Ht	0.15	0.19	0.34
N6	−0.05	0.19	−0.80

transition vector (TV), which yields very concisely the essentials of the chemical process under study,[51] are obtained with a calculation of normal modes.

The development of the theory of kinetic isotope effects (KIE) is usually made within the transition state theory. According to this theory, thermal equilibrium among the molecules of the TS and between them and the reactants is assumed. For KIE calculations, we have considered the potential energy surfaces in those regions near the transition state and the activated precursor complex (APC), as defined in previous papers.[24,50,52] Thus, KIE are obtained from the partition functions of the APC and TS for selected isotopic species. To find them, the geometrical parameters and vibrational frequencies for the geometries of APC and TS are calculated, using molecular partition functions evaluated by means of the ideal gas, rigid rotor, and harmonic approximations. KIE are calculated with a modified Bigeleisen equation[53] used by Klinman:[54]

$$\frac{k}{k'} = \exp\left(\frac{hc}{2kT}\right)\left[\sum_{\mathrm{APC}}^{3N-6}(\omega_i - \omega_i') - \sum_{\mathrm{TS}}^{3N-7}(\omega_i^{\ddagger} - \omega_i^{\ddagger\prime})\right]$$

where the primed quantities correspond to the isotopic species, k and k' are rate constants, ω_i and $\omega_i^{\ddagger}$ in m^{-1} are frequencies of the APC and transition structure, respectively; h, c, and $\boldsymbol{k}$ are Planck's constant, the speed of light in vacuum and Boltzmann's constant, respectively; T is the temperature in Kelvin. This equation has been previously used by us for model systems of lactate dehydrogenase[19b] and formate dehydrogenase.[20]

3. Results and Discussion

In Tables 1 and 2, the geometry and TV of the TS, obtained with AM1, PM3 and HF/3-21G methods, for the molecular model system **I**, are reported. In Tables 3 and 4, the geometry and TV of the TS, obtained with AM1, HF at 3-21G, 4-31G, 6-31G and 6-31G* basis set levels, BP86, and BLYP methods, for the reduced model **II**, are reported. The optimized geometries are available from the authors on request.

The results obtained are weakly dependent on the computing methods and model systems. The distances between the donor and the acceptor centers of both fragments are in the range 2.6−2.8 Å. The forming N6−Ht bond is shorter than the breaking C4−Ht, and the donor−hydride−acceptor bond angle, C4−Ht−N6, is found in the range of *ca.* 160° for the **I** molecular model system and *ca.* 147° for the model **II**. Similar results have been found by us[16,19,20,23] and other authors[14,15,18,55] for related HT steps in other studies of model systems on different enzymes. A schematic view of TS geometries, including the atoms numbering, is given in Figures 2 and 3.

The calculated TSs for both models were found to be in an endo conformation. This result is in agreement with the recent work of Houk *et al.*,[15d] who demonstrated that, from an energetical point of view, the TS prefers an endo rather than an exo conformation, with a bent disposition of the hydrogen in the bridge. If we divide the molecular system into three parts: the donor and acceptor rings and the Ht, the preference of the endo conformation can be understood in terms of the overlap between the highest occupied molecular orbital (HOMO) of these three parts at TS. Due to the fact that the donor and acceptor systems are conjugated, the stability of this conformation reflects a degree of σ aromaticity: the in plane orbitals involved in bond making and breaking are isoconjugate with the π orbitals of both donor and acceptors systems, which would not be present in the exo arrangement. In Figure 4, a representation of the favorable HOMO overlap in TS, obtained at HF/3-21G level, is depicted for model **I**. Similar results were found in the model formed by cyclopropene and azirinium cation mimicking both partners.[56]

