Calculated Molecular Structures Of Anion Ubiquinone In The Gas Phase, In Solvents And In The Q_a Binding Site Of Purple Bacteria Reaction Centers

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Abstract

Structural properties of ubiquinone one anion radical (UQ_1^-) are studied in the gas phase and in Q_A binding site of purple bacteria reaction center using Gaussian 03. Polarizable continuum model (PCM) and Our own N-layered quantum mechanics + molecular mechanics (ONIOM) methods have been used to optimize the UQ_1^- molecule in solvent and in the Q_A binding site of purple bacteria Rhodobactor sphaeroides reaction centers. The UQ_1^- molecule exist four equivalent conformations of methoxy groups in the gas phase and solvents. However, all four conformations reduce to one in the Q_A binding site of the purple bacteria reaction centers. Both carbonyl (C=O) bond lengths are similar in all four conformations in the gas phase and in solvents. However, C4=O bond is slightly longer than C1=O bond in the Q_A binding site. This result infers that Q_A binding site impacts asymmetric interaction on the carbonyl groups of the quinone molecule.

Keywords: *purple bacteria, Q_A binding, ubiquinones, vibrational frequency.*

Introduction

Ubiquinones (UQ_n: 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone) play an important role in biological electron and proton transfer processes that occur in both respiration and photosynthesis [1]. In photosynthetic reaction centers from purple bacteria, two UQ molecules, called Q_A and Q_B, act as terminal electron acceptors [2]. In purple bacterial reaction centers (PBRCs) from *Rhodobacter sphaeroides*, Q_A and Q_B are both ubiquinone-10 (UQ_{10}) molecules. Q_A and Q_B have very different functions, however. Q_A is an intermediary cofactor involved in transferring electrons from bacteriopheophytin to Q_B, while Q_B couples electron and proton transfer processes [3, 4]. The very different redox functions of Q_A and Q_B is testimony to the flexibility of UQs in biological processes. Since Q_A and Q_B are both UQ_{10} molecules, it is clearly the nature of pigment-protein interactions that modulate UQ_{10} functional properties in PBRCs. It is elucidation of these structure function properties of UQs that is at the heart of much current research in photosynthesis.

Fig. 1 shows the structure and numbering scheme for UQ_n. The subscript, *n*, refers to the number of isoprene units in the chain at position 6. Virtually all protein bound UQs contain a poly-isoprene chain. Importantly, experimental FTIR spectra of UQs have been shown to be independent of the number of isoprene units in the chain at position 6, *so long as there is at least one isoprene unit* [5].

Few computational studies aimed at modeling the vibrational properties of ubiquinone (UQ) [6] and ubisemiquinones ($UQ^{\bar{}}$) [7, 8] have been undertaken. The work that has been undertaken [7, 9] is limited in one way or another, for example tail-less quinone models in only the gas phase were considered, using relatively low-levels of theory. Most recent studies [8] in the Q_A binding site gave more emphasis on the effect of non-heme iron and its ligands than the surrounding amino acids on the ubisemiquinone. In this manuscript structural properties of anion ubiquinone one have been studied in the gas phase, in dichloromethane and in the Q_A binding site of Rb. sphaeroides reaction centers.

Materials and methods

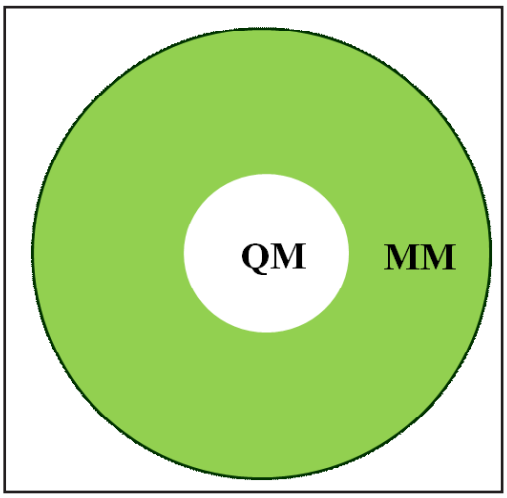
1. Gas and Solvent Phase Calculations

Molecular geometry optimizations and harmonic vibrational frequency calculations of anion ubiquinone were performed using hybrid DFT methods, employing the B3LYP functional and the 6-31+G(d) method within Gaussian 03 [10]. Minimum energy conformations of anion ubiquinones were explored by

calculating single point energy for 10^0 steps of 2- and 3- methoxy dihedral angles. The constrained minimum energy conformers were further optimized in the gas phase and in dichloromethane using PCM method [11] without any constrained.

