Vibrational Spectrum and Torsional Potential of 2-Methoxy-3-methyl-1,4-benzoquinone

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Stable conformations and vibrational spectra of 2-methoxy-3-methyl-1,4-benzoquinone were calculated using density functional methods. Two stable conformers have been found which differ in their vibrational spectrum. Although the calculated infrared spectra of the two conformers differ with respect to several modes, a definite assignment of the experimentally available bands to one of the two conformers is difficult. Protein−chromophore interactions have been studied by modeling quinone−H_2O, quinone−Li^+, and quinone−NH_4^+ complexes. A complexation with water already considerably affects the relative energies of the two conformers and the torsional barrier for the rotation of the methyl part of the methoxy group. In fully optimized quinone−water complexes, vibrational modes in the $C=O$ and $C=C$ stretching mode region are affected through the complexation. Complexation with a positively charged counter ion dramatically changes the energetics of the system and changes the former minimum energy conformation into a saddle point. Vibrational frequencies are more strongly affected than upon complexation with a water molecule.

Introduction

Quinones play an important role in the electron transfer processes of photosynthetic bacterial reaction centers. After light excitation of a bacteriochlorophyll dimer (*special pair*) an electron is transferred via a bacteriopheophytin molecule to a primary quinone Q_A and to a secondary quinone Q_B. The secondary quinone accepts two electrons through this pathway, and from the cytoplasmic side of the protein it receives two protons. Q_BH_2 thus formed leaves the reaction center. Detailed reviews of the primary electron-transfer steps of photosynthesis can be found in refs 1−3.

Quinones at the Q_A and Q_B sites of bacterial reaction centers have been successfully investigated by using FTIR spectroscopy.[4−11] Reconstitution of reaction centers with isotopically labeled quinones allowed to separate the IR absorptions of the quinones from those of the protein and revealed a world of information about protein−chromophore interactions.[7−11] A proper interpretation of IR spectroscopic data, however, requires a detailed understanding of the vibrational spectrum of quinones.

It has been demonstrated in the past that density functional methods can accurately predict frequencies of $C=C$ and $C=O$ vibrations of *p*-benzoquinone[12,13] as well as of methoxy- or methyl-substituted *p*-benzoquinone.[14,15] In the case of 2,3-dimethoxy-1,4-benzoquinone,[14] which served as a model molecule for ubiquinones, we have shown that stable conformations predicted by quantum chemical methods which account for correlation effects (DFT, MP2) and simple semiempirical calculations were qualitatively different. We were furthermore

able to explain differences in bond lengths and bond stretching force constants among the different stable conformers in terms of a resonance model which involves the two methoxy substituents.

In a previous communication we have reported on the mesomeric resonance effect of methoxy groups on the carbonyl frequencies in ubiquinones.[16] In the case of 2-methoxy-3-methyl-1,4-benzoquinone we suggested that the two experimentally detected vibrational modes at 1669 and 1653 cm^{-1} are caused by a planar orientation of the methoxy group. The downshift from 1681 cm^{-1} in 2-methoxy-1,4-benzoquinone to 1669 cm^{-1} in 2-methoxy-3-methyl-l,4-benzoquinone was related to an effect of the added methyl group at C3 and not to a disruption of mesomeric resonance effects caused by an out-of-plane orientation of the methoxy group. The suggestion of a planar conformation of the methoxy group, however, disagrees with semiempirical calculations of this molecule.[17]

It has previously been demonstrated that the electron affinity of a quinone can be tuned through the orientation of methoxy substituents.[17−20] Notably, an experimentally detected redox potential difference of ∼70 mV measured between the two chemically identical ubiquinones Q_A and Q_B in *Rhodobacter sphaeroides*[21] had been attributed to different conformations of the methoxy groups in the protein binding sites.[22] Intermolecular interactions could be one reason for a reorientation of methoxy groups. Furthermore, electrostatic interactions between the quinone and the protein could be one reason for the large differences observed in the vibrational properties of ubiquinone in the two binding sites of the reaction center. While the quinone in the Q_B site is only slightly perturbed compared to solution,[11] the chemically identical quinone in the Q_A site exhibits an

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TABLE 1: Numbering and Definition of Internal Coordinates[a]

number	coordinate	description
2,5	R2, R5	C=C str
1,3,4,6	R1, R3, R4, R6	C−C str
7,10	R7, R10	C=O str
8,13	R8, R13,	C−O str
9	R9	C−C^{methyl} str
11,12,14,15,16,17,18,19	R11, R12, R14, R15, R16, R17, R18, R19	C−H str
20	α7	C−O−C bend
21,22,23	α8, α9, α10	H−C−O bend
24,25,26	α11, α12, α13	H^{methyl}−C−C bend
27	τ7	C−O−C−C tors
28,29,30	τ14, τ15, τ16	H−C−O−C tors
31,32,33	τ17, τ18, τ19	H−C−C−C tors
34,36	δ1, δ3	C=O wag (out-of-plane)
35	δ2	C−O wag (out-of-plane)
37	δ4	C−C wag (out-of-plane)
38,39	δ4, δ5	C−H wag (out-of-plane)
40	$6^{-1/2}$ (α1 − α2 + α3 − α4 + α5 − α6)	ring def (in-plane)
41	$12^{-1/2}$ (2α1 − α2 − α3 + 2α4 − α5 − α6)	ring def (in-plane)
42	2^{-1} (α2 − α3 + α5 − α6)	ring def (in-plane)
43,46	$2^{-1/2}$ (β1 − γ1), $2^{-1/2}$ (β4 − γ4)	C=O def (in-plane)
44	$2^{-1/2}$ (β2 − γ2)	C−O def (in-plane)
45	$2^{-1/2}$ ($2^{-1/2}$ (β3 − γ3)	C−C def (in-plane)
47,48	$2^{-1/2}$ (β5 − γ5), $2^{-1/2}$ (β6 − γ6)	C−H def (in-plane)
49	$6^{-1/2}$ (τ1 − τ2 + τ3 − τ4 + τ5 − τ6)	ring tors
50	2^{-1} (−τ2 + τ3 − τ5 + τ6)	ring tors
51	$12^{-1/2}$ (−τ1 + 2τ2 − τ3 − τ4 + 2τ5 − τ6)	ring tors

