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Valentin Gogonea
Cleveland State University, V.GOGONEA@csuohio.edu

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Potential Energy Surface of the Reaction of Imidazole With Peroxynitrite: Density Functional Theory Study

VALENTIN GOGONEA

Introduction

Nitric oxide (NO) is a small radical molecule with a complex biochemistry and many implications in cellular activity, such as its role in the regulatory mechanisms of the cardiovascular system, as a neurotransmitter, and in the body's immune-defense system [1]. Understanding the chemistry of NO, the mechanism of NO synthesis, and the reaction of NO with different molecular targets in living cells (e.g., superoxide ion, $O_2^{\cdot -}$) is an essential first step toward controlling its activity.

Peroxynitrite ($ONOO^-$) is one of NO metabolites at physiological pH ($pK_a = 6.8$) and results from the reaction of NO with $O_2^{\cdot -}$ (both species have unpaired electrons) [2]:

In the bloodstream, $ONOO^-$ can rearrange to nitrate (NO_3^-) via peroxynitrous acid [2], or it can react with carbon dioxide or thiols [3]. Peroxynitrite can damage DNA by strand breaking or nitration of guanine. It has been shown experimentally that $ONOO^-$ converts guanine to 8-nitroguanine in solution [4, 5]. Peroxynitrite can also be involved in oxidation of, e.g., amines, sulfides, and alkenes (E° for the half-reaction, $ONOO^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$, is +1.6 V at pH 7) [6, 7].

In addition to the extensive experimental investigation of $ONOO^-$ reactivity, recent publications show a growing interest in understanding the electronic structure, spectroscopic properties, and reactivity of $ONOO^-$, using theoretical tools. The first

Hartree–Fock (HF) self-consistent-field (SCF) calculation on the $ONOO^-$ molecule was reported by Shen et al. [8], who studied the $ONOO^-$ –water complex. In another study, Krauss [9] used the multiconfiguration self-consistent field (MCSCF) Hamiltonian to investigate the rotational barrier and electronic spectra of $ONOO^-$. Later Tsai et al. [10] employed Møller–Plessett second-order perturbation theory (MP2), coupled cluster (CC), and density functional theory (DFT) to calculate the vibrational frequencies of $ONOO^-$ molecule and compared them with the Raman spectra reported earlier [11]. Tsai and coworkers concluded that including electron correlation through high-level ab initio theories (MP2, CC, MCSCF) is essential for an accurate description of $ONOO^-$ electronic structure.

Houk et al. [6, 7] studied the mechanism of amines, sulfides, and alkenes oxidation by peroxynitrous acid using DFT (Becke3–Lee–Yang–Parr (B3LYP) functional [12, 13] with the 6-31G(*d*) basis set). These investigators located transition state structures (activated complexes) for the two-electron oxidation reaction and an activated form of peroxynitrous acid, assumed to be essential for the potent oxidative chemistry of peroxynitrous acid and its derivatives. In addition, they calculated the structure of the CO_2 – $ONOO^-$ adduct and proposed a mechanism for the acceleration of oxidation by $ONOO^-$ in the presence of CO_2 .

More recently, using DFT, Pfeiffer et al. [14] studied various neutral, ionic, and free radical species involved in the $ONOO^-$ chemistry in aqueous solution. Solvation effects were modeled by adding a water molecule to anions and wrapping them in a

polarized dielectric continuum [15]. From these results, standard reaction Gibbs energies were calculated for a series of reactions involved in peroxynitrite chemistry. For example, the standard reaction Gibbs energy, $\Delta G^\circ(\text{aq.})$, for the homolysis of peroxynitrous acid calculated at the G3MP2B3//PCM/B3LYP/cc-pvtz level of ab initio theory is 46.4 kJ mol^{-1} , in good agreement with the experimentally determined value of $56.9 \pm 1.7 \text{ kJ mol}^{-1}$ [16] obtained from experimentally determined standard Gibbs energies of formation ($\Delta_f G^\circ$).

It was shown experimentally that guanine is chemically modified by peroxynitrite through the insertion of a nitro ($-\text{NO}_2$) or nitroso ($-\text{NO}$) group in its five-member ring leading to 8-nitro- or 8-nitrosoguanine [4, 5]. The nitrogen atom of peroxynitrite binds to carbon C_7 of guanine (C_2 of imidazole). Thus, the reaction mechanism of peroxynitrite with imidazole in gas phase and solution was first examined; the potential energy surface (PES) obtained for the latter reaction was then used as a starting point for studying the reaction of peroxynitrite with guanine in gas phase and solution. This strategy was adopted because many of the intermediates and activated complexes (transition state structures) from the reaction of peroxynitrite with guanine may have essentially the same geometry as those found in the reaction of peroxynitrite with imidazole. In this case, the computational time required for completing the investigation of the reaction mechanism of guanine nitration by peroxynitrite would be lowered significantly. To the best of our knowledge, there is no theoretical study that reports on the reaction mechanism of imidazole nitration by ONOO^- . Thus, the present study focuses first on the investigation of the reaction mechanism of imidazole nitration by peroxynitrite in gas phase because this reaction is a good model for guanine nitration by peroxynitrite.

