

## Research Article

# On the Importance of Water Molecules in the Theoretical Study of Polyphenols Reactivity toward Superoxide Anion

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Received 9 June 2014; Accepted 24 July 2014; Published 14 September 2014

Academic Editor: John R. Sabin

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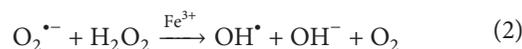
Numerous studies have shown the benefits of a diet rich in fruits and vegetables. These benefits are partly due to the radical scavenging properties of polyphenols contained in fruits and vegetables since polyphenols can fight against an excess of radicals which goes along inflammation in a certain number of diseases. This pathological state, called oxidative stress, results from the aerobic condition of human organism when OH radical, hydrogen peroxide, superoxide anion, or peroxynitrite is produced in excess. If hydrogen peroxide is easily handled by human defense against radicals, the other radicals can cause damage to biological constituents like lipids, cell membranes, and other biomolecules. This paper is devoted to the theoretical study of the interaction of superoxide anion ( $O_2^{\bullet-}$ ) with a very potent radical scavenger, 1,2,4,6,8-pentahydroxynaphthalene. The importance of hydration of superoxide radical for the reactivity is analyzed. Potential energy surfaces (PES) are calculated for different number of water molecules around the radical and it is shown that the transition barrier vanishes when complete hydration with six water molecules is explicitly handled. The nature of the reactivity is determined by using the natural bond orbital (NBO) analysis.

## 1. Introduction

The production of radicals in the body is a consequence of the aerobic metabolism of the organism [1]. Free radical reactive oxygen species (ROS) can be produced during mitochondrial dysfunction or in pathophysiological conditions. Superoxide anion is one of the most important and biologically relevant ROS radicals in living organisms. It is formed from one-electron reduction of oxygen. It is much less reactive and much more selective than hydroxyl radical which reacts with most biomolecules at a nearly diffusion-controlled rate ( $10^{10} M^{-1}s^{-1}$ ). The lifetime of superoxide in biological systems is typically a few seconds. It can react with another superoxide anion to give hydrogen peroxide or with nitric oxide to form a very potent oxidant, peroxynitrite. Superoxide anion is also produced by an enzyme, NADPH, in phagocytes to kill invading pathogens. Although  $O_2^{\bullet-}$  is not a strong microbicidal [1], it is essential for bacterial killing. It is a source of hydrogen peroxide,  $H_2O_2$ , which in presence of released iron could form hydroxyl radical via the Fenton mechanism:



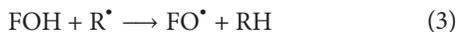
or the Haber-Weiss mechanism:



There are several pathological cases where overproduction of superoxide leads to tissue damage, in particular in ischemic episodes [2]. The deprivation of oxygen, a consequence of severe restriction in blood flow, leads to the conversion of xanthine dehydrogenase, an enzyme which participates in the degradation of purines, in another form of enzyme: xanthine oxidase. During the degradation of xanthine in uric acid, xanthine dehydrogenase uses a cofactor, NADP<sup>+</sup>, as an electron acceptor. In xanthine oxidase, some sulphur bridges are broken and the cavity where NADPH should react is partly obstructed. Only small molecules like oxygen can enter and capture electrons to form superoxide and hydrogen peroxide. During the reperfusion after an ischemic episode, xanthine oxidase transforms oxygen into superoxide, which leads to oxidative stress and tissues damage. One way to protect against this damage is to use scavengers of peroxide and inhibitors of the enzyme.

Oxidative stress is also the consequence of depletion of dietary antioxidants (vitamins A, C, and D, flavonoids, and

carotenoids) and micronutrients indispensable to the proper functioning of antioxidant enzymes. A lot of studies have tried to characterize the antioxidant properties of natural compounds. The flavonoid family has been particularly investigated. These compounds can scavenge radicals either by hydrogen atom transfer or by charge transfer:



In the first case, the reactivity is governed by the OH bond dissociation enthalpy (BDE). If the flavonoid BDE is lower than the BDE of RH, the reaction is favored.

In the second case, the thermodynamic parameter which describes the reaction is the electron transfer enthalpy (ETE) [3].