[a] Valence coordinates are defined in Figure 1.

unusually large downshift of one of the C=O modes[7−10] as well as a drastic change in the coupling of this C=O mode with C=C modes.[8]

In this contribution we investigate the structure and vibrational spectrum of 2-methoxy-3-methyl-1,4-benzoquinone by using state of the art density functional methods with different aims in mind. Consideration of correlation effects in the applied quantum chemical method is expected to give a more accurate prediction of possible stable conformations with respect to the orientation of the methoxy group than had been achieved by using semiempirical methods.[16,17] At the stationary points, vibrational spectra are calculated and compared to new experimental data. Although 2-methoxy-3-methyl-1,4-benzoquinone is a suitable model molecule for quinones found in photosynthetic reaction centers, it is also a relatively simple molecule since it contains only one flexible methoxy group which can interact with the π-system of the quinone ring. We have therefore used this molecule for carrying out first studies of quinone−protein interaction by modeling simple quinone−water, quinone−Li$^+$, and quinone−NH$_4^+$ complexes.

Methods

Experimental Methods. 2-Methylresorcinol was monomethylated with CH$_3$I/K$_2$CO$_3$[23] to give 2-methoxy-3-methylphenol which reacted with the diazonium salt formed from sulfanilic acid NaNO$_2$/HCl leading to the diazonium acid derivative. The latter was reduced with H$_2$ on Pd/C to obtain 4-amino-2-methyl-3-methoxyphenol which was oxidized with K$_2$Cr$_2$O$_7$[24] to give 2-methoxy-3-methyl-1,4-benzoquinone in 15% overall yield.

The IR absorption spectrum of the quinone was measured as a thin film between two CaF$_2$ plates using a Nicolet 60SX FTIR instrument operated at a resolution of 4 cm^{-1}.

Methods of Calculation. Semiempirical, ab initio, and DFT calculations were carried out with the program package *Gaussian 94*.[25] In semiempirical calculations, the PM3[26] method was applied. Correlation effects have been taken into account either by using perturbation theory at the MP2 level or by applying
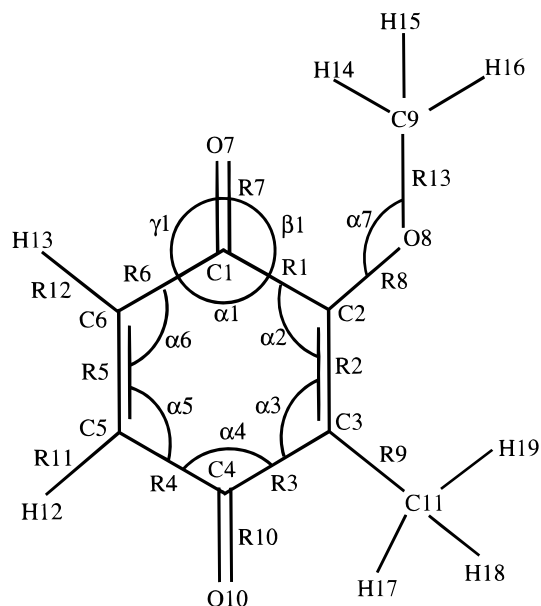
density functional methods. In all DFT calculations, the exchange functional of Becke[27] combined with the gradient corrected correlation functional of Perdew[28] were used. We have applied the standard 3-21G and 6-31G**[29] basis sets in most of the calculations. We denote these calculations as PM3, MP2/3-21G, BP86/3-21G, and BP86/6-31G**, respectively. In some calculations, we have added diffuse functions to the 6-31G** basis set (6-31++G**).[30] For the calculation of the torsional energy profiles presented in Figures 2 and 4, the dihedral angle which defines the orientation of the methoxy group was constrained to selected values while all other structural parameters were fully optimized. For the estimation of solvent effects on the relative energies, the *Cosmo*[31] module of *Gaussian 98*[32] was used.

In the calculations of the potential energy profile of quinones complexed with H$_2$O or Li$^+$, only the distance between the additional molecule or ion and the carbonyl group adjacent to the methoxy group was optimized. The oxygen or lithium was kept on the extension of the corresponding C=O bond. Two quinone−water complexes have afterwards been fully optimized in order to ensure to obtain optimal hydrogen bonding. The two optimized quinone−water complexes were used for modeling quinone−NH$_4^+$ complexes.
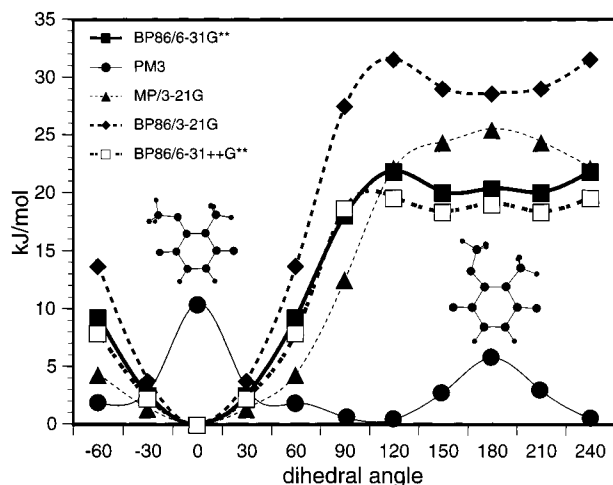
The quantum chemically derived matrix of the cartesian force constants was then read into the program GAMESS[33] which was employed to compute normal modes, force constants of internal coordinates, and the total energy distribution to assign the normal vibrations.[34,35] Internal coordinates for the normal coordinate analysis were defined as proposed by Liu at al.[36] for benzene (see Table 1 and Figure 1). IR spectra from the results of the *Gaussian 94* calculations have been generated with the program G92ToSpec.[37] Molecular structures have been displayed using MacMolPlot.[38]

Results and Discussion

Structures and Energetics. Calculated potential energy profiles for a rotation of the methoxy group around the C−O

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Figure 1. Definition of valence internal coordinates of 2-methoxy-3-methyl-1,4-benzoquinone.