Methods

The PES for the reaction of imidazole with peroxynitrite was first mapped at the HF level of ab initio theory using the 6-31G(*d*) basis set. Next, the PES obtained from HF calculations was used as a starting point for DFT calculations (B3LYP Hamiltonian), which were carried out with double-zeta basis sets ranging from 6-31G(*d*) to 6-31++G(*d,p*), and the triple-zeta basis set 6-311G(*d*). It was found that more than one-half of the stationary points of the HF-derived PES are also stationary points on

the B3LYP-derived PES as well. The nature of the stationary points (minima, transition states, local maxima) was verified by performing a normal mode analysis. All energy minimum structures were confirmed to have zero imaginary frequencies, while the transition state structures (activated complexes) were confirmed to have a single imaginary frequency.

The reaction path from reactants (imidazole and peroxynitrite) to products was constructed by connecting the transition states with the intermediates they led to as follows. First, the transition state structure (activated complex) was slightly distorted along the eigenvector corresponding to the imaginary frequency in both directions. Second, the resulting structures were subsequently geometry optimized, and the optimum-geometry structures obtained were compared with the structures of the intermediates, assumed to be joined by that particular transition state.

Exploratory calculations for the reactants and the rate-limiting activated complex were performed in dielectric continuum (the dielectric constant of water) using the Poisson equation. All calculations (in gas phase and solution) were performed with the quantum chemistry Jaguar program [17].

Results and Discussion

Peroxynitrite chemically modifies imidazole at its carbon atom C_2 (positioned between the two nitrogen atoms). During this reaction, the hydrogen atom bonded to C_2 is extracted by the nitroso oxygen atom ($\text{ON}-$) of peroxynitrite and passed over to the terminal oxygen of peroxy moiety ($-\text{O}-\text{O}$) in what appears to be a proton-hopping transport mechanism.

PES OVERVIEW

Investigation of the PES for imidazole nitration by peroxynitrite at the HF/6-31G(*d*) level of ab initio theory reveals the existence of three reaction intermediates (I_1 , I_4 , and I_5 ; Schemes 1a and 1b; and Table I), and five activated complexes, which correspond to the following transition states: TS_1 (Fig. 1), TS_2 (Fig. 2), TS_5 , TS_7 (Fig. 3), and TS_8 , (see also Schemes 1a and 1b, and Table I). Intermediate I_1 connects TS_2 with TS_5 , I_4 connects TS_5 with TS_7 , and I_5 connects TS_7 with TS_8 . The reaction products found at this level of theory are water and 2-nitroimidazolidine (P_3 ; see Fig. 7). The exploration

TABLE I

Relative energies (with respect to reactants) of products, intermediates, and activated complexes involved in the reaction mechanism of imidazole with peroxyxynitrite.

