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Tuning Cofactor Redox Potentials: The 2-Methoxy Dihedral Angle Generates a Redox Potential Difference of >160 mV between the Primary (Q_A) and Secondary (Q_B) Quinones of the Bacterial Photosynthetic Reaction Center

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Supporting Information

ABSTRACT: 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 *Rhodobacter sphaeroides*. ^{13}C hyperfine sublevel correlation measurements of the 2-methoxy in the semiquinone states, SQ_A and SQ_B, were compared with quantum mechanics calculations of the ^{13}C couplings as a function of the dihedral angle. X-ray structures support dihedral angle assignments corresponding to a redox potential gap (ΔE_m) between Q_A and Q_B of ~180 mV. This is consistent with the failure of a ubiquinone analogue lacking the 2-methoxy to function as Q_B in mutant reaction centers with a ΔE_m of ≈160−195 mV.

Type II reaction centers (RCs) from photosynthetic bacteria and oxygenic organisms contain two quinones that function in series as electron acceptors.[1] In many cases, the two quinones are chemically identical, yet forward electron transfer from the primary quinone, Q_A, to the secondary quinone, Q_B, is thermodynamically favorable by 60−75 meV.[2] In RCs from *Rhodobacter sphaeroides*, which utilizes ubiquinone, reconstitution studies show that only quinones with a 2-methoxy group are able to function as both Q_A and Q_B. This was most clearly demonstrated using two synthetic analogues of ubiquinone in which one of the two methoxy groups or the other was replaced with a methyl (2-methoxy-3,5-dimethyl-6-tetraisoprenyl-1,4-benzoquinone and 3-methoxy-2,5-dimethyl-6-tetraisoprenyl-1,4-benzoquinone, abbreviated 2-MeO-Q and 3-MeO-Q, respectively). Both can fully reconstitute Q_A function, but only 2-MeO-Q was able to support Q_B activity; 3-MeO-Q showed no Q_B activity.[3]

The tuning of cofactor redox potentials by the protein is universal and can be extreme. It is often readily accounted for by the local electrostatic potential provided by the protein.[4] This is sufficient for electron transfer in photosystem II RCs in which plastoquinone, which has no methoxy groups, binds and functions in both quinone sites,[5] but it cannot account for the unique requirement of a 2-methoxy group to simultaneously restore Q_A and Q_B activity in *Rb. sphaeroides* RCs. An additional factor evidently resides with the methoxy group itself, and the methoxy dihedral angle has been suggested to have a strong influence on the redox midpoint potential (E_m) of benzoquinones.[6] 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, but when the methoxy is in plane, the oxygen p orbitals can conjugate with the π-system of the quinone causing the donation of electrons to the ring. Previous computational studies showed that rotating one methoxy group alters the electron affinity by up to 0.25 eV,[7] in very good agreement with our own calculations.[8] Earlier results showed that rotation of both methoxy groups of 2,6-dimethoxy-1,4-benzoquinone altered the electron affinity by up to 0.4 eV.[9]

Applying this to the reaction center quinones is hampered by a lack of adequate information about the methoxy orientations in Q_A and Q_B, as the numerous available X-ray structures of RCs yield a wide range of values.[10] To address this, we recently conducted hyperfine sublevel correlation (HYSCORE) measurements of the semiquinone radicals (SQ_A and SQ_B) in RCs containing ubiquinone labeled with ^{13}C at the two methoxy groups and the single methyl of the ring. We identified the hyperfine coupling constants, a_{iso}, of the 2-methoxy groups in Q_A and Q_B and compared these measured values to quantum mechanically calculated a_{iso} values as a function of the 2-methoxy dihedral angle. The angles determined were then compared to the computed relationship between the dihedral angle and the resulting electron affinity.[8]

Comparison of ^{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][8] defines four possible combinations for dihedral angle θ ($C_mO_mC_2C_1$) in the two SQs (Table 1 and Figure S2 of the Supporting Information).

In our previous analysis, we discussed two of the pairs in Table 1, i.e., i and iv, in which the 2-methoxy dihedral angles of

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Table 1. Estimated Angles of the 2-Methoxy Conformation in SQ_A and SQ_B and Corresponding Differences in Electron Affinity (EA) and Redox Potential (E_m)

	θ_A (deg)	θ_B (deg)	ΔEA (eV)	ΔE_m (mV)
i	45	75	0.04	40
ii	45	135	−0.13	−130
iii	155	75	0.18	180
iv	155	135	0.05	50

both quinones are on the same side of the perpendicular. This comparison yielded a contribution by the different 2-methoxy angles to the ΔE_m between Q_A and Q_B of ∼50 mV.[8] This is a substantial fraction of the experimental ΔE_m of 60−75 mV.[2] However, it is not large enough to account for the complete absence of electron transfer with 3-MeO-Q, which lacks the 2-methoxy group.[3]

Consideration of methoxy angle pairs ii and iii in Table 1 shows significantly larger E_m differences than for pairs i and iv. Pair ii indicates that the 2-methoxy group makes an unfavorable contribution of −130 mV to ΔE_m, while pair iii shows a favorable contribution of 180 mV. The latter value is consistent with the complete failure to support Q_B function without a 2-methoxy group, e.g., 3-MeO-Q, but some independent evidence of this assignment is needed.