Figure 2. Torsional energy profiles for a rotation of the methoxy group.

TABLE 2: Calculated Structural Parameters (BP86/6-31G**, in Å) of the Two Stable Conformers of 2-Methoxy-3-methyl-1,4-benzoquinone[a]

| | 2-methoxy-3-methyl-benzoquinone | | | |
parameter	conformer 1	conformer 2	2-methoxy-1,4-benzoquinone	1,4-benzo-quinone[b]
R1	1.503	1.524	1.512	1.490
R2	1.379	1.379	1.372	1.355
R3	1.478	1.492	1.463	1.490
R4	1.494	1.490	1.496	1.490
R5	1.350	1.350	1.352	1.355
R6	1.486	1.480	1.485	1.490
R7	1.241	1.234	1.239	1.239
R8	1.357	1.353	1.349	
R9	1.504	1.518		
R10	1.245	1.245	1.244	1.239
R13	1.447	1.437	1.449	

[a] Included are also the data of 1,4-benzoquinone and 2-methoxy-1,4-benzoquinone. The latter molecule has its methoxy group oriented toward the adjacent carbonyl group. [b] Reference 12.

The torsional barrier for a conformational change from conformation **1** to conformation **2** and the relative energies of the two conformers depend strongly on the basis set. The smaller 3-21G basis set overestimates the torsional barrier by nearly 10 kJ/mol or 45% compared to the BP86/6-31G** calculation. It also seems to considerably overestimate the energy difference between the two conformers. Addition of diffuse functions to the 6-31G** basis set, however, does not significantly affect the energetics. The energy difference between the two stable conformations is 20.0 kJ/mol at the BP86/6-31G** level of theory and agrees well with the corresponding calculated energy difference between conformers A and B of 2,3-dimethoxy-1,4-benzoquinone.[14] The calculated energy difference can be expected to depend on the medium due to different solvent effects. We have estimated solvation energies by using the Cosmo method.[31] The calculations predict a reduction of the energy difference between the two conformers in polar environments. In a solvent with a high dielectric constant such as water, conformer **1** is predicted to be still about 11.5 kJ/mol more stable than conformer **2**.

Structural parameters of the two stable conformers are listed in Table 2. Upon addition of a methoxy group to 1,4-benzoquinone in the orientation found in conformer **1**, different effects have to be taken into account. As we have demonstrated in detail in our previous contribution on the structure and vibrational spectrum of 2,3-dimethoxy-1,4-benzoquinone,[14] a mesomeric resonance effect becomes active which weakens bonds R10 and R2 and strengthens bonds R3 and R8. Furthermore, resonance structures which are already present in 1,4-benzoquinone are perturbed. This results in a strengthening of bond R7 and a weakening of bond R1.[14]

These two phenomena are able to explain the behavior of R7 when going from 180° to 90° (see Figure 4 of ref 14). The further reduction of this force constant upon an ongoing rotation of the methoxy group towards the adjacent carbonyl group, however, disagrees with the proposed resonance phenomena. We have postulated that these findings are caused by a weak internal hydrogen bonding which weakens R7 and strengthens R1 and R3.[39] When a methoxy group which points towards the adjacent carbonyl group is added to 1,4-benzoquinone, the combination of these effects results in the following structural changes: Compared to 1,4-benzoquinone, bonds R1 and R2 are elongated by 0.022 and 0.017 Å, respectively, while bond R3 is shortened by 0.027 Å. Smaller changes are found for bonds

bond are presented in Figure 2. Like in previous investigations[14] semiempirical methods predict different stable conformations compared to correlated methods like MP2 or DFT. While two nonplanar energy minima, separated by a small barrier, are predicted by the semiempirical PM3 method, DFT calculations predict as the more stable conformation a planar one which has the methoxy group pointing towards the adjacent C=O group, corresponding to a dihedral angle τ of 0°. The less stable conformation is slightly nonplanar in the vicinity of $\tau = 180°$. The deviation from planarity of about 25° of this conformer is most likely caused by steric interactions between the methoxy group and the methyl group at carbon C3. The two identical nonplanar minima are separated by a very small barrier at 180°. Upon consideration of the zero point energy by using all nonimaginary frequencies (3N-6 modes for the optimized structure and 3N-7 modes for the saddle point), the nonplanar energy minima disappear in favor of the planar conformation at $\tau = 180°$. In the following discussion of structural parameters, vibrational frequencies, and force constants, we therefore compare the two planar configurations which we will denote as conformer **1** (methoxy group points towards the adjacent C=O bond) and conformer **2** (methoxy group points towards the methyl substituent).

R4 (+0.006 Å), R5 (−0.003 Å), R6 (−0.005 Å), and R10 (+0.005 Å). Adding the structural effects we have found upon addition of a methyl group to 1,4-benzoquinone,[15] i.e., a shortening of R1 by 0.007 Å and an elongation of R2 and R3 by 0.006 and 0.014 Å, respectively, reveals in good approximation the structural parameters we have calculated for conformer **1** of 2-methoxy-3-methyl-1,4-benzoquinone.

