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Hydrogen bonding between histidine and lignin model compounds or redox mediators as calculated with the DFT method. Effects on the ease of oxidation †

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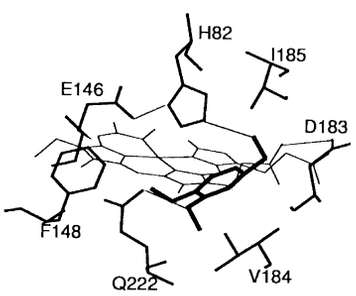
Using the Density Functional Theory method, the effect of hydrogen bonding between imidazole (IM) and ten benzyl alcohol derivatives (BA) on the ionization potentials of the latter is calculated. IM is used as a model for histidine, which is found in the reaction sites of laccases and lignin peroxidases, and the BA-derivatives serve as lignin model compounds. A marked decrease (~15 kcal mol^{-1}) is found for the IP's of the BA-derivatives when paired with IM. This should facilitate the one-electron oxidation of BA in the reaction site of the enzyme. The same effect is found for the known redox mediators violuric acid, 1-hydroxybenzotriazole and *N*-hydroxyacetanilide which are assumed to enter the reaction site of the enzymes. Furthermore, upon one-electron oxidation the strength of the H-bond from BA to IM is considerably increased and in the case of the mediators this effect is so pronounced that the relevant proton shifts from them to IM. If this occurs in the active site of the enzyme then the oxidized redox mediators are released into the aqueous phase in their neutral form rather than as radical cations (deprotonation of the radical cations). The oxidation power of the neutral radical mediators, however, is too low to initialize oxidation of lignin. A more likely reaction pathway is oxidation of the substrates *via* hydrogen abstraction. The pertinent bond dissociation energies are similar for the BA-derivatives and the redox mediators, which in principle allows the reaction to occur.

Introduction

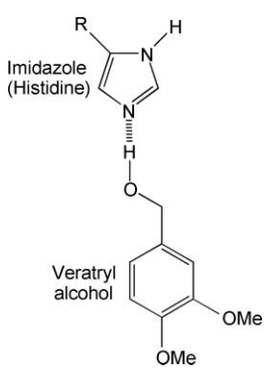
Lignin comprises some 15 to 30% of woody plant cell walls, making it the most abundant aromatic polymer on this planet.[1] Lignin is not a desired component in paper and it must therefore be removed from the original pulp-mass and, in a final step, the pulp has to be bleached in order to obtain white paper. Conventional lignin removal methods, relying on chlorine or chlorine based chemicals, are not acceptable due to environmental reasons and hence the pulp and paper industry is under pressure to develop more environmentally friendly delignification processes. Biodegradation of the lignin polymer using enzymes, *e.g.*, laccases and peroxidases, is a promising potential solution to this problem.[2,3] Many research papers have appeared on the structure of laccase[4–9] and lignin peroxidase[10–13] with the aim of shedding light on the enzymatic degradation mechanism of lignin. For both laccase and peroxidase, the amino acid histidine, which contains the imidazole (IM) moiety, is present in the reaction centers of the enzymes and it has been postulated[11] that hydrogen bonding exists between the lignin substrate and the IM of histidine as shown in Schemes 1 and 2.[14]

As seen above, the OH group of veratryl alcohol is positioned to H-bond with His-82. A close-up of this picture is shown in Scheme 2.

Therefore the question arises whether this kind of hydrogen bonding affects the redox reactions initiating the lignin degradation process. Furthermore, redox mediators, *i.e.*, small redox-active substrates, are known to increase the efficiency of enzymatic lignin degradation *via* indirect oxidation.[15–18] Many of these mediators are able to form hydrogen bonds, similar to the lignin model compounds, thus the same question can



Scheme 1 View of the complex between veratryl alcohol and lignin peroxidase as suggested by Gold *et al.*[11] In the foreground, veratryl alcohol is highlighted with thicker lines. The thin lines indicate hydrogen bonds.[14]



Scheme 2 Close-up picture of the postulated (Gold *et al.*[11]) hydrogen bonding between imidazole (= IM, as a model for histidine) and veratryl alcohol (serving as a lignin model compound).

be asked, *i.e.*, does the hydrogen bonding affect the redox properties of these mediators? With this scenario it is assumed that the redox mediators are one-electron oxidized in the reaction site of the enzyme, like the lignin (model) substrate.