2. **ONIOM (Our own N-layered Integrated molecular Orbital + molecular Mechanics) for optimizing pigment molecule in the protein binding site**

The UQ molecule is optimized in the Q_A binding site of Rb. sphaeroides RC taken from crystal structure 1AIJ [12] using ONIOM method [13]. The UQ molecule is optimized at quantum level (high level) and the rest of molecules at molecular level (low level). The high level calculation is performed using DFT with B3LYP functional and 6-31+G(d) basis set. Electronic embedding of the protein binding site on the quinone molecule can be obtained using AMBER level of calculation at the low level and Gaussian key word ONIOM = embed. ONIOM layer and Q_A site model prepared for ONIOM calculation is shown in Figure 1.



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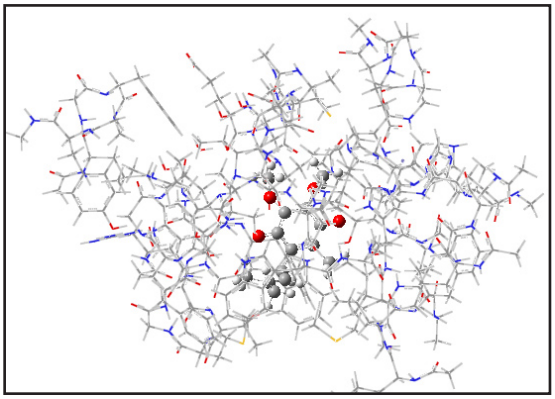

B

Figure 1 A) ONIOM layer, B) 10Å sphere of Q_A binding site prepared for ONIOM calculation. The Q_A molecule is treated at high level and the rest of the molecules are treated at molecular level. This binding site consists of one ubiquinone molecule, 49 amino acids, 7 water molecules and one non-heme iron atom.

Results

1. UQ structure and numbering

Fig. 2 shows the structure and numbering scheme for UQ_n. The subscript, *n*, refers to the number of isoprene units in the chain at position 6. The ubiquinone molecule has two carbonyl groups at C1 and C4 positions, two methoxy groups at positions at C2 and C3, a methyl group at C5 position and an isoprene unit at C6 position. In this work we have removed the part of the tail after the first isoprene unit.

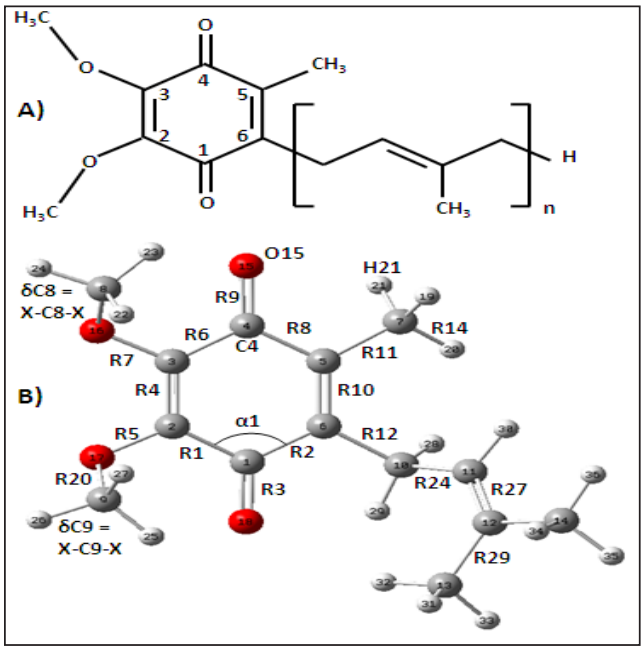

Figure 2 (A) Structure and numbering scheme for UQ_n. The subscript *n* refers to the number of isoprene units associated with the chain attached at position 6. (B) Energy minimized UQ_1 model used in calculations. Atom numbering scheme and brief representations of internal coordinates are also shown. R, α and δ represent for bond stretching, angle bending and combination of angle bending at a vertex atom respectively.