Species ^a	ΔE^b (kcal mol ⁻¹)	ΔE (ZPE) ^c (kcal mol ⁻¹)	ν^d (cm ⁻¹)	Species ^a	ΔE^b (kcal mol ⁻¹)	ΔE (ZPE) ^c (kcal mol ⁻¹)	ν^d (cm ⁻¹)
I ₁				TS ₂			
HF/6-31G(d)	31.1	32.6		HF/6-31G(d)	101.4	100.4	222 <i>i</i>
B3LYP/6-31G(d)	16.9	19.4		B3LYP/6-31G(d)	86.0	85.7	184 <i>i</i>
B3LYP/6-31+G(d)	20.3	21.9		B3LYP/6-31+G(d)	85.4	84.6	135 <i>i</i>
B3LYP/6-31+G(d,p)	18.3	20.5		B3LYP/6-31+G(d,p)	84.4	84.2	142 <i>i</i>
B3LYP/6-31++G(d,p)	18.0	20.5		B3LYP/6-31++G(d,p)	84.1	84.1	144 <i>i</i>
B3LYP/6-311G(d)	18.7	21.2		B3LYP/6-311G(d)	85.5	85.2	167 <i>i</i>
I ₂				TS ₃			
HF/6-31G(d)	11.9	14.4		B3LYP/6-31G(d)	23.0	24.6	209 <i>i</i>
B3LYP/6-31G(d)	13.4	15.1		B3LYP/6-31+G(d)	26.5	27.2	180 <i>i</i>
B3LYP/6-31+G(d)	12.6	12.9		B3LYP/6-31+G(d,p)	24.4	25.7	180 <i>i</i>
B3LYP/6-31+G(d,p)	10.1	11.0		B3LYP/6-31++G(d,p)	24.1	25.7	184 <i>i</i>
B3LYP/6-31++G(d,p)	9.8	11.1		B3LYP/6-311G(d)	25.0	26.6	193 <i>i</i>
B3LYP/6-311G(d)	13.7	15.1		TS ₄			
P ₁				HF/6-31G(d)	38.7	38.0	1355 <i>i</i>
HF/6-31G(d)	30.7	32.5		B3LYP/6-31G(d)	27.4	25.8	1312 <i>i</i>
B3LYP/6-31G(d)	18.4	19.3		B3LYP/6-31+G(d)	23.5	21.1	1461 <i>i</i>
B3LYP/6-31+G(d)	9.4	9.7		B3LYP/6-31+G(d,p)	20.1	18.4	1355 <i>i</i>
B3LYP/6-31+G(d,p)	7.9	8.8		B3LYP/6-31++G(d,p)	19.8	18.4	1356 <i>i</i>
B3LYP/6-31++G(d,p)	7.5	8.8		B3LYP/6-311G(d)	27.4	25.8	1445 <i>i</i>
B3LYP/6-311G(d)	16.0	17.1		TS ₅			
I ₃				HF/6-31G(d)	38.4	39.5	191 <i>i</i>
B3LYP/6-31G(d)	13.3	15.3		B3LYP/6-31G(d)	14.7	16.3	138 <i>i</i>
B3LYP/6-31+G(d)	16.7	17.7		B3LYP/6-31+G(d)	17.4	18.0	76 <i>i</i>
B3LYP/6-31+G(d)	14.6	16.2		B3LYP/6-31+G(d,p)	15.4	16.6	81 <i>i</i>
B3LYP/6-31++G(d,p)	14.4	16.3		B3LYP/6-31++G(d,p)	15.2	16.7	79 <i>i</i>
B3LYP/6-311G(d)	15.1	17.2		B3LYP/6-311G(d)	16.1	17.6	79 <i>i</i>
I ₄				TS ₆			
HF/6-31G(d)	19.5	22.0		B3LYP/6-31G(d)	20.8	22.1	380 <i>i</i>
B3LYP/6-31G(d)	20.7	22.1		B3LYP/6-31+G(d)	24.0	22.9	880 <i>i</i>
B3LYP/6-31+G(d)	23.1	24.2		TS ₇			
P ₂ (I ₅)				HF/6-31G(d)	29.4	28.7	1418 <i>i</i>
HF/6-31G(d)	26.9	28.8		B3LYP/6-31G(d)	24.3	23.7	827 <i>i</i>
B3LYP/6-31G(d)	22.0	23.5		B3LYP/6-31+G(d)	27.4	26.0	987 <i>i</i>
B3LYP/6-31+G(d)	23.3	23.9		B3LYP/6-31+G(d,p)	25.5	24.6	869 <i>i</i>
B3LYP/6-31+G(d,p)	22.7	23.6		B3LYP/6-31++G(d,p)	25.1	24.5	874 <i>i</i>
B3LYP/6-31++G(d,p)	22.3	23.5		B3LYP/6-311G(d)	27.7	26.8	1131 <i>i</i>
B3LYP/6-311G(d)	22.2	23.7		TS ₈			
P ₃				HF/6-31G(d)	43.4	44.0	704 <i>i</i>
HF/6-31G(d)	-61.2	-60.2		B3LYP/6-31G(d)	25.5	26.7	450 <i>i</i>
B3LYP/6-31G(d)	-58.2	-56.2		B3LYP/6-31+G(d)	26.0	26.4	343 <i>i</i>
B3LYP/6-31+G(d)	-58.5	-57.4		B3LYP/6-31+G(d,p)	25.1	26.0	349 <i>i</i>
B3LYP/6-31+G(d,p)	-60.7	-58.8		B3LYP/6-31++G(d,p)	24.8	25.9	341 <i>i</i>
B3LYP/6-31++G(d,p)	-60.9	-58.6		B3LYP/6-311G(d)	26.9	28.0	440 <i>i</i>
B3LYP/6-311G(d)	-58.8	-56.7					
TS ₁							
HF/6-31G(d)	0.3	0.4	47 <i>i</i>				
B3LYP/6-31G(d)	0.5	0.9	75 <i>i</i>				

^a Product (P), intermediate (I), transition state structure (TS).