The calculated BDE of flavonoids correlates very well with the inhibition of low-density lipoprotein oxidation indicating that lipid peroxide scavenging [4] is done by hydrogen atom transfer with no barrier. On the contrary, superoxide anion scavenging by these natural molecules seems more complex. Experimental results [5–8] sometimes give opposite results even in nonenzymatic assays where there is no competition with enzyme inhibition. Theoretical calculations can be of some help in the study of the conditions of reactivity. Dhaouadi et al. [9] have performed a DFT study of the reaction of quercetin with superoxide radical. Their calculations were done in gas phase. The reaction had no barrier but was characteristic of a proton transfer instead of hydrogen atom transfer as expected. Due to the anionic character of superoxide radical, one may question the importance of superoxide hydration in the reactivity. This is the purpose of this paper: studying the influence of explicit hydration on the reactivity of polyphenols with superoxide anion. In order to simplify the study, the reaction has been analyzed with one of the best known radical scavengers: 1,2,4,6,8-pentahydroxynaphthalene (PNH). This compound has been designed [10] for its very low BDE of 57 kcal/mol (vitamin C: 68 kcal/mol, quercetin: 72 kcal/mol). It is a weak acid: the deprotonated form is preponderant at physiological pH. The study will be limited to the reaction at the most labile hydroxyl group of PNH. It is divided into two parts: first, the electronic and free energies of reactants and products are calculated with an increasing number of water molecules around superoxide radical. The reaction consists in a stretching of the OO bond of superoxide and a large increase of the OH bond length of the hydroxyl group. To characterize the reaction, potential energy surfaces (PES) have been constructed in function of these two coordinates. Then, in a second part, analysis of natural bond orbitals tries to give a better insight into the conditions of spontaneous reactions.

## 2. Methods

The attack of superoxide anion on 1,2,4,6,8-pentahydroxynaphthalene (PHN) has been studied only on the most labile hydrogen on position 4 [10] by modifying the solvation of

superoxide anion with explicit water molecules. In addition, the effect of the bulk solvent has been taken into account through the field polarized continuum model (PCM) which mimics the bulk solvent by creating a solute cavity via a set of overlapping spheres [11, 12]. The importance of the water molecules surrounding superoxide anion has been studied by varying their number and positions. It was impossible to investigate all the possible configurations for the water molecules. Two different configurations among the most stable ones have been chosen in order to determine the importance of geometry of the reactants.

This study has been carried out by using Gaussian 09 package [13] within density functional theory. Two functionals have been tested, cam-B3lyp, developed by Yanai et al. [14], which contains long range corrections, and wB97XD [15], which includes empirical dispersion. cam-B3lyp has the advantage to lead to a better localization of the charges, which can avoid artifacts in calculations with superoxide anions. In the reactivity studied in this paper, the two reactants have a negative charge and there is no possibility of a charge transfer artifact. This is why it has been possible to test also the wB97XD functional to evaluate the possible dispersion effects. All the calculations have been performed by using the 6-311+G(d,p) basis set.

Harmonic vibrational frequencies of reactants and products were calculated with the same functional and basis set in order to verify that they are true minima.

The potential energy surfaces (PES) describing the reactivity of 1,2,4,6,8-pentahydroxynaphthalene with superoxide anion solvated by a certain number of water molecules have been obtained by calculating the electronic energy of the adducts in function of the two coordinates involved in the reaction: the OH bond length of the hydroxyl bond and the OO bond length of superoxide anion. The electronic energies have been obtained by optimizing the geometry with these two fixed coordinates. The OO bond was varied from 1.31 Å (or 1.32 Å depending on adduct initial geometry) to 1.47 Å by step of 0.03 Å. The OH bond was varied from 0.99 Å to 1.8 Å by step of 0.1 Å. Near the transition state, the steps were lowered to 0.05 Å.

In order to characterize precisely the transition state, the different PES have been parameterized with the Surfer software [16] by using the local polynomial gridding method with polynomial of order 3.

## 3. Results

*3.1. Effect of Explicit Hydration of Superoxide Anion on the Barrier to Reaction.* Table 1 displays the electronic energies differences between the reactants and the transition states or the products. Even if there are some discrepancies between DFT functional results or water molecules conformations, a general trend emerges: the addition of water molecules stabilizes the products and decreases the barrier to reaction. With six or more molecules there is no more barrier, the reaction is barrierless.

*No Water Molecule.* Figure 1 displays the geometry of reactants and products with no water molecule. The conformation

TABLE 1: Electronic energies differences between the reactants and transition state or products. For two to four water molecules, two conformations have been tested. The free energies are given relative to the most stable reactants.

