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## **Bis-Coenzyme Q<sub>0</sub>: Synthesis, Characteristics, and Application**

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In memory of Xiuwen Wang

**Abstract:** A methylene-bridged biscoenzyme  $Q_0$ , bis(2,3-dimethoxy-5-methyl-l,4-benzoquinone)methane (Bis-CoQ<sub>0</sub>), that shows intramolecular electronic communications has been synthesized for the first time. By employing electrochemical, in situ UV/ Vis, and electron paramagnetic resonance (EPR) spectroelectrochemical techniques, the unstable reduced intermediate species—monoradicals, diamagnetic dianions and tetraanions of Bis-CoQ<sub>0</sub>—have been observed. The electron-transfer process can be de-

### Introduction

Compounds with multiredox centers have received considerable attention because of their unique and tunable electrochemical properties.<sup>[1–4]</sup> By coupling two or more redoxactive sites in a molecule through suitable bridges, its electrochemical properties could be changed due to intramolecular electronic communication between redox-active sites.<sup>[5–8]</sup> Typical electroactive compounds, such as ferrocenes and quinones, have been widely investigated with a view toward their potential optoelectronic and antioxidation applications, and much work has focused on coupling two ferrocenyl or quinonyl groups to construct bis-ferrocenes or bis-quinones.<sup>[9–12]</sup> Coupled bis-ferrocenes or bis-quinones have often exhibited different redox behaviors compared to their original monomeric ferrocenes or quinones.

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fined as a three-step reduction process with a total of four electrons in solution in CH<sub>3</sub>CN. The chemical reaction in the third redox step process was confirmed by variable temperature cyclic voltammetry. In an aprotic CH<sub>3</sub>CN solution, the peak potential separation between electron-transfer steps dimin-

**Keywords:** coenzymes • cyclic voltammetry • electrochemistry • electronic communication • hydrogen bonds ished sequentially with increasing concentration of water. The hydrogenbonding interactions between water and the electrochemically reduced intermediates of Bis-CoQ<sub>0</sub> can be estimated by peak potential shifts. The electronic communications of Bis-CoQ<sub>0</sub> may have been blocked when one reduction peak was observed with proper quantities of water in CH<sub>3</sub>CN solution. The antioxidant defense capacity of Bis-CoQ<sub>0</sub>-protected cells has also been assessed.

Coenzyme  $Q_0$  (2,3-dimethoxy-5-methyl-1,4-benzoquinone; CoQ<sub>0</sub>), shown in Scheme 1, is an intermediate in the syntheses of coenzyme Qs, which are the most common redoxactive compounds that display the typical electronic properties of quinones. These naturally occurring compounds fulfill several biological functions in a living cell. It participates in electron and proton transport and adenosine triphosphate (ATP) synthesis in the mitochondrial respiratory chain. It is well documented that CoQ<sub>0</sub> undergoes two sequential oneelectron reductions that correspond to the formation of the radical anions and dianions in nonaqueous solvent systems.<sup>[13]</sup> Covalent coupling of two CoQ<sub>0</sub> units may result in favorable redox properties and widen its application window.

In this study, the compound bis(2,3-dimethoxy-5-methyll,4-benzoquinone)methane (Bis-CoQ<sub>0</sub>) has been synthesized for the first time. We developed a practical synthetic route to synthesize Bis-CoQ<sub>0</sub> for possible applications in biocatalysis, antioxidation, and antitumor activity. For most potential antioxidation agents, their biological functions are intimately related to their electrochemical properties.<sup>[14]</sup> The electron-transfer process is a fundamental issue for metabolic reactions in living beings. Understanding the electrontransfer process of a potential drug is therefore always a top priority. For that reason, we have tried to understand the



Scheme 1. Synthesis of methylene-bridged Bis-CoQ $_0$ .

electrochemical properties and electron-transfer processes as far as we could. The electrochemical experiments showed that in nonaqueous solutions Bis-CoQ<sub>0</sub> exhibited two pairs of reversible redox peaks and one pair of irreversible redox peaks, thus indicating intramolecular electronic communications. UV/Vis and EPR spectroelectrochemistry experiments further supported the existence of intramolecular electronic communications. The chemical reaction in the irreversible redox process was also confirmed by variable-temperature cyclic voltammetry (VT-CV). Due to the fact that most of the quinones and their intermediates as well as reduced forms exist inside hydrophobic cell membranes, it is to our advantage to study their electrochemical properties in aprotic organic solvents, which are useful in mimicking the nonpolar environment in the cell.<sup>[15]</sup> The hydrogen-bonding in-