The above postulated internal hydrogen bonds between the methoxy substituent and the adjacent carbonyl group should of course also exist in one of the conformers of 2-methoxy-1,4-benzoquinone. Although hydrogen bonds stabilize a molecule, this conformer has a higher energy than that having the methoxy group pointing away from the carbonyl group.[19] How can this contradiction be explained? The calculation predicts distances between O−C−H protons and the carbonyl group of 2.50 Å. For comparison, we have calculated the structure of a 3-methyl-1,4-benzoquinone−methanol complex. Here, we find corresponding distances of 3.12 Å. Because of steric restrictions, the methoxy protons are too close to the carbonyl oxygen in the less stable conformer of 2-methoxy-1,4-benzoquinone. This causes a repulsion and raises the energy.

After rotation of the methyl group of the methoxy group of 2-methoxy-3-methyl-1,4-benzoquinone from 0° (conformer **1**) to 180° (conformer **2**) we find the following effects on these bond lengths: bonds R1, R3, and R9 are elongated by 0.021, 0.014, and 0.014 Å, respectively, and bonds R13 and R7 are shortened by 0.010 and 0.007 Å, respectively. According to the simple concepts of mesomeric resonances which are frequently applied in the discussion of vibrational spectra of quinones, there is no reason why the effect of a pure resonance mechanism should differ considerably in conformers **1** and **2**. The predicted structural effects are therefore most likely due to the loss of internal hydrogen bonding. More difficult to understand are the changes found for bonds R9 and R13. Since the conformation of the methyl group is similar in both conformers, the change in R9 is thus most likely also caused by the conformational change of the methoxy group. The lengthening of bond R13 in conformer **1** might also be caused by internal hydrogen bonding. No similar effect, however, could be found in the planar conformation of 2,3-dimethoxy-1,4-benzoquinone.[14]

In 2,3-dimethoxy-1,4-benzoquinone the planar conformation which is stabilized through internal hydrogen bonds is predicted to correspond to the most stable conformer. The predicted vibrational spectrum for this conformer, however, clearly disagrees with experimental data. We explained these findings by assuming that the weak internal hydrogen bonds are broken when energetically favorable hydrogen bonds with solvent molecules or with polar groups in a protein can be formed.[14] A corresponding behavior could also be expected in the case of conformation **1** of 2-methoxy-3-methyl-1,4-benzoquinone. We will demonstrate in the section entitled Modeling of Protein Chromophore Interactions, that a planar conformation of a methoxy group which is stabilized through internal hydrogen bonding is indeed lost upon interaction with a polar or charged group or atom.

Vibrational Spectra and Force Constants. Calculated infrared spectra of the two conformers are depicted in Figure 3. Both conformers show three intensive infrared absorptions in the frequency region between 1500 and 1700 cm^{-1}. The high energy mode in this region is 22 cm^{-1} higher in energy in conformer **2** than in conformer **1**. The splitting between the two high energy modes in the frequency region of the C=C and C=O stretching modes is 19 cm^{-1} in conformer **1** and 38 cm^{-1} in conformer **2**. This difference might serve as a tool for
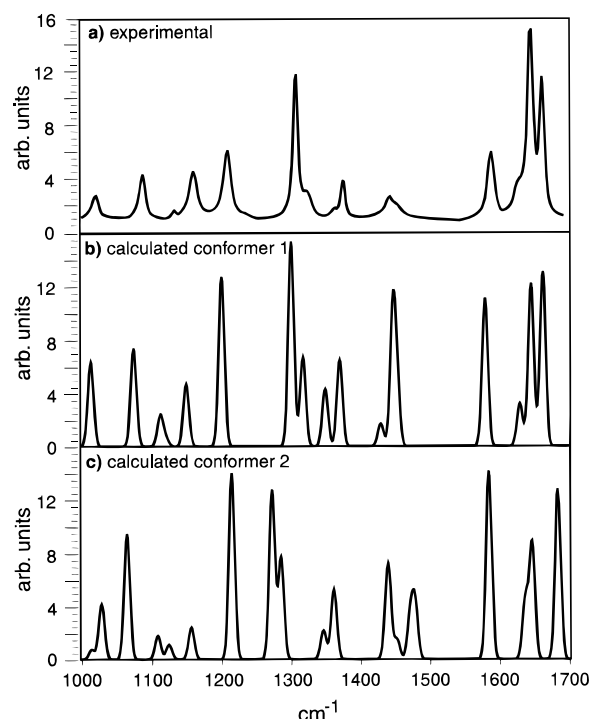
Figure 3. Experimental infrared spectrum and calculated infrared spectra of the two stable conformers of 2-methoxy-3-methyl-1,4-benzoquinone. The IR absorption spectrum of the quinone was measured as a thin film between two CaF$_2$ plates at a resolution of 4 cm^{-1}.

discriminating between the two structures in experimental spectra. Mode decompositions of the more intensive absorptions are listed in Table 3. In both conformers, the high energy mode in the 1500 to 1700 cm^{-1} region corresponds to a predominant C1=O7 mode, followed by a mixed C4=O10/C5=C6 mode, a predominant C4=O10 mode, and a predominant C2=C3 mode. The mode at 1627 cm^{-1} of conformer **1** contains contributions of both C=O modes while no similar mixing is found in conformer **2**.

