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Letter

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The 2-Methoxy Group Orientation Regulates the Redox Potential Difference between the Primary (Q_A) and Secondary (Q_B) Quinones of Type II Bacterial Photosynthetic Reaction Centers

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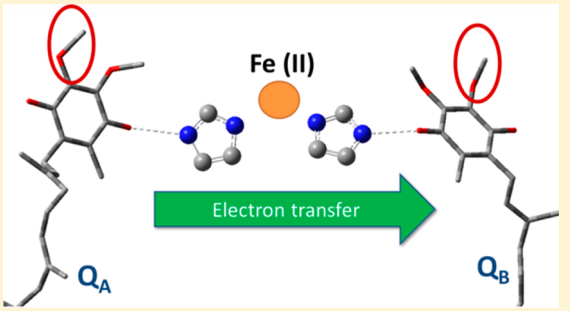
Supporting Information

ABSTRACT: Recent studies have shown that only quinones with a 2-methoxy group can act simultaneously as the primary (Q_A) and secondary (Q_B) electron acceptors in photosynthetic reaction centers from purple bacteria such as *Rb. sphaeroides*. ^{13}C HYSCORE measurements of the 2-methoxy group in the semiquinone states, SQ_A and SQ_B, were compared with DFT calculations of the ^{13}C hyperfine couplings as a function of the 2-methoxy dihedral angle. X-ray structure comparisons support 2-methoxy dihedral angle assignments corresponding to a redox potential gap (ΔE_m) between Q_A and Q_B of 175−193 mV. A model having a methyl group substituted for the 2-methoxy group exhibits no electron affinity difference. This is consistent with the failure of a 2-methyl ubiquinone analogue to function as Q_B in mutant reaction centers with a ΔE_m of ∼160−195 mV. The conclusion reached is that the 2-methoxy group is the principal determinant of electron transfer from Q_A to Q_B in type II photosynthetic reaction centers with ubiquinone serving as both acceptor quinones.



SECTION: Biophysical Chemistry and Biomolecules

Type II reaction centers (RCs) from anoxygenic and oxygenic photosynthetic RCs contain two quinones Q_A and Q_B that function in series as electron acceptors (Figure 1).[1,2] Following charge separation in the RC, Q_A is one-electron-reduced, generating the semiquinone radical form SQ_A, which then transfers the electron to Q_B, forming SQ_B. In the anoxygenic species *Rhodobacter (Rb.) sphaeroides*, and many others, Q_A and Q_B are chemically identical ubiquinones, and yet forward electron transfer is thermodynamically favorable by 60−75 mV.[3]

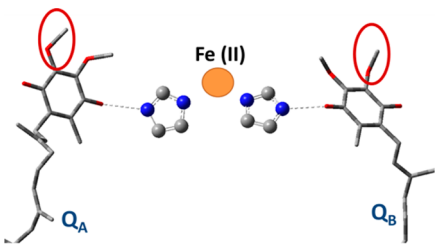


Figure 1. Q_A and Q_B quinones in the *Rb. sphaeroides* RC (coordinates from PDB ID: 3I4D). Hydrogen bond acceptance by the O4 atom of each quinone from the imidazole group N_δ of His-M219 (Q_A) and His-L190 (Q_B) is illustrated, as well as the Fe(II) atom that bridges the imidazoles. The 2-methoxy group of each quinone is circled in red.

The tuning of cofactor redox potentials is critically important to biological function and can often be accounted for by the electrostatic environment provided by the protein solvation.[4] This appears to be sufficient for electron transfer in oxygenic Photosystem II (PS II), where plastoquinone is active in both quinone sites. However, it cannot account for the unique ability of ubiquinone and other 2-methoxy-containing quinones to simultaneously fulfill Q_A and Q_B activity in *Rb. sphaeroides* RCs.[5] This was clearly demonstrated using two synthetic analogues of ubiquinone in which one or the other of the two methoxy groups was replaced by a methyl group, 2-methoxy-3,5-dimethyl-6-tetraisoprenyl-1,4-benzoquinone (2-MeO-Q) and 3-methoxy-2,5-dimethyl-6-tetraisoprenyl-1,4-benzoquinone (3-MeO-Q). Both can fully reconstitute Q_A function, but only

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2-MeO-Q was also active as Q_B; 3-MeO-Q was completely inactive.[6] This points to a factor unique to the 2-methoxy group in determining functionality in the Q_B site.