#### **Abstract in Chinese:**

本文首次报道了由亚甲基相连的双辅酶 Qo 化合物:双(2,3-二甲氧基-5-甲基-1,4-苯醌)亚甲基的合成:并采用电化学,现场紫外/可见和电子顺磁共振光谱 电化学技术,对双辅酶 Qo 及其所有还原产物:单自由基阴离子、反磁二价阴离 子和四价阴离子性质进行表征。在乙腈溶液中其循环伏安电化学性质为三步四 电子的氧化还原过程。这一性质表明双醌内部有强烈的相互作用力。并通过变 温循环伏安法,确认了第三步的电化学氧化还原过程中伴随了化学反应。在含 有微量水的乙腈溶剂体系中,各电子转移的电位差随着乙腈溶剂中水含量的增 加而减少。其水和还原产物形成的氢键强度可通过峰电位的位移来计算。当加 入适量水观察到一个还原峰时,双醌内强烈的分子内作用力消失。此外,本文 还考察了双辅酶 Qo 在细胞内的抗氧化能力。 teractions between water and the electrochemically reduced intermediates of Bis-CoQ<sub>0</sub> in an aprotic organic solvent have been well studied. The electronic communications of Bis-CoQ<sub>0</sub> may be blocked in a solution of CH<sub>3</sub>CN with a moderate concentration of water. The three-step reduction process with a total of four electrons transferred gradually converts into a two-step, two-electron one. In addition, the protective effect of Bis-CoQ<sub>0</sub> against hydrogen peroxide induced oxidative damage to HeLa cells was also investigated.

### **Results and Discussion**

### Synthesis

Bis-CoQ<sub>0</sub> was synthesized according to Scheme 1. CoQ<sub>0</sub> was reduced by using KBH<sub>4</sub> in methanol at 0°C for 10 min to give **1**. Compound **1** was treated with base and then methylated by using dimethyl sulfate at room temperature to give compound **2** in 69% yield. A mixture of **2**, trioxane, and InCl<sub>3</sub> was stirred at 120°C for 6 h to give the methylenebridged **3** in 93% yield. Silver oxide in 6M HNO<sub>3</sub> was used to regenerate the oxidized quinone (Bis-CoQ<sub>0</sub>). After purification by column chromatography, Bis-CoQ<sub>0</sub> was obtained in 53% overall yield. The structure of compound Bis-CoQ<sub>0</sub> was confirmed by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, MS, and elemental analysis.

### Electrochemical Behavior of Bis-CoQ<sub>0</sub>, CoQ<sub>0</sub>, and CoQ<sub>0</sub>-CH<sub>3</sub> in Nonaqueous CH<sub>3</sub>CN

To understand the electrochemical behavior of Bis-CoQ<sub>0</sub>,  $CoO_{0}$ and 2,3-dimethoxy-4,5-methyl-l,4-benzoquinone (CoQ<sub>0</sub>-CH<sub>3</sub>) they were compared by means of cyclic voltammetry. Figure 1a shows the CV results at a glassy carbon (GC) working electrode in nonaqueous acetonitrile (CH<sub>3</sub>CN) that contained 0.15 M tetrabutylammonium perchlorate (TBAP) after complete removal of oxygen by purging with dry nitrogen for ten minutes. CoQ<sub>0</sub> and CoQ<sub>0</sub>-CH<sub>3</sub> displayed two pairs of typical reversible redox peaks of quinones at  $-1.02 \text{ V} (\text{E}^{\text{I}}_{_{1_{h}}}), -1.65 \text{ V} (\text{E}^{\text{II}}_{_{1_{h}}}), \text{ and } -1.11 \text{ V} (\text{E}^{\text{I}}_{_{1_{h}}}),$  $-1.78 \text{ V} (\text{E}^{\text{II}_{1/2}})$  versus ferrocene/ferrocinium (Fc/Fc<sup>+</sup>), respectively. Bis-CoQ<sub>0</sub> exhibited two sets of reversible redox peaks at  $-1.02 \text{ V} (\text{E}^{\text{Ia}_{1/2}})$  and  $-1.20 \text{ V} (\text{E}^{\text{Ib}_{1/2}})$  and one set of irreversible redox peaks at  $-1.73 \text{ V} (\text{E}^{\text{IIa,b}_{1/2}})$ . The two possible responses from tether-identical redox sites are attributed to the presence and absence of electronic communications.<sup>[16]</sup> Tethered systems that do not display electronic communication will exhibit the same behavior as nontethered redox probes. In the case of quinones, Figure 1a shows a clear difference in the CV redox peaks of CoQ<sub>0</sub> and Bis-CoQ<sub>0</sub>, which is consistent with the different redox states of Bis-CoQ<sub>0</sub> that are in electronic communication with each other.