Experimentally, modes at 1670, 1654, 1635, and 1596 cm^{-1} are observed (see Figure 3a). These bands are in good agreement with previous data[40,17] except that the mode at highest energy corresponds to a clear intensive absorption and that the mode at 1635 cm^{-1} is only a weak shoulder. The three main bands had been assigned to the symmetrical C=O stretching mode, the antisymmetrical C=O stretching mode, and a C=C stretching mode, respectively.[17]

In a previous contribution we compared the IR spectra of 2-methoxy-1,4-benzoquinone and 2-methoxy-3-methyl-1,4-benzoquinone.[16] We concluded that the shift from 1681 cm^{-1} in 2-methoxy-1,4-benzoquinone to 1670 cm^{-1} in 2-methoxy-3-methyl-1,4-benzoquinone is not primarily due to a disruption of mesomeric resonance effects but more likely corresponds to a 6 to 12 cm^{-1} frequency downshift generally observed upon addition of a methyl group to 1,4-benzoquinone.[40] We assumed 2-methoxy-3-methyl-1,4-benzoquinone to correspond to conformer **1**. This comparison between the two molecules is, however, to some extent questionable since the methoxy group has different orientations in the more stable conformer of 2-methoxy-1,4-benzoquinone and in conformer **1** of 2-methoxy-3-methyl-1,4-benzoquinone. In the following, we first discuss a direct comparison of experimental and calculated downshifts and frequencies. We then investigate the effects we expect upon addition of a methyl group to C3 of 2-methoxy-1,4-benzoquinone.

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TABLE 3: Mode Decomposition of Selected Infrared Modes of the Two Stable Conformers of 2-Methoxy-3-methyl-1,4-benzoquinone

	conformer 1		
mode	mode decomposition	exp.	Δ
1661	69% C1=O7 + 10% C5=C6	1670	9
1642	35% C4=O10 + 33% C5=C6	1654	12
1627	52% C4=O10 + 23% C5=C6 + 14% C1=O7	1635	8
1573	59% C2=C3 + 14% C5=C6	1596	23
1448	54% OCH$_3$-bending	1448	0
1360	82% CCH$_3$-bending	1379	19
1344	40% CH^{ring} bending + 32% CC + 11% ring bending		
1320	43% CC + 18% HC^{ring} bending		
1291	21% HC^{ring} bending + 19% CC + 18% C^{ring}O^{methoxy}	1310	19
1199	20% CC + 17% C^{ring}O^{methoxy} + 21% HC^{ring} bending	1210	11
1148	50% OCH$_3$-bending	1161	13
1114	23% HC^{ring} bending + 23% CC + 22% C^{ring}C^{methyl}		
1068	27% HC^{ring} bending	1087	19
1014	23% C^{ring}C^{methyl} + 16% CC	1019	5

	conformer 2		
mode	mode decomposition	exp.	Δ
1683	85% C1=O7	1670	13
1645	58% C5=C6 + 11% C4=O10	1654	9
1634	72% C4=O10 + 16% C=C	1635	1
1581	53% C2=C3 + 12% C5=C6	1596	15
1470	54% ring-CH$_3$ torsion		
1432	40% OCH$_3$-bending	1448	16
1361	86% CCH$_3$-bending	1379	18
1345	58% CH^{ring} bending + 20% CC		
1284	55% CC + 14% HC^{ring} bending		
1271	21% C^{ring}O^{methoxy} + 20% CC + 10% ring bending	1310	39
1216	24% C^{ring}O^{methoxy} + 14% CC + 12% HC^{ring} bending	1210	6
1157	64% OCH$_3$-bending	1161	4
1105	36% HC^{ring} bending + 19% CC + 15% C^{ring}C^{methyl}		
1062	37% HC^{ring} bending + 16% CC	1087	25
1026	26% OC^{methoxy} + 22% C^{ring}C^{methyl}	1019	7

A visual, straightforward comparison of the patterns (frequencies and intensities) of the experimental spectrum with those of the calculated spectra of conformers **1** and **2** suggests that the calculated spectrum of conformer **1** is more similar to the experimental spectrum than that of conformer **2**, notably above 1550 cm^{-1}. The two modes at higher energy are calculated at 1661 and 1642 cm^{-1} for conformer **1** and at 1683 and 1645 cm^{-1} for conformer **2**, respectively, compared to experimental bands at 1670 and 1654 cm^{-1}. The splitting of these two experimental absorptions of 16 cm^{-1} agrees better with the calculated splitting of 19 cm^{-1} of conformer **1** than with that of 38 cm^{-1} of conformer **2**. A comparison of experimental and calculated spectra in the 1500 to 1700 cm^{-1} region in terms of *RMS* deviations results in a better agreement for conformer **2** (12.6 cm^{-1}) than for conformer **1** (16.5 cm^{-1}). The largest deviation (23 cm^{-1}) is found for the CC mode of conformer **1** at 1573 cm^{-1}. This large deviation together with the very small deviation of only 1 cm^{-1} for the mode at 1634 cm^{-1} of conformer **2** might, however, dominate this comparison. A corresponding comparison for the spectral region between 1000 and 1700 cm^{-1}, on the other hand, results in a better agreement for conformer **1** (14.9 cm^{-1}) than for conformer **2** (18.2 cm^{-1}). The largest deviations are now found for conformer **2** (25 and 39 cm^{-1}). According to the mode decompositions, the previously proposed assignment to symmetrical and antisymmetrical carbonyl stretching vibrations is not appropriate since no strong coupling of the two carbonyl stretching modes is predicted by the calculation. Only the mode at 1627 cm^{-1} of conformer **1** shows some coupling between the two C=O modes.