The orientation that a methoxy group makes with the quinone ring plane has been previously investigated with regard to its influence on the quinone electron affinity and resultant redox potential (E_m) value.[7−9] Qualitatively, it can be reasoned that when the methoxy group is out of the plane of the quinone ring, the main influence is the electron-withdrawing nature of the electronegative oxygen, leading to a relatively increased electron affinity. When the methoxy is in plane, the oxygen p orbitals can conjugate with the π-system of the quinone, causing electron donation to the ring, leading to a decreased electron affinity. The orientations of the methoxy groups for the Q_A and Q_B sites should, in principle, be obtainable from the atomic-level structural information available from crystal structure determinations on RC preparations. However, this is precluded by a lack of conformity on the methoxy orientations in Q_A and Q_B in the numerous available X-ray structures.[10]

We have recently introduced an additional method of estimating methoxy group orientation by using hyperfine sublevel correlation (HYSCORE) measurements of the semiquinone radicals (SQ_A and SQ_B) in RCs containing ubiquinone ^{13}C-labeled at the two methoxy groups.[11,12] The 2-methoxy groups in Q_A and Q_B were shown to give rise to quite distinct ^{13}C isotropic hyperfine coupling (hfc) values with the magnitude of SQ_B exceeding that of SQ_A. Comparison of these couplings with quantum mechanically predicted values for a small model (6-methyl-ubisemiquinone) as a function of the methoxy orientation demonstrated that the larger value for SQ_B could be at least qualitatively explained by a more out-of-plane orientation of the 2-methoxy group compared with that of SQ_A. As this was also associated with a higher electron affinity value, the higher redox potential of the Q_B ubiquinone was easily rationalized.[11,12] However, other computational approaches have indicated that the midpoint potential difference between Q_A and Q_B can be accounted for by classical electrostatics,[13] such that the added effect of the 2-methoxy group orientation would be in excess of the experimental difference of 60−75 mV.

To address this, in this Letter we carry out a full quantitative analysis of the methoxy group orientation using a larger model (Figure S1a, Supporting Information), computed over the full range of ±180°. This allows us to fully explore the complete orientation dependence of the methoxy group and directly compare with experimental determinations. We also investigate models for 2-MeO-Q and 3-MeO-Q (Figures S1b and S1c, Supporting Information), which have been instrumental in experimentally demonstrating the key role played by the 2-methoxy group in controlling the redox potential of the ubiquinone.

The theoretical dependence of the 2-methoxy isotropic ^{13}C hfc on the methoxy orientation for our ubisemiquinone model is shown in Figure 2. The ^{13}C couplings (A_{iso}) for the 2-methoxy group in SQ_A (1.3 MHz) and SQ_B (5.7 MHz, adjusted to the same unpaired spin density (0.11) on C_2)[12] are indicated by the solid horizontal lines. This defines four possible dihedral angles in the two SQs (see Figure 2 legend). In a survey of X-ray structures at resolutions of at least 2.8 Å, the average values for the 2-methoxy dihedral angles ($C_mO_mC_2C_1$) of Q_A and Q_B were Q_A = +139 ± 25° and Q_B = −90 ± 9°, showing that the 2-methoxy group is located on opposite sides of the ring for the two quinone sites and that the Q_A site quinone has a 2-methoxy