In order to gain further insight into the reaction mechanism of enzyme induced lignin degradation and that of redox mediation, in this work we calculated, using the Density Functional

† Electronic supplementary information (ESI) available: Fig. S1: comparison of bond lengths and angles of violuric acid with those of the known crystal structure, Tables S1–S6: single point and zero point vibrational energies. See http://www.rsc.org/suppdata/ob/b3/b314991a/

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Theory (DFT) method, how the hydrogen bonding with IM affects the ionization potentials (IP) of the lignin substrates and of the redox mediators. In addition, the energies contained in the hydrogen bond bridges and their changes upon one-electron oxidation are calculated.

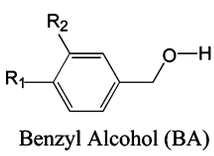
Computational methods

The energy calculations and geometry optimizations were performed with the GAUSSIAN 98 program package[19] utilizing unrestricted DFT. The functional of Lee *et al.* was used for the correlation part[20] and Becke's combined three parameter functional was used for the exchange part (B3LYP).[21,22] The basic split valence standard 6-31G(d,p) basis set[23] was used for the geometry optimization and frequency analysis. In all cases the normal modes of the molecular motions revealed no imaginary frequencies for the calculated structures, which is in support of them representing a minimum on the potential energy surface. The zero-point vibrational energies (ZPE) were scaled according to Wong (0.9804).[24] Subsequent single-point electronic energy calculations were performed at the B3LYP level with Pople's triply split valence and polarized 6-311G(2df,p) basis set. The basis set superposition error (BSSE) was calculated and taken into account by using the Counterpoise Theory for the hydrogen bonding energies.[25,26]

Results and discussion

The results of the single-point electronic energy calculations and ZPEs of the calculated structures of lignin model compounds, the redox mediators and the hydrogen bonded pairs are listed in Tables S1–S3 (Electronic Supplementary Information, ESI†). Also, the BSSE corrections are given in Tables S1–S2.

Imidazole (IM) is used as a model for histidine and ten derivatives of benzyl alcohol (BA), substituted on the *meta*- and/or *para*-positions, are taken as lignin substrates. The BA-derivatives are depicted in Scheme 3.



Benzyl Alcohol (BA)

i. R_1=H, R_2=H
ii. R_1=NH_2, R_2=H
iii. R_1=Cl, R_2=H
iv. R_1=CN, R_2=H
v. R_1=OH, R_2=H
vi. R_1=CH_3, R_2=H
vii. R_1=OCH_3, R_2=H
viii. R_1=H, R_2=OCH_3
ix. R_1=OCH_3, R_2=OCH_3
x. R_1=NO_2, R_2=H

Scheme 3 Benzyl alcohol (BA) and its derivatives used in this work (ix = veratryl alcohol).

Three known redox mediators are considered, *i.e.*, violuric acid (VIO), 1-hydroxybenzotriazole (HBT) and *N*-hydroxyacetanilide (NHA). Additionally, 2-aminopurine (2AP) is investigated. The radical cation of 2AP has been successfully used as a one-electron oxidant in the DNA double helix.[27] The molecular structures of the redox mediators as well as 2AP are depicted in Scheme 4.[28]

Ionization potentials (IP)

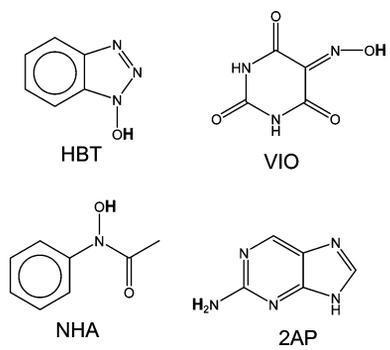
From the values in Tables S1–S3 (see ESI†), IP's were derived according to the procedures described in Foresman and Frisch.[29,30] The calculated IP's are shown in Table 1 for the single BA-derivatives as well as their paired structures with IM.