The normal mode analysis of TS for the **I** model yields a relatively high imaginary frequency: 1838i and 2042i cm^{-1} for AM1 and PM3, respectively, and 1847i cm^{-1} for HF/3-21G, mainly associated with the breaking/forming bonds. The negative curvature of the quadratic region arises from the negative value of force constant associated with the C4−Ht distance. The atomic displacements in the TV correspond to the hydride transfer step for the reaction catalyzed by GR. The motion corresponds to Ht from C4 to N6, showing large amplitudes not only for the degrees of freedom describing the primary transfer, Ht, but also in the angle controlling the position of secondary hydrogen, H5. This fact suggests that the primary and secondary isotope effects could be strongly coupled. In order to demonstrate this hypothesis, primary, secondary, and primary and secondary kinetic isotope effects (PKIE, SKIE and PSKIE, respectively) have been calculated with the AM1 method for model **I** and with HF/6-31G for model **II**. The results can be compared with available experimental data.

The calculated $k_{H,H}/k_{D,H}$ (primary deuterium KIE) are 4.14 and 4.15, while the $k_{H,H}/k_{T,H}$ (primary tritium KIE) are 7.21 and 7.31, for the model systems **I** and **II**, respectively. These results are in very good agreement with the experimental values (3.99 ± 0.13 and 7.26 ±

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Table 2. Imaginary Frequency (cm^{-1}), Eigenvalue, Force Constants in au (*F*), and the Corresponding Eigenvector (*C*) Associated with the Unique Negative Eigenvalue for the TS of the Molecular Model I

	AM1,[a] 1838.34i, −0.210045		PM3,[a] 2042.89i, −0.303713		HF/3-21G,[a] 1847.63i, −0.246703	
	C	F	C	F	C	F
C4−Ht	0.826	−0.076	0.762	−0.111	0.780	−0.066
C4−N6	−0.329	0.069	−0.474	−0.019	−0.322	0.083
C3−C4	−0.108	1.773	−0.106	1.536	−0.156	1.286
N6−C7	0.196	1.121	0.197	0.883	0.242	0.879
C7−C8	−0.107	0.780	−0.085	0.739	−0.090	0.719
Ht−C4−N1	−0.115	0.432	−0.160	0.309	−0.120	0.491
H5−C4−N1	0.271	0.142	0.224	0.116	0.289	0.176

[a] Method; imaginary frequency, eigenvalues.

Table 3. Selected Geometrical Parameters and Net Atomic Charges for the Transition Structures of the Molecular Model II

	AM1	HF/3-21G	HF/4-31G	HF/6-31G	HF/6-31G*	BP86/6-31G*	BLYP/6-31G*
distances (Å)							
C4−Ht	1.729	1.750	1.739	1.735	1.753	1.727	1.732
N6−Ht	1.125	1.154	1.163	1.166	1.162	1.191	1.198
C4−N6	2.714	2.791	2.782	2.781	2.812	2.800	2.814
C3−C4	1.431	1.432	1.432	1.437	1.433	1.451	1.456
N6−C7	1.402	1.405	1.393	1.394	1.386	1.402	1.407
bonds angles (deg)							
Ht−C4−N1	106.5	100.6	104.1	105.1	103.4	103.4	105.9
H5−C4−N1	163.3	166.1	163.9	163.3	163.8	161.7	159.8
C4−Ht−N6	143.1	147.2	146.1	146.2	148.8	146.7	147.0
net atomic charges							
N1	−0.35	−0.93	−0.91	−0.91	−0.74	−0.54	−0.52
C4	−0.26	−0.36	−0.20	−0.33	−0.33	−0.32	−0.26
Ht	0.20	0.27	0.29	0.29	0.29	0.27	0.24
N6	−0.17	−0.76	−0.75	−0.74	−0.66	−0.51	−0.49

Table 4. Imaginary Frequency (cm^{-1}), Eigenvalue, Force Constants in au (*F*), and the Corresponding Eigenvector Components (C) Associated with the Unique Negative Eigenvalue for the TS of the Molecular Model II