	Number of water molecules	Transition state	Products	Relative reactants free energy	Relative products free energy
<b>0</b>	in cam-B3lyp	15 kcal/mol	-4 kcal/mol		-3.4 kcal/mol
	in wB97XD	15 kcal/mol	-4 kcal/mol		-3.1 kcal/mol
<b>1</b>	in cam-B3lyp	8 kcal/mol	-9 kcal/mol	0.7 kcal/mol	-8.7 kcal/mol
		4 kcal/mol	-10 kcal/mol		-10 kcal/mol
	in wB97XD	8 kcal/mol	-9 kcal/mol	0.6 kcal/mol	-8.1 kcal/mol
		6 kcal/mol	-9 kcal/mol		-8.8 kcal/mol
<b>2</b>	in cam-B3lyp	+8 kcal/mol	-17 kcal/mol	1.8 kcal/mol	-11.2 kcal/mol
		+4 kcal/mol	-14 kcal/mol		-13.9 kcal/mol
	in wB97XD	+9 kcal/mol	-16 kcal/mol	1.1 kcal/mol	-13.2 kcal/mol
		+7 kcal/mol	-11 kcal/mol		-14.4 kcal/mol
<b>3</b>	in cam-B3lyp	+5 kcal/mol	-17 kcal/mol	1.9 kcal/mol	-13.5 kcal/mol
		+6 kcal/mol	-17 kcal/mol		-12.8 kcal/mol
	in wB97XD	+9 kcal/mol	-16 kcal/mol	0.5 kcal/mol	-14.7 kcal/mol
		+9 kcal/mol	-19 kcal/mol		-15. kcal/mol
<b>4</b>	in cam-B3lyp	+1.6 kcal/mol	-21 kcal/mol	0.6 kcal/mol	-18.1 kcal/mol
		+4 kcal/mol	-21 kcal/mol		-17.2 kcal/mol
	in wB97XD	+3 kcal/mol	-21 kcal/mol	1 kcal/mol	-17.4 kcal/mol
		+9 kcal/mol	-21 kcal/mol		-17.6 kcal/mol
<b>6</b>	in cam-B3lyp	No barrier	-24 kcal/mol		
	in wB97XD		-20 kcal/mol		
			-23 kcal/mol		
			-21 kcal/mol		

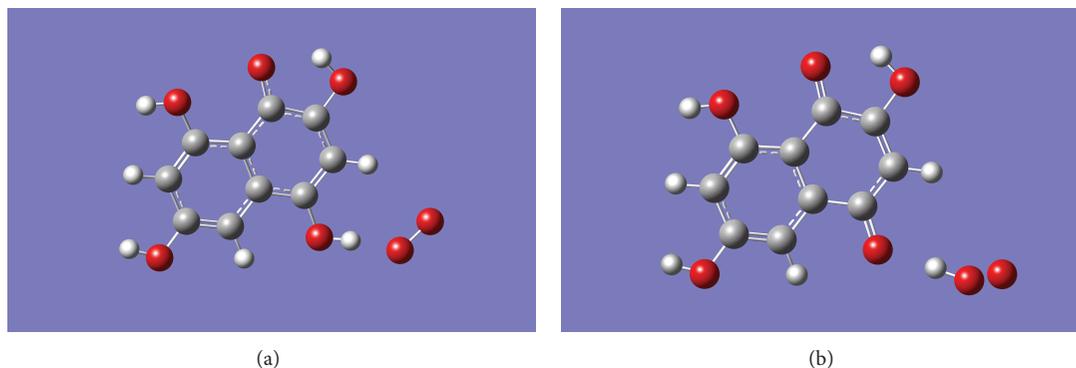


FIGURE 1: Optimized geometry of reactants (a) and products (b) with no explicit water molecules.

of PNH depicted in Figure 1 corresponds to the first step of acid-base proton exchange with water. This conformation may be followed by a rotation of the hydroxyl group on position 1 which leads to the more stable conformation studied in [10]. The choice of conformer has few effects on reactivity. The reactants adducts have the same geometry, with the exception of the hydroxyl position. In the reactants adduct, the superoxide anion is more or less situated in the plane of the substituted naphthalene. The addition of the hydrogen atom to the radical modifies the dihedral angle between the OO bond and the PHN plane which becomes 70 degrees. To attain the products, it is necessary to stretch both OO and OH bonds. The OO bond length varies from 1.32 to 1.479 Å and

the OH bond length from 1 to 1.837 Å. The transition state geometry is analogous to that of products with a OO bond almost perpendicular to the PHN plane. However, the lengths of OO and OH bonds are intermediate: respectively, 1.37 and 1.42 Å (Table 2). Without water molecule, the reaction cannot be complete since it lacks a proton in order to obtain hydrogen peroxide. However the products  $\text{HOO}^-$  and PHN radicals are more stable by 3 kcal/mol than the reactants for the PNH conformer depicted in Figure 1. For the other more stable conformer, the difference in free energy is lower: 1 kcal/mol. This discrepancy in the energy difference between the two possible conformers decreases with the number of water molecules. It is negligible with six water molecules.

TABLE 2: Geometry of transition state.

Number of water molecules		OO bond	OH bond
0	in cam-b3lyp	1.37	1.42
	in wB97xd		
1	in cam-b3lyp	1.38	1.21
		1.36	1.12
	in wb97xd	1.37	1.23
		1.40	1.09
2	in cam-b3lyp	1.37	1.33
		1.36	1.09
	in wb97xd	1.38	1.42
		1.37	1.15
3	in cam-b3lyp	1.36	1.16
		1.38	1.16
	in wb97xd	1.37	1.43
		1.37	1.37
4	in cam-b3lyp	1.37	1.06
		1.40	1.03
	in wb97xd	1.37	1.05
		1.36	1.28