In the following paragraph we discuss how the addition of a methyl group to C3 of 2-methoxy-1,4-benzoquinone affects the vibrational spectrum. Experimentally, a downshift of 11 cm^{-1}

has been found upon addition of a methyl group to 2-methoxy-1,4-benzoquinone. With the previously calculated high energy C=O stretching mode at 1687 cm^{-1} for the more stable conformer of 2-methoxy-1,4-benzoquinone,[14] the calculation predicts downshifts of 26 (2-methoxy-1,4-benzoquinone → conformer **1**) and 4 cm^{-1} (2-methoxy-1,4-benzoquinone → conformer **2**). The latter result agrees thus better with the experimental data. The calculations therefore suggest that in order to achieve a downshift of 11 cm^{-1}, a small conformational change of the methoxy group is most likely needed. For comparison, we have also calculated the vibrational spectrum of 2-methoxy-1,4-benzoquinone in its less stable conformation with the methoxy group pointing toward the adjacent carbonyl group. We find a vibrational frequency for the high energy C=O stretching mode of 1665 cm^{-1} for this conformer. Upon addition of a methyl group, we thus find in both conformers a downshift of only 4 cm^{-1} when no additional conformational changes are allowed. Our calculations agree with previous work[40] in their prediction of a downshift of the C=O stretching mode upon addition of a methyl group; they however suggest that the size of 6 to 12 cm^{-1} is most likely overestimated.

In the spectral region between 1000 and 1500 cm^{-1} the experimental spectrum exhibits a strong absorption at 1310 cm^{-1} and weaker bands at 1448, 1379, 1210, 1161, 1087, and 1019 cm^{-1}. The most intensive band at 1310 cm^{-1} most likely corresponds to the calculated band at 1291 cm^{-1} of conformer **1** or to the mode at 1271 cm^{-1} of conformer **2**. Both modes have contributions of HC^{ring} and ring bending modes and of CC stretching modes. The modes at 1448 and 1379 cm^{-1} agree with calculated vibrations at 1448 and 1360 cm^{-1} (conformer **1**) or at 1432 or 1361 cm^{-1} (conformer **2**). The modes at higher energy are both predominant OCH$_3$ bending modes while the modes around 1360 cm^{-1} are predominant CCH$_3$ bending modes. The band at 1210 cm^{-1} can most likely be assigned to the calculated modes at 1199 cm^{-1} (conformer **1**) or at 1216 cm^{-1} (conformer **2**). Both modes contain CC stretching, C^{ring}O^{methoxy}, and HC^{ring} bending character. The remaining bands at 1161, 1087, and 1019 cm^{-1} can either be assigned to vibrations of conformer **1** determined at 1148, 1068, and 1014 cm^{-1} or to modes of conformer **2** at 1157, 1062, and 1026 cm^{-1}. The vibrations at 1148 and 1155 cm^{-1} are predominant OCH$_3$ bending modes. The modes around 1060 and 1020 cm^{-1} are strongly mixed modes which show some HC^{ring} bending, OC^{methyl} stretching, CC stretching, and C^{ring}C^{methyl} stretching character. Our analysis shows that for most vibrations in this frequency region, the mode decomposition is very similar in both conformers.

Although the similarity of the calculated spectra of the two conformers does obviously not allow an unambiguous assignment of the experimental infrared absorptions to one conformer or the other, we suggest after the preceding analysis of our data that conformer **1** rather than conformer **2** corresponds to the structure investigated by FTIR spectroscopy of the liquid quinone. Because of the slightly different mode decompositions of the vibrations in the 1500 to 1700 cm^{-1} region of the two conformers, isotope labeling might help to discriminate more clearly between the two structures. ^{18}O and ^{13}C labeling of the carbonyl oxygens and ring carbons, respectively, are listed in Table 4. The table illustrates that in this spectral region different shifts can be expected upon ^{18}O labeling of both carbonyl oxygen atoms in the two modes at higher energy as well as upon complete ^{13}C labeling of all ring carbons in some of the modes. Slightly different shifts can also be expected upon ^{13}C labeling of carbons C1 or C4. Unfortunately, no experimental

TABLE 4: Calculated Isotope Shifts (in cm^{-1}) upon ^{18}O and ^{13}C Labeling

		conformer **1**		
ν	$^{18}O_{C=O}$	$^{13}C^{ring}$	$^{13}C_{C1}$	$^{13}C_{C4}$
1661	40	44	50	0
1642	−6	48	−2	6
1627	30	50	−8	32
1573	0	57	0	1
		conformer **2**		
ν	$^{18}O_{C=O}$	$^{13}C^{ring}$	$^{13}C_{C1}$	$^{13}C_{C4}$
1683	35	42	41	0
1645	9	61	−4	2
1634	28	38	3	31
1581	3	55	1	7

TABLE 5: Calculated Force Constants of the CC and CO Bonds (in mdyn/R) of the Two Conformers of 2-Methoxy-3-methyl-1,4-benzoquinone[a]

|	2-methoxy-3-methyl-1,4-benzoquinone			
bond	conformer **1**	conformer **2**	2-methoxy-1,4-benzoquinone	1,4-benzoquinone[b]
R1	4.152	4.022	4.002	4.309
R2	7.777	7.801	7.957	8.687
R3	4.762	4.593	4.866	4.309
R4	4.306	4.428	4.217	4.309
R5	8.892	8.987	8.816	8.687
R6	4.420	4.587	4.425	4.309
R7	11.274	11.704	11.338	11.400
R8	6.551	6.527	6.756	
R9	4.639	4.380		
R10	11.005	11.006	11.028	11.400
R13	4.536	4.595	4.494	

[a] Also included are calculated bond stretching force constants of 1,4-benzoquinone and 2-methoxy-1,4-benzoquinone. The latter molecule has its methoxy group oriented toward the adjacent carbonyl group. [b] Reference 12.

data about isotope shifts of 2-methoxy-3-methyl-1,4-benzoquinone is currently available.

Calculated force constants of the two conformers are listed in Table 5. For comparison, calculated force constants of 1,4-benzoquinone and 2-methoxy-1,4-benzoquinone are included in the table. We note that the force constants of both conformers of 2-methoxy-3-methyl-1,4-benzoquinone are generally in better agreement with those of 2-methoxy-1,4-benzoquinone than with those of 1,4-benzoquinone. In agreement with our discussion of structural properties, we can thus treat the added methyl group basically as a perturbation of 2-methoxy-1,4-benzoquinone. Among the two conformers, deviations are mainly found for the force constants of R7 and R9. As discussed above, the weakening of R7 in conformer **1** is attributed to the formation of internal hydrogen bonds.[14,39] No such simple explanation could yet be found for the behavior of bond R9. Smaller changes in the force constants of bonds R1, R4, and R6, are mainly caused by resonance effects.