	AM1,[a] 816.94i, −0.034841		HF/3-21G,[a] 1051.72i, −0.069887		HF/4-31G,[a] 1300.03i, −0.100145		HF/6-31G,[a] 1346.83i, −0.106109		HF/6-31G*,[a] 1316.36i, −0.107453		BP86/6-31G*,[a] 710.82i, −0.026097		BLYP/6-31G*,[a] 831.15i, −0.031908	
	C	F	C	F	C	F	C	F	C	F	C	F	C	F
C4−Ht	0.906	0.019	0.870	0.003	0.864	−0.024	0.862	−0.029	0.852	−0.021	0.902	0.048	0.925	−0.006
C4−N6	0.172	0.162	0.043	0.148	−0.103	0.117	−0.125	0.112	−0.101	0.118	0.192	0.128	0.132	0.107
C3−C4	−0.065	0.627	−0.112	0.504	−0.120	0.523	−0.121	0.526	−0.122	0.519	−0.068	0.427	−0.074	0.409
N6−C7	0.073	0.674	0.141	0.494	0.147	0.534	0.147	0.542	0.168	0.547	0.050	0.452	0.060	0.433
Ht−C4−N1	−0.074	1.124	−0.130	1.087	−0.126	1.065	−0.121	1.056	−0.136	0.953	−0.117	0.860	−0.114	0.827
H5−C4−N1	0.239	0.107	0.331	0.129	0.337	0.340	0.340	0.131	0.247	0.124	0.252	0.104	0.257	0.107

[a] Method, imaginary frquency, eigenvalue.

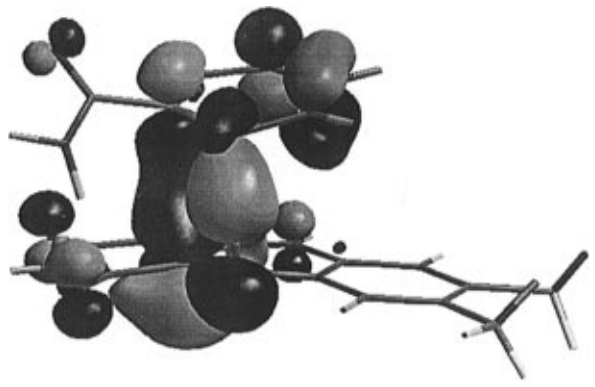

Figure 4. Representation of the favorable HOMO overlap in TS obtained at the HF/3-21G level for the model **I**. The structure is oriented in the same way as for Figure 2. Black and grey lobes represent the two different phases of the HOMO.

0.34 for primary deuterium and tritium KIE, respectively), reported by Vanoni *et al.*,[6b] for the spinach glutatione reductase enzyme when NADH and (4*S*)-[4-^{n}H]NADH were used as substrates; *n* is equal to 2 for deuterium and 3 for tritium KIE. The calculated deuterium SKIE ($k_{H,H}/k_{H,D}$) are 0.90 and 0.98, the calculated tritium SKIE ($k_{H,H}/k_{H,T}$) are 0.83 and 0.95, the calculated deuterium PSKIE ($k_{H,H}/k_{D,D}$) are 3.75 and 4.22, and the calculated tritium PSKIE ($k_{H,H}/k_{T,T}$) are 6.31 and 7.56 for the model systems **I** and **II**, respectively. It should be noted that these results show that the magnitude of isotope effects at multiple positions are no longer independent of one another, for example: SKIE ≠ PSKIE/PKIE[57] either for deuterium or tritium.

Although for the TS of the reduced molecular model **II** the values of the imaginary frequencies are lower: 817i (AM1), 1052i (HF/3-21G), 1300i (HF/4-31G), 1347i (HF/6-31G), 1316i (HF/6-31G*), 711i (BP86/6-31G*), and 831i cm^{-1} (BLYP/6-31G*), the corresponding eigenvector is associated with the same parameters. In this case, the unique negative eigenvalue results from the cross terms in the force constant matrix. The minimal set of coordinates capable of producing the TS is the hydrogen advance and the rehybridization coordinates at both the acceptor, N6, and donor, C4, centers. This result is obtained by an analysis of the TV.