*One Water Molecule.* The most stable geometry of the reactants with one water molecule is different according to the functional used for calculations. However in all cases the free energy difference is low (between 0.6 and 0.7 kcal/mol). In the most stable conformation for wB97XD, the water molecule forms a bridge between the two other molecules (Figure 2(a)). The superoxide anion and water molecule are in a perpendicular plane with respect to PNH. The products keep approximately the same pattern with the water molecule more inclined. The water does not give a proton to  $\text{OOH}^-$  anion but its OH bond is slightly stretched with a length of 1.02 Å. In the transition state conformation (Figure 2(b)), the pattern is conserved with the hydrogen atom approximately in the middle of the two oxygen atoms. This transition state corresponds to a saddle with a OO bond length of 1.38 Å and OH bond length near 1.21 Å for cam-b3lyp functional. The other stable conformation with the cam-b3lyp functional (Figure 2(c)) has the water molecule at the opposite side of superoxide anion. The free energies of the two configurations are similar but their electronic energies are different by approximately 4 kcal/mol. This configuration leads to a more exothermic reaction (Table 1) with a lower barrier. The transition state geometry of this configuration is more similar to that of the reactants since the OH bond length is shorter by 0.8 Å. Thus, from the comparison between these two configurations, it can be concluded that the position of the water molecule at the end of the superoxide anion is important in lowering the barrier height. However, it is not sufficient to complete the reaction since, in the products, the water does not give a proton to the  $\text{OOH}^-$  molecule. However, the bond length of the hydroxyl group situated near the  $\text{OOH}^-$  anion is greater than usual: 1.06 Å instead of 1.02 Å. As in the former case with no water molecule, the conformation of PNH has few effects on reactivity: with the

most stable conformation, only a slightly lower difference between the electronic energies is calculated.

*Two Water Molecules.* Adducts with two water molecules are numerous and it was not possible to study all the possibilities. However two configurations similar to the former with one water molecule have been chosen in order to verify if the geometry influence on barrier height was still effective. In the first configuration, the two water molecules formed a bridge between PNH and superoxide: one is in the plane of PNH and the other is perpendicular. However, after the reaction has occurred, the bridge in the PNH plane no longer exists and the conformation of the reactants corresponding to the product geometry (Figure 3(a)) is slightly more stable than the geometry with the two bridges. Thus the reactivity was studied with this conformation as initial state. The addition of a water molecule forming a hydrogen bond on the oxygen atom of reactive hydroxyl group leads to a better stabilisation of the products. The barrier height remains of the same order of magnitude. The second studied configuration still possesses a water molecule forming a bridge between the reactants. The second water is situated at the end of superoxide (Figure 3(b)). Contrary to what has been calculated for adducts with one water molecule, this configuration is largely favoured since it possesses a lower free energy by 1.8 kcal/mol. The comparison of the results with one or two water molecules shows that addition of the water molecule does not modify the barrier height in the two configurations. However, the position of the saddle varies differently. In the first configuration (Figure 3(a)) the saddle point is further from initial state with OO and OH bonds length of 1.37 and 1.33, Å respectively. In the second configuration it is the other way round: the bridge formed by the water molecule diminishes the OH bond distance of the saddle to 1.09 Å. One has to notice that positioning the water molecule toward the lower oxygen atom of superoxide on the opposite side of the hydroxyl group leads to similar results.

*Three Water Molecules.* From Table 1 it can be observed that the addition of a third molecule around superoxide anion and the hydroxyl group of PNH does not modify fundamentally the transition state barrier height or the stabilisation of the products. The two configurations that have been chosen are depicted in Figure 4. In the two cases, the upper oxygen atom of superoxide is linked to two water molecules. The lower oxygen atom is linked to the hydroxyl PNH group only in the first configuration. It is approached by another water molecule in the second. This second configuration is more stable by 1.9 kcal in cam-b3lyp.

*Four Water Molecules.* The two configurations with four water molecules were built by adding water at the lower oxygen atom of superoxide in the configurations with three water molecules (Figure 5). As a consequence, the difference in free energy between the two configurations decreases to 0.6 kcal/mol. There are a further stabilization of the products and a lowering of the barrier height in the first configuration. With the cam-b3lyp functional, in the transition state, the hydroxyl OH bond has a short length, close to that of the initial state one.