^b The difference between the total energy in vacuum of a certain PES species (P, I, TS) and the reactant (R).

^c The difference in energy includes zero point energy (ZPE) correction.

^d Imaginary frequencies.

FIGURE 1. Geometry of the low-lying transition state TS_1 that connects reactants states (distances given in Å). Imidazole and peroxydinitrite are in the same plane (C_s symmetry), and the terminal O of peroxy group makes a hydrogen bond with the hydrogen atom bonded to N_1 of the imidazole molecule. Levels of theoretical calculations: (a) HF/6-31G(d), (b) B3LYP/6-31++G(d,p), (c) B3LYP/6-311G(d). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of PES using DFT: B3LYP Hamiltonian with the largest basis sets employed in this study, double zeta 6-31++G(d,p) and triple zeta 6-311G(d), produced three reaction intermediates (I_1 , I_2 , and I_3 ; Schemes 1a and 1b; Table I, Fig. 3) and seven activated complexes (which correspond to the following transition states: TS_1 , TS_2 , TS_3 , TS_4 , TS_5 , TS_7 , and TS_8 , Schemes 1a and 1b, Table I, Figs. 1, 2, and 5). The reaction products found at this level of theory are either (a) hydroxide ion and 2-nitroimidazole (P_1 ; Fig. 4), (b) hydrogen superoxide ion (HOO^-) and 2-nitrosoimidazole (P_2 ; Fig. 6), and water and 2-nitroimidazolidine (P_3 ; Fig. 7). The reaction intermediate I_5 found at HF/6-31G(d) corresponds to reaction products P_2 at the DFT level. DFT calculations with smaller basis sets [6-31G(d), 6-31++G(d,p)] produced an additional activated complex (TS_6) corresponding to a transition state for transferring a proton between the terminal oxygen of $-O-O$ moiety and N_1 of imidazole (data not shown). The quantities presented and discussed in this section are those calculated at the B3LYP/6-31++G(d,p) level of DFT theory, unless another level is specified.

TRANSITION STATE TS_1

Both imidazole and peroxydinitrite are planar molecules. Initially, the peroxydinitrite molecule is posi-

tioned in such a way that the terminal oxygen of its peroxy moiety ($-O-O$) points toward the hydrogen atom bonded to N_1 of imidazole (1.472 Å). While in "reactants state" (R), peroxydinitrite and imidazole molecules are not in the same plane. The nitration reaction starts after peroxydinitrite and imidazole molecules arrange themselves in the same plane (the reactant arrangement has C_s symmetry; Fig. 1). In this arrangement, the hydrogen bond between the hydrogen atom bonded to N_1 and the terminal oxygen of $-O-O$ group increases from 1.472 to 1.598 Å, while the distance between C_2 and peroxydinitrite nitrogen shortens to 3.447 Å (Fig. 1). This arrangement corresponds to a low-lying transition state [0.9 kcal/mol above the "reactant state" (R) with an imaginary frequency of 75 cm^{-1} calculated at B3LYP/6-31G(d)]. Because this transition state was found at both the HF/6-31G(d) and

FIGURE 2. Geometry of the transition state TS_2 (distances given in Å). This transition state corresponds to the rate-determining step of the reaction and connects TS_1 with the first reaction intermediate (I_1). The hydrogen atom formerly bonded to C_2 of imidazole is now bonded to the nitroso oxygen of peroxydinitrite. Levels of theoretical calculations: (a) HF/6-31G(d), (b) B3LYP/6-31++G(d,p), (c) B3LYP/6-311G(d). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIGURE 3. Geometry reaction intermediate: I_2 (distances given in Å). This intermediate is formed from the activated complex TS_3 . At DFT level of calculation, a hydroxide ion separates, leaving an O-protonated 2-nitroimidazole intermediate that quickly rearranges to 2-nitroimidazole and eliminates a hydroxide ion. Levels of theoretical calculations: (a) HF/6-31G(*d*), (b) B3LYP/6-31++G(*d,p*), (c) B3LYP/6-311G(*d*). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

B3LYP/6-31G(*d*) levels of calculation, it was not examined whether it exists on the PES obtained with a larger basis set. TS_1 connects to reactant states corresponding to the peroxyxynitrite molecule symmetrically placed above and below the plane of the imidazole molecule.

