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Time-Resolved Resonance Raman Spectroscopic Studies on the Radical Anions of Methyl-1,4-benzoquinone and 2,6-Dimethyl-1,4-benzoquinone

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Time-resolved resonance Raman spectroscopy (TR3) and density functional calculations have been used to study the effect of asymmetric substitution of methyl group on the structure and vibrational spectra of the radical anion of benzoquinone. The asymmetrically substituted benzoquinones that have been studied are methyl-1,4-benzoquinone (MBQ) and 2,6-dimethyl-1,4-benzoquinone (2,6 DMBQ). Specific vibrational mode assignments have been made to all the vibrational frequencies recorded in the experiment. From the structural changes upon reduction to the radical anion, it has been observed that the unpaired electron in the antibonding π^* orbital is more localized on the C═O bond proximal to the methyl group which results in increase in proximal carbonyl bond length compared to the distal bond. The coupling between the C═O bonds has been analyzed on the basis of the (DFT results) shift in the vibrational frequency upon isotopic substitution of carbonyl carbon and oxygen atoms. Comparison of the (^{18}O) isotopic shift and shift in their C═O stretching frequencies between MBQ and 2,6 DMBQ suggests in the case of 2,6 DMBQ that coupling of the C═O modes with other internal coordinates is considerable for the symmetric C═O stretch compared to asymmetric C═O stretch. Further studies based on site-specific isotopic substitution of the carbonyl carbon and oxygen atoms suggest that symmetric C═O stretch is predominantly from the proximal carbonyl bond and the extent of contribution from the proximal carbonyl bond increases in case of 2,6 DMBQ. The asymmetric C═O stretch has slightly more contribution from the distal carbonyl stretch than the proximal.

Introduction

Quinones play a vital role as acceptors in the electron-transfer reaction found in the photosynthetic bacterial reaction center.[1−3] The reduced intermediates of these biologically relevant quinones have been of considerable interest, in recent times, and therefore have been investigated using transient absorption and Raman methods.[4−17] Interestingly, most of the quinones in the bacterial reaction center bear methyl group substituents and a side chain adjacent to each of the carbonyl group. It is relevant, thus, to study the influence of these groups in bringing changes in the electronic and vibrational structure of the reduced state of quinone. In particular, Fourier transform infrared difference spectroscopy has been extensively used to investigate the nature of binding of quinones to the proteins in the reaction center (RC).[11−17] These measurements have revealed asymmetrical bonding interaction of the neutral quinone with the protein in the Q_A site, whereas, the radical anions have symmetrical bonding interaction.[11] The bonding interaction observed in the radical anion has been ascribed to strong hydrogen bonds between the protein and the two carbonyl of the semiquinone. Further comparison of the FTIR double difference spectra for the RC's containing Vitamin K_1 with those containing a similar quinone system, 2,3-dimethyl-1,4-naphthoquinone, reveals that the side chain does not perturb the binding of these quinones to the RC site.[11] Previously in a computational study, Nonella and co-workers have studied the effect of substituent on the structure and vibrational spectra of ground-state benzoquinone,[18,19] where two types of resonance mechanism have been considered in order to explain the changes observed in the structure of the quinone imparted by the substituent. One of the resonance mechanisms occurs due to internal hydrogen bonding between the substituent hydrogen and the carbonyl oxygen and the second is caused by interaction of the substituent p-orbital with the delocalized π-system of the quinone.[19] The nature and extent of such interactions are expected to influence the structure of the radical anion intermediate, since the addition of an electron in the reduced state of quinone brings a large structural change with respect to the ground state. Density functional calculations on the ground state of methyl substituted benzoquinones have shown that in the case of asymmetric substitution the presence of asymmetry has been reflected in the force constant of C═O bonds and on the nature of vibrational spectra.[18] In the case of the radical anion of ubiquinone the orientation of methoxy groups relative to each other affects the vibrational spectra and force constant of quinone vibrations.[33] In this context it is important to undertake a systematic study on how the structural changes of the radical anion are induced by the asymmetrical substitution. In this paper, a time-resolved resonance Raman spectroscopy (TR3) technique has been used to study vibrational spectra of radical anions of methyl-1,4-benzoquinone (MBQ) and 2,6-dimethyl-1,4-benzoquinone (2,6 DMBQ) generated photochemically in water. Thus the objectives of this paper are to understand the influence of asymmetric substitution (a) on the symmetric and asymmetric C═O frequencies, (b) on the mode composition of the proximal and distal C═O modes, and (c) on the overall structure of the radical anions, both by TR3 experimental and DFT computation studies. The DFT studies have been extended with particular emphasis on identifying the vibrational frequencies by isotopic substitu-

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tion. These results are expected to be very useful to interpret the in-vivo photosynthetic bacterial studies (in particular, structure−function relationship of quinone Q_A and quinone Q_B).

