Control of One- versus Two-Electron Reduction of Ubiquinone via Redox-Dependent Recognition

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Redox-active organic cofactors such as quinones and flavins are important species in biological systems, playing vital roles in redox and electron-transfer processes. An important determinant of the function of these redox centers is the noncovalent interactions between apoenzyme and cofactor. While much has been learned through study of the biomolecular systems, model studies provide a critical next step in understanding the chemical behavior of biological systems.[1] Model studies have the potential to isolate and quantify specific interactions, providing detailed insight into the role of enzyme−cofactor interactions in modulating biological redox processes.[2,3]

Quinones are featured in a wide range of biological redox processes.[4] The bacterial photosynthetic reaction center provides an extensively studied example.[5] In this reaction center, light-induced single-electron transfer proceeds from a bacteriochlorophyll donor via a series of donor−acceptor species to a tightly bound primary ubiquinone ($\mathbf{Q_a}$). From here, the quinone radical anion of $\mathbf{Q_a}$ transfers the electron to a more loosely bound secondary ubiquinone ($\mathbf{Q_b}$). In contrast to the single-electron shuttle $\mathbf{Q_a}$, the radical anion of $\mathbf{Q_b}$ is protonated and further reduced to the hydroquinone form (Scheme 1).[6,7]

The photosynthetic reaction center controls the roles played by the two quinones in what can be best described as a one- versus two-electron gate mechanism,[8] mediated by noncovalent interactions of the quinones with specific protein functionality.[9] In addition to the control of electron movement, proton transfer is an intrinsic feature of this gating process. Recent biophysical studies of the photosynthetic reaction center have explored the nature of the second electron transfer to the $\mathbf{Q_b}$ radical.[10] While it has been postulated that this occurs via a proton-coupled mechanism in which formation of the neutral radical enhances the second electron-transfer process,[11] there is still considerable uncertainty as to the exact mechanism of quinone reduction.

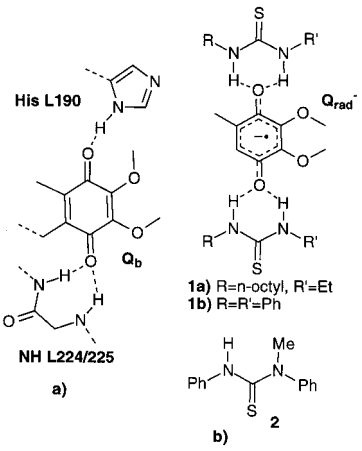
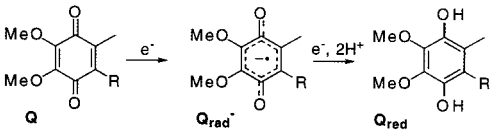


Figure 1. (a) $\mathbf{Q_b}$ binding site of the *Rhodobacter sphaeroides* photosynthetic reaction center.[14] (b) $\mathbf{Q_0}$−receptor **1** complex (shown here for $\mathbf{Q_{rad}^-}$) and nonchelating control **2**.

Scheme 1. Ubiquinone 10 ($\mathbf{Q_a}$, $\mathbf{Q_b}$) (R = Decaisoprene) and Ubiquinone 0 ($\mathbf{Q_0}$) (R = H)



To provide insight into the role of specific protein−cofactor interactions in defining the redox properties of quinone cofactors, we have created simple thiourea-based receptors **1**.[12,13] These receptors provide specific hydrogen bonds to the carbonyl/phenolate oxygen of quinones, mimicking the hydrogen bonding provided by the active sites of the quinoproteins. We report here the ability of these receptors to control the reduction of ubiquinone 0 ($\mathbf{Q_0}$, Figure 1) via both recognition-mediated proton transfer and specific stabilization of the reduced form, directly analogous to their quinoenzyme prototypes.

The effects of interaction with receptors **1** on the redox chemistry of $\mathbf{Q_0}$ were investigated electrochemically using cyclic voltammetry (CV). One-electron reduction of $\mathbf{Q_0}$ to the radical anion $\mathbf{Q_{rad}^-}$ was fully reversible, with an $E_{1/2}$ of −1.13 V (vs ferrocene)[15] in CH_2Cl_2 (Figure 2, trace a). Addition of dialkyl-

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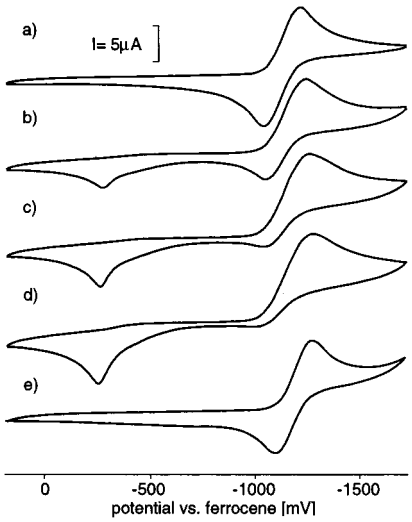
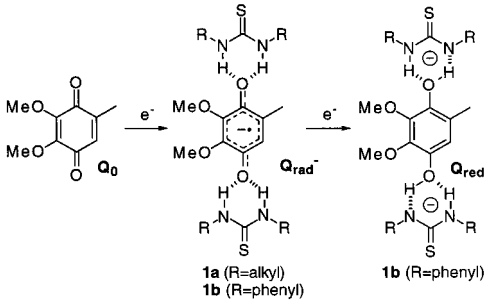


Figure 2. Cyclic voltammetry of $\mathbf{Q_0}$ [10^{-3} M] in CH_2Cl_2: (a) $\mathbf{Q_0}$ alone; (b) $\mathbf{Q_0}$ with 1 equiv of **1a**; (c) $\mathbf{Q_0}$ with 2 equiv of **1a**; (d) $\mathbf{Q_0}$ with 4 equiv of **1a**; (e) $\mathbf{Q_0}$ with 4 eq. control **2**. Tetrabutylammonium perchlorate carrier (0.1 M); scan rate 200 mV/s; $T = 23$ °C.

