Redox Potentials of Quinones in Aqueous Solution: Relevance to Redox Potentials in Protein Environments

Hiroshi Ishikita and Keisuke Saito

Abstract Quinones serve as redox-active cofactors in photosynthetic reaction centers. To understand the energetics of electron transfer along the electron transfer pathways in protein environments, the redox potentials (E_m) of the cofactors in water versus normal hydrogen electrode (NHE) are required. However, ubiquinone, menaquinone (phylloquinone), and plastoquinone, which are found in photosynthetic reaction centers, have insoluble hydrophobic isoprene side chains, and thus far only E_m in dimethylformamide (DMF) versus saturated calomel electrode (SCE) had been reported. Recently, E_m in water versus NHE was reported for the quinone species of photosynthetic reaction centers. These results confirmed that $E_m(Q/Q^{\bullet-})$ in water versus NHE was more relevant to $E_m(Q/Q^{\bullet-})$ in protein environments than $E_m(Q/Q^{\bullet-})$ in DMF versus SCE. It has also been demonstrated that E_m for one-electron reduction can also be calculated based on the lowest unoccupied molecular orbital (LUMO) level of the quinone molecules.

Keywords Bacterial photosynthetic reaction centers · *Blastochloris viridis* · Cytochrome b_6f · Cytochrome bc_1 · Photosystem II · *Rhodobacter sphaeroides*

1 Introduction

Quinones are redox-active cofactors in many photosynthetic reaction centers. Ubiquinone serves as an electron acceptor at the Q_A and Q_B binding sites in photosynthetic reaction centers of purple bacteria (PbRC) from *Rhodobacter sphaeroides*

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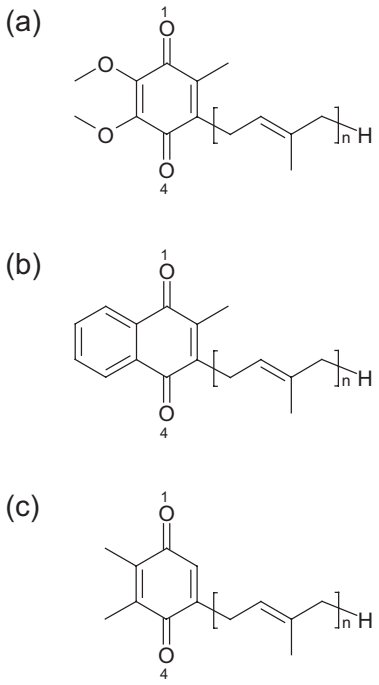

Fig. 1 Molecular structures of (**a**) ubiquinone (n = 10), (**b**) menaquinone and phylloquinone (n = 3 to 9), and (**c**) plastoquinone (n = 6 to 9), where n is the number of isoprene units

and as an electron donor in cytochrome bc_1. Menaquinone (vitamin K_2) is the acceptor at the Q_A site in PbRC from *Blastochloris viridis*, whereas phylloquinone (vitamin K_1) is the active center at the A_{1A} and A_{1B} sites in photosystem I (PSI). Plastoquinone serves as an electron acceptor at the Q_A and Q_B sites in photosystem II (PSII) (Fig. 1) (Robinson and Crofts 1984; Rutherford et al. 1984; Okamura et al. 2000; Brettel and Leibl 2001; Wraight 2004) and as an electron donor in cytochrome b_6f.

Prince et al. measured the redox potential for one-electron reduction, $E_m(Q/Q^{\bullet-})$, of 1,4-quinones, including ubiquinone, menaquinone (phylloquinone), and plastoquinone, in dimethylformamide (DMF) versus saturated calomel electrode (SCE) (Prince et al. 1983). Swallow also measured $E_m(Q/Q^{\bullet-})$ for small 1,4-quinones in water versus normal hydrogen electrode (NHE) (Swallow 1982). As mentioned (Kishi et al. 2017), experimentally measured $E_m(Q/Q^{\bullet-})$ in DMF versus SCE can be practically converted to $E_m(Q/Q^{\bullet-})$ in water versus NHE by adding 480 mV.