TR3 spectroscopy has been found to be an ideal technique to study reactive intermediates, particularly radical anions of quinones.[5,9,20,21] Although a traditional time-resolved absorption method has been used to study such intermediates, it provides information only on the kinetics and electronic spectra of the intermediates with little or no information on the structural aspects. On the other hand, TR3 spectroscopy can be used to understand the vibrational structure of these intermediates as well.

Density functional theories have been successfully applied in the past for assigning the experimental vibrational frequencies of the ground state and transient species.[22−38] BP86 is one of the well-known hybrid HF/DF functionals which has been well demonstrated to give good agreement with the experimental vibrational frequencies. This method has been successfully applied to the case of benzoquinone[36] and other substituted quinones such as methyl[18,19] and methoxy substituted benzoquinone.[33] Thus, the observed TR3 spectra and structure have been analyzed with the help of the density functional (BP86) calculation. In addition, DFT studies have also been extended to systematically investigate the nature of the mode coupling of the carbonyl modes from the shift in the vibrational frequencies upon ^{18}O and ^{13}C isotopic substitution of the carbonyl carbon and oxygen atoms.

Methods

Experimental Procedure. The experimental apparatus and procedures used for the TR3 spectroscopy have been described in detail previously.[9] Briefly the fourth harmonic 266 nm output of a Nd:YAG laser (DCR-11) was used as a photoexcitation pump source. The probe wavelength at 416 nm was obtained from a homemade H_2 Raman shifter in order to study the photogenerated intermediates. The laser pulses were about 8−10 ns in temporal width and energies of about 1.0 and 0.5 mJ, respectively, for the pump and probe. The delay between the laser pulses was provided by the standard DG535 delay generator. A SPEX 1404 double monochromator was used with two 600 groves grating to disperse the scattered light. A liquid nitrogen cooled CCD (Princeton instrument) with 576 × 376 pixels was used as a multichannel detector. 2,6 DMBQ and MBQ were obtained from Aldrich. The water used was of Millipore grade and sodium nitrite was of analytical grade and used as received. The recorded Raman spectra were calibrated using known solvent bands as reference and the spectral resolution is estimated as ±5 cm^{-1}.

Computational Methods. The hybrid density functional (HF/DF) calculations were performed with Gaussian-94/DFT program on an IBM RS-6000 computer system. The calculations for the radical anion of MBQ and 2,6 DMBQ were carried out employing open shell wave functions and 6-31G(d,p) basis sets. The exchange functional used in the various DFT calculations was a gradient corrected functional of Becke (B).[39] The correlation functionals include the gradient corrected functional of Perdew (P86).[40] Geometry optimization was carried out employing Berny's optimization algorithm. The bond orders were calculated at the optimized geometries using natural bond orbital analysis. The vibrational frequencies and the corresponding normal modes were evaluated for the optimized geometries using analytical differentiation algorithms contained within the program. The normal modes are using the corresponding potential energy distributions (PED) and isotopic shifts.

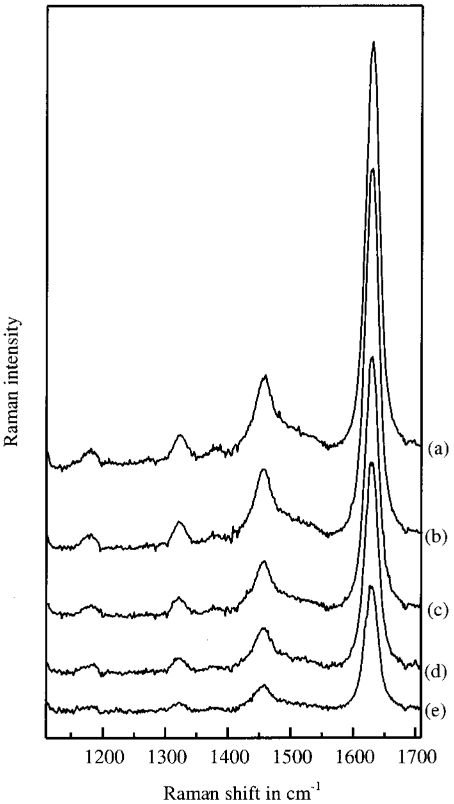

Figure 1. Time-resolved resonance Raman spectra of 2,6-dimethyl-1,4-benzoquinone radical anion from 1 × 10^{-3} M H_2O solution containing 4 × 10^{-2} M $NaNO_2$ time delays (λ_{pump} 266 nm, λ_{probe} 416 nm): (a) 30 ns, (b) 130 ns, (c) 1.1 μs, (d) 2.1 μs, (e) 12.1 μs.