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The difference in the values for the imaginary frequency of the two models can be due to the complex molecular system **I** in which the relative movement of donor and acceptor partners is more restricted than in model **II**. The vectors corresponding to the second and third eigenvalues (below 100 cm^{-1}) for the latter model are associated with the dihedral angle N1−C4−N6−N9 describing the relative displacement of both rings, revealing that this movement is also quite free. This result is calculation method independent, in agreement with the work of Korzeniewski *et al.*[58] showing the utility of semiempirical methods for deriving initial vibrational modes of experimental systems.

There has been much discussion about the effects of the ring puckering on HT process.[15,18,21,59−62] Our calculations show that the rings are quite flexible with small barriers to inversion, in agreement with related theoretical[21,63] and experimental studies.[64,65] The puckering in the TS can be easily achieved from the nearly planar structure during the HT. The two rings are slightly puckered in boatlike conformation in model **II** while in complex model **I** the reduced isoalloxazine ring has lost its planarity due to the fact that the central ring adopts a boat conformation, allowing the lone pairs of the two nitrogen atoms in this ring to be directed toward the oxidized nicotinamide.

The motion between the hydride donor C4 and acceptor N6 is quite free. Distortion away from TS geometry along the axes defined by both centers is accompanied by a small rise in energy. In particular, an elongation of 0.1 Å for this distance carried out in model **I** produces an energy increase of only 0.4 kcal/mol at the HF/3-21G level. The X-ray crystallographic data for GR by Pai and Schulz[13a] show that the intermolecular distance between the C4 of 1,4-dihydronicotinamide and the N6 of the isoalloxazine moiety is 3.5 Å. The fact that the active site imposes strong constraints on the relative orientation and distance of the hydride donor−acceptor centers lets us to carry out a simulation study using the HF/6-31G* level in model **II**: constraining the C4−N6 distance, we can obtain a set of constrained TSs from 2.7 to 3.5 Å, thereby calculating a ridge on the potential energy surface defined by these TSs with a relative energy with respect to the fully optimized TS in the range from 0.2 to 5.9 kcal/mol.

The overall electron density distribution is an essential element in the complete description of the TS for the formal hydride ion transfer, however, the electronic nature of the HT step remains controversial.[66−72] Currently, three possible mechanisms have been proposed and discussed relative to the experimental kinetic information:[66−72] (i) transfer of two electrons and a proton in three separate steps and orders, (ii) electron and hydrogen transfer or its reverse, and (iii) the one-step hydride ion transfer all along the reaction path. Since this issue is hard to settle experimentally,[66−72] theoretical work can help in solving this important problem. Our previous results[16,19,20,23,73] on models of the dehydrogenase enzyme family show that two mechanisms are possible: a hydride ion mechanism and an electron transfer accompanied by a hydrogen atom migration. Within the diabatic approach used in our work,[16a,73] the electron-hydrogen atom mechanism appears to be a plausible path. On the other hand, Bertrán *et al.*[74,75] have carried out an extensive analysis of the hydride transfer in different model systems in terms of valence bond structures to understanding the coupling between the hydrogen motion and the electronic shift along the reaction pathway for HT step.[76]

We have performed a natural population analysis of model **I** at the HF/3-21G level, and the results indicate that the charge transfer is fairly large (0.6 au) and the atomic charge on the Ht being transferred is 0.3 au. Similar results have been obtained by Gready *et al.*[22] These facts indicate that what has previously been called a hydride is more a hydrogen atom than a pure hydride at the TS. The Ht motion and the charge transfer are coupled, acting as a real charge transporter. These data give us a clue related to the nature of the mechanism; because the Ht motion and the charge transfer are coupled, it can be said that an inner sphere electron transfer takes place.[75]