Modeling of Protein Chromophore Interaction. In the case of 2,3-dimethoxy-1,4-benzoquinone, a planar conformation with both methoxy groups pointing toward the corresponding proximal carbonyl groups had been predicted to correspond to the most stable conformer.[14] The calculated infrared spectrum of this conformer, however, did not agree with experimental data. We suggested, that this conformation is stabilized through internal hydrogen bonds which, in solution or in a protein, are replaced by energetically more favorable hydrogen bonds. We have now studied for 2-methoxy-3-methyl-1,4-benzoquinone how hydrogen bonding or interaction with an external charge
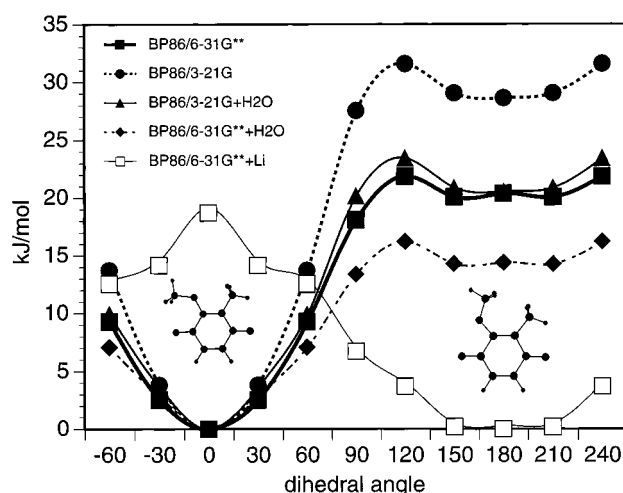
Figure 4. Torsional energy profiles for a rotation of the methoxy group in the free quinone and in quinones interacting with a water molecule or a lithium ion.

affects energetics and vibrational spectra. Torsional energy profiles for the rotation of the methoxy group with and without complexation with water or Li$^+$ are shown in Figure 4. For the quinone−water complex, in a first step only the distance between the two fragment was optimized (see Methods of calculation). The weak hydrogen bond results in a reduction of the torsional barrier of 6 kJ/mol or about 25%. An energy lowering of similar size is also found for the less stable conformer. While the complexation with water significantly affects the energetics of the system, vibrational frequencies determined for these structures, which have been optimized under the application of geometrical constraints, are not much affected. Stronger effects on the vibrational frequencies are however found after complete minimization of the quinone−water complexes. Optimized structures of two fully optimized complexes are shown in Figure 5. In the case of conformer **1** (Figure 5a), the methoxy group is moved out of the ring plane by about 15°. Infrared active modes are found at 1655, 1640, 1634, 1623, and 1572 cm^{-1}. The mode at 1655 cm^{-1} is a predominant C1−O7 stretching mode. The C4−O10 stretching mode is distributed among the modes at 1640, 1634, and 1623 cm^{-1}. The bending mode of the water molecule is not predominantly contained in one of these modes but mixes into several modes. In the structure derived from conformer **2** (Figure 5b) the complexing water molecule disturbs the structure even more and rotates the methoxy group 43° out of the ring plane. Frequencies at 1665, 1662, 1644, 1634, and 1589 cm^{-1} are calculated for this structure. The mode at 1665 cm^{-1} corresponds to a nearly pure vibration of the complexing water molecule. The modes at 1662 and 1644 cm^{-1} are assigned as predominant stretching vibrations of the C=O bonds C1−O7 and C4−O10, respectively. The modes at 1634 and 1589 cm^{-1} are mixed modes containing mainly C=C character. Allowing optimal hydrogen bonding to the interacting molecule does thus destroy the planar conformation of the methoxy groups and causes considerable shifts in the vibrational spectrum.

The potential energy profile of 2-methoxy-3-methyl-1,4-benzoquinone interacting with a positively charged group, modeled by adding a lithium ion, is also presented in Figure 4. The external charge causes a strong perturbation of the system. The formerly more stable conformation with the methoxy group pointing toward the carbonyl group becomes a saddlepoint and the formerly less stable conformation around 180° corresponds now to the energy minimum. In the fully optimized structure,

Vibrational Spectrum of 2-Methoxy-3-methyl-1,4-benzoquinone

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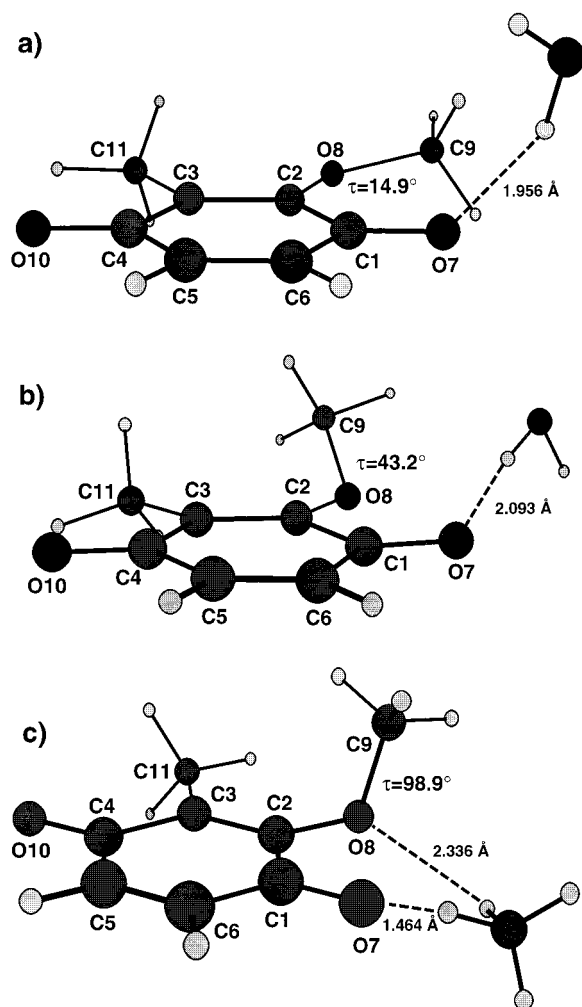
Figure 5. Fully optimized structures of 2-methoxy-3-methyl-1,4-benzoquinone–water and 2-methoxy-3-methyl-1,4-benzoquinone–NH$_4^+$ complexes. The angle τ is defined with respect to the ring plane, independent of the actual orientation.