Results and Discussion

The radical anions of both the quinones (2,6 DMBQ and MBQ) are generated upon reducing the triplet state of quinones by nitrite ion. This method has commonly been used to generate radical anion in the case of other quinones.[5,9,41,42] Solution containing the quinone and sodium nitrite in water is photoexcited by pump laser at 266 nm and the resonance Raman spectrum is recorded using 416 nm (probe laser) which corresponds to the absorption maximum of the radical anion for both the quinones. The typical TR3 spectra at various time delays for the radical anion of 2,6 DMBQ are shown in Figure 1. The spectra obtained at 50 ns delay between the pump and probe for both the 2,6 DMBQ and MBQ radical anion are shown in Figure 2a and Figure 2b, respectively. The radical anion bands in the case of 2,6 DMBQ are observed at 1176, 1320, 1453, 1503, and 1628 cm^{-1}, whereas for MBQ, radical anion bands are present at 1157, 1235, 1438, 1510, and 1625 cm^{-1}. The TR3 spectra obtained for these quinones are similar to the resonance Raman spectra of benzoquinone radical anion[41] except for the fact that the presence of substitutent leads to additional bands (resonance enhanced totally symmetric modes). The assignments of the radical anion vibrational frequencies have been carried out with the help of the results obtained from density functional calculation and also by comparison with the reported data on benzoquinone, as discussed below.[36]

The possibility of occurrence of TR3 bands due to a protonated radical is unlikely in these spectra since pK_a for protonation is 4.45 in the case of MBQ and 4.75 for 2,6 DMBQ.[10] Under our experimental conditions of pH 6.0 the formation of a protonated radical is not expected. To confirm this, TR3 measurements on 2,6 DMBQ have also been carried out as a test case at a lower pH. As shown in Figure 2c, the

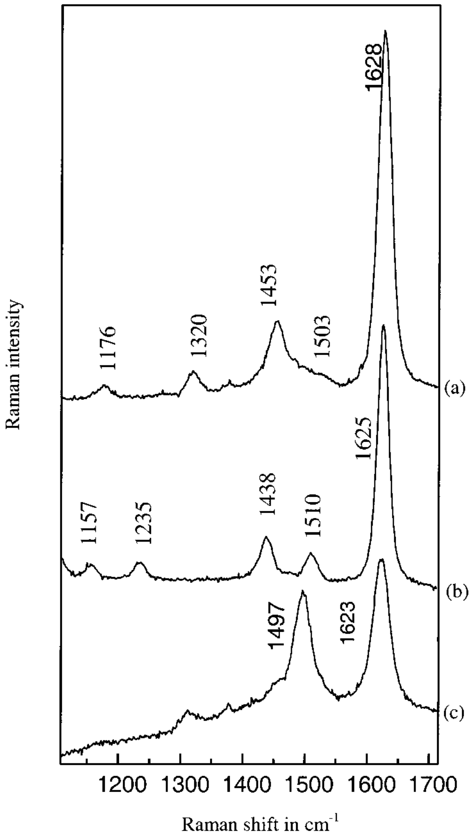

Figure 2. (a) Time-resolved resonance Raman spectra of 2,6-dimethyl-1,4-benzoquinone radical anion from 1 × 10^{-3} M saturated solution containing 4 × 10^{-2} M $NaNO_2$. (b) Time-resolved resonance Raman spectra of methyl-1,4-benzoquinone radical anion from 1 × 10^{-3} M H_2O solution containing 4 × 10^{-2} M $NaNO_2$ (λ_{pump} 266 nm, λ_{probe} 416 nm) at 50 ns delay between the pump and probe laser. (c) Time-resolved resonance Raman spectra of 2,6-dimethyl-1,4-benzoquinone (1 × 10^{-3} M) H_2O solution containing 4 × 10^{-2} M $NaNO_2$ and slight amount of sulfuric acid (λ_{pump} 266 nm, λ_{probe} 416 nm) at 50 ns delay between the pump and probe.

