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^{1}H, ^{13}C, and ^{17}O Isotropic and Anisotropic Hyperfine Couplings for the Plastosemiquinone Anion Radical

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Abstract: Hybrid density functional calculations (B3LYP) are performed for the plastosemiquinone anion radical in its free form and in models to simulate its hydrogen bonding environment in alcohol solvents. ^{1}H, ^{13}C, and ^{17}O isotropic and anisotropic hyperfine couplings are calculated. The effect of hydrogen bonding interactions in the alcohol solvent model is shown to lead to a redistribution of spin density from the oxygen atoms to the carbon atoms of the carbonyl groups. ^{1}H calculated hyperfine couplings are compared with previous assignments based on ENDOR measurements. A rearrangement of the experimental assignments for the methyl group couplings is indicated and a reinterpretation of the ^{1}H hydrogen bonding data is presented.

Introduction

Quinones are ubiquitous to living systems. Plastoquinone is found in higher plants and algae where it is a vital electron-transfer agent in the initial electron-transfer reactions of photosynthesis. In the Photosystem II reaction center it is believed that two plastoquinone molecules act in concert to accept electrons generated via photoejection from a specialized chlorophyll *a* molecule (see ref 1 for a review). The primary acceptor plastoquinone, Q_a, is reduced after initial charge separation to give rise to a semiquinone anion radical. This primary acceptor is then believed to reduce a second plastoquinone molecule Q_b, giving rise to a second plastosemiquinone anion radical. Although chemically identical quinone molecules, one functions as a one-electron gate while the other accepts two electrons to become reduced to the quinol form.

The methods of choice for examining the properties of semiquinone radicals are Electron Paramagnetic Resonance (EPR) based techniques. As such EPR, ENDOR, TRIPLE, and ESEEM studies have been performed on the plastosemiquinone anion radical in both alcohol solvents and also on the Q_a radical form of Photosystem II.[2−6] All these methods are mainly concerned with the observation of hyperfine couplings arising from the interaction of the unpaired spin density distribution of the radical with the nuclear centers of the radical itself and the surroundings. So far hyperfine couplings to the ^{1}H nuclei of the radical itself and the surrounding hydrogen bonding protons have been reported in addition to some ^{14}N hyperfine couplings from surrounding amino acid residues for the Q_a radical of Photosystem II.

For the in vivo radical the spectra are inevitably of the powder type with contributions to the spectra from all possible radical orientations. Both isotropic and anisotropic contributions are present which in theory should provide a wealth of electronic structure information but in practice leads to severe spectral overlap. Although a variety of techniques such as deuteration and sample orientation can lead to some spectral simplification it is still difficult to unambiguously assign spectral features to specific positions.

We have shown in recent studies the outstanding success of the hybrid density functional electronic structure calculation in predicting spin density distributions and isotropic and anisotropic hyperfine couplings for *p*-benzosemiquinone and durosemiquinone anion radicals.[7−11] Excellent agreement between calculated and experimentally determined hyperfine couplings is found for these well-characterized radicals. Crucial information regarding the changes occurring in the electronic structure on hydrogen bond formation at the carbonyl oxygen atoms have also been reported.

In this report we extend our calculations to study the plastosemiquinone anion radical of Figure 1. The effect of hydrogen bonding is examined via hydrogen bonding models with two and four methanol molecules. Isotropic and anisotropic hyperfine couplings are reported for ^{17}O, ^{13}C, and ^{1}H. The calculated couplings allow us to reexamine the reported experimental ^{1}H ENDOR spectra and correct some erroneous assignments. It is clearly imperative to have a correct description of the in vitro radical before attempting to deduce electronic structure variations brought about by in vivo binding to the protein.

Methods

The models used for the density functional calculations are shown in Figure 2. All geometries were first optimized with use of the PM3 method and these complexes were then used to calculate hyperfine couplings by using UB3LYP/EPR-II as described in detail previously.[9,10]

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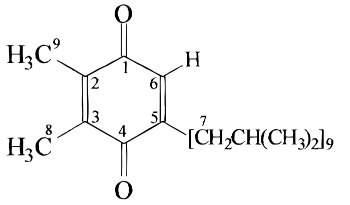

Figure 1. Structural formula and numbering scheme of plastoquinone-9 (2,3-dimethyl-5-solanesyl-1,4-benzoquinone).