TRANSITION STATE TS_2

The first step in the reaction is the transfer of a hydrogen atom from C_2 to the nitroso oxygen of peroxyxynitrite (ON—) coupled with the formation of a covalent bond between C_2 and N (of peroxyxynitrite). The structure and relevant bond lengths of the activated complex corresponding to TS_2 are shown in Figure 2. We were unable to locate a transition state for transferring the hydrogen atom from C_2 to N (of ONOO[−]); thus, it appears that this

transfer occurs simultaneous to the formation of the bond between C_2 and N. In the activated complex corresponding to TS_2 (Fig. 2), the hydrogen atom is already transferred to the nitroso oxygen (O—H: 0.985 Å and C_2 —H: 2.154 Å), N is at 2.243 Å far from C_2 , and the hydrogen bond between H(—N₁) and the terminal oxygen of —O—O group disappears (3.30 Å). TS_2 is the transition state for the rate-determining step of this reaction (Fig. 8). The activation energy is 101.4 kcal/mol [calculated at HF/6-31G(*d*) and 84.1 kcal/mol at B3LYP/6-31++G(*d,p*)], which is much higher than what an enzymatic reaction usually can afford. The calculated activation energy varies little with the quality of the basis set used (Table I). TS_2 connects TS_1 with the first reaction intermediate on the reaction path (I_1 , Scheme 1, Table I, Fig. 8). In I_1 , peroxyxynitrite nitrogen is bonded to C_2 (1.441 Å). HF and DFT give different structures for I_1 (Scheme 2). In the DFT structure, the hydrogen atom originally connected to N₁ is now bonded to the terminal oxygen of —O—O group, while in the HF structure this

FIGURE 4. Geometry for reaction products: hydroxide ion and 2-nitroimidazole (P_1 , distances given in Å). These products are formed from the activated complex TS_4 after a proton is transferred from the nitroso oxygen of peroxyxynitrite to N₃ of imidazole. Levels of theoretical calculations: (a) HF/6-31G(*d*), (b) B3LYP/6-31++G(*d,p*), (c) B3LYP/6-311G(*d*). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIGURE 5. Geometry of the transition state TS_7 (distances given in Å). This transition state involves an intramolecular proton transfer between two oxygen atoms of peroxyxynitrite (the nitroso oxygen and the terminal oxygen atom of $-O-O$ group). On HF-PES, TS_7 connects I_4 and I_5 , and on DFT-PES it connects I_3 and P_2 . Levels of theoretical calculations: (a) HF/6-31G(d), (b) B3LYP/6-31++G(d,p), (c) B3LYP/6-311G(d). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

hydrogen atom is still bonded to N_1 . We were unable to find a transition state structure for transferring this hydrogen atom from N_1 to O in this stage of the reaction. I_1 is located 20.5 kcal/mol above the reactant state (Table I).

TRANSITION STATES TS_3 , TS_4 , AND TS_5

I_1 intermediate has enough energy (~ 20 kcal mol^{-1} more than the reactants) to proceed with a series of conformational changes that lead to another intermediate on HF-PES (I_4) and two intermediates (I_2 and I_3) and products hydroxide ion and nitroimidazole (P_1) on DFT-PES. I_1 and I_2 are joined by TS_3 , I_2 and P_1 are joined by TS_4 (Scheme 1a), and I_2 and I_3 are joined by TS_5 (Scheme 1b). On HF-PES, I_1 and I_4 are joined by TS_5 ; TS_3 does not exist on HF-PES (Fig. 8). TS_3 is 5 kcal mol^{-1} above I_1 , and its structure is very similar to I_1 and I_2 (data not shown). The C_2-N bond is slightly smaller in

TS_3 (1.424 Å) and I_2 (1.407 Å) than in I_1 (1.441 Å), and the $C_2-N-O-O$ dihedral angle changes from 80° (I_1) to 30° (TS_3) and -73° (I_2). I_2 is 9.4 kcal mol^{-1} more stable than I_1 (Table I). From I_2 , the reaction path bifurcates, leading to two transition states: TS_4 and TS_5 . TS_4 on HF- and DFT-PES is 26.8 and 10 kcal mol^{-1} above I_2 , respectively (Table I). For simplicity, TS_4 and P_1 on HF-PES are not shown in Figure 8. Products P_1 are 30.7 (HF-PES) and 7.5 kcal mol^{-1} (DFT-PES) above reactants. TS_5 on DFT-PES is 5.6 kcal mol^{-1} above I_2 , and its structure is similar to I_2 and I_3 (Scheme 1b). The main geometric aspect that distinguishes them is the N_1-C_2-N-O dihedral angle (O is the nitroso oxygen): -173° (I_2), -130° (TS_5), and -77° (I_3). The intermediate I_3 is 5.2 kcal mol^{-1} less stable than I_2 (Table I). On DFT-PES, TS_5 is about one-half the height of TS_4 ; thus, the gas phase reaction proceeds predominantly following the path through TS_5 , which leads to products P_2 and P_3 . The transition states TS_5 on HF-PES is mainly distinguished from the one found on DFT-PES by the fact that the hydrogen atom originally connected to N_1 is still bonded to N_1 , while in TS_5 found on DFT-PES, this hydrogen atom is now connected to the terminal