TR3 bands for ketyl radical of 2,6 DMBQ obtained upon slight addition of acid are observed at 1623 and 1497 cm^{-1}, which are distinct from the radical anion spectrum shown in Figure 2a. The 1623 cm^{-1} band is shifted by 5 cm^{-1} with respect to the intense band in the radical anion spectrum, which is comparable to the shift observed for the C=C stretching frequencies of the benzoquinone ketyl radical with respect to the radical anion.[36]

Geometrical Structure. The structural parameters of interest for the ground state and radical anion of benzoquinone, MBQ ($MBQ^{\bullet-}$) and 2,6 DMBQ (2,6 $DMBQ^{\bullet-}$), are given in Table 1 along with the available X-ray diffraction data of the ground state of benzoquinone. The structural parameters calculated by Nonella[18] and co-workers for the ground state of MBQ and 2,6 DMBQ have been included in the table for comparison with their geometrical parameters for the radical anion. The geometrical parameters are defined in Figure 3. These are obtained from the optimized equilibrium geometry corresponding to D_{2h} symmetry in case of benzoquinone, C_s symmetry for MBQ, and C_{2v} symmetry for 2,6 DMBQ.

A close examination of the bond distance obtained for $MBQ^{\bullet-}$ and 2,6 $DMBQ^{\bullet-}$ reveals that the effect of methyl substitution results in inequivalence between the C=O bonds proximal and distal with respect to the methyl groups. Further, the bond length difference between the two carbonyls increases due to the presence of the second methyl group in the case of 2,6 $DMBQ^{\bullet-}$.

TABLE 1: Optimized Geometrical Parameters[a] of *p*-Benzoquinone, Methyl-1,4-benzoquinone, 2,6-Dimethyl-1,4-benzoquinone, and Radical Anions Obtained at BP86/6-31G(d,p) Level

	bond length (Å)					
	benzoquinone		methyl-1,4-benzo quinone		2,6-dimethylbenzo quinone	
structural parameter	ground state	radical anion	ground state[c]	radical anion	ground state[c]	radical anion
C_2-O_1	1.239 (1.222)[b]	1.277	1.240	1.278	1.240	1.280
C_6-O_8	1.239 (1.222)	1.277	1.240	1.277	1.242	1.277
C_4-C_5	1.355 (1.322)	1.382	1.361	1.384	1.359	1.385
C_3-C_7	1.355 (1.322)	1.382	1.354	1.382	1.359	1.385
C_2-C_3	1.490 (1.477)	1.459	1.488	1.457	1.503	1.466
C_2-C_4	1.490 (1.477)	1.459	1.504	1.471	1.503	1.466
C_6-C_7	1.490 (1.477)	1.459	1.490	1.460	1.484	1.459
C_6-C_5	1.490 (1.477)	1.459	1.483	1.461	1.484	1.459

[a] For the definition of parameters, see Figure 1. [b] X-ray diffraction values from ref 48. [c] Ref 18.

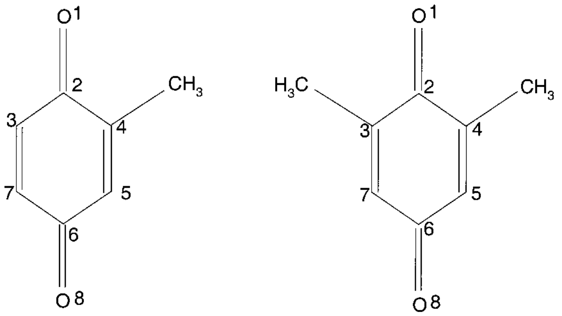

Figure 3. Numbering of the atoms for both methyl-1,4-benzoquinone and 2,6-dimethyl-1,4-benzoquinone.

The bond length of the proximal C_2-O_1 bond length is greater than the distal C_6-O_8 bond. The force constant calculation of the neutral methyl substituted quinones has shown that the C=O bond strength for the proximal carbonyl bond is more than that of the distal.[19] The changes induced by the methyl group in the neutral state have been explained by the interplay of resonance mechanism and hydrogen bonding interaction between the methyl hydrogen and the proximal carbonyl group.[19] The bond length changes of C=O bond upon reduction to the radical anion indicate that the methyl subsitituent localizes the unpaired electron in the antibonding π^* orbital preferentially on the proximal carbonyl group. For example, for the radical anion of 2,6 DMBQ the proximal C=O bond length (C_2-O_1) is elongated by 0.040 Å, whereas change in the distal carbonyl bond (C_6-O_8) (0.035 Å) is comparatively less. In the case of MBQ the C_2-O_1 and C_6-O_8 bond length increases by 0.038 and 0.037 Å, respectively, upon reduction to the radical anion. The overall bond length changes upon reduction to the radical anion are similar to the changes induced by the singly occupied antibonding π^* in the case of benzoquinone. It is noted from density functional calculations on the ubisemiquinone radical anion in the Q_A binding site model of *Rb. sphaeroides*, that hydrogen bonding interaction of the carbonyl group with a doubly protonated imidazole leads to redistribution of spin density from the carbonyl oxygen atom to the carbonyl carbon atom of the semiquinone.[44] In our calculation the spin densities obtained from Mulliken population analysis for radical anion of methylated quinones have shown different spin density distribution on the carbon and oxygen atoms for the two carbonyl groups. Compared to the distal carbonyl spin density distribution, the proximal carbonyl shows increased spin density on the C_2 carbon atom, which is accompanied by the decrease in the spin density of the O_1 atom. The asymmetric spin density