2. Calculated structure of anion UQ_1

Ubiquinone can have various conformations in the gas phase, in solvents and in protein binding sites [7, 14, 15]. The systematic way of exploration of neutral ubiquinone conformers was explained in elsewhere [14]. We have also applied similar technique to find the possible conformers of anion UQ_1.

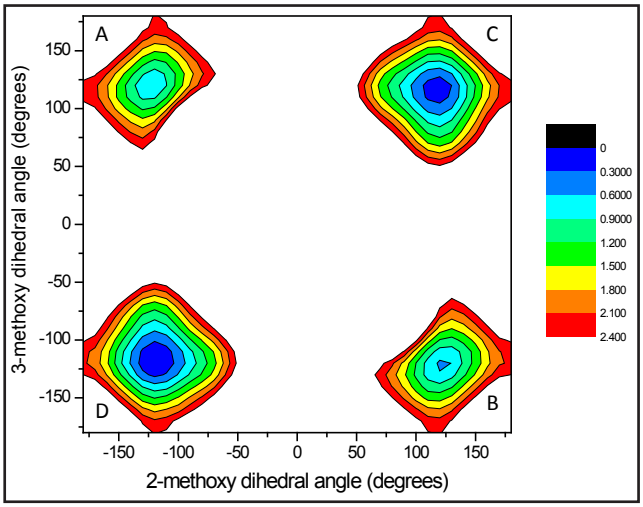

Figure 3 Calculated optimized energy (in kcal/mol) of ubisemiquinone1 for all C2 (C3-C2-O17-C9) and C3 (C4-C3-O16-C8) dihedral angles. The energy axis was shifted so that the lowest energy conformer was set to zero.

Contour plot in Figure 3 shows that there are at least four minimum energy conformers of anion UQ_1 at room temperature. In the gas phase calculation the conformer D with both methoxy angles equal to -120^0 has the lowest energy. Conformer C with both methoxy dihedral angles equal to 120^0 has nearly equal energy to the conformer D. Other two slightly higher energy conformers are A and B which have 2- and 3-methoxy dihedral angles -/+ 120^0 and +/- 120^0.

To find the actual minimum energy anion UQ_1 conformers in the gas phase, the molecule is geometry optimized without any constrains at the same level of theory with the starting geometry chosen for 2- and 3-methoxy dihedral angles -/+ 120^0 and +/- 120^0. Calculated geometry parameters and relative energy of anion UQ_1 are shown in Table 1. As shown in Table 1, the anion UQ_1 conformer again D has the lowest energy in the gas phase. Conformer C has slightly higher energy than conformer D. Conformers A and B have energy nearly equal to thermal energy at room temperature (0.592 kcal/mol) compared to the energy of conformer D. Molecular geometry slightly differs in solvent from the gas phase geometry. The conformer C has the lowest energy in dichloromethane. The energy of conformer B is closer to the energy of conformer C than the energies of conformers A and D.

The dihedral angles of the methoxy groups of the anion UQ_1 conformers in the gas phase are found within +/- 5^0 values from the corresponding dihedral angles calculated with the constrained dihedral angles. However, methoxy group dihedral angles of anion UQ_1 in dichloromethane differ by +/- 20º from the gas phase values and they are now closer to 90º. Surprisingly, the 2-methoxy group dihedral angle rotated a lot in the Q_A binding site of Rb. sphaeroides RC. The -35º orientation of 2-methoxy group is not expected from the contour plot shown in Figure 3. This result clearly implies that the Q_A binding site has an impact on the anion UQ molecule.

The bond lengths differ by less than 0.001Å among different conformers in the gas phase and in solvent. The calculated carbonyl bond lengths in this work are mid-way between previously calculated values [7, 16]. The C=C and C=O bond lengths in anion UQ in the gas phase and dichloromethane are shorter than the corresponding values in the Q_A binding site. The observed variation of C-C and C-O bonds is negligible to change properties of ubisemiquinone molecule. The UQ tail angle also does not vary among the conformers in the gas phase and in solvent but is 4^0 higher in the Q_A binding site than in the gas phase.

Table 1 A) Calculated bond lengths, tail angle, methoxy dihedral angles and the relative energy of anion UQ_1

conformers in the gas phase.