2 E_m for Quinones in Water and in Protein Environments

Kishi et al. reported the $E_m(Q/Q^{\bullet-})$ values in water versus NHE as −163 mV for ubiquinone, −260 mV for menaquinone (phylloquinone), and −154 mV for plastoquinone (Table 1) by quantum chemical calculation of the free energy difference

Table 1 Experimentally measured $E_m(Q/Q^{\bullet-})$ (exp.) versus SCE (Prince et al. 1983; Swallow 1982) and calculated $E_m(Q/Q^{\bullet-})$ (calc.) versus NHE (Kishi et al. 2017)

	E_m in DMF (vs. SCE)		E_m in water[a] (vs. NHE)	
	exp. [b]	calc. [c]	exp.	calc. [c]
Ubiquinone-1	−611	−633	*n.d.*	−260
Menaquinone-1	*n.d.*	−738	*n.d.*	−260
Menaquinone-2	−709	−736	*n.d.*	−256
Plastoquinone-1	−640	−626	*n.d.*	−154

n.d. not determined
[a]pH 7
[b]Ref. (Prince et al. 1983)
[c]Ref. (Kishi et al. 2017)

between the neutral state Q and the reduced state $Q^{\bullet-}$ in the aqueous phase (Kishi et al. 2017). Before that study, it was a matter of debate whether $E_m(Q/Q^{\bullet-})$ for quinone in DMF could be relevant to calculate the E_m values in photosynthetic reaction centers when using theoretical approaches, namely, electrostatic calculations. Notably, in electrostatic calculations, only the difference between the E_m of quinone in bulk water and quinone in the protein environment can be computed. Thus, to obtain, for example, $E_m(A_1)$ in PSI, it is necessary to determine the $E_m(Q/Q^{\bullet-})$ for phylloquinone in a reference system (preferentially in water) and add the calculated E_m difference.

Previously, to calculate $E_m(A_1)$ in PSI, Ptushenko et al. used $E_m(Q/Q^{\bullet-}) = -800$ mV for phylloquinone in DMF versus NHE, by assuming a liquid junction potential between SCE in DMF and NHE in water (Ptushenko et al. 2008). However, it should be noted that $E_m(Q/Q^{\bullet-})$ for quinones in the two systems differs by 600 mV even in the absence of the liquid junction potential, i.e., the discrepancy between the $E_m(Q/Q^{\bullet-})$ values in the two systems cannot be explained by the liquid junction potential, as previously demonstrated (Kishi et al. 2017).

Although not clearly stated by Ptushenko et al. (2008), it seems likely that in their computational model, the electrostatic interaction between the PSI protein environment and the A_1 phylloquinone molecule was originally underestimated and that they needed the unusually low E_m value of −800 mV for phylloquinone as a reference, mainly to reproduce the reported low $E_m(A_1)$ in PSI (e.g., −810 mV (Vos and van Gorkom 1990), −754 mV (Iwaki and Itoh 1994), and −700 mV (Brettel and Leibl 2001)). Using the unusually low E_m value of −800 mV for phylloquinone in DMF versus NHE allowed them to conveniently match their calculated value to the reported low $E_m(A_1)$ value. However, using the unusually low E_m value would simultaneously cause a problem in reproducing the $E_m(Q_A)$ of −150 mV for the same quinone species (menaquinone) in PbRC (Brettel and Leibl 2001). That is, they must explain how the PbRC protein environment is able to increase $E_m(Q/Q^{\bullet-}) = -800$ mV in DMF versus NHE for phylloquinone to −150 mV at the Q_A site in the PbRC protein environment. Obviously, this is impossible in the PbRC protein electrostatic environment, as demonstrated in numerous electrostatic calculations

(Rabenstein et al. 1998; Ishikita and Knapp 2004; Zhu and Gunner 2005). It seems plausible that the E_m values measured in water versus NHE (−260 mV for menaquinone (phylloquinone)) (Kishi et al. 2017) are more relevant to the E_m values in proteins than the E_m values measured in DMF versus SCE (unless the proteins are solvated in DMF).