The first reduction peak of  $\text{CoQ}_0$  at -1.06 V is split into two reduction peaks for Bis-CoQ<sub>0</sub> at -1.06 and -1.24 V. The large redox peak splitting ( $\Delta E_{1/2} = 0.18$  V) indicates that strong intramolecular electronic communications exist be-



Figure 1. a) CV values obtained at a GC electrode (3 mm in diameter) at  $22\pm2$ °C at 0.05 V s<sup>-1</sup> for 0.5 mM CoQ<sub>0</sub>, CoQ<sub>0</sub>-CH<sub>3</sub>, and Bis-CoQ<sub>0</sub> in non-aqueous CH<sub>3</sub>CN that contained 0.15 M TBAP. b) Experimental (black line) and simulated (colored line) square-wave voltammetry (SWV) values of CoQ<sub>0</sub>, CoQ<sub>0</sub>-CH<sub>3</sub>, and Bis-CoQ<sub>0</sub>. Increment E=0.004 V, frequency = 15 Hz.

tween the methylene-bridged quinonyl moieties of Bis- $CoQ_0$ . The second reduction peak of Bis-CoQ<sub>0</sub> is at a more negative potential than the first reduction peak of CoQ<sub>0</sub>-CH<sub>3</sub> at -1.15 V. The CV results suggest that the two electrons are captured successively by the two quinonyl moieties and a more negative potential is needed for the second electron transfer. This phenomenon can be understood like this: once Bis-CoQ<sub>0</sub> accepts one electron on one quinonyl moiety at -1.06 V, the electronic behavior of the other quinonyl moiety is very similar to that of CoQ<sub>0</sub>-CH<sub>3</sub>. It is known that the electron-acceptor capability is significantly enhanced when the side chain of  $CoQ_0$  is substituted by an electronwithdrawing moiety, but is decreased with an electron-donating moiety. So the electron-acceptor capability of CoQ<sub>0</sub>-CH<sub>2</sub>-R (R is a substituent) is significantly decreased when the side chain of -CH<sub>2</sub>- is coupled by an electron-donating moiety of semiquinone radicals of CoQ<sub>0</sub>. Therefore the second reduction peak potential of Bis-CoQ<sub>0</sub> is more negative than that of CoQ<sub>0</sub>-CH<sub>3</sub>. Further reduction to the dianions of  $CoQ_0$  occurs at -1.69 V, which is a more positive potential than the tetranions of Bis-CoQ<sub>0</sub> (-1.81 V). For any radicals of quinonyl moieties, the other  $-CH_2$ -coupled quinonyl radical moiety is electron donating, thereby suggesting that a certain degree of stabilization is gained by the methylene bridge. The dianions of  $CoQ_0$ -CH<sub>3</sub> at -1.82 V are at a similar potential region to the Bis-CoQ<sub>0</sub> tetranions. The final reduction step to the tetranions of Bis-CoQ<sub>0</sub> implies a redox system; once at the diamagnetic dianions stage, it behaves as if it were a nontethered redox system. The electron-transfer process is totally different to bis-quinone without electronic communications, for which only two reduction peaks are observed. Each of two quinonyl groups of Bis-CoQ<sub>0</sub> accept one electron respectively, then the dianions of Bis-CoQ<sub>0</sub> convert to tetranions by accepting the other two electrons almost simultaneously.