TABLE 2: DFT Vibrational Frequencies (cm^{-1}) and Isotopic Shifts (δ, δ_1, δ_2, δ_3, δ_4)^a (cm^{-1}) of Methyl-1,4-Benzoquinone Radical Anion (MBQ$^{\bullet-}$) As Obtained by BP86/6-31G(d,p) Calculations (experimental values in parentheses)

sym	$^{12}C^{16}O$	PED(%)	δ	δ_1	δ_2	δ_3	δ_4
a′	1118 (1157)	δCH (25), (C−C)$_1$ (43)	2	0	1	3	1
a′	1183	C−C (30), δCH (51)	2	1	1	12	1
a′	1211 (1235)	δCH (17), C−C (69)	0	0	0	1	22
a′	1313	δCH (62), C−C (15)	6	3	3	2	1
a′	1351	δmethylCH (80), (C−C)$_1$(13)	0	0	0	0	0
a′	1396	C−C (55), δCH (26)	1	1	1	9	8
a′	1443	δHCH (42), δmethylCH (17)	1	0	1	1	1
a′	1471 (1438)	CO (39), C=C (44) (C_2O_1 (22), C_6O_8 (17))	21	13	10	15	12
a′	1505 (1510)	CO (22), C=C (43) (C_2O_1 (11), C_6O_8 (11))	7	6	6	10	10
a′	1522	CO (57), C−C (25) (C_2O_1 (27), C_6O_8 (30))	19	6	6	9	10
a′	1612 (1625)	C=C (42), C−C (23)	3	1	2	5	6

^{a} $\delta_1 = \nu^{12}C^{16}O - {}^{12}C_2{}^{18}O_1$, $\delta_2 = \nu^{12}C^{16}O - {}^{12}C_6{}^{18}O_8$, $\delta_3 = \nu^{12}C^{16}O - {}^{13}C_2{}^{16}O_1$, $\delta_2 = \nu^{12}C^{16}O - {}^{13}C_6{}^{16}O_8$, $\delta = \nu^{12}C^{16}O - {}^{12}C^{18}O$.

TABLE 3: DFT Vibrational Frequencies (cm^{-1}) and Isotopic Shifts (δ, δ_1, δ_2, δ_3, δ_4)^a of 2,6-Dimethyl-1,4-benzoquinone Radical Anion (2,6 DMBQ$^{\bullet-}$) As Obtained by BP86/6-31G(d,p) Calculations (experimental values in parentheses)

sym	$^{12}C^{16}O$	PED(%)	δ	δ_1	δ_2	δ_3	δ_4
a$_1$	1156 (1176)	δCH (19), (C−C)$_1$(53)	4	0	4	0	0
a$_1$	1296	δCH (50), C−C (16)	5	3	2	0	1
a$_1$	1351 (1320)	δmethylCH (78), (C−C)$_1$(15)	0	0	0	1	0
a$_1$	1442	δHCH (68), δmethylCH (14)	2	0	2	0	2
a$_1$	1490 (1453)	CO (69), C=C (13) (C_2O_1 (50), C_6O_8 (19))	26	21	12	28	16
a$_1$	1517 (1503)	CO (60), C−C (21) (C_2O_1 (20), C_6O_8 (40))	19	3	9	6	14
a$_1$	1613 (1628)	C=C (43), C−C (20)	2	1	1	4	5

^{a} $\delta_1 = \nu^{12}C^{16}O - {}^{12}C_2{}^{18}O_1$, $\delta_2 = \nu^{12}C^{16}O - {}^{12}C_6{}^{18}O_8$, $\delta_3 = \nu^{12}C^{16}O - {}^{13}C_2{}^{16}O_1$, $\delta_2 = \nu^{12}C^{16}O - {}^{13}C_6{}^{16}O_8$, $\delta = \nu^{12}C^{16}O - {}^{12}C^{18}O$.