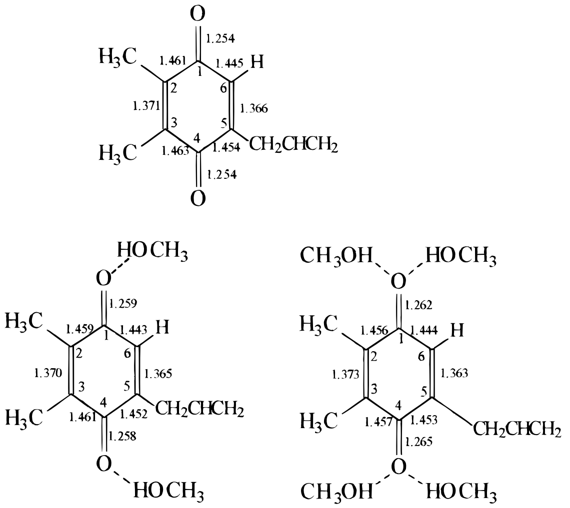

Figure 2. Plastoquinone models used for density functional calculations: top, "free plastoquinone" PQ; bottom left, PQ-2CH_3OH; and bottom right, PQ-4CH_3OH. For all models the isoprenyl chain has been reduced to one unit. Experimental studies have shown, as expected, that the length of this chain has negligible influence on the EPR/ENDOR spectral characteristics.[14] Key internal bond distances are also given in angstroms. All hydrogen bond lengths are between 1.77 and 1.79 angstroms.

All density functional calculations were performed with GAUSSIAN 94(12) and PM3 calculations and graphical surface generation were carried out with SPARTAN.[13]

Results and Discussion

Spin Densities. Hyperfine couplings arise from the interaction of the nuclear magnetic moments with the unpaired spin density distribution of the radical. Contours of positive spin density ($\alpha-\beta$) of PQ are plotted for four values, 0.01, 0.0075, 0.005, and 0.002 e/au^3, in Figure 3. The 0.002 contour shows where most of the positive spin density is located in the radical. The "tighter" contours, in particular the 0.01 contour, provide an excellent visualization of regions of concentrated spin density, which mainly influences the anisotropic hyperfine coupling constant.[9,10] The 0.01 e/au^3 contour clearly demonstrates that the C3 position of plastosemiquinone represents a region of low positive spin density compared with the other ring carbon positions. This does not occur as a result of the isoprenoid unit at the 5 position as identical spin density plots to those shown in Figure 3 are also observed for the trimethylsemiquinone anion radical (unpublished data). The introduction of hydrogen bond donors at the oxygen atoms, Figure 4, leads to an increase in spin density concentration at the C1 and C4 atoms. This is similar to what has been observed for the *p*-benzosemiquinone and durosemiquinone anion forms[9,10,11] where it has been shown that hydrogen bonding to the oxygen atoms of the semiquinone leads to a redistribution of spin density from the oxygen atoms of the carbonyl groups to the carbon atoms. No significant changes, on hydrogen bond formation, are obvious for the C2, C3, C5, and C6 positions.

^{13}C and ^{17}O Anisotropic Hyperfine Couplings. Table 1 shows the anisotropic and isotropic hyperfine coupling constants calculated for the free radicals of Figure 2. The anisotropic hyperfine couplings are a direct reflection of the spin density contours in Figure 4. Depending on the sign of the nuclear magnetic moment, positive (excess α) spin density will give rise to positive or negative hyperfine couplings. For the ^{17}O nucleus, which has a negative magnetic moment, excess α spin gives rise to a negative hyperfine coupling and excess β spin will give rise to a positive isotropic hyperfine coupling, whereas for ^1H and ^{13}C the magnetic moment is positive and the opposite holds. For the oxygen atoms large absolute anisotropic hyperfine coupling values are observed for both, reflecting the large concentration of spin density as shown in Figures 3 and 4. The axial symmetry of the spin density surrounding the oxygen nucleus is reflected in the axial tensor values observed. The ^{13}C anisotropic tensor values for the ring carbon atoms again broadly reflect the contours of spin density surrounding these atom positions in Figure 4. The largest values are observed for the C4 carbon atom. The C1 values are less than the C4 values, which is again in agreement with Figures 3 and 4. Broadly similar values are observed for the C2, C5 and C6 positions. The C3 values are noticeably smaller than these values and is of course a reflection of the low concentration of spin at this position demonstrated in Figures 3 and 4.