Ubisemiquinone Parameters	UQ^- Conformers in the Gas Phase			
	A	B	C	D
R3(C1=O)	1.273 Å	1.273 Å	1.273 Å	1.273 Å
R9(C4=O)	1.272 Å	1.272 Å	1.271 Å	1.271 Å
R4(C2=C3)	1.380 Å	1.380 Å	1.379 Å	1.379 Å
R10(C5=C6)	1.384 Å	1.384 Å	1.384 Å	1.384 Å
R1(C1-C2)	1.457 Å	1.457 Å	1.456 Å	1.457 Å
R6(C3-C4)	1.457 Å	1.458 Å	1.457 Å	1.457 Å
R2(C1-C6)	1.461 Å	1.461 Å	1.461 Å	1.461 Å
R8(C4-C5)	1.459 Å	1.459 Å	1.459 Å	1.459 Å
R5(C2-O)	1.380 Å	1.381 Å	1.381 Å	1.381 Å
R7(C3-O)	1.380 Å	1.380 Å	1.380 Å	1.380 Å
R27(C11=C12)	1.345 Å	1.345 Å	1.345 Å	1.345 Å
C6-C10-C11	113.4^0	113.5^0	113.5^0	113.5^0
C3-C2-O-CH3	-121.8^0	120.8^0	116.7^0	-117.3^0
C2-C3-O-CH3	122.4^0	-123.2^0	118.3^0	-119.0^0
ΔE (kcal/mol)	0.5366	0.6457	0.1062	0

Table 1 B) Calculated bond lengths, tail angle, methoxy dihedral angles and the relative energy of anion UQ_1 conformers in dichloromethane and in Q_A binding site of purple bacteria RCs.

Ubisemiquinone Parameters	In Q_A Binding Site		UQ^- Conformers in Dichloromethane (DCM)			
	Q_ACS	Q_A^-	A	B	C	D
R3(C1=O)	1.234 Å	1.272 Å	1.274 Å	1.275 Å	1.274 Å	1.274 Å
R9(C4=O)	1.232 Å	1.281 Å	1.276 Å	1.276 Å	1.276 Å	1.276 Å
R4(C2=C3)	1.404 Å	1.382 Å	1.378 Å	1.379 Å	1.377 Å	1.378 Å
R10(C5=C6)	1.419 Å	1.383 Å	1.385 Å	1.385 Å	1.386 Å	1.386 Å
R1(C1-C2)	1.400 Å	1.463 Å	1.457 Å	1.456 Å	1.455 Å	1.456 Å
R6(C3-C4)	1.405 Å	1.449 Å	1.454 Å	1.455 Å	1.454 Å	1.453 Å
R2(C1-C6)	1.413 Å	1.460 Å	1.461 Å	1.461 Å	1.461 Å	1.462 Å
R8(C4-C5)	1.407 Å	1.452 Å	1.459 Å	1.458 Å	1.458 Å	1.459 Å
R5(C2-O)	1.393 Å	1.370 Å	1.380 Å	1.381 Å	1.381 Å	1.380 Å
R7(C3-O)	1.394 Å	1.383 Å	1.381 Å	1.380 Å	1.381 Å	1.381 Å
R27(C11=C12)	1.380 Å	1.445 Å	1.345 Å	1.345 Å	1.345 Å	1.345 Å
C6-C10-C11	113.03^0	117.22^0	113.0^0	113.0^0	113.0^0	112.9^0
C3-C2-O-CH3	-57.06^0	-35.28^0	-113.6^0	111.2^0	101.4^0	-107.7^0
C2-C3-O-CH3	109.54^0	123.10^0	110.2^0	-112.3^0	102.8^0	-103.9^0
C1=O---N_{Ala}	2.837 Å	2.790 Å				
C4=O---N_{His}	2.788 Å	2.798 Å				
ΔE (kcal/mol)			0.336	0.099	0	0.282

Parameter values for Q_A were taken from purple bacteria crystal structure 1AIJ [12]. CS= crystal structure values; C=O---N = hydrogen bonding distance between carbonyl oxygen and alanine backbone or histidine side chain nitrogen.