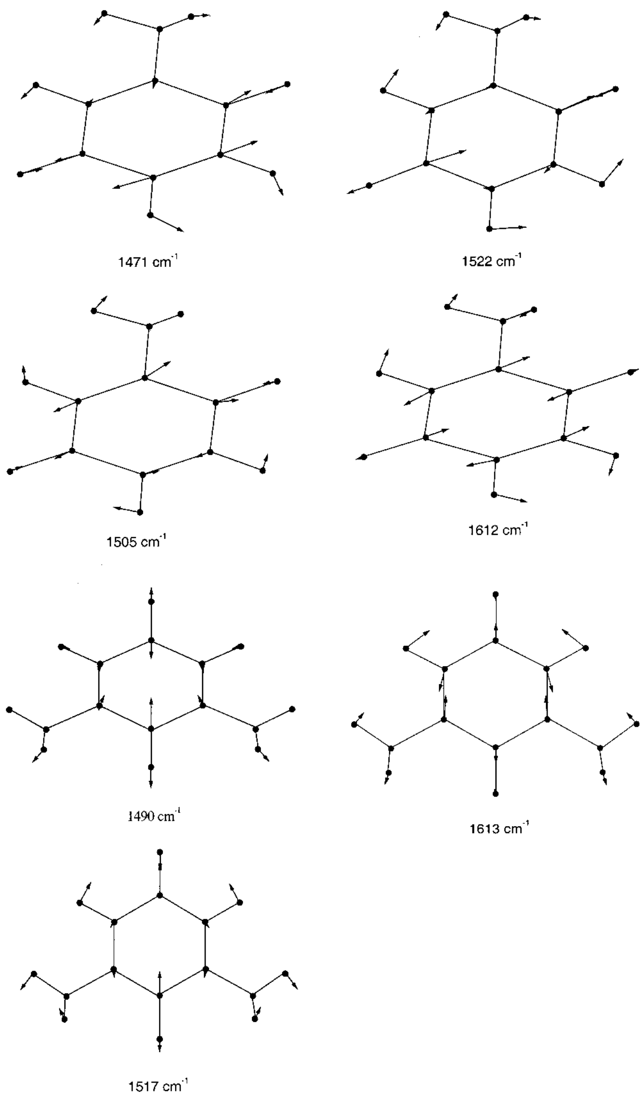

Figure 4. Calculated atomic displacements of normal modes involving C=O and C=C motions and their frequencies (cm^{-1}) of methyl-1,4-benzoquinone and 2,6-dimethyl-1,4-benzoquinone.

distribution suggests hydrogen-bonding interaction between the proximal carbonyl and the methyl hydrogen atoms. In the case of 2,6 DMBQ the effect of hydrogen bonding interaction of the second methyl group enhances the polarization of the spin density from the O$_1$ to the C$_1$ atom, suggesting the through bond effect of methyl groups on the proximal carbonyl.

Vibrational Frequencies and Assignments. The calculated vibrational frequencies, PED, and isotopic shifts along with observed experimental frequencies of MBQ$^{\bullet-}$ and 2,6 DMBQ$^{\bullet-}$ are given in Tables 2 and 3, respectively. The experimental vibrational bands are compared with the calculated vibrational frequency for totally symmetric a′ modes in the case of MBQ$^{\bullet-}$ (C_s symmetry) and totally symmetric a$_1$ modes in the case of 2,6 DMBQ$^{\bullet-}$ (C_{2v} symmetry). From DFT calculation the atomic displacements of the vibrational modes involving C=O, C=C stretch for MBQ$^{\bullet-}$, and 2,6 DMBQ$^{\bullet-}$ are shown in Figure 4. From Figure 4 it can be seen that the two C=O bonds in the case of MBQ$^{\bullet-}$ contract (symmetric $\nu_{C=O}$) for the 1471 and 1505 cm^{-1} whereas for the 1522 cm^{-1} one contracts and the other is elongated (asymmetric $\nu_{C=O}$). It is resonable to expect the symmetric $\nu_{C=O}$ intensities to be enhanced in the resonance Raman spectrum. With this rationale, we assign the observed band at 1438 cm^{-1} to the calculated symmetric $\nu_{C=O}$ at 1471 cm^{-1}. Although the experimental band at 1510 cm^{-1} matches well with the calculated one at 1522 cm^{-1}, it is unreasonable to assign because it relates to asymmetric vibrations of the C=O bonds. Therefore this band has been assigned to the calculated vibration at 1505 cm^{-1}. The calculated isotopic shifts for the symmetric (1471 cm^{-1}) and asymmetric (1522 cm^{-1}) carbonyl stretching (upon ^{18}O labeling of both the carbonyl oxygen atoms) of 21 cm^{-1} and 19 cm^{-1} clearly indicates that they are predominantly C=O stretch. These shifts agree well with the isotopic shift of 24 cm^{-1} of the carbonyl mode at 1452 cm^{-1} observed experimentally for the benzoquinone radical anion in solution.[46] The weakening of the C=O bond following reduction to the radical anion is consistent with the downshift of the calculated symmetric and asymmetric C=O stretching frequency to 191 and 143 cm^{-1}, respectively, compared to the BP86 results reported for its ground state.[18] The most intense band for MBQ$^{\bullet-}$ is observed at 1625 cm^{-1}. It is noted from previous studies reported on benzoquinone that the modes due to in-phase coupling of the C=O and C=C stretches are more intense compared to the out-of-phase coupling modes.[29] Therefore from the present computational results the in-phase coupled motion (see Figure 4) of the C=C and C=O stretches at 1612 cm^{-1} have been assigned to the intense TR3 band at 1625 cm^{-1} having predominantly C=C stretch. The calculated (observed) Raman bands at 1211 cm^{-1} (1235 cm^{-1}) and 1118 cm^{-1} (1157 cm^{-1}) correspond to the calculated (observed) modes of *p*-benzoquinone[36] at 1207 cm^{-1} (1271 cm^{-1}) and 1111 cm^{-1} (1161 cm^{-1}), respectively.