FIGURE 6. Geometry for reaction products: hydrogen superoxide ion (HOO^-) and 2-nitrosoimidazole (P_2). This structure is an intermediate (I_5) on the HF-PES. Levels of theoretical calculations: (a) HF/6-31G(d), (b) B3LYP/6-31++G(d,p), (c) B3LYP/6-311G(d). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIGURE 7. Geometry for reaction products: water and 2-nitrosoimidazole (P_3). Levels of theoretical calculations: (a) HF/6-31G(*d*), (b) B3LYP/6-31++G(*d,p*), (c) B3LYP/6-311G(*d*). Products P_3 are the most stable species on the potential energy surface (at both HF and DFT levels) for the gas phase reaction. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

oxygen atom of the —O—O group. The intermediate I_4 (HF-PES) is very similar to I_3 (DFT-PES), with the exception of the same hydrogen atom originally bonded to N_1 . This hydrogen atom is bonded to the terminal oxygen atom of the —O—O group in I_3 and to N_1 in I_4 . Calculations performed at the DFT level with smaller basis sets [6-31G(*d*) and 6-31+G(*d*)] also produced a transition state for transferring the proton back from the terminal oxygen of —O—O group to N_1 (TS_6 , Table I). This transition state was not located with larger basis sets, so it was not included in Scheme 1 and Figure 8.

TRANSITION STATE TS_7

The next step in the reaction following the path going through TS_5 is the transfer of the hydrogen atom (originally bonded to C_2) from the nitroso oxygen to the terminal oxygen of —O—O group (Fig. 5). In the activated complex corresponding to TS_7 , the hydrogen atom originally bonded to C_2 is now equally distanced from both the nitroso oxygen and the terminal oxygen of —O—O group (O—H: 1.227 Å and 1.218 Å, respectively). The hydrogen atom originally bonded to N_1 (which is

bonded to the terminal oxygen of —O—O group in I_3) is now bonded to N_1 in TS_7 . Again, we were unable to locate a transition state (TS_6) for transferring this proton back (from the terminal O to N_1) when larger basis sets were used at the DFT level. This transition state (TS_6) may exist, but its electronic structure may be unusual, which could explain the convergence difficulties observed with larger basis sets. The transition state TS_7 is 8.2 kcal mol⁻¹ above I_3 (Table I). On HF-PES, TS_7 joins I_4 with I_5 . In I_5 , the transferred hydrogen atom makes a hydrogen bond with the nitroso oxygen [1.634 Å at HF/6-31G(*d*)] and the —OOH group is still part of the peroxyimide moiety (ON—O: 1.437 Å at HF/6-31G(*d*)). At DFT level this —N—O— bond is elongated (1.851 Å), suggesting the formation of two reaction products at this stage: 2-nitrosoimidazole and hydrogen superoxide ion (P_2 , Fig. 5). The O—O bond is 1.409 Å in I_5 (HF-PES) and 1.450 Å in P_2 (DFT-PES), which shows that the negative charge begins to localize on the —OOH moiety and, due to electron–electron repulsion, the O—O bond length begins to increase. The 2-nitrosoimidazole/hydrogen superoxide ion adduct is 7.2 kcal mol⁻¹ less stable than I_3 (Table I).

FIGURE 8. Potential energy surface for imidazole nitration by peroxyimide in gas phase. Complete reaction pathways from reactants **R** to different products (P_1 , P_2 , and P_3) found at different levels of quantum mechanical calculations. Levels of theoretical calculations: (a) ■■■■■■, HF/6-31G(*d*), (b) ■■■■■■, B3LYP/6-31++G(*d,p*), (c) ■■■■■■, B3LYP/6-311G(*d*). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

SCHEME 1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TRANSITION STATE TS_8

Transition state TS_8 leads to the formation of the reaction products: water and 2-nitroimidazole (P_3 , Fig. 7). In the activated complex corresponding to TS_8 , the hydrogen atom bonded to the terminal oxygen of the peroxy group forms a hydrogen bond with the nitroso oxygen [1.722 Å at B3LYP/6-31++G(*d,p*) and 2.448 Å at HF/6-31G(*d*)], and the hydrogen atom bonded to N_1 makes a hydrogen bond with the terminal oxygen of the peroxy group