The major effect of hydrogen bonding is a decrease in the absolute value of the anisotropic hyperfine coupling values for the oxygen atoms and a corresponding increase in the C1 and C4 values. This is a direct reflection of the spin density transfer from the carbonyl oxygen atoms to the carbonyl carbons demonstrated in Figure 4. Relatively minor changes are observed for the other ring carbon positions. An increase in the number of hydrogen bond donors from one to two, PQ-2CH_3OH to PQ-4CH_3OH, leads to a corresponding decrease in the oxygen values with a corresponding increase in the carbon carbonyl values.

^{13}C and ^{17}O Isotropic Hyperfine Couplings. The isotropic hyperfine couplings arise due to the presence of finite spin at the nucleus in question. This spin at the nuclei of the oxygen and carbon atoms in this radical arises via spin polarization by the π spin density of Figures 3 and 4. Polarization by an atom's own π spin leads to excess α or positive spin at the nuclear position whereas polarization by neighboring spin leads to excess β or negative spin.[9] Depending on the sign of the nuclear magnetic moment this will give rise to positive or negative isotropic hyperfine couplings. For the ^{17}O nucleus, which has a negative magnetic moment, excess α spin gives rise to a negative hyperfine coupling and excess β spin will give rise to a positive isotropic hyperfine coupling, whereas for ^1H and ^{13}C the magnetic moment is positive and the opposite holds. For the oxygen atom the spin polarization arises mainly from the large spin density at its own position, and this excess α spin gives rise to a negative ^{17}O isotropic hyperfine coupling, Table 1. The C1 and C4 carbons, especially for PQ, will have a strong dependence of neighboring spin densities on the isotropic hyperfine couplings. For C1 the atom's own π spin density will contribute positively to its isotropic hyperfine coupling. Negative contributions will come from the C2 and C6 positions.

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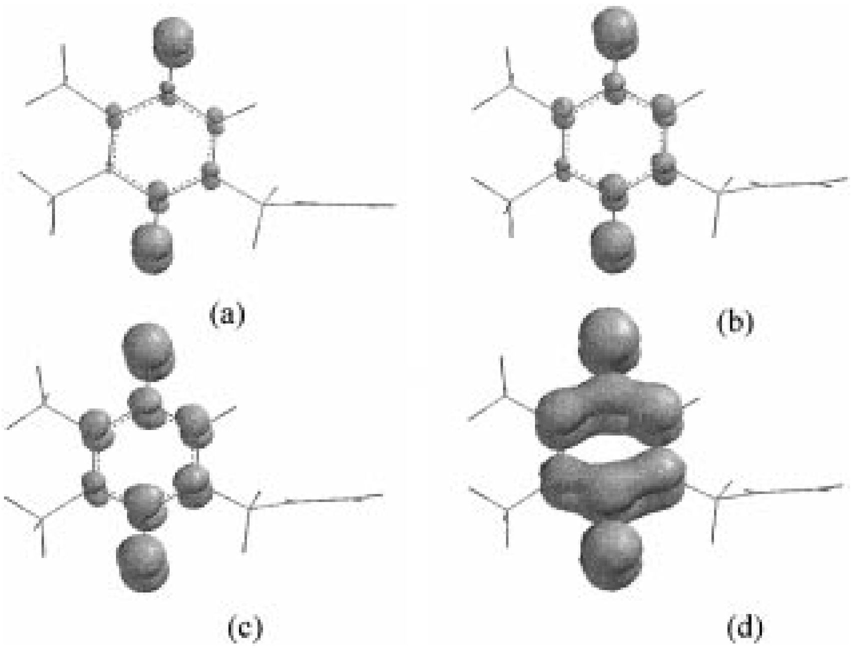

Figure 3. The (a) 0.01, (b) 0.0075, (c) 0.005, and (d) 0.002 e/au^3 spin density (α-β) contours for PQ.