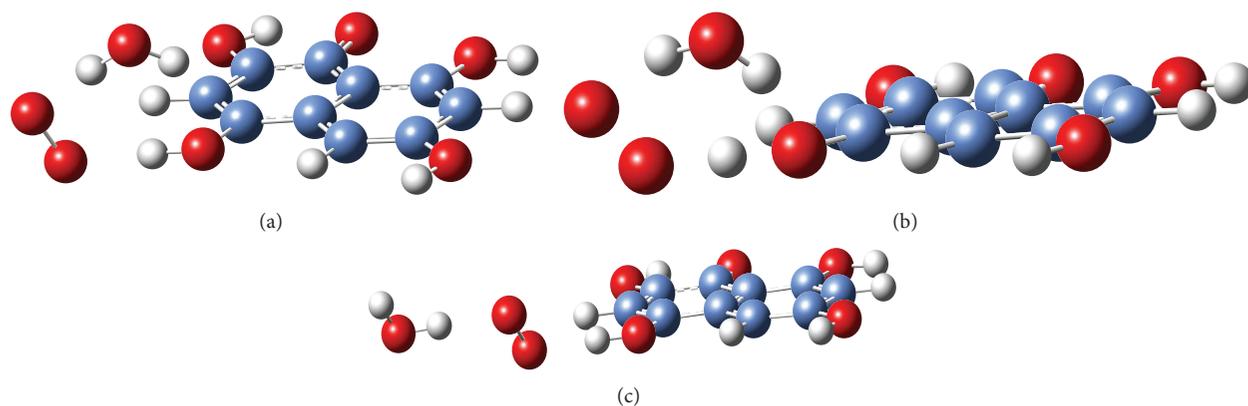


FIGURE 2: (a) Optimized geometry of the first conformation of reactants with one water molecule; (b) transition state conformation corresponding to the configuration (a); (c) optimized geometry of the second conformation of reactants with one water molecule.

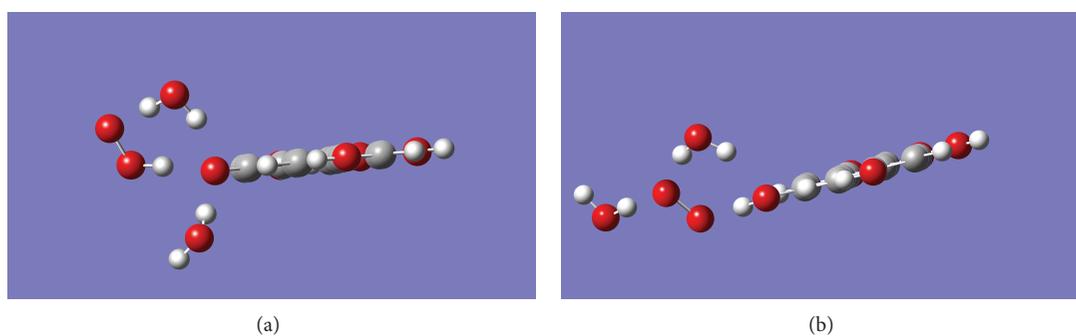


FIGURE 3: Optimized geometries with two water molecules: (a) optimized geometry of the products in the first configuration; (b) optimized geometry of the reactants in the second tested configuration.

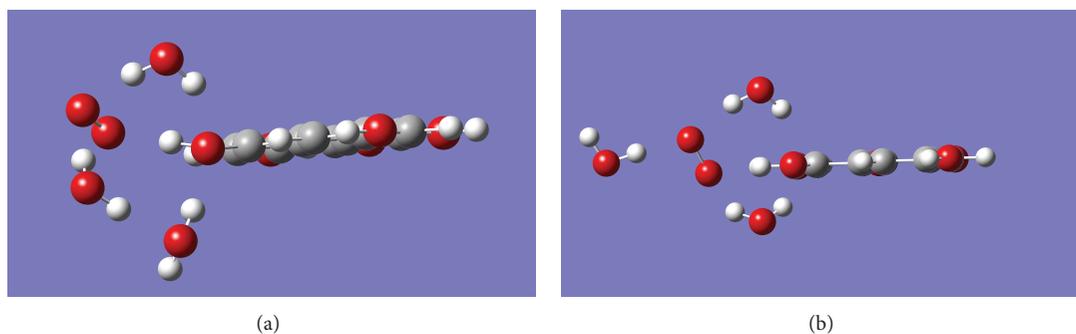


FIGURE 4: Optimized geometry of reactants in the two tested configurations with three water molecules.

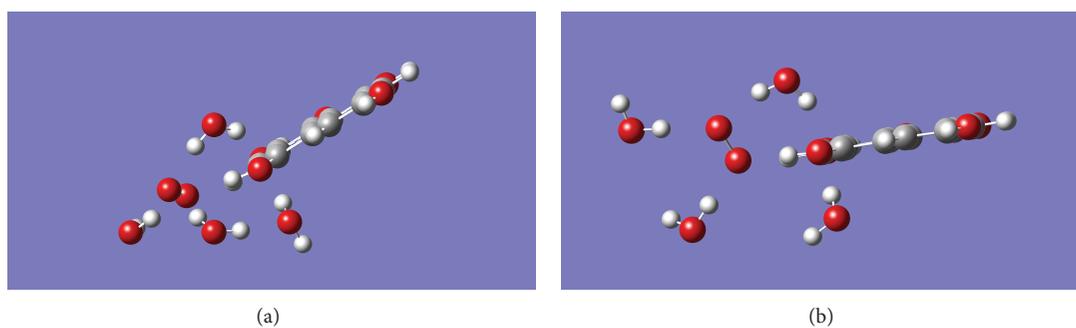


FIGURE 5: Optimized geometry of reactants in the two tested configurations with four water molecules.