[2.075 Å at B3LYP/6-31++G(*d,p*) and 1.960 Å at HF/6-31G(*d*)]. The O—O bond length in TS_8 is 1.525 Å at B3LYP/6-31++G(*d,p*) and 1.712 Å at HF/6-31G(*d*). This bond breaks while water is formed from the HO group and the hydrogen atom bonded to N_1 . On the DFT-PES, TS_8 is 2.4 kcal mol⁻¹ above P_2 (Table I). Products P_3 are 58.6 kcal mol⁻¹ more stable than the reactants (R), and 82.1 kcal mol⁻¹ more stable than P_2 . Products P_2 can still form in substantial amount if hydrogen superoxide ion can separate quickly from 2-nitrosoimidazole. The experimental data confirm that a nitroso product is obtained during guanine nitration [4, 5]. Figure 7 shows the products P_3 completely formed. Both have C_{2v} symmetry, as expected.

SCHEME 2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

REACTION IN SOLUTION

The puzzling high activation energy barrier for the reaction of peroxynitrite with imidazole in vacuum raises the question of the role of solvation, or of the adjacent six-member ring of guanine (through resonance effects), or both, in lowering the activation energy of the reaction of guanine with peroxynitrite. To elucidate the role of solvation and the six-member ring of guanine on the activation energy, exploratory calculations were performed for the reaction of 9-methylguanine with peroxyni-

TABLE II

Relative energies (with respect to reactants) of the activated complexes with the highest activation energy (TS_2), involved in the reaction of imidazole and 9-methylguanine with peroxynitrite in solution.

Species	ΔE^a (kcal mol ⁻¹)	ΔE (ZPE) ^b (kcal mol ⁻¹)	ΔG_s^c (kcal mol ⁻¹)	ν^d (cm ⁻¹)
Imidazole				
B3LYP/6-31+G(d,p)	95.6	94.6	11.3	215i
9-Methylguanine				
B3LYP/6-31G(d) ^e	69.9	68.7	—	132i
B3LYP/6-31G(d)	83.4	81.3	14.3	185i

^a The difference in total energy in solution between the activated complex (TS_2) and the reactant (R).

^b The difference in total energy includes zero point energy (ZPE) correction.

^c The difference in the solvation free energy between TS_2 and R .

^d Imaginary frequencies.

^e In vacuum.

trite in vacuum and the reaction of imidazole and 9-methylguanine with peroxynitrite in the dielectric continuum (using the dielectric constant of water). Because of the complexity of the PES for the reaction of imidazole with peroxynitrite in vacuum, these exploratory calculations were limited to reactants (R) and the rate-limiting activated complex (TS_2). The calculations of R and TS_2 for the reaction of imidazole with peroxynitrite in solution were carried out at the B3LYP/6-31+G(d,p) level of DFT. Surprisingly, these calculations (Table II) show that the activation energy of the rate-limiting step (94.6 kcal mol⁻¹) of the imidazole nitration in solution is 10.4 kcal mol⁻¹ higher than in the gas phase (Table I). The solvation free energy (ΔG_s) of R is 11.3 kcal mol⁻¹, larger than ΔG_s of TS_2 (Table II). Table III shows the distribution of electric charge on the imidazole and peroxynitrite moieties for R and TS_2 in gas phase and solution. The electrostatic potential (ESP)-derived charges calculated by Jaguar, are those introduced by Breneman and Wiberg [18]. In R , 85% of the -1 e.u. electric charge of the system is located on the peroxynitrite moiety when the reaction occurs in the gas phase (Table III), and 96% when the reaction occurs in solution. In TS_2 57% of the -1 e.u. electric charge is located on the peroxynitrite moiety when the reaction occurs in gas phase, and 50% when the reaction occurs in solution. Because the charge in TS_2 is evenly distributed between the imidazole and peroxynitrite moieties, the activated complex is destabilized with respect to the reactant (11.3 kcal mol⁻¹) when the reaction is performed in solution. We tried to use an explicit water molecule as a bridge between imidazole and peroxynitrite to facilitate the proton transfer from

C_2 carbon of imidazole to peroxynitrite oxygen, but we were unsuccessful in locating a transition state or intermediate. The water molecule pushes the reactants away, and the hydrogen atom bound to C_2 carbon is not acidic enough to form a hydrogen bond with the water oxygen and thus to initiate a proton transfer from imidazole to peroxynitrite. In addition, it seems that the peroxynitrite nitrogen must be in close proximity to C_2 carbon (<3 Å) for the nitration to proceed.

Table II shows that in the reaction of 9-methylguanine with peroxynitrite, the adjacent fused six-

TABLE III

Comparison of the partial charge distribution in the activated complex, TS_2 , and the reactant (R) for the reaction of imidazole and 9-methylguanine with peroxynitrite in vacuum and solution.