Experimental IP values are known for BA and *p*-chloro-BA. The published adiabatic IP's for BA lie between 190.5 and 210.8 kcal mol^{-1} and the vertical ones between 210.1 and 219.8 kcal mol^{-1}.[31] In comparison, the calculated adiabatic value for BA is 193.4 kcal mol^{-1}, which lies within the measured numbers. However, the calculated vertical value for BA is smaller by up to 5.2% when compared to the experimental values (see Table 1). Therefore, the IP's of BA were further calculated, again using the B3LYP method, however, with a larger Pople basis set (6-311G++(3df,3pd)) for both the optimization/vibrational and energy calculations. The results are 195.9 kcal mol^{-1} for the adiabatic value and 201.6 kcal mol^{-1} for the vertical value, which represents an only 1.3% difference from the numbers calculated with the procedure described in the computational methods section (see Table 1). The experimental number for *p*-chloro-BA, *i.e.*, adiabatic 197.6 kcal mol^{-1}, is 2.6% different to the calculated value in Table 1. Unfortunately no other experimentally obtained IP's of the BA-derivatives were found. Concerning IM, its adiabatic IP is known to be 203.2 ± 0.2 kcal mol^{-1} [31] and the calculated value is 198.8 kcal mol^{-1}, which is a 2.2% difference. The vertical experimental value is 202.5–206.6 kcal mol^{-1},[31] which agrees very well with the calculated number of 204.7 kcal mol^{-1}.

Table 1 The IP's of the BA-derivatives and their paired structures with IM in kcal mol^{-1}

	Single		Pairs	
BA-derivatives	Adiabatic	Vertical	Adiabatic	Vertical
BA	193.4	199.1	174.4	183.9
4-Amino-BA	164.3	175.5	151.7	166.0
4-Chloro-BA	192.4	201.2	175.1	184.6
4-Cyano-BA	203.4	207.6	183.1	191.2
4-Hydroxy-BA	179.7	188.5	164.6	175.5
4-Methyl-BA	186.3	195.5	169.2	179.2
4-Methoxy-BA	174.9	184.2	160.6	172.9
3-Methoxy-BA	176.7	186.3	163.4	174.3
3,4-Dimethoxy-BA[a]	162.1	168.4	150.8	164.0
4-Nitro-BA	207.9	220.3	187.3	194.8

[a] Also known as veratryl alcohol.



Scheme 4 The redox mediators violuric acid (VIO), 1-hydroxybenzotriazole (HBT) and *N*-hydroxyacetanilide (NHA) as well as 2-aminopurine (2AP). The protons which form hydrogen bonds with IM are depicted bold.

To further check the validity of the IP calculations, benzene and some of its simple substituted derivatives with known IP's were investigated and the results are shown in Table 2.

As is evident, the computed IP's in a vacuum are up to 10% lower than the measured values reported in the literature. This may be caused by the "over-correlation" with the electrons, which is known to occur with the B3LYP method.[32] To check the B3LYP method even further the larger 6-311G++(3df,3pd) basis set was used to calculate the IP values of benzene. The use of this basis set revealed 208.9 kcal mol^{-1} (adiabatic) and 214.2 kcal mol^{-1} (vertical), which represents only 0.7% difference from the "worst" values presented in Table 2. Often, the DFT method used here produces IP's which are ~5% lower than the experimental numbers.[33,34] Thus, this "intrinsic" deviation of the calculated *vs.* experimental values has to be kept in mind when interpreting the results presented. We also used the Hartree–Fock method to generate the IP's for BA, using the same basis sets as described in the computational methods

Table 2 The IP's of benzene and its derivatives in a vacuum (kcal mol^{-1})

Molecules	Calculated		Experimental[a]	
	Adiabatic	Vertical	Adiabatic	Vertical
Benzene	207.4	212.7	212.2–215.8	212.2–214.5
Chlorobenzene	203.3	207.7	207.3–209.8	209.2–209.6
Benzonitrile	220.0	229.6	221.4–225.3	223.7–226.0
Nitrobenzene	220.8	228.4	221.4–234.3	227.4–236.6
Toluene	196.9	201.6	199.9–205.0	203.4–207.5
Aniline	171.1	178.5	172.5–192.6	184.9–186.8
Phenol	189.0	193.2	193.0–200.4	197.4–201.8
Anisole	183.0	187.9	188.6–198.3	190.2–195.1
1,2-Dimethoxybenzene	168.0	173.8	179.9	188.4

[a] Taken from ref. 31.

section. This method produced *significantly* lower values (~25 kcal mol^{-1}) than B3LYP and further calculations using this method were therefore abandoned.