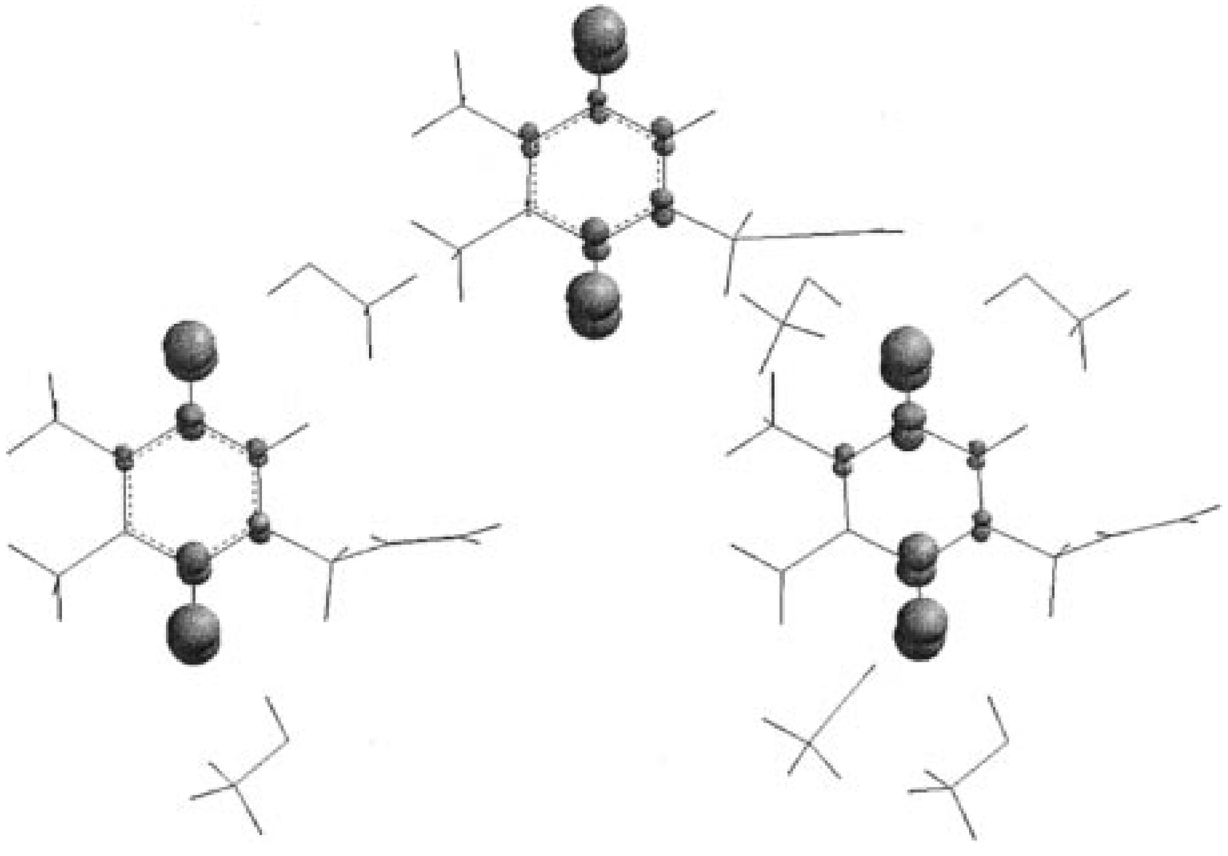

Figure 4. The 0.01 e/au^3 spin density contours for PQ (top), PQ-2CH_3OH (bottom left), and PQ-4CH_3OH (bottom right).