Square-wave voltammetry (SWV) was used to understand the reduction process in detail. As shown in Figure 1b, the half-peak width of the Bis-CoQ<sub>0</sub> peaks Ia and Ib are equal to that of peak I for CoQ<sub>0</sub> and CoQ<sub>0</sub>-CH<sub>3</sub>, whereas the halfpeak width II for Bis-CoQ<sub>0</sub> is larger than that of peak II for both  $CoQ_0$  and  $CoQ_0$ -CH<sub>3</sub>. Thus, the Bis-CoQ<sub>0</sub> peak II could be split into IIa and IIb. CoQ<sub>0</sub> and CoQ<sub>0</sub>-CH<sub>3</sub> exhibited the expected two peaks (I and II), attributed to two oneelectron redox reactions. Integration of peak I and II of  $CoQ_0$  shows that the area of peak II is equal to 94% of the area of peak I, and CoQ<sub>0</sub>-CH<sub>3</sub> shows about 70%. The theoretical result should show peak area ratios of 1:1 for two one-electron redox reactions. The deviation from 1:1 peak areas is explained by invoking a chemical reaction of the radical anions into an electrochemically inert species. The assumption of a complexation reaction in which anion radicals react to the dimer was in excellent agreement with the experimental curves.[17]

Bis-CoQ<sub>0</sub> displayed a more interesting result. The first reduction (peak I) of Bis-CoQ<sub>0</sub> to the dianions is split into two peaks (Ia and Ib) due to the electronic communication. The integrated areas of the peak Ia and Ib are almost equal. Peak II for Bis-CoQ<sub>0</sub> was expected to be integrated to the sum of peaks Ia and Ib; however, the area of peak II was only 44% of the sum of Ia and Ib. This suggested that a similar chemical reaction occurs for Bis-CoQ<sub>0</sub> as for CoQ<sub>0</sub>.<sup>[15]</sup> All half-widths of the peaks and their areas are shown in the Supporting Information.

Figure 2 illustrates the influence of the potential scan rate v on the cyclic voltammograms at a glassy carbon (GC) electrode in a solution of 0.15 M TBAP in nonaqueous CH<sub>3</sub>CN that contains 0.2 mM of Bis-CoQ<sub>0</sub> at a scan rate of 0.02, 0.04, 0.06, 0.08, 0.1, 0.15, and  $0.20 \text{ Vs}^{-1}$ . The scanning potential range was -0.4 to -2.4 V. Inspection of the curves reveals that an increase in the scan rates increases the current of all the redox peaks of Bis-CoQ<sub>0</sub>. The first (Ia) and second (Ib) redox peak potentials have no significant shifts. The third (IIa,b) reduction peak shifted to more negative potentials. The ratio of the reduction peak current to the oxidation peak current for the first (Ia) and second (Ib) redox process is close to unity ( $\approx$ 1). However, the ratio of peak currents ( $i_{pa}/i_{pc}$ ) for the third (IIa,b) pair of peaks is found to be



Figure 2. CV values at a GC electrode (3 mm in diameter) of 0.2 mM Bis-CoQ<sub>0</sub> in nonaqueous CH<sub>3</sub>CN that contained 0.15 M TBAP at 0.02, 0.04, 0.06, 0.08, 0.1, 0.15, and 0.2 Vs<sup>-1</sup>. The inset shows the plots of peak currents Ia (**■**), Ib ( $\bigtriangledown$ ), and IIa,b ( $\bigcirc$ ) of Bis-CoQ<sub>0</sub>,  $i_p$ , versus the square root of the scan rate,  $\nu^{1/2}$ .

about 0.77. The ratio of peak currents and the peak potential shifts indicate that third redox process is irreversible.

All the redox peak currents increase linearly with the square root of the scan rate,  $v^{\frac{1}{2}}$ ; the corresponding linear equations are  $i_{\text{pala}}$  ( $\mu$ A)=19.59 $v^{\frac{1}{2}}$  (Vs<sup>-1</sup>), (R=0.999);  $i_{\text{pcla}}$  ( $\mu$ A)=-20.22 $v^{\frac{1}{2}}$  (R=0.999);  $i_{\text{palb}}$  ( $\mu$ A)=16.765 $v^{\frac{1}{2}}$  (Vs<sup>-1</sup>), (R=0.999);  $i_{\text{pclb}}$  ( $\mu$ A)=-15.78 $v^{\frac{1}{2}}$  (R=0.999);  $i_{\text{palla,b}}$  ( $\mu$ A)=21.27 $v^{\frac{1}{2}}$  (Vs<sup>-1</sup>), (R=0.997);  $i_{\text{pclIa,b}}$  ( $\mu$ A)=-21.03 $v^{\frac{1}{2}}$  (R=0.996). The redox peak currents,  $i_p$ , were enhanced with the square root of the scan rate on the glassy carbon electrode. The linear equation is inserted into Figure 2, which indicates a diffusion-controlled electrode process. For a diffusion-controlled electrode process are and the concentration of redox species in the following equation [Eq. (1)]:<sup>[18]</sup>