Scheme 2



receptor **1a** to $\mathbf{Q_0}$ resulted in a 15 mV less negative quinone reduction potential, while maintaining full electrochemical reversibility. This behavior is indicative of redox-enhanced hydrogen bonding and stabilization of $\mathbf{Q_{rad}}^-$ through complexation with receptor **1a** (Scheme 2), a feature previously observed in flavin, naphthalimide, and *o*-quinone systems.[16]

In contrast to dialkylurea receptor **1a**, addition of the more acidic diarylurea receptor **1b** dramatically changed the redox pathway of $\mathbf{Q_0}$ (Figure 2, traces b−d). Upon the addition of **1b** a second oxidation wave appears at −0.3 V vs ferrocene; in the presence of 2 equiv of **1b** the original reoxidation wave has all but disappeared, and the area of the reduction peak has increased approximately 2-fold. This change in the CV of $\mathbf{Q_0}$ is quite similar to that observed upon addition of strong proton donors such as excess trifluoroacetic acid,[17] where an ece-type mechanism occurs. In this mechanism, a one-electron reduction is followed by protonation and a second one-electron reduction, leading to the formation of the hydroquinone species, $\mathbf{Q_{red}}$. The presence of at least 2 equiv of **1b** thus leads to two-electron reduction of $\mathbf{Q_0}$ to $\mathbf{Q_{red}}$. Further evidence for the requirement of 2 equiv of **1b** to achieve complete two-electron reduction of $\mathbf{Q_0}$ was obtained from quantitative EPR measurements (Figure 3).[18] Addition of 1 equiv of **1b** during bulk electrolysis of $\mathbf{Q_0}$ produced an approximately 50% reduction in radical concentration, with the addition of 2 equiv required for almost complete loss of radical signal.

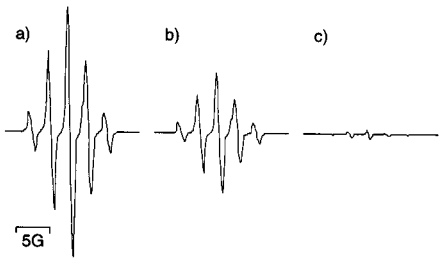


Figure 3. Quantitative EPR after bulk electrolysis of $\mathbf{Q_0}$ [2×10^{-4} M] in CH_2Cl_2, 0.1 M TBAP carrier electrolyte, $V = 1200$ mV vs ferrocene: (a) $\mathbf{Q_0}$ alone, $[\mathbf{Q_{rad}}^-] = 1.4 \times 10^{-4}$ M (68% radical yield); (b) $\mathbf{Q_0}$ with 1 equiv of **2b**, $[\mathbf{Q_{rad}}^-] = 7.7 \times 10^{-5}$ M (38% radical yield); (c) $\mathbf{Q_0}$ with 2 equiv of **2**, $[\mathbf{Q_{rad}}^-] = 1.2 \times 10^{-6}$ M (0.6% radical yield).

Significantly, addition of even 4 equiv of thiourea **2**, with comparable acidity to **1b**, but incapable of chelation, did not affect the fully reversible reduction of $\mathbf{Q_0}$ to $\mathbf{Q_{rad}}^-$ (Figure 2, trace e). This established that specific redox-dependent recognition is required for conversion of the quinone reduction process from one to two electrons.

These experimental results support a recognition-mediated proton transfer from receptor **1b** to reduced $\mathbf{Q_0}$,[19] with ultimate formation of a complex between $\mathbf{Q_{red}}$ and two molecules of receptor **1b** (Scheme 2).[20] This recognition-mediated proton transfer enables direct two-electron reduction and selectively stabilizes the fully reduced $\mathbf{Q_{red}}$.

In summary, we have demonstrated that specific recognition of quinones by receptors can be used to regulate proton-coupled electron-transfer processes, providing direct control of the switching between one- and two-electron processes. In these host−guest complexes, hydrogen bonding to the quinone allows direct two-electron reduction to occur via facilitated proton transfer. This recognition-mediated gating of the reduction process is a plausible mechanism for biological quinone reduction. Application of time-resolved spectroscopic methods to further elucidate the mechanism of two-electron reduction of quinones in the presence of these receptors is underway, and will be reported in due course.

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(18) The signals of the sample and a TEMPO standard (10^{-4} M in toluene) were recorded simultaneously in a TE 104 dual cavity; the concentration of the sample was calculated from the two double integrals.

(19) Given the rapidity the proton-transfer event (proton transfer was observed at scan rates up to 240 V/s), it is difficult to determine electrochemically whether proton transfer follows or precedes the second electron transfer. Preliminary calculations predict a low-barrier hydrogen bond between receptor **1b** and $\mathbf{Q_{rad}}^-$, which effectively means that the proton is shared within the hydrogen bonding network. This would present a pathway intermediate between proton transfer occurring before as opposed to after the second one-electron reduction. We are currently exploring this intriguing mechanistic possibility using density functional methodology.

(20) Since there is little recognition of oxidized $\mathbf{Q_0}$ by receptor **1b** ($K_a < 1\ M^{-1}$ determined via ^1H NMR titrations in $CDCl_3$), the affinity must be greatly enhanced upon reduction of the quinone, another example of redox-enhanced molecular recognition.[16]