When the IP's for the BA-derivatives (Table 1) are compared to their IM-paired counterparts it is apparent that a considerable lowering is affected by the hydrogen bond formation. The magnitude of this reduction in IP lies between 11.3 and 20.6 kcal mol^{-1} for the adiabatic values. Electron withdrawing substituents on BA, *e.g.*, –NO_2 and –CN, lead to the most pronounced lowering of IP upon pairing whereas with the electron donating groups this effect is less. Thus, hydrogen bond formation with IM has the smallest effect on veratryl alcohol (3,4-dimethoxy-BA).

The effect of H-bonding could, in principle, be interpreted in terms of hydrogen bonding increasing the electron density on the BA-derivative, leading to a lower IP. An alternative explanation, which however relates only to the adiabatic potentials, involves the assumption that the H-bonding effect is due to lowering the energy of the *product*, *i.e.* the one-electron oxidized complex by stronger H-bonding or even partial proton transfer between the partners. A consequence of this picture is that the H-bonding energy holding the partners together should be larger when the pair is one-electron oxidized than when in the parent state.[35] This was checked by looking at the H-bond strengths (see next paragraph).

In Table 3 are contained the calculated IP's of the redox mediators VIO, HBT, NHA and 2AP, with and without H-bonding with IM. It is evident that, as for the BA-derivatives, hydrogen bonding with IM lowers the IP's for all the mediators. This lowering lies between 17.7 and 62.9 kcal mol^{-1}.

To summarize, the hydrogen bonding to IM reduces the energy needed to one-electron oxidize a lignin model compound or a redox mediator. If this type of effect is also present when these substrates are bound in the reaction site of laccases or peroxidases, the lowering of IP (which facilitates their one-electron oxidation) may enable the reaction to take place at all. For instance, the oxidation potential of veratryl alcohol in aqueous solution has been determined to be 1.36 V/NHE.[39] In comparison, the oxidation potential of "compound I" (the primary oxidant in ligninase) can be estimated[40] to be close to 1.44 V.[41] On this basis, there is only 0.08 V of driving force (1 V = 23.06037 kcal mol^{-1}) for the oxidation of veratryl alcohol *non*-bonded to an imidazole moiety. This driving force would increase to 0.57 V (neglecting the difference in environment between water and the reactive site of ligninase) upon H-bond formation (see Schemes 1 and 2) between the substrate and the enzyme. It is clear that this kind of hydrogen bonding would be highly advantageous for facilitating lignin oxidation by the enzyme.

So far the effects of the environment have been ignored in the calculations. This may be excused by the fact that the dielectric constant is not known for the reaction cavity of the pertinent enzymes. Nevertheless, as a test, we investigated the effects of water environment with Tomasi's Polarized Continuum Model (PCM)[43,44] using B3LYP/6-311G(2df,p) on the optimized structure of benzene. Benzene was used because an estimate of its IP in water is known based on experimental data. The calculated IP in water was obtained by subtracting from the gas phase adiabatic IP value the solvation energy differences of the radical cation and its parent compound.[45] In the value given, the hydration energy of the ejected electron (e^-_{aq}), which is reported to be −27.7 kcal mol^{-1},[46] has been taken into account. The ionization potential of benzene in water was estimated[47] as 138.4 ± 11.5 kcal mol^{-1} and the number presented here is 132.5 kcal mol^{-1}, *i.e.* only 4% difference. When, however, a hydrogen bonded system was placed in a continuum, very disappointing and nonsensical results were obtained,[48] and further use of PCM was abandoned. In principle, alternatives are the QM/MM method or the Car–Parrinello[49] approach. These methods are very CPU demanding and presently we do not have the capabilities needed in order to perform these types of calculations.