$$i_{\rm p} = 0.446 n F c A (D v n F / RT)^{\frac{1}{2}}$$
 (1)

in which *D* is the diffusion coefficient of the Bis-CoQ<sub>0</sub> and its intermediate species, *c* is its molar concentration, *n* is the number of electrons that participated in the redox reaction, *A* is the active area of the electrode, and the other variables have conventional meanings. The value of diffusion coefficients of Ia, Ib, and IIa,b of Bis-CoQ<sub>0</sub> are  $2.625 \times 10^{-5}$ ,  $1.615 \times 10^{-5}$ , and  $3.61 \times 10^{-6}$  cm<sup>2</sup>s<sup>-1</sup>, respectively.

To confirm the electron-transfer mechanism, the molecular structures of the intermediates and final reduced species were explored by using in situ UV/Vis spectroelectrochemical techniques. The UV/Vis spectra were recorded during the reduction of 0.2 mm Bis-CoQ<sub>0</sub> in nonaqueous CH<sub>3</sub>CN that contained 0.15 m TBAP in an optically transparent thinlayer electrochemical cell (optical path length is  $(0.4 \pm 0.1)$  mm). As shown in Figure 3left, before reduction Bis-CoQ<sub>0</sub> exhibited the characteristic  $\pi$ - $\pi$ \* and n- $\pi$ \* electronic



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Figure 3. Left: The UV/Vis spectra of  $CoQ_0-CoQ_0$  (line a),  $CoQ_0-CoQ_0^-$  (line b),  $CoQ_0^--CoQ_0^-$  (line c) and  $CoQ_0^{2-}-CoQ_0^{2-}$  (line d). Right: EPR spectra of  $CoQ_0-CoQ_0^-$  at -1.10 V (line a) and -1.30 V (line b).

transitions of the benzoquinonyl rings at 275 and 400 nm, as shown in Figure 3left a. With an applied potential at -1.10 V (one-electron reduction), the main absorption band at 275 nm decreased and a new broad absorption band appeared at 625 nm, as shown in Figure 3left b, which is assigned to the  $n-\pi^*$  absorption the of radical anions (CoQ<sub>0</sub>- $CoQ_0^{-}$ .<sup>[19]</sup> This absorption profile indicates that one of the quinonyl groups is still in its oxidized form and the other quinonyl ring is in its reduced radical anionic form. With further reduction at -1.30 V, the absorption band at 275 nm completely disappeared whereas the absorption band at 625 nm was retained. It signifies the absence of the quinonyl group and the presence of one radical anion on each quinonyl group. Other new bands appeared at 306 and 350-450 nm, as shown in Figure 3left c. The radical anions convert to diamagnetic dianions (CoQ<sub>0</sub><sup>.-</sup>-CoQ<sub>0</sub><sup>.-</sup>). At −1.90 V, all the benzoquinonyl diamagnetic dianions bands disappeared and only the characteristic absorption band for the tetranions of Bis-CoQ<sub>0</sub> at 284 nm was left, as shown in Figure 3left d. If the diamagnetic dianions of Bis-CoQ<sub>0</sub> just accept one electron at this potential, at which one of the quinonyl groups is in its reduced dianionic form and the other quinonyl ring is still in its reduced radical anionic form, the absorption of radicals of Bis-CoQ<sub>0</sub> cannot disappear completely. Therefore the absorption band at 284 nm indicated that at -1.90 V, diamagnetic dianions of Bis-CoQ<sub>0</sub> accept two electrons and convert to tetranions  $(CoQ_0^{2-})$  $CoQ_0^{2-}$ ).