This fact would be more understandable when considering E_m of Q_B near the protein bulk surface in PbRC and PSII. $E_m(Q/Q^{\bullet-})$ is −154 mV for plastoquinone in water versus NHE, which is more consistent with $E_m(Q_B) = +90$ mV versus NHE in PSII determined using spectroelectrochemistry (Kato et al. 2016) than $E_m(Q_B) \approx -750$ mV in DMF versus NHE (Kishi et al. 2017). Again, these results confirm that $E_m(Q/Q^{\bullet-})$ in water versus NHE is more relevant to $E_m(Q/Q^{\bullet-})$ in protein environments than $E_m(Q/Q^{\bullet-})$ in DMF versus SCE.

3 Alternative Approach for Calculating E_m of Quinones and Other Cofactors

There are other approaches for calculating E_m of redox-active groups isolated in a solvent, including quinone molecules. As the basis of quantum chemistry, the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy levels are associated with E_m for one-electron oxidation and for one-electron reduction, respectively (Watanabe and Kobayashi 1991). Indeed, the experimentally measured E_m for ten 1,4-quinones in dimethylformamide (DMF) versus SCE (Prince et al. 1983) is strongly correlated with the LUMO level of quinone in the neutral state in the aqueous phase (coefficient of determination $R^2 = 0.97$, Fig. 2).

The MO-based approach presented herein requires quantum chemical calculation of the neutral state only, whereas the previous approach reported by Kishi et al. (2017) requires quantum chemical calculation of both the neutral and reduced states. The strong correlation between the calculated E_m values and the LUMO energy levels indicates that the E_m values are in fact determined by the molecular structures in the neutral states (prior to reduction of the quinones) and that structural changes that may be induced in response to reduction of the quinones are negligibly small in terms of E_m. This approach can also be applied to other redox-active cofactors, e.g., chlorophylls (Watanabe and Kobayashi 1991).

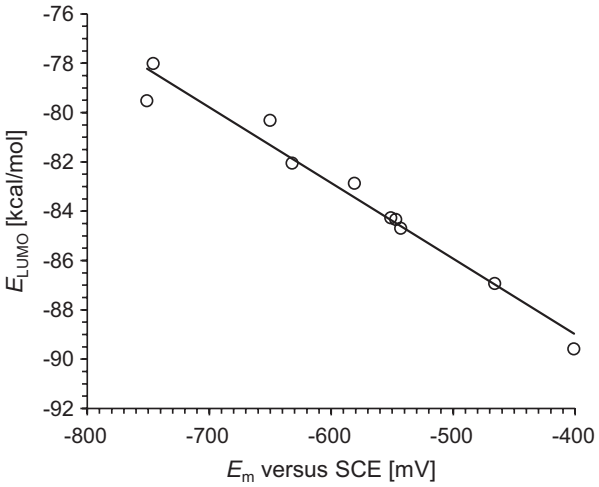

Fig. 2 Correlation between experimentally measured E_m for ten 1,4-quinones in dimethylformamide (DMF) versus saturated calomel electrode (SCE) (Prince et al. 1983) and calculated LUMO energy level (E_{LUMO}) for neutral quinones (coefficient of determination $R^2 = 0.97$). The 1,4-quinones shown are 1,4-benzoquinone, methyl-1,4-benzoquinone, 2,3-dimethyl-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, trimethyl-1,4-benzoquinone, tetramethyl-1,4-benzoquinone, 1,4-naphthoquinone, 2-methyl-1,4-naphthoquinone, and 2,3-dimethyl-1,4-naphthoquinone. E_{LUMO} can be calculated using a quantum chemical approach. We employed the restricted density functional theory (DFT) method with the B3LYP functional and 6-31g++** basis sets for Q ($S = 0$), using the Gaussian (Frisch et al. 2004) program code with the PCM method for DMF. Solvent molecules were considered implicitly, using the SCRF = water option and the SCRF = dimethylformamide option with dielectric constants of 78.355 for water and 37.219 for DMF (i.e., default values). (For further details, including the atomic coordinates, see ref. Kishi et al. 2017)

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