Confirmation that the 2-methoxy group makes a substantially larger contribution to the E_m gap between Q_A and Q_B comes from mutants of the Q_A site that lower the E_m of Q_A (Figures 1

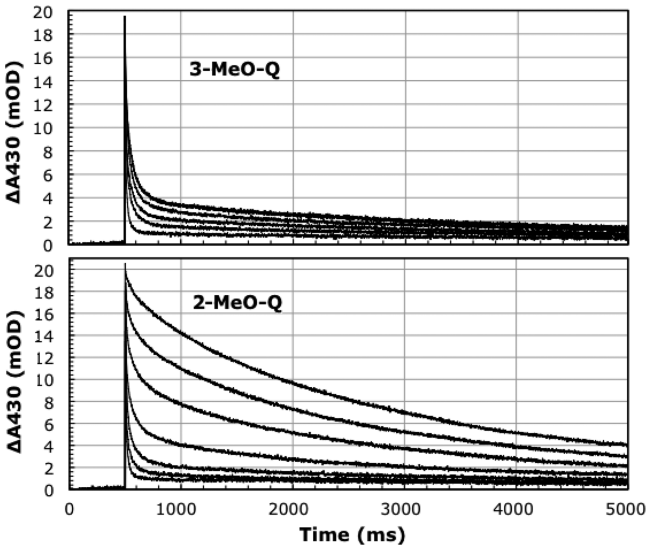

Figure 1. Kinetics of the back reaction for 2- and 3-MeO-Q reconstituted in M265IT RCs with ubiquinone as Q_A (1Q-RCs). Top: 3-MeO-Q concentrations of 0, 0.5, 1, 2, 4, and 16 μM. Bottom: 2-MeO-Q concentrations of 0, 0.5, 1, 2, 4, and 8 μM. Conditions: ∼1 μM M265IT RCs, 10 mM Tris (pH 7.8), and 0.1% LDAO.

and 2). Mutation of isoleucine M265 to threonine (mutant M265IT) decreases the E_m of Q_A by 100−120 mV by a mechanism that does not involve the methoxy groups.[11] This greatly increases ΔE_m, the driving force for electron transfer from Q_A^- to Q_B. However, in polar mutants of M265, 3-MeO-Q is still completely inactive as Q_B (Figure 1).

The kinetics of the back reaction in Figure 1 (charge recombination) reflect the activity of Q_A (initial amplitude) and Q_B (fraction slow phase, ΔS) (Figure 2). 2-MeO-Q fully

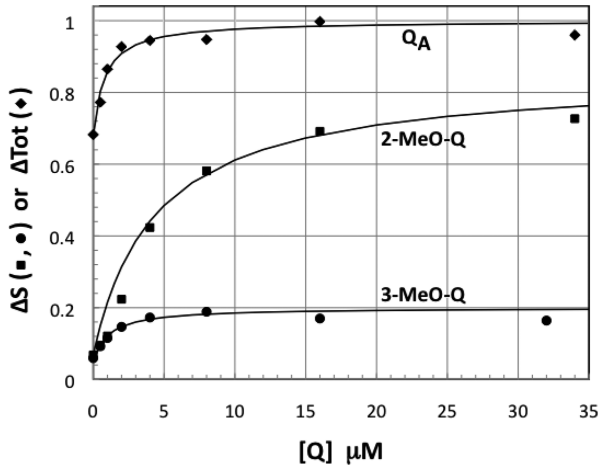

Figure 2. Titration curves for initial (Q_A activity) and slow phase (ΔS) amplitudes. The fitted curves are for K_d values of 1 μM (Q_A), 4 μM (2-MeO-Q, ΔS), and 1 μM (3-MeO-Q, ΔS).

reconstitutes both. However, 3-MeO-Q shows no true ΔS restoration; the small increase seen in the slow phase reflects reconstitution of Q_A, which is partially depleted in these mutant preparations. Some extraneous Q-10 is also present and functions as Q_B when Q_A is restored by 3-MeO-Q (note that the apparent affinity for ΔS is the same as for Q_A; K_d ∼ 1 μM).

Taking into account the 60−75 mV favorable ΔE_m for ubiquinone in wild-type RCs, we found the failure of 3-MeO-Q in M265IT mutant RCs indicates that its E_m in the Q_B site is more than 160−195 mV lower than that of ubiquinone. It is reasonable to assume that other influences on E_m are not significantly affected by the substitution of one methoxy group with a methyl.

From a survey of more than 20 X-ray structures at resolutions of at least 2.8 Å (range of 1.8−2.8 Å), the average values for the methoxy dihedral angles of Q_A and Q_B are listed in Table 2.[10] Note that the dihedral angles for the 2-methoxy

Table 2. Average Values for the Methoxy Dihedral Angles (degrees) of Q_A and Q_B from X-ray Structures

quinone	2-MeO	3-MeO
Q_A	139 ± 25	77 ± 8
Q_B	90 ± 9	88 ± 20

groups of Q_A and Q_B are quite distinct, while those for the 3-methoxy group are similar. The 2-methoxy angles are most consistent with pair iii derived from the ^{13}C HYSCORE data and quantum mechanics calculations (Table 1), giving support for this assignment. This would provide a calculated contribution of ∼180 mV to the redox potential gap between the quinones.

Other factors, e.g., electrostatics, hydrogen bonds, etc., undoubtedly contribute (either positively or negatively) to the net difference in midpoint potential, but the data presented here clearly indicate a large, favorable role for the 2-methoxy group in setting the functional redox potential gap between Q_A and Q_B. The HYSCORE and computational analysis show that this effect is implemented through different dihedral angles for Q_A and Q_B. These are presumably determined by interactions with the environment of the binding sites. For Q_B, the methoxy dihedral angles are likely restricted by hydrogen bond(s) to the 2-methoxy oxygen from the peptide NH group of Gly-L225

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and/or Thr-L226, accounting for a fairly narrow distribution (Table 2); for Q_A, the constraints are by steric interactions with nonpolar groups, although a weak hydrogen bond from Ala-M249 is also possible.[10]

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and Figures S1 and S2. 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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