Table 3 The IP's (in kcal mol^{-1}) of the redox mediators VIO, HBT and NHA and 2AP as singles and as pairs with IM

Mediators	Single		Pair	
	Adiabatic	Vertical	Adiabatic	Vertical
HBT	193.7	197.9	158.5	182.6
NHA	178.0	185.9	139.4	171.4
VIO	217.6	223.2	154.7	194.2
2AP	177.0	182.8	159.3	168.5

Hydrogen bonding energies

The hydrogen bonding energy of an H-bonded pair is defined as the difference in energy between the fully optimized H-bonded pair and the sum of the energies of the two individually optimized components making up the pair, with ZPE corrections taken into account (*e.g.*, for the IM–BA pair: Hydrogen bond energy = E(IM–BA) − E(IM) − E(BA)). This value is therefore an estimate of the total hydrogen bond strength holding the components together. The calculated hydrogen bond energies of IM with the BA-derivatives and the redox mediators as derived from the energies shown in Tables S1–S2† and corrected for their BSSE, are reported in Tables 4 and 5.

As seen from Table 4, in all cases upon one-electron oxidation, the hydrogen bonding energy is increased and this increase lies between 10.5 and 24.0 kcal mol^{-1}. The hydrogen bond energy is increased upon one-electron oxidation independent of whether the substituents on BA are electron donating or withdrawing. In all these cases, the increase is larger than for the *un*substituted BA (see Δ in Table 4).[50]

Table 4 The BSSE-corrected hydrogen bonding energies/kcal mol^{-1} of BA–IM "dimers". Δ is the energy change in the hydrogen bond upon one-electron oxidation

Pair	Parent	Oxidized	Δ
BA–IM	8.6	19.1	10.5
p-(Amino)BA–IM	6.4	20.3	13.9
p-(Chloro)BA–IM	7.6	26.1	18.5
p-(Cyano)BA–IM	8.0	32.0	24.0
p-(Hydroxy)BA–IM	6.7	24.0	17.3
p-(Methyl)BA–IM	6.7	25.5	18.8
p-(Methoxy)BA–IM	6.6	22.6	16.0
m-(Methoxy)BA–IM	7.1	21.7	14.6
m,p-(Dimethoxy)BA[a]–IM	6.3	19.2	12.9
p-(Nitro)BA–IM	8.5	31.7	23.2

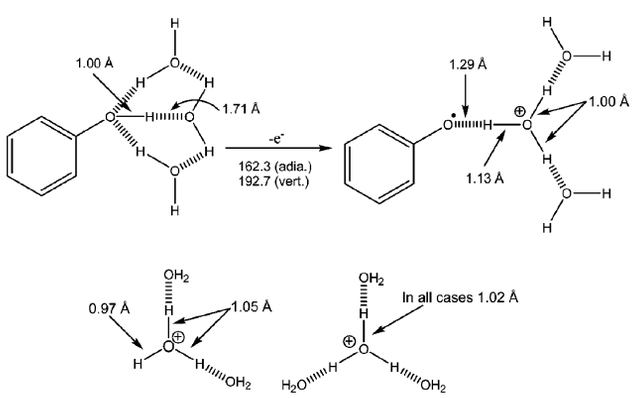
[a] Also known as veratryl alcohol.

Table 5 The BSSE-corrected hydrogen bonding energies of IM and the redox mediators. Δ is the energy change in the hydrogen bonds upon one-electron oxidation. Note, here the comparison is between two different H-bonds, *i.e.*, not the same H-bond as for the BA-derivatives (Table 4)

Mediators	Parent	Oxidized	Δ
HBT–IM	15.5	19.7	4.2
NHA–IM	10.4	25.6	15.2
VIO–IM	15.5	10.7	4.8
2AP–IM	6.5	33.6	27.1

The strengthening of hydrogen bonds, effected by ionization, is known from DNA base pairs (see refs 37,38 and refs therein). This effect is also evidenced by the phenol–water system, used as a model which we computed with the 6-31++G(d,p) basis set.[51] For further comparison, the structures of two protonated water clusters and the length of their valence bonds are shown.

The calculated adiabatic IP of "naked" phenol turns out as 189.0 kcal mol^{-1} (see Table 2), but that of the H-bonded phenol $\cdots$ $3H_2O$ cluster is only 162.3 kcal mol^{-1}. As can been seen from Scheme 5, upon one-electron oxidation deprotonation from the phenol moiety to the water cluster occurs. Furthermore, the oxygen lone pairs on the resulting neutral phenoxyl radical appear to lose their ability to form H-bonds.