For further validation of mechanism, an in situ EPR spectroelectrochemical study, a versatile technique for the detecting paramagnetic species  $CoQ_0$ - $CoQ_0^-$ , was conducted under the same conditions. By applying a reductive voltage at -1.10 V as shown in Figure 3right a, the three-line spectra of  $CoQ_0$ - $CoQ_0^-$  was obtained in nonaqueous CH<sub>3</sub>CN. The values of hyperfine coupling constants, *a*, were obtained by simulation. The main coupling constant for hydrogen atoms at the methylene-bridged group (triple, a=3.10) suggested that the radical anion is mainly located at the 4-position of the quinonyl ring. When applying a further reduction at

-1.30 V, the monoradical (CoQ<sub>0</sub><sup>--</sup>-CoQ<sub>0</sub>) obtained one more electron to produce the diamagnetic dianion (CoQ<sub>0</sub><sup>--</sup>-CoQ<sub>0</sub><sup>--</sup>). The monoradical EPR signal gradually decreased and vanished eventually. This phenomena indicated that the radical anions convert to diamagnetic dianion (CoQ<sub>0</sub><sup>--</sup>-CoQ<sub>0</sub><sup>--</sup>).

The temperature-dependent CV values of Bis-CoQ<sub>0</sub> are shown in Figure 4. Variable-temperature experiments were performed with a nonisothermal electrochemical cell. The reference electrode was kept at constant temperature (( $20 \pm 0.1$ ) °C), whereas the half cell that contained the working electrode and the junction to the reference electrode were kept under thermostatic control with a water bath; its temperature was varied from 7 to 57 °C, as shown in Figure 4.

Unlike the kinetics of a homogeneous reaction, the peak potentials do not correspond to the thermodynamic formal potentials  $(E^{\circ})^{[20]}$  In a heterogeneous reaction, the redox behaviors are remarkably influenced by temperature. The first and second apparent standard potentials have no obviously negative shifts, whereas the third ones have distinc-



Figure 4. CV values at a GC electrode (3 mm in diameter) of 0.2 mM Bis-CoQ<sub>0</sub> in nonaqueous CH<sub>3</sub>CN that contained 0.15 M TBAP at 280, 290, 300, 310, 320, and 330 K. The thermodynamic formal potentials  $E^{\circ}_{Fe/Fe^+} = 0$  V.

tively negative shifts with an increase in temperature. The negative shifts are about 0.62 V from 7 to 57 °C. The striking potential shifts of the third reduction can be explained by complexation reactions. The anion radicals react to dimer spontaneity. The dimer accepts electrons more easily than diradical anions. As the temperature increases, the dimerization equilibrium constant decreases,<sup>[17f]</sup> so the third peak potential is most positive at low temperature and becomes less positive as temperature increases.

With this experimental configuration, the reaction entropy for Bis-CoQ<sub>0</sub> ( $\Delta S^{\circ'}(rc)$ ) is given by Equation (2):<sup>[21]</sup>

$$\Delta S^{\circ\prime}(\mathrm{rc}) = S^{\circ\prime}(\mathrm{red}) - S^{\circ\prime}(\mathrm{ox}) = nF(\mathrm{d}E^{\circ\prime}/\mathrm{d}T) \tag{2}$$

Thus,  $\Delta S^{\circ\prime}(\mathbf{rc})$  was determined from the slope of the plot of  $E^{\circ\prime}$  versus temperature, as shown in Figure 5, which turns out to be linear under the assumption that  $\Delta S^{\circ\prime}(\mathbf{rc})$  is con-



Figure 5. The third (IIa,b) redox formal potentials of Bis-CoQ,  $E^{\sigma}_{Ha,b}$  versus *T* plots.

stant over the limited temperature range investigated. With the same assumption, the enthalpy change  $(\Delta H^{\circ'}(\text{rc}))$  was obtained from the Gibbs–Helmholtz equation, namely, as the negative slope of the  $E^{\circ'}/T$  plotted versus 1/T. The  $E^{\circ'}$ values show a monotonic linear decrease with increasing temperature from 280 to 330 K.  $\Delta S^{\circ'}(\text{rc})$  and  $\Delta H^{\circ'}(\text{rc})$  are -1158 and 66 670 J mol<sup>-1</sup>, respectively.