The calculated endo form of TS fits at the active site of GR enzyme without steric hindrances. According to the current view, the value of TS characterization for understanding enzyme catalysis is widely used,[77] and the dominant concept is related to the selective binding to TS[78−81] proposed in the Pauling's seminal works.[78−80] This idea has been used by Lerner, Benkovic, and Schultz[82] to demonstrate experimentally that a practical entry into designed catalysis is provided by antibodies raised against stable TS analogs of chemical reactions. In recent years, based on this approach, a new field has emerged with the expressed aim to select reaction types and substrates.[82−85] In this sense, this is a highly interdis-

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ciplinary field, ranging from the study of biological systems via synthetic chemistry to computational chemistry. In particular, simulation of the functions of the enzymes should provide novel biomimetic methods, and hence useful strategies for organic synthesis can be explored.[86]

4. Final Remarks

Quantum chemical characterization of TSs may be rationalized to discuss enzyme-catalyzed reactions. This study represents a modelization of the molecular mechanism for the HT step in the GR enzyme. The results presented in this paper are obtained by means of the AM1 and PM3 semiempirical procedures, *ab initio* at the HF level with 3-21G, 4-31G, 6-31G, and 6-31G* basis sets and BP86 and BLYP as density functional methods with the 6-31G* basis set. A gas phase study of isolated substrate and cofactors species permits several relative conformations of the reactants, but in the real enzyme, the active site residues in the enzyme would impose directional constraints on the location of the reactants. Therefore, we have used two model systems considering the essential residues in their X-ray crystallographic positions. The results can be summarized as follows:

(i) The obtained endo conformation, with the transferred Ht located in an almost bent arrangement between donor and acceptor centers, associated to the geometry for the TS, results in optimal frontier orbital interaction. As the components of TVs suggested, primary and secondary kinetic isotope effects are strongly coupled. Calculated deuterium and tritium PKIEs are in very good agreement with experimental data.

(ii) Natural population analysis indicates that there is a considerable charge transfer accompanying the Ht motion.

(iii) Our calculations point out that the geometry of TSs for the hydride transfer step in different model systems obtained with different computing methods are transferable and invariant. The reaction partners in the active site are oriented in such a way that they are prepared for the TS along the reaction pathway of the HT step. The above results can be used as a guide to build up putative TSs in hydride transfer reactions. Molecular graphics and computer-assisted simulations are complementary techniques to apply, from the present quantum mechanical calculations, for the study of real biological systems.

(iv) The TS can be docked at the active site of the enzyme without bumping with the molecular walls while the associated reactants or products need important molecular deformation to adapt them at the corresponding active site. The primary function of the enzyme is to trap the reactant by molding it into a geometry resembling as much as possible the one of the TS.

(v) There exists a minimal set of geometrical parameters with a TS which describes the essentials of the chemical interconversion step in a given enzyme mechanism, and the corresponding TV, which contains the fundamental information relating reactive fluctuations patterns, is an invariant feature. For model molecular systems undergoing reactions similar to those catalyzed by enzymes, the characterization of TS on quantum mechanical PES may help disentangle the essential electronic factors. The hypothesis behind this work is that the electronic properties of the minimal molecular model sustaining the TS are robust features in so far as structure and fluctuation issues are concerned; the effects produced by the inclusion of other fragments surrounding the minimal model are not expected to change properties related with the energy derivatives, so that the TS character might be invariant. The comparative analysis of the semiempirical, DFT, and *ab initio* results obtained with two different molecular models prove the geometrical invariance of the fragments. These results were also found in the study of the molecular mechanism for the oxidation of methanol by pyrroloquinoline quinone.[87]

(vi) It seems reasonable to assume that this work should yield reliable results in studies of HT on the GR enzyme. It is also clear that a more complete investigation incorporating the entire enzymatic environment should be conducted to verify our results and to examine the validity of different methods and models. In this respect, solvent effects calculations are in progress and will be reported elsewhere.

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