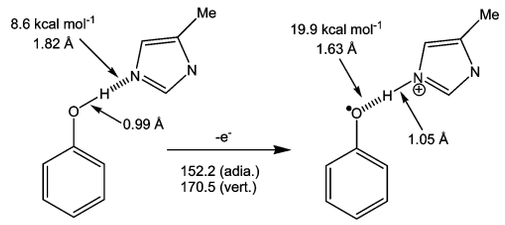


Scheme 5 One-electron oxidation of phenol bonded to a water cluster consisting of three water molecules and, in comparison, pertinent water clusters and their bond lengths.

Replacing water as the proton acceptor by 2-methylimidazole leads to the analogous results shown in Scheme 6.

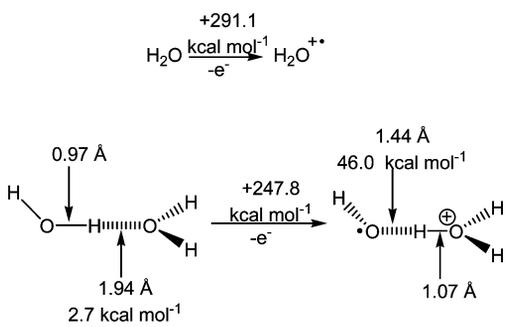
Since the O–H bond dissociation energy of phenol is 85 kcal mol^{-1},[54] from the numbers in Scheme 6 it is evident that one-electron oxidation of phenol, when H-bonded to IM, leads to a weakening of the O–H bond by $\approx$ 65 kcal mol^{-1}, while increasing the H–N bond energy from 8.6 to 81.4 kcal mol^{-1}.[55]

On the way to demonstrating the generality of this phenomenon we looked at the effect of cluster formation on the IP



Scheme 6 One-electron oxidation of phenol bonded to 2-methylimidazole.

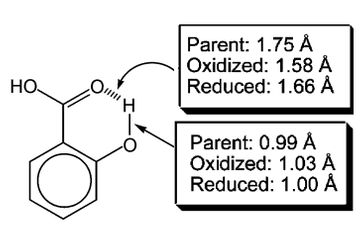
of a water molecule, using B3LYP/6-311++G(3df,3pd) (the biggest Pople basis set available). This basis set is so large that the BSSE is very small and it is thus not necessary to employ the Counterpoise method. The results are shown in the Scheme 7 below:



Scheme 7 One-electron oxidation of water and a water dimer.

The adiabatic IP of a single water molecule in the gas phase is calculated as 291.1 kcal mol^{-1}. The experimentally derived number is 291.04 $\pm$ 0.01 kcal mol^{-1},[31] the same as the calculated value. If the water radical cation is given the chance to deprotonate to a neighboring water, the IP drops by 43.3 kcal mol^{-1} (see Scheme 7). In a recent paper, Novakovskaya and Stepanov[57] arrived at a very similar value (41.6 kcal mol^{-1}), using, however, the UHF method and the 4-31G basis set. It thus appears that proton transfer to a neighboring Brønsted base from a one-electron oxidized (weak) organic or inorganic acid may in fact be a *general* way to increase the oxidizability of that acid.

Furthermore, in order to find an example for the changes in an H-bond upon oxidation (and also on reduction) in an *intra*molecular situation, we calculated the H-bond length in *o*-hydroxybenzoic acid. Here the B3LYP method is used as before with the 6-31++G(d,p)[58] basis set.[59] This basis set contains diffuse functions, which allows a better representation of the H-bond. The changes in the H-bond length upon a one-electron change in oxidation state of the molecule are depicted in Scheme 8.