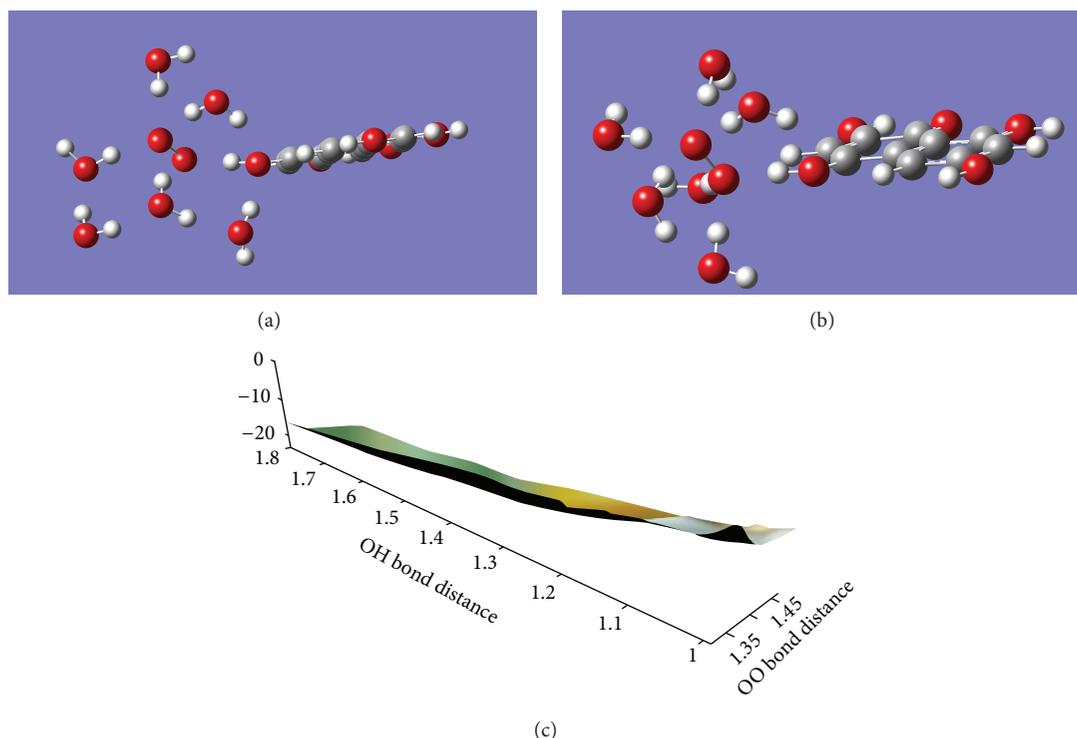


FIGURE 6: Optimized geometries with six water molecules: (a) Reactant configuration which leads to a reactivity with a low barrier; (b) reactant configuration which leads to spontaneous reactivity; (c) PES for the first configuration. The ordinates correspond to electronic energy difference (in kcal/mol). The axis is given in Å.

*Six Water Molecules.* With six water molecules (Figure 6) there are no more barriers for two configurations. The reaction is spontaneous and the reactants geometry does not correspond to a minimum. It has been calculated by freezing the two OO and OH bonds. In these two configurations the upper oxygen atom of superoxide anion is approached by four water molecules. In the first case one water molecule is linked to the lower oxygen atom, in the second case two water molecules form hydrogen bonds in addition to the hydroxyl group of PNH (Figure 6(b)). In a third configuration (Figure 6(a)), the water molecule is displaced from the lower oxygen atom of superoxide versus the oxygen atom of the hydroxyl group. In that case, the reaction is no more spontaneous but has a very low barrier. Thus, the number of water molecules pointing toward the oxygen of superoxide has some importance in the spontaneous character of the reactivity. However, if dynamic effects had been included in the model, the reaction would have taken place also in the third configuration since the barrier is very low (Figure 6(c)).

Thus the hydration of superoxide modifies its reactivity with PNH. This study could not be done thoroughly with MP2 method but it has been verified that this result was not dependent on the method of calculation. In MP2 also, with one or two water molecules, the reaction passes through a barrier. It is spontaneous with six water molecules.

It has been shown that the first layer of water molecules around superoxide radical indirectly participates in the reaction. What about the molecules around PNH? Calculations have been done with a complete layer of water molecules

around the reactant. For these calculations, the two sets of water molecules, around superoxide and around PNH, have been described with two basis sets. Reactants and six water molecules around superoxide were described with the 6-311+G(d,p) basis set. The other 43 water molecules were described with the low basis set 3-21+G. In this case also, the reactivity is spontaneous. The difference in electronic energy between reactants and products still increases.