The large spin on the neighboring positions gives rise to an overall negative hyperfine coupling for C1 in PQ. For C4 a similar situation exists. Here, however, the lower π spin at the C3 position can be expected to lead to a less negative contribution to the C4 isotropic coupling. The slightly higher ^{13}C isotropic coupling for C4 of −2.8 G vs the −3.8 G value for C1 reflects this fact.

On hydrogen bond formation the analysis of the spin density plots and the anisotropic hyperfine couplings showed that spin density is transferred from the carbonyl oxygens to the carbonyl carbons. Both of these will lead to an increased positive contribution to the carbonyl carbon ^{13}C isotropic hyperfine coupling. This is indeed the case for the ^{13}C values of Table 1 for C1 and C4 in PQ-2CH_3OH and PQ-4CH_3OH. For PQ-2CH_3OH both the C1 and C4 values increase by 1 G from the PQ values. For PQ-4CH_3OH the C1 value is further increased by 0.9 G whereas a further increase of 1.4 G is observed for C4. For the C2, C3, C5, and C6 positions small isotropic hyperfine coupling values are observed. Again the isotropic hyperfine coupling for these positions will be determined by

Table 1. ^{13}C and ^{17}O Calculated Anisotropic (T) and Isotropic Hyperfine Couplings (G) for the Radical Complexes of Figure 2

	PQ		PQ-2CH$_3$OH		PQ-4CH$_3$OH	
atom	T_{11} T_{22} T_{33}	A_{iso}	T_{11} T_{22} T_{33}	A_{iso}	T_{11} T_{22} T_{33}	A_{iso}
O1	−28.0	−7.0	−26.3	−7.2	−24.0	−6.8
	13.9		13.0		11.9	
	14.1		13.3		12.1	
O4	−27.6	−7.0	−26.6	−7.2	−25.1	−7.2
	13.7		13.2		12.4	
	13.9		13.4		12.6	
C1	4.8	−3.8	6.3	−2.8	7.8	−1.9
	−1.7		−2.5		−3.3	
	−3.1		−3.8		−4.4	
C4	6.2	−2.8	7.8	−1.8	9.8	−0.4
	−2.4		−3.3		−4.4	
	−3.8		−4.5		−5.4	
C6	5.4	0.1	5.0	−0.3	4.6	−0.5
	−2.6		−2.5		−2.3	
	−2.7		−2.6		−2.4	
C3	3.6	−0.8	3.4	−1.0	3.2	−1.3
	−1.8		−1.6		−1.5	
	−1.9		−1.8		−1.7	
C5	5.5	0.1	5.4	−0.1	4.8	−0.5
	−2.7		−2.6		−2.3	
	−2.9		−2.8		−2.5	
C2	4.9	0.5	4.7	0.3	4.9	0.3
	−2.4		−2.3		−2.4	
	−2.5		−2.3		−2.5	
C7	0.3	−1.6	0.3	−1.5	0.3	−1.3
	−0.1		−0.1		−0.1	
	−0.2		−0.2		−0.2	
C8	0.3	−1.1	0.3	−1.1	0.3	−1.0
	−0.1		−0.1		−0.1	
	−0.2		−0.2		−0.2	
C9	0.3	−1.5	0.3	−1.4	0.3	−1.4
	−0.1		−0.1		−0.1	
	−0.2		−0.2		−0.1	

its own π spin and the π spin on the neighboring carbon atoms. The increase in spin density on the C1 and C4 atoms caused by hydrogen bonding will lead to increased polarization by this neighboring spin density on all these isotropic couplings. This will increase the negative contribution to the isotropic couplings for C2, C3, C5, and C6. On increasing the number of hydrogen bond donors, i.e., PQ-2CH$_3$OH to PQ-4CH$_3$OH, all these couplings decrease, Table 1.

For the two methyl group carbons C7, C8 and the C9 atom of the isoprenoid unit the anisotropic couplings are low, reflecting the absence of π spin directly on these carbons. The isotropic coupling is negative, reflecting spin polarization by the nearest ring carbon atom's π spin density. Very similar values have been reported previously by us for the methyl group carbons in the durosemiquinone radical[11] which showed excellent agreement with experimental determinations.