we find a Li$^+$–O7 distance of 1.76 Å. The vibrational spectrum is more affected by the external charge than after complexation with a water molecule. Modes are calculated at 1648 (87% C4=O10), 1643 (57% C5=C6), 1609 (77% C1=O7), and 1562 cm^{-1} (50% C2=C3 + 14% C5=C6). The absorption at 1609 cm^{-1} is predicted to correspond to the most intensive IR mode, followed by the bands at 1562 and 1648 cm^{-1}. Using the optimized quinone–water complexes for modeling quinone–NH$_4^+$ complexes resulted in a stable structure which is shown in Figure 5c. Again, the planarity of the methoxy group is lost because of the complexation. The ammonium ion bridges the carbonyl and methoxy oxygen atoms. The calculated C=C and C=O modes are 1674 (22% C4=O10), 1670 (67% C4=O10), 1636 (50% C5=C6 + 21% C2=C3), 1590 (44% C2=C3 + 18% C5=C6 + 12% C1=O7), and 1563 cm^{-1} (47% C1=O7). The C1=O7 stretching mode is thus even more downshifted than in the lithium complexes. The dihedral angle of the methoxy group is close to 90°. This makes the resonance effect inefficient and shifts the frequency of the C4=O10 mode up to 1670 cm^{-1}. Interaction of a carbonyl moiety with a positively charged group thus mainly affects the proximal carbonyl group and lowers its frequency by around 70–100 cm^{-1}. The modeled protein–chromophore interaction also nearly completely decouples the C=C and C=O modes. Even though the presently investigated molecule is not a 2,3-dimethoxy substituted

1,4-benzoquinone, it is interesting to note that the modes calculated at 1648, 1643, and 1609 cm^{-1} of 2-methoxy-3-methyl-1,4-benzoquinone interacting with a positive charge might correspond to observed vibrational modes at 1660, 1628, and 1601 cm^{-1} of ubiquinone at the Q$_A$ site of *R. sphaeroides*.[7,10] Our calculations would thus support the suggestion that the positively charged iron–histidine complex which points toward the C1=O7 bond of Q$_A$ is responsible for the downshift to 1601 cm^{-1} of the corresponding mode.

Conclusion

We have presented quantum chemical calculations of the structure and the vibrational spectrum of 2-methoxy-3-methyl-1,4-benzoquinone. Two energy minima with respect to the orientation of the methoxy group exist in the free molecule. In the more stable conformation (conformer **1**), the methoxy group is in the ring plane and points toward the proximal carbonyl group. In the less stable conformation the methoxy group shows a dihedral angle of ~155°. This minimum and the corresponding minimum at 205° are separated by a small barrier. This barrier is so small that after consideration of the zero point energy, the minimum energy is found for the in-plane orientation of the methoxy group (conformer **2**). Although our analysis of calculated and experimental infrared spectra is slightly in favor of conformer **1**, the similarity of the calculated spectra of the two conformers and the lack of experimental isotope shifts make it difficult to unambiguously assign the available data to one conformer or the other.

We have also investigated the energetics of the orientation of the methoxy group in quinones interacting with either a neutral water molecule (hydrogen bonding) or a positively charged ion. Hydrogen bonding lowers the torsional barrier for a rotation of the methoxy group by ~30%. Also, the energy difference between the two stable conformations becomes smaller upon hydrogen bonding. In the 1500 to 1700 cm^{-1} region, quinone infrared frequencies are only weakly affected owing to the complexation with water. These findings change when the quinone–water complexes are fully optimized. Both stable complexes become nonplanar with respect to the methoxy group orientation and the vibrational modes are shifted.

Interaction with a positively charged group dramatically affects the energetic of the methoxy group rotation. The formerly stable conformation with the methoxy group pointing toward the adjacent carbonyl group becomes a saddle point. The new energy minimum is close to that of the previously less stable (conformer **2**), but the methoxy group has a dihedral angle of 15.3° with respect to the ring plane.

The calculations demonstrate how sensitive the energetics of the methoxy group orientation as well as relative energies of stable conformations can depend on protein–chromophore interactions. These results are a confirmation of previously stated speculations that the different properties of Q$_A$ and Q$_B$ in the photosynthetic reaction center of *R. sphaeroides* could be caused by different methoxy group orientations due to differences in protein–chromophore interactions.[17]

The strong effect of even weak interactions on torsional barriers and relative energies demonstrated in this work lets one suspect that an application of purely classical simulation techniques must be limited for such systems. On one hand, a classical simulation can give more insight on the formation of hydrogen bonds in a solvent than our very limited models of interacting systems. On the other hand, the subtle effects of the environment on the electronic structure of the quinone will not be accounted for in a classical force field. A suitable way to

extend our work would be to apply QM/MM hybrid methods invented about ten years ago[41,42] which have currently been extended to the use of DFT methods combined with classical force fields.[43−49]

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