The resonance Raman bands of 2,6 DMBQ$^{\bullet-}$ are observed at 1628 cm^{-1}, 1503 cm^{-1}, 1453 cm^{-1}, 1320 cm^{-1}, and 1176 cm^{-1}. The atomic displacement of the modes obtained from calculation have shown that the 1517 cm^{-1} band relates to asymmetric C═O stretching vibration whereas in the 1490 cm^{-1} band the carbonyl groups vibrate symmetrically. Thus the strong band at 1453 cm^{-1} has been assigned to symmetric C═O stretch at 1490 cm^{-1}. Although enhancement of asymmetric C═O stretch is not pronounced in resonance Raman since the asymmetric 1517 cm^{-1} vibrational band has a_1 symmetry which is not symmetry forbidden, the weak band observed at 1503 cm^{-1} should then correspond to the calculated asymmetric C═O stretch at 1517 cm^{-1}. The calculated isotopic shifts (upon ^{18}O labeling of both the carbonyl oxygen atoms) for the symmetric (1490 cm^{-1}) and asymmetric (1517 cm^{-1}) of 26 cm^{-1} and 19 cm$^{-1,}$ respectively, clearly indicate that they are predominantly C═O stretch. Interestingly, the calculated asymmetric C═O stretch is shifted to lower wavenumber by 5 cm^{-1} compared to those of MBQ$^{\bullet-}$, whereas in symmetric stretch the observed/calculated vibrational frequencies are shifted to higher wavenumber by 15/19 cm^{-1}. From the magnitude of the shift it is inferred that the second methyl group has considerable influence on the symmetric C═O stretch compared to the asymmetric C═O stretching frequency. On the basis of these observations it is suggested that the symmetric C═O stretch is more susceptible to the changes in the electronic structure of the quinone brought by the substituent compared to the asymmetric C═O stretch. Shift of the carbonyl stretching frequencies upon isotopic substitution of both carbonyl oxygen (^{18}O) shows higher shift for the symmetric C═O stretching frequencies of 2,6 DMBQ$^{\bullet-}$ (26 cm^{-1}) compared to MBQ$^{\bullet-}$ (21 cm^{-1}). The calculated asymmetric and symmetric C═O stretching frequencies of 2,6 DMBQ$^{\bullet-}$ are shifted to lower wavenumber by 142 and 151 cm^{-1} compared to the ground state. The shift in the asymmetric C═O stretch is similar to the case of benzoquinone whereas symmetric C═O stretch shows lower shift compared to benzoquinone. The observed band at 1320 cm^{-1} for 2,6 DMBQ$^{\bullet-}$ has been assigned to the calculated (1351 cm^{-1}) C−H bending vibrations of the methyl group whereas the corresponding band is not observed in the resonance Raman spectra of MBQ$^{\bullet-}$. The most intense band at 1628 cm^{-1} has been assigned to the calculated C═C stretch at 1613 cm^{-1}. Compared to carbonyl stretching frequency the perturbation imposed on the C═C stretching frequency is less.