^{1}H Hyperfine Couplings and Comparison with Experimental Determinations. Experimental proton EPR and ENDOR/TRIPLE measurements have been performed on the plastosemiquinone anion radical. EPR measurements in the liquid state have been available for some time,[2] and the hyperfine couplings have been confirmed by recent high-resolution ENDOR and TRIPLE resonance measurements.[4,14] In liquid solution the rapid tumbling of the radical leads to an averaging out of the anisotropic contributions and the experimental observable is the isotropic value. Experiments have also been performed in frozen alcohol and Photosystem II reaction center preparations.[3,4] Here the immobilized state freezes in the anisotropic contributions and the overall (isotropic + anisotropic) coupling is the experimental observable. While for the simple *p*-benzosemiquinone radical an excellent analysis of proton tensors is possible with use of powder ENDOR,[15] for the more substituted forms severe overlapping of spectral lines renders assignments difficult. This is particularly the case for PQ, emphasizing the need for accurate theoretical predictions to aid assignments.

Figure 5. Side view of PQ-4CH$_3$OH after energy minimization demonstrating the out of plane orientation for the two hydrogen bond donors to the O4 oxygen atom on the left.

In Table 2 we compare the calculated proton values of this study with the experimental values. We take the PQ-4CH$_3$OH model to represent the radical state in alcohol solvents. The ring α proton tensor is predicted quite accurately. This tensor is very similar to the values for the unsubstituted *p*-benzosemiquinone radical and the experimental determination is quite reliable. Our calculated values for the methyl groups suggest that they have been incorrectly assigned in the experimental study. From our analysis above it is clear that the 3 position is a relatively low spin density position for PQ. This was reflected in the ^{13}C anisotropic hyperfine coupling of this position discussed above. The spin density on the protons of the methyl groups arises due to hyperconjugation at the C2 and C3 positions. The lower π spin at C3 is reflected in the lower hyperfine couplings for this position compared with the methyl group at C2.

Of particular interest from a biological perspective is the detection of ^{1}H couplings from the hydrogen bonded protons. These were first detected for the *p*-benzosemiquinone anion radical[15] with use of powder ENDOR spectroscopy and are characterized by an essentially dipolar axial tensor having an $A_{\parallel}$ value of 6.0 ± 0.3 MHz and an $A_{\perp}$ value of 2.8 ± 0.3 MHz. When the semiquinone form contains bulky substituents near the quinone carbonyl groups such as durosemiquinone which has four methyl group rotors, the hydrogen bond cannot form in the ring plane, which is the most stable arrangement for the unsubstituted *p*-benzosemiquinone form. Instead the hydrogen bond is forced out of the ring plane and the hydrogen bonds form both above and below the quinone ring plane.[11] Moving the hydrogen bond out of the ring plane has a dramatic influence on the predicted hydrogen bonding hyperfine coupling values.[11] The principal effect is the introduction of a negative isotropic hyperfine coupling for the hydrogen bonded proton. For PQ-4CH$_3$OH the orientation of the hydrogen bond donors after energy minimization is shown in Figure 5. Whereas the two hydrogen donating atoms near the O1 atom are close to the ring plane, the hydrogen donors near the O4 oxygen atom are forced out of the ring plane as demonstrated previously for durosemiquinone.[11] For the PQ radical in alcohol solvents,[3,14] the experimental ENDOR observables attributed to hydrogen bonded protons are a broad band covering a hyperfine coupling

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Table 2. Methyl Group, α, and Methylene Group ^{1}H Calculated Total and Isotropic Hyperfine Couplings (MHz)[a]