### 3.2. Study of Frontier Molecular Orbitals

*3.2.1. Frontier Molecular Orbitals of the Reactants.* In order to understand the effect of hydration on reactivity, the frontier molecular orbital occupancies of the reactants have been pictured. The natural orbital occupancies of the reactants with no water molecule can be divided into two types. For some electronic levels, electrons are only localized on PNH in molecular orbitals similar to PNH ones. They are delocalized on all the heavy atoms of the molecule. The corresponding alpha and beta electronic states have almost the same energy. Other molecular orbitals correspond to the orbitals of superoxide. Their energies are indicated in italic numbers in Table 3. This is the case of HOMO-1 which is localized on both superoxide and the hydroxyl group of PNH. In this case, the alpha and beta electronic level energies are different. The SOMO has a relatively low energy next to HOMO-4. As a consequence, there is a mixing of the occupancies of the alpha electrons in the two levels (Figure 7(a)). Addition of a small number of water molecules

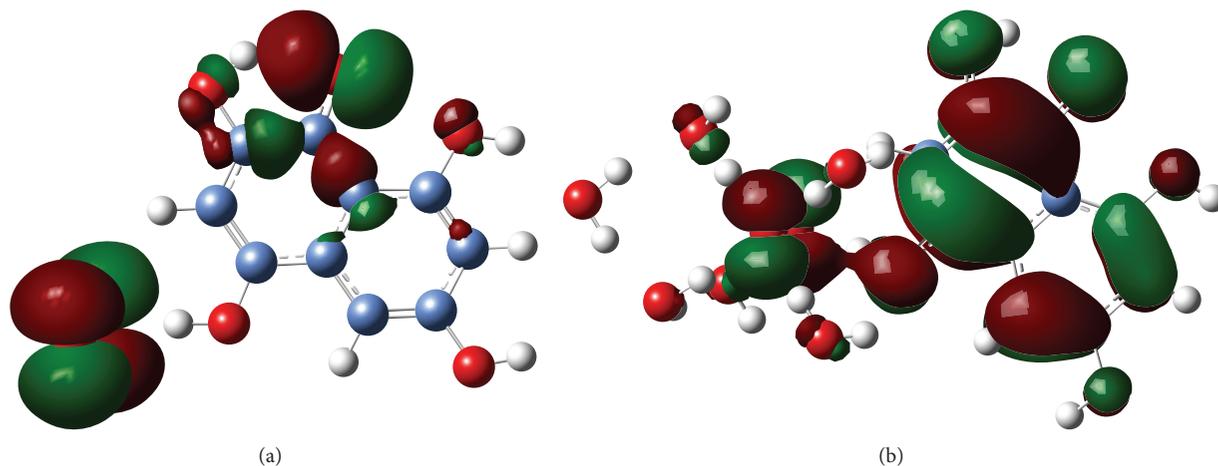


FIGURE 7: (a) SOMO of reactants with no water molecule; (b) HOMO alpha of reactants with six water molecules.

does not modify thoroughly the localization of the molecular orbital occupancies localized on PNH. It stretches on oxygen atom of bounded water molecules but in a very limited manner. On the contrary, superoxide orbitals are largely delocalized on bonded water molecules. The mixing between SOMO and alpha HOMO-4 becomes less important when increasing the number of water molecules since the energy difference between the levels increases. With four molecules, the SOMO is almost pure. However, there is a mixing between the three lowest alpha electronic levels of Table 3. For these three levels, the molecular orbital occupancies are extended on both superoxide and PNH.

With six water molecules, for the case with a very low barrier, the pattern continues: the molecular orbital occupancies are principally localized either on PNH and bounded water molecules or hydrated superoxide. There is no delocalization due to resonances between levels. But in the two other cases, the delocalization due to resonance is moved to the two highest occupied orbitals of alpha electrons. Their occupancies are extended on all the reactants (Figure 7(b)). Moreover, the corresponding alpha and beta electrons are not localized on the same sites. There is no more similitude between alpha and beta level energies. The spontaneous reactivity is correlated with this high delocalization of the highest occupied levels.

It is also possible to characterize the molecular orbital occupancies of the two reactants: the hydrated superoxide and PNH. The occupied frontier orbitals of the hydrated superoxide in the frozen geometry corresponding to reactant are delocalized also on water molecules directly bounded to the anion. This is not the case of the LUMO. With one or two water molecules, this molecular orbital is only localized on superoxide. For three or four molecules, delocalization begins but on water situated orthogonally to the  $\text{OH}\cdots\text{O}$  axis. With six molecules, the delocalization occurs on the water molecules parallel to the  $\text{OH}\cdots\text{O}$  axis in the configuration with a low barrier. When the number of water molecules directly bounded to the superoxide increases, the delocalization progressively extends to all water molecules. Moreover, addition of water molecules around the anion