There are reports concerning the nature of coupling of the carbonyl modes for asymmetrically substituted quinones. Meyerson[47] has interpreted the splitting in the IR spectra of asymmetrically substituted quinones as due to independent fundamental vibrations of either proximal or distal carbonyl groups. In the case of 2-methy-1,4-naphthoquinone the B3LYP computed results suggest that the carbonyl bands are split by 5 cm^{-1} and it has been interpreted that the split bands correspond to in-phase (symmetric) or out-of-phase (antisymmetric) vibration of the carbonyl stretching frequencies.[34] With the help of site-specific isotopic substitution (^{18}O and ^{13}C of the proximal and distal carbonyl bonds) we have investigated the nature of coupling of the carbonyl modes for the asymmetrically substituted MBQ$^{\bullet-}$ and 2,6 DMBQ$^{\bullet-}$. The isotopic shift upon site-specific ^{18}O and ^{13}C isotopic labeling of MBQ$^{\bullet-}$ and 2,6 DMBQ$^{\bullet-}$ are given in Table 2 and Table 3, respectively. Labeling separately O_1 and O_8 for MBQ$^{\bullet-}$ results in shift of 13 and 10 cm^{-1}, respectively, of the 1471 cm^{-1} symmetric C═O stretch. In the case of 2,6 DMBQ$^{\bullet-}$ the corresponding shifts for the 1490 cm^{-1} symmetric stretch are 21 and 12 cm^{-1}. From this it is apparent that the vibrational frequency undergoes more shift upon isotopic substitution of the oxygen atom of the proximal carbonyl bond. The difference in the isotopic shifts between the proximal and distal substitution are more pronounced in the case of 2,6 DMBQ$^{\bullet-}$. The isotopic shift upon ^{13}C also shows pronounced effect for $^{13}C_2$ labeling compared to $^{13}C_6$. Consequently it follows that the effect of added methyl substituent proximal to one of the carbonyl group results in a large contribution of the proximal carbonyl stretching to the overall symmetric C═O stretching vibrational frequency. The PED obtained for the symmetric C═O stretching frequencies also indicates higher contribution from the carbonyl bond proximal to the methyl group. In contrast to this, the corresponding isotopic shifts in the case of asymmetric C═O stretching vibration reveal slightly more contribution from distal carbonyl stretch. This is evident in case of 2,6 DMBQ$^{\bullet-}$ which shows $^{18}O_1$ isotopic shift of 3 cm^{-1} and $^{18}O_8$ isotopic shift of 9 cm^{-1} for 1517 cm^{-1} asymmetric C═O stretch. When the carbonyl carbon atoms are substituted by ^{13}C, the isotopic shift for $^{13}C_2$ labeling and $^{13}C_6$ are 6 and 14 cm^{-1}, respectively. In the case of MBQ$^{\bullet-}$, the asymmetric vibrational frequency at 1522 cm^{-1} is identically affected by labeling ^{18}O at O_1 and O_8 oxygen atoms and ^{13}C labeling at C_2 and C_6 carbon atoms. Therefore we infer that the presence of asymmetry is reflected more in the symmetric C═O stretch compared to the asymmetric C═O stretch.

Conclusions

TR3 spectroscopy and density functional calculation have been used to study the effect of asymmetric substitution of the methyl group on the structure and vibrational spectra of the radical anion of benzoquinone. Compared to the ground state, the radical anions of MBQ and 2,6 DMBQ show reverse ordering of the bond length between the proximal and distal carbonyl bonds. The proximal carbonyl bond shows increase in bond length compared to the distal. Bond length changes of C═O bond upon reduction to the radical anion reveal that the π^* antibonding character is more localized on the proximal carbonyl bond compared to the distal bond. The hydrogen-bonding interaction between the methyl hydrogen and the proximal carbonyl group is evident from the polarization of the spin density from the proximal carbonyl oxygen to the carbon atom bonded to it. Assignments of the experimental TR3 bands with the calculated ones have been made on the basis of isotopic shift and PED analysis. The additional second methyl substitutent in 2,6 DMBQ$^{\bullet-}$ results in more shift in the vibrational frequency for the symmetric stretch compared to asymmetric C═O stretch. From the shift in the vibrational frequency upon (^{18}O) isotopic substitution of both the carbonyl oxygen atoms it has been found that the additional methyl substituent does not change the isotopic shift for the asymmetric C═O stretch whereas the isotopic shift for symmetric C═O stretch is affected. Therefore it has been suggested that the second methyl substitutent in case of 2,6 DMBQ$^{\bullet-}$ affects the coupling of the C═O modes with other internal coordinates more for symmetric C═O stretch compared to asymmetric C═O stretch. The results obtained from the site-specific isotopic labeling of the carbonyl oxygen and carbon atoms have shown that the C═O modes for symmetric C═O stretch are less coupled to each other and it is predominantly from the proximal carbonyl group and the contribution from the proximal C═O mode increases considerably in case of 2,6 DMBQ$^{\bullet-}$. The isotopic shift, which reveals the presence of asymmetry of the methylated quinones in the isolated state, might be considerable for these kind of quinones when reconstituted in the RC because of strong interaction with the protein. So comparison of isotopic shift and vibrational spectra of the asymmetrically substituted quinone in in vivo and in vitro will help to understand the interaction of the quinones with the protein in the photosynthetic reaction center.

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