	PQ		PQ-2CH$_3$OH		PQ-4CH$_3$OH	
	A_{11} A_{22} A_{33}	A_{iso}	A_{11} A_{22} A_{33}	A_{iso}	A_{11} A_{22} A_{33}	A_{iso}
CH$_3$(2)	8.0	5.5	8.0	5.5	8.8(6.7)	6.2
	4.8		4.6		5.4(3.7)	
	3.8		3.8		4.5(3.7)	
CH$_3$(3)	6.2	3.7	6.2	3.6	6.2(8.6)	3.7
	2.9		2.8		2.9(5.3)	
	2.0		1.9		2.0(5.3)	
CH$_2$(4), a	13.8	11.3	14.3	11.7	19.1	16.3
	10.4		10.8		15.2	
	9.5		10.0		14.5	
CH$_2$(4), b	4.7	1.2	4.9	1.4	−0.7	−0.8
	0.1		0.2		−0.8	
	−1.1		−0.9		−0.8	
H(6)	−1.5	−7.4	−1.1	−7.1	−0.7(−1.3)	−6.6
	−9.6		−9.4		−9.0(−8.0)	
	−11.3		−10.8		−10.1(−9.0)	

[a] For the methyl groups the average value for the three protons is given to compare with the experimental data where rotation of the methyl group leads to a similar averaging effect. Experimental values (14) are given in parentheses along with the PQ-4CH$_3$OH values.

Table 3. ^{1}H Calculated Hyperfine Couplings for the Hydrogen Bonding Protons of PQ-4CH$_3$OH of Figures 2 and 5[a]

atom	A_{11} A_{22} A_{33}	A_{iso}
H(a)	6.3	0.3
	−2.7	
	−2.7	
H(b)	6.5	−0.7
	−4.2	
	−4.4	
H(c)	7.4	−1.2
	−5.7	
	−5.4	
H(d)	6.5	0.2
	−2.8	
	−3.1	

[a] All values are given in MHz. Hydrogens labeled a and d are hydrogen bond donors to O1, b and c are hydrogen bond donors to O4.

range of 7.0 to 3.5 MHz, a relatively sharp band at 2.9 MHz, and a band near the matrix at approximately 1.5 MHz. These were assigned in ref 14 as follows: the broad band was assigned to the $A_{||}$ component of the out of plane and in plane hydrogen bonds while the smaller couplings at 1.5 and 2.9 MHz were ascribed to the $A_{\perp}$ component of the out of plane hydrogen bond and in plane hydrogen bond, respectively.

The calculations of this study would indicate that the broad ENDOR band contains *all* components of the out of plane hydrogen bonding interaction in addition to the $A_{||}$ component of the in plane hydrogen bond. For PQ, in plane hydrogen bonding can occur at the O1 atom, but the presence of two bulky substituents at the O4 atom forces hydrogen bonding interactions out of the ring plane, see Figure 5. We would therefore attribute the broad band observed experimentally to an overlap of the tensors for the hydrogen bonded donors to the O4 carbonyl and the $A_{||}$ of the O1 hydrogen bond donors. The band at 2.9 MHz corresponds to the $A_{\perp}$ components of the O1 in plane hydrogen bond donors. As mentioned above in the experimental studies an ENDOR band was also observed at 1.5 MHz, which was attributed to the perpendicular component of the out of plane hydrogen bond protons. In ref 14 this was given a negative sign and used to derive a positive isotropic coupling value for the out of plane hydrogen bonding interaction. Our values in Table 3 disagree with this assignment. For out of plane hydrogen bonding Table 3 shows clearly that a negative isotropic coupling value is expected. For all hydrogen bonds no total principal hyperfine coupling value is predicted having an absolute value less than 2.7 MHz, Table 3. With the benefit of the density functional calculations of this study we can eliminate the assignment of a 1.5 MHz hyperfine coupling ENDOR band to an out of plane hydrogen bond. This band is likely to occur, *not* from the protons directly hydrogen bonded to the semiquinone carbonyl group, but from a second coordination shell of alcohol molecules.

Conclusions

We have calculated the ^{17}O, ^{13}C, and ^{1}H isotropic and anisotropic hyperfine couplings for the plastosemiquinone anion radical. The usefulness of hybrid density functional methods for hyperfine coupling assignment is demonstrated. Our calculated hyperfine couplings allow us to propose a reassignment of experimental hyperfine couplings for the methyl groups and hydrogen bonded protons. It should now be possible to use hybrid density functional methods to study models of the plastosemiquinone radical in its Photosystem II reaction center environment and combine these studies with EPR/ENDOR studies to investigate the effect of protein binding on the semiquinone electronic structure.

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