### **Reaction Mechanism**

By taking into consideration the experimental data from CV, SWV, UV/Vis, and EPR spectroelectrochemistry analyses, the electron-transfer process of Bis-CoQ<sub>0</sub> (CoQ<sub>0</sub>-CoQ<sub>0</sub>) in nonaqueous CH<sub>3</sub>CN that contained 0.15 M TBAP could be suggested as follows:

### Electrochemical Behavior of Bis-CoQ<sub>0</sub> in CH<sub>3</sub>CN with Varying Concentrations of Water

For a better understanding of the hydrogen-bonding interactions between water and the electrochemically reduced inElectrochemical Reaction

$CoQ_0 CoQ_0$	+	e	$CoQ_0 CoQ_0^{-}$	peak Ia	
$CoQ_0 CoQ_0^{-}$	+	e- 💶 🚬	$\operatorname{CoQ}_0^{-}\operatorname{CoQ}_0^{-}$	peak Ib	
$CoQ_0^{-}CoQ_0^{-}$	+	e	$\operatorname{CoQ}_0^{2^-}$ $\operatorname{CoQ}_0^{2^-}$	peak IIa	
$\operatorname{CoQ}_0^{-}\operatorname{CoQ}_0^{2^-}$	+	e- 💶	$CoQ_0^{2^-}CoQ_0^{2^-}$	peak IIb	
Chemical & Electrochemical Reaction of Peak IIa,b					
$2\text{CoQ}_0^-\text{CoQ}_0^ \leftarrow$ $\text{CoQ}_0^{2^-}\text{CoQ}_0^{2^-}$ + $\text{CoQ}_0^-\text{CoQ}_0$					
$CoQ_0^{2^-}CoQ_0^{2^-}$	+ Cc	$Q_0 CoQ_0 =$	Bis-Co	$Q_0$ -Bis-Co $Q_0$	
Bis-CoQ <sub>0</sub> -Bi	s-Co	$Q_0 \Big]^{+-} + e^{-} + e^{-}$	Bis-Co	$Q_0$ -Bis-Co $Q_0$	

termediates of Bis-CoQ<sub>0</sub> in an aprotic organic solvent, the electrochemical properties of Bis-CoQ<sub>0</sub> and its intermediate species, monoradicals (CoQ<sub>0</sub>-CoQ<sub>0</sub><sup>-</sup>), diamagnetic dianions (CoQ<sub>0</sub><sup>-</sup>-CoQ<sub>0</sub><sup>-</sup>), and tetraanions (CoQ<sub>0</sub><sup>2-</sup>-CoQ<sub>0</sub><sup>2-</sup>), were studied in CH<sub>3</sub>CN that contained 0.15 m TBAP with varying concentrations of water. With the addition of water into the CH<sub>3</sub>CN, which results in hydrogen-bonding interactions, the three reduction peaks shifted positively. The third reduction peak shifted most strongly and merged into the second one (as shown in Figure 6); the new second one shifted more strongly and finally merged into the first one. The representative cyclic voltammograms are given in Figure 8.

According to the experimental results, it can be concluded that all the Bis-CoQ<sub>0</sub> reduced species have hydrogen-bonding interactions with water in CH<sub>3</sub>CN. Relative to the first and second reduction peaks, a large positive potential shift for the third reduction peak was observed. It would suggest that the tetraanions have much stronger hydrogen-bonding interactions with water.

Theoretical calculations found that the oxygen atoms of monoquinones do have an increasing negative-charge density during electrochemical reduction from the neutral quinones to their radicals and then to their dianions.<sup>[22]</sup> Since tetraanions of bis-quinones have large negative-charge densities with two dianions of quinones, it would be expected that the oxygen atoms with higher negative-charge densities in the tetraanions will have a stronger interaction with water molecules than those in the diradical anions. To treat the situation more quantitatively, the reaction formula can be expressed in the form of Equations (3)–(8):

$CoQ_0-CoQ_0 + e^-$	$\frown$ CoQ <sub>0</sub> CoQ <sub>0</sub>	(3)
$CoQ_0-CoQ_0^{-} + aH_2O$	$\leftarrow$ CoQ <sub>0</sub> -CoQ <sub>0</sub> <sup>-</sup> (H <sub>2</sub> O) <sub>a</sub>	(4)
$CoQ_0-CoQ_0^{-}(H_2O)_a + e^{-}$	$\leftarrow$ CoQ <sub>0</sub> -CoQ <sub>0</sub> (H <sub>2</sub> O) <sub>a</sub>	(5)
$\operatorname{CoQ}_0^{-}\operatorname{CoQ}_0^{-}(\operatorname{H}_2\operatorname{O})_a + (b-a)\operatorname{H}_2\operatorname{O}$	$\frown$ CoQ <sub>0</sub> -CoQ <sub>0</sub> (H <sub>2</sub> O) <sub>b</sub>	(6)
$\operatorname{CoQ}_{0}^{\bullet}-\operatorname{CoQ}_{0}