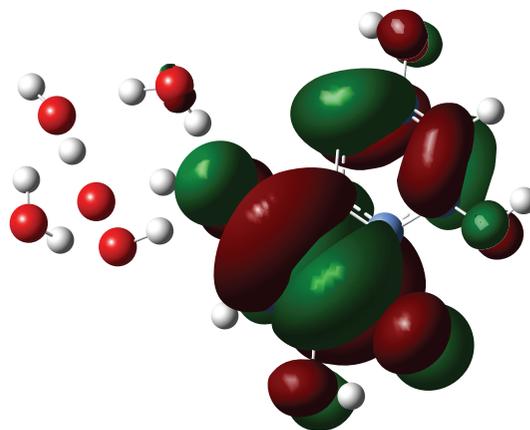


FIGURE 8: SOMO of the products with four water molecules.

lowers the energies. In particular, the LUMO beta has a positive or slightly negative value for all the configurations with a barrier. It becomes frankly negative in the two other configurations with no barrier (Table 3). Thus, the existence of a the first layer of water molecules around superoxide leads to a decrease of the electronic level energies, in particular the beta LUMO, and facilitates its interaction with the HOMO of PNH ( $-5.3$  eV). As a consequence, the reactants highest occupied orbitals are largely delocalized.

**3.2.2. Characterization of the Products.** The SOMO of the products is depicted in Figure 8. It has the same localization whatever the number of water molecules. Its large delocalization on PNH is an indication of the relatively good stability of PNH radical. Thus, the reaction corresponds to a hydrogen atom transfer as expected. The second part of the reaction, proton transfer from water molecule to  $\text{OOH}^-$ , is never achieved even with six water molecules around  $\text{OOH}^-$ . It is well known that water is a locally structured medium and proton transfer a collective motion. Thus one layer of water molecules around superoxide is not sufficient for achieving

TABLE 3: (a) Energies of occupied natural orbitals of reactants (in eV). (b) Energies of frontier orbitals of hydrated superoxide with the frozen geometry corresponding to the most stable reactants (in eV).

		(a)							
Number of water molecules		0	1	2	3	4	6	6 without barrier	
Alpha		-5,05562	-5,14922	-5,17616	-5,26704	-5,80906	-5,31466	-6,14293	
		-6,73175	-7,10344	-7,30725	-7,35405	-7,90831	-7,3886	-6,2349	
		-7,24929	-7,28874	-7,48602	-7,61064	-8,23483	-7,80682	-6,37993	
		-7,67676	-7,72002	-7,7358	-7,78124	-8,33633	-7,86777	-8,06994	
		-7,70506	-7,77199	-7,79757	-7,84355	-8,35429	-8,03892	-8,46394	
							-8,92134		
Beta		-5,05562	-5,14976	-5,17643	-5,26677	-5,80906	-5,31439	-6,2134	
		-6,28769	-6,66645	-7,0561	-7,18398	-7,7758	-7,3886	-6,37503	
		-7,25065	-7,28847	-7,30725	-7,35432	-7,90831	-7,60139	-7,83621	
		-7,67757	-7,72057	-7,73608	-7,77988	-8,33361	-7,80954	-8,19184	
		-7,72601	-7,77526	-7,79811	-7,84355	-8,35075	-7,87049	-8,86937	
							-8,92842		
SOMO		-7,78396	-8,08355	-8,39592	-8,49578	-9,04488	-9,00515		
		(b)							
Number of water molecules		0	1	2	3	4	6	6 without barrier	6 without barrier
Alpha									
	HOMO	-6,16089	-6,54183	-6,96059	-7,06725	-7,32466	-7,40466	-8,80897	-9,18256
	LUMO	3,40479	2,39584	2,08973	1,63587	1,4005	1,10445	1,39043	1,28513
Beta									
	HOMO	-5,70322	-6,08987	-6,51326	-6,6221	-6,8844	-6,95569	-8,41224	-8,80135
	LUMO	0,55944	0,29632	-0,0068	-0,19428	-0,3072	-0,63263	-2,14823	-2,68644
SOMO		-7,29201	-7,59159	-7,93525	-8,06804	-8,24	-8,50585	-9,81655	-9,94662

the proton transfer. It needs at least two supplementary layers for the stable position of the proton to be nearer to  $\text{OOH}^-$  than to the water molecule.

#### 4. Conclusion

This study has evidenced the importance of solvent water molecules in the reactivity of superoxide radical with polyphenols. Both barriers to reaction and reaction free energies depend on the number of explicit water molecules around superoxide radical. PNH possesses a sufficiently low BDE for the reaction to be energetically favored even with no water molecule. But, in this case, the calculated reaction free energy is low. It is multiplied by a factor five to six between the two cases: no or four water molecules. The first layer of surrounding water molecules is essential for the reactivity: it lowers the energies of the electronic excited states of hydrated superoxide anion and facilitates the reaction. Thus the reactivity seems to depend on at least two important factors: the BDE of the polyphenol hydroxyl group and the environment of that hydroxyl group. Indeed, this environment can thoroughly modify the number of hydrating water molecules. A better understanding of the second point is essential. It will be the starting point for a further upcoming study.

#### Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

#### Acknowledgment

The calculations have been made with computers purchased with the funds of the Région Aquitaine, France.

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