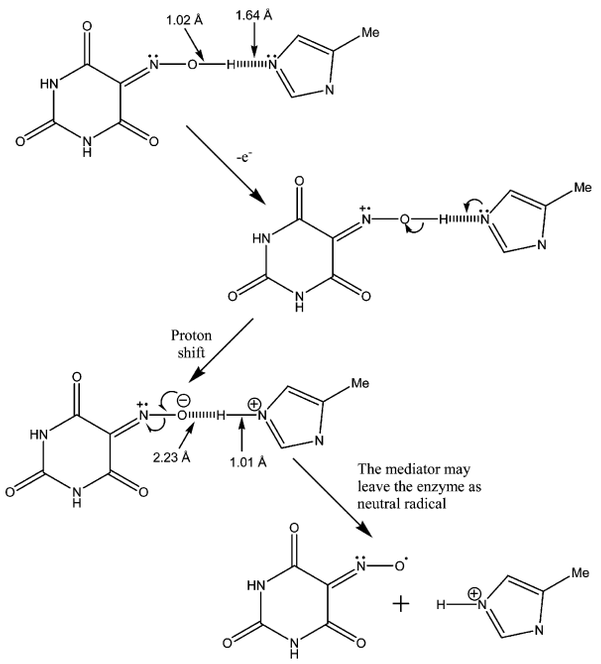


Scheme 8 The changes of the intramolecular H-bond and O–H covalent bond lengths in *o*-hydroxybenzoic acid upon one-electron oxidation (⟶radical cation) and reduction (⟶radical anion).

As seen, the H-bond lengths to the proton acceptor are considerably shortened when oxidation or reduction occurs whereas the O–H covalent bond is only affected to a small extent. Again, it is likely that the effects presented above reflect a *general* phenomenon.

To return to lignin decomposition, in Table 5 are listed the hydrogen bonding energies between IM and the redox mediators shown in Scheme 4.

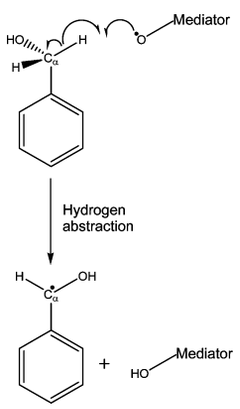
Unlike for the benzyl alcohols (Table 4), the one-electron oxidation results in an essentially complete proton shift from the mediators to IM. This follows from the increases in bond distance between the oxygen atoms in the hydroxyl groups of the mediators from ~1.0 Å to 1.6–2.2 Å, when an electron is removed from the systems, and the decreases in bond length between the nitrogen atom, in the imidazole rings, and the protons from 1.6–2.0 Å to ~1.0 Å. This is visualized in Scheme 9 for VIO and IM.



Scheme 9 One-electron oxidation of the VIO-IM pair and a possible mechanism of the subsequent proton shift from $VIO^{\bullet+}$ to IM.

This proton shift from the redox mediators to IM means that the one-electron oxidized redox mediators are converted to *neutral* radicals. This character of the deprotonated mediators as *neutral* radicals makes oxidation of lignin substrates by an electron transfer an unlikely event since, as compared to radical cations, neutral radicals have *low* reduction potentials.[60] In contrast, with hydrogen abstraction the homolytic bond breaking energy of the relevant bonds plays the main role, not the oxidation/reduction potentials of the pertinent structures. In such a reaction, a carbon-centered neutral radical of the lignin substrate would be formed, as shown in Scheme 10.

Galli *et al.*[17,18,61–63] have demonstrated experimentally that indeed hydrogen abstraction (HAT) is an important mechanism



Scheme 10 The proposed hydrogen abstraction from BA.

in the oxidative degradation of lignin involving laccase and redox mediators.

In order to get thermochemical information on such reactions, we calculated the homolytic bond dissociation energies for a series of relevant compounds.

Bond dissociation energies (BDE)

If a hydrogen abstraction occurs from a BA-derivative to a neutral redox mediator radical (Scheme 10), then, in order for the reaction to be exothermic, the BDE's of the mediators have to be larger than those of the bond homolytically broken. The BDE's of the C_α-H bonds for the BA-derivatives and the O–H/N–H bonds for the mediators were calculated by subtracting the single-point energies of the neutral radicals from the energy of the parent molecule corrected for their ZPEs, *e.g.*, for BA: BDE = E(BA) − E(BA(− H)$^\bullet$) − E(H$^\bullet$ [= 0.5 a.u.]). The energies are obtained from Tables S1–S4. † The resulting BDE's of the BA-derivatives and the mediators are given in Table 6.