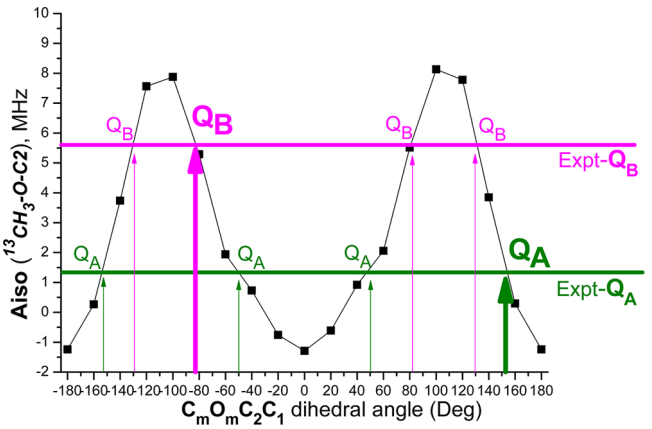

Figure 2. Variation in the 2-methoxy $^{13}C_m$ isotropic hfc as a function of the $C_mO_mC_2C_1$ dihedral angle for the model shown in Figure S1a (Supporting Information). The estimates for the 2-methoxy dihedral angles, giving agreement with experimental determinations, are indicated by green (Q_A) and pink (Q_B) vertical arrows, Q_A (−155°, −50°, 50°, 155°) and Q_B (−130°,-82°, 82°,130°). The best agreement with X-ray $C_mO_mC_2C_1$ dihedral angle values are highlighted in bold vertical arrows. Experimental Q_A and Q_B 2-methoxy ^{13}C hfc values for the ubsemiquinone radical are indicated as solid horizontal lines.

orientation relatively closer to the ring plane.[10] One can see that the best agreement when comparing our estimated dihedral angles with the experimental X-ray range is +155° for SQ_A and −82° for SQ_B. These are shown in Figure 2 by the solid vertical arrows.

Figure 3 gives the variation in electron affinity value as a function of the 2-methoxy dihedral angle. Again, the

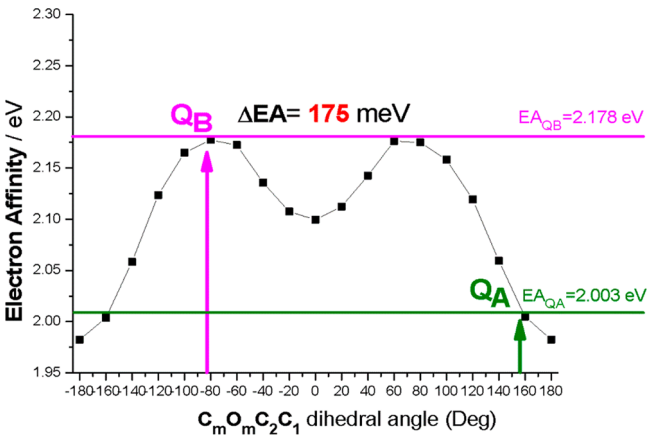

Figure 3. Variation in EA as a function of the $C_mO_mC_2C_1$ dihedral angle for the model shown in Figure S1a (Supporting Information). Bold vertical arrows indicate dihedral angle values for SQ_A and SQ_B estimated from Figure 2. The 3-methoxy dihedral angles ($C_mO_mC_3C_4$) for the Q_A and Q_B points are, respectively, −63.5 and −66.1°. The horizontal lines are the EA values (Q_A and Q_B) for ubiquinone.

orientations corresponding most closely to the crystal structure analysis are indicated by the vertical arrows. For the dihedral angles given above, the Q_B site quinone is estimated to have an electron affinity 175 meV higher than that of Q_A. This is similar to our previous calculated value using a smaller model and restricted scan.[11,12] Also included in Table 1 is the ΔEA value calculated when the 3-methoxy group ($C_mO_mC_3C_4$) is fixed at its midrange value from the crystal structure analysis, −77° for Q_A and +88° for Q_B.[10] This leads to an elevation of the ΔEA

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Table 1. Electron Affinity Difference (ΔEA, $Q_B - Q_A$) for Model Ubisemiquinone (2,3-diMeO-Q: 2,3-dimethoxy-5-methyl-6-isoprenyl-1,4-benzoquinone) and Corresponding Monomethoxy Structures 3-MeO-Q (3-methoxy-2,5-dimethyl-6-isoprenyl-1,4-benzoquinone) and 2-MeO-Q (2-methoxy-3,5-dimethyl-6-isoprenyl-1,4-benzoquinone)[a]

	Electron Affinity Difference[b] (ΔEA/meV)	
	A	B
2,3-diMeO-Q	175	193
2-MeO-Q	175	175
3-MeO-Q	0	10