Species	Charges ^a			
	ONOO ^{-b}	Imidazole ^b	ONOO ^{-c}	Me-guanine ^c
Vacuum				
R	-0.8477	-0.1523	-0.8138	-0.1862
TS_2^d	-0.5700	-0.4300	-0.3524	-0.6476
Solution				
R	-0.9576	-0.0424	-1.4643	0.4643
TS_2^d	-0.5020	-.4980	-0.4031	-0.5969

^a The partial atomic charges (in e.u.) are summed up for peroxynitrite and the imidazole/guanine, respectively.

^b Calculated at B3LYP/6-31+G(d) level.

^c Calculated at B3LYP/6-31G(d) level.

^d The hydrogen, atom initially bound to C_2 carbon in R , is bound to the single oxygen of peroxynitrite in TS_2 . Consequently, the partial charge of this hydrogen atom is counted to peroxynitrite in TS_2 .

member ring of guanine does have a favorable effect on the rate-limiting step of nitration by lowering the activation energy ($68.7 \text{ kcal mol}^{-1}$) with $15.5 \text{ kcal mol}^{-1}$ [B3LYP/6-31-G(d)], with respect to that of imidazole reaction in gas phase. However, the activation energy for the reaction of 9-methylguanine with peroxynitrite in solution ($81.3 \text{ kcal mol}^{-1}$) is higher than the gas phase activation energy by $12.6 \text{ kcal mol}^{-1}$. As in the case of imidazole nitration in solution (Table III), the activated complex is destabilized with respect to reactants ($14.3 \text{ kcal mol}^{-1}$) when 9-methylguanine reacts with peroxynitrite in solution.

The discrepancy between the calculated activation energy for the reaction of guanine with peroxynitrite in solution and the ease with which this reaction occurs experimentally raises the possibility that proton tunneling may be the cause for the low activation energy inferred from experiment. The probability of proton tunneling is directly related to the thickness of the activation energy barrier, which in turn can be modulated by the structural details of the reactants (e.g., substituents on the imidazole ring and the adjacent six-member ring of guanine).

Conclusions

Peroxynitrite chemically modifies imidazole at the C_2 carbon atom. During the reaction, the hydrogen atom bonded to C_2 is extracted by the nitroso oxygen atom of peroxynitrite and is passed over to the terminal oxygen of peroxy moiety ($-\text{O}-\text{O}$) of peroxynitrite in what appears to be a proton-hopping transport mechanism.

The investigation of the mechanism for this reaction in vacuum using quantum mechanical calculations reveals a complex PES [at B3LYP/6-31++G(d,p)] with three intermediates, seven transition states, and six possible reaction products: (i) hydroxide ion and 2-nitroimidazole, (ii) hydrogen superoxide ion and 2-nitrosoimidazole, and (iii) water and 2-nitroimidazolidine. The most stable products obtained in the gas phase reaction are water and 2-nitroimidazolidine (P_3), which are $60.9 \text{ kcal mol}^{-1}$ more stable than the reactants. Hydrogen superoxide ion and 2-nitrosoimidazole (P_2) are $22.3 \text{ kcal mol}^{-1}$, and hydroxide ion and 2-nitroimidazole (P_1) are $7.5 \text{ kcal mol}^{-1}$ less stable than the reactants. P_2 can still be produced in a substantial amount if these products separate fast and the recombination probability decreases. The transition

state (TS_4) leading to P_1 is about twice as high as TS_5 , which gives a ratio of P_3 to P_1 concentrations of about 7:1 (without considering P_2). The transition state corresponding to the rate-determining step (TS_2) is $84.1 \text{ kcal mol}^{-1}$ above the reactant state (R), which makes the activation energy too high for an enzymatic reaction. Exploratory calculations performed for the reaction of imidazole with peroxynitrite in solution (dielectric continuum), on the one hand, and for the reaction of 9-methylguanine with peroxynitrite in gas phase and solution, on the other, show no significant decrease in the activation energy due to resonance effects of the adjacent fused six-member ring of guanine or solvation. In solution, the reactants are stabilized with respect to the activated complex because of the pronounced charge separation between the imidazole (guanine) and peroxynitrite moieties, which increases the activation energy with respect to the gas phase reaction. These unexpected results lead to the speculation that proton tunneling may be at the origin of the low activation energy observed experimentally for the reaction of guanine with peroxynitrite. Peroxynitrite is one of NO metabolites at physiological pH (resulting from its reaction with O_2^-) that is assumed to play a role in DNA damage. Understanding the reaction mechanism of guanine nitration by peroxynitrite (one of the chemical pathways for damaging DNA in cells), will help in elucidating the biological role of this extremely reactive molecule.

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