No empirical values were found for the BDE's calculated here. Thus, in order to check on the validity of the calculations three simple model compounds were chosen with experimentally known BDE's. These are methanol (CH_3O–H), methylamine (CH_3NH–H) and ethane (CH_3CH_2–H). The experimental number for methanol is 102 ± 2 kcal mol^{-1} [64] and the calculated value, using the same procedure as for the mediators is 98.4 kcal mol^{-1}, *i.e.* 3.5% difference. For methylamine the experimental value is 92 ± 3 kcal mol^{-1} [64] and the calculated one is 95.3 kcal mol^{-1}, the same difference as for methanol. Finally, ethane has 98 ± 1 kcal mol^{-1} [64] and the theoretical number is 98.7 kcal mol^{-1}, *i.e.* only 0.7% difference. By using a larger basis set (6-311G++(3df,3pd)) the correlation with the experimental numbers was slightly improved, *i.e.*, methanol 100.2 kcal mol^{-1}, methylamine 96.3 kcal mol^{-1} and ethane 98.3 kcal mol^{-1}. Using, however, the Møller–Plesset [65] method with the same basis sets as described in the computational methods section, values ~25 kcal mol^{-1} lower than the experimental ones were obtained.

Additional values were calculated for the aromatic compounds shown in Table 7, for which experimental values are

Table 6 The BDE values of the BA-derivatives and the mediators

Systems	BDE/kcal mol^{-1}
BA	77.8
p-(Amino)BA	79.2
p-(Chloro)BA	78.3
p-(Cyano)BA	76.1
p-(Hydroxyl)BA	79.2
p-(Methyl)BA	78.9
p-(Methoxy)BA	79.2
m-(Methoxy)BA	78.8
m,p-(Dimethoxy)BA	77.8
p-(Nitro)BA	75.9
HBT	78.0
NHA	78.1
VIO	68.0
2AP	98.2

Table 7 The BDE's (in kcal mol^{-1}) of benzene and its derivatives in vacuum. The bonds investigated are C_6H_5–H, C_6H_5NH–H, C_6H_5O–H and $C_6H_5CH_2$–H

Molecules	Calculated	Experimental[a]
Benzene	110.4	110.9 ± 2
Aniline	88.0	88.0 ± 2
Phenol	83.1	86.5 ± 2
Toluene	87.1	88.0 ± 1

[a] Taken from ref. 56.

available. The conclusion can again be drawn that the DFT method produces values in good agreement with the experimental values.

On the basis of the values in Table 6, the difference in BDE between the BA-derivatives and the HBT and NHA mediators is very small but 2AP has a ~20 kcal mol^{-1} higher value. Interestingly, VIO has a lower BDE by ~10 kcal mol^{-1} than the BA-derivatives, which excludes this reaction on thermochemical grounds. However, VIO is deprotonated on its hydroxy moiety[66,67] at physiological pH, thus the BDE of the *endo*cyclic N–H is the one which counts. It is calculated as 79.0 kcal mol^{-1}, which is a value similar to that for HBT and NHA (see Table 6). It is known experimentally that HBT, NHA and VIO react with lignin model compounds upon enzymatic treatment.[15–18] On the basis of Table 6 this reaction can only take place with C–H bonds with BDE's ≤ 78 kcal mol^{-1}. C–H BDE's in the deoxyribose moiety in DNA are calculated as ~90 kcal mol^{-1}[68,69] as well as measured[70] in the ribose model tetrahydrofuran. Using these values for the C–H BDE's in cellulose it is apparent that no hydrogen abstraction can occur from cellulose moieties to the deprotonated redox mediators. On this basis the mediators should show selectivity in favor of interaction with the lignin but not the cellulose component of wood.

Summary

The DFT calculations reveal that hydrogen bonding between IM and the potential substrates, *i.e.*, the BA-derivatives or the mediators, leads to a marked lowering in their IP's. The reason for this is the considerable strengthening of the H-bonds upon one-electron oxidation as compared to the parent pair. Examples have been presented which indicate that this H-bonding effect on IP's may be a *general* phenomenon. Such hydrogen bond bridges in redox enzymes would increase the driving force of the oxidation process. Similarly, proton shift from the redox mediators to IM upon one-electron oxidation has been found to be likely, as a result of which the enzyme may release the mediators as *neutral* radicals to the aqueous phase. The neutral radicals of the redox mediators, having low oxidation power, are unlikely to be able to one-electron oxidize the aromatic components of lignin, however, hydrogen abstraction from C_α– of the lignin model compounds to the redox mediator neutral radicals is a possibility.

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