[a]See Figure S1, Supporting Information. [b]These electron affinities were obtained by re-optimizing the geometry while keeping the 2-methoxy dihedral angles fixed at their values from Figure 2 (Q_A: 2-MeO ($C_mO_mC_2C_1$) = +155°; Q_B: 2-MeO ($C_mO_mC_2C_1$) = −82°) and the 3-methoxy dihedral angles (3-MeO ($C_mO_mC_3C_4$)) kept at either their optimized values (column **A**: 3-MeO −63.5° (Q_A) and −66.1° (Q_B)) or at the mid-range crystal structure values (column **B**: 3-MeO −77° (Q_A) and +88° (Q_B)).

value to 193 meV compared with 175 meV using the optimized 3-methoxy dihedral angle values. This illustrates, as expected, that the 3-methoxy orientation influences the electron affinity as well but that the orientation of this group is similar for both Q_A and Q_B, in contrast to the 2-methoxy group where each has a significantly different orientation.

The favorable electron affinity difference (ΔEA), resulting from the 2-methoxy orientation difference in Q_A and Q_B, is significantly larger than the experimentally measured ΔE_m of 60−75 mV in wild-type RCs. This implies, somewhat surprisingly, that the protein-solvation contribution to the electron-transfer reaction may be at least 100 mV, unfavorable for Q_A to Q_B electron transfer in the wild type. This is in striking contrast to electrostatic calculations, which imply a favorable solvation influence.[4] This could be due to either an overestimation of the EA difference using our theoretical model or incorrect parametrization in the electrostatic calculations. To further explore this, we have calculated the electron affinity values for 2-MeO-Q and 3-MeO-Q quinone models (Figures S1b and S1c, Supporting Information). The values are given in Table 1. Replacement of the 3-methoxy group by a methyl group (2-MeO-Q) leads to the same EA difference value, 175 meV, while replacement of the 2-methoxy group by methyl (3-MeO-Q) effectively eliminates the EA difference, demonstrating its crucial contribution. The essentially same electron affinity for Q_A and Q_B (ΔEA = 0 or 10 meV) predicted for the 3-MeO-Q model explains the lack of Q_B reduction observed experimentally for this quinone upon substitution in wild-type RCs. In contrast, the maintenance of electron transfer for 2-MeO-Q in wild-type RCs is readily accounted for by an electron affinity difference very similar to that exhibited by the native ubiquinone (ΔEA = 175 or 193 mV).

Of special relevance are the data obtained for a mutant with isoleucine replaced by threonine at residue M265 in the Q_A site. Here, the E_m of Q_A is decreased by 100−120 mV by a mechanism that does not involve the methoxy groups.[3] No electron transfer from Q_A to Q_B is observed experimentally for the 3-MeO-Q substituted form.[11] Thus, even though a favorable site ΔE_m value of 100−120 mV has been engineered to facilitate Q_A to Q_B electron transfer, the quinone lacking the 2-methoxy group is still unable to manifest electron transfer. Only an out-of-plane-oriented 2-methoxy group can elevate the electron affinity of Q_B sufficiently to overcome a net unfavorable site solvation effect and render electron transfer from Q_A thermodynamically favorable. Strong corroboration comes from a recent experimental study in which naphthoquinones were tested for Q_B activity.[14] These quinones, lacking methoxy groups, were found to exhibit Q_B redox potentials 60−100 mV more negative than expected by comparison with the native ubiquinone and were only reducible when a low-potential quinone was present in the Q_A site.

The heterodimeric RCs present in PS II and purple bacteria are believed to have evolved from a common homodimeric system, with efficient Q_A to Q_B electron transfer providing a key driving force.[1] For bacteria, this was accomplished using the 2-methoxy group of its ubiquinone. In PS II, which uses plastoquinone, lacking methoxy groups, an alternative mechanism is required. Most simply, this would be the local electrostatic environment, as proposed in previous electrostatic calculations.[15] A recent theoretical study suggested that a complex switching mechanism using tyrosine residues and bicarbonate may also influence Q_A to Q_B electron transfer in PS II.[16]

■ ASSOCIATED CONTENT

Supporting Information

Computational models and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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