Discussions

Properties of ubiquinone molecule depend on the methoxy group orientation, redox state and interaction with the surrounding molecules [7, 14-22]. The rotational flexibility of methoxy groups is an inertial property of ubiquinone molecule which determines conformation and various properties of the molecule. In principle the dihedral angle of methoxy groups can be varied from 0 to 360 degrees. However, calculations show that the minimum energy state structures have either $0^0/ \pm 120^0$, $\pm 120^0/ 0^0$ or $\pm 120^0/ \pm 120^0$ in the neutral UQ giving rise to at least eight conformers [14] in the gas phase or in solvents. Contour plot in Figure 3 shows that minimum energy ubisemiquinone conformers localized near both methoxy angles closer to +/- 120º in the gas phase. Out of four conformers C and D have lower energy than conformers A and B in the gas phase. However, polarity of the solvent causes the methoxy groups orient more perpendicularly to the ubiquinone ring in solvent than in the gas phase and also the minimum energy conformers changes to B and C. We have not observed non-symmetric conformation with isoprene unit as observed by Marco

Nonella for ubisemiquinone without isoprene unit [7]. The non-symmetric ubisemiquinone conformer b observed by Marco Nonella has more than 5 times thermal energy compared to the symmetric conformers at ambient temperature 298 K. The non-symmetric ubisemiquinone conformer has much less probability to be observed at 298 K in solvent, however.

Conformer A is preferred by the Q_A binding site of Rb. Sphaeroides RC in the neutral state [15]. The 2- and 3- methoxy dihedral angles of UQ respectively change by -10º and -28º on reduction in the Q_A binding site, however, this modification does not alter overall conformation of UQ molecule. The increase in C=C and C=O bond length increases the size of the quinone ring on reduction of UQ molecule. The increase in the size of UQ molecule on the other hand decreases the hydrogen bonding distance between carbonyl oxygen and corresponding nitrogen from the protein binding site. The C4=O bond is longer than C1=O bond despite longer C4=O---N-His M219 hydrogen bonding than C1=O--- N-Ala M260 hydrogen bonding. This result clearly suggests that the protein binding site has asymmetric interaction with quinone carbonyl groups in the Q_A binding site of Rb. sphaeroides reaction centers. One of the major asymmetric effects causes from the doubly ionized iron atom, which pulls the negatively charged oxygen in the C4=O group causing the increase in bond length.

Because of molecular symmetry, the C2=C3 and C5=C6 stretching vibrations as well as C1=O and C4=O stretching vibrations in ubisemiquinone couple to give in phase and out of phase vibrations. The in phase vibration is Raman active [23] and the out of phase vibration is infrared active [24]. The in phase C=C vibration of ubisemiquinone gives an intense Raman band around 1607 cm^{-1} in solvents [22]. The change in the size of the C=C bond length of ubisemiquinone in Q_A site is not enough to alter Raman band position from the solvent values. Similar sizes of two C=O bonds of ubisemiquinone are responsible to an intense infrared band in solvents [17]. However, asymmetric interaction of protein binding site resulted C4=O bond longer than C1=O bond in Q_A site of Rb. sphaeroides reaction centers. These two carbonyl stretching vibration should also couple to either with C=C asymmetric vibration or methyl bending vibrations to give three intense infrared bands of ubisemiquinone in Q_A binding site [25, 26].

Conclusion

Ubisemiquinone ring C=C and carbonyl groups, which are responsible to the intense Raman and infrared bands, can be calculated from the optimized molecule. Size of UQ_1^- ring C=C bond lengths are similar in the gas phase, polar solvent and the Q_A binding site of purple bacteria reaction centers. This result is coherent with the experimental Raman spectra of ubisemiquinone [22]. Both carbonyl bonds of UQ_1^- similarly increases in size in polar solvent compared to the gas phase values. However, C4=O bond length is longer than C1=O in the Q_A binding site of Rb. sphaeroides reaction centers. This result shows that Q_A binding site asymmetrically interacts with two carbonyl groups of ubiquinone. The inequivalence between two carbonyl bonds is also responsible to the splitting of intense infrared band in the Q_A binding site [17, 25, 26].

Acknowledgements

I would like to thank Dr. Gary Hastings for providing me guidance and technical support through the IBM System p5 supercomputer at Georgia State University. This computer was acquired through a partnership of the Southeastern Universities Research Association and IBM, with additional support from the Georgia Research Alliance.

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Abbreviations: Density functional theory, DFT; difference spectra/spectrum/spectroscopy, DS; Fourier transform infrared, FTIR; infrared, IR; integral equation formalism; IEF; purple bacterial reaction centers, PBRCs; polarizable continuum model, PCM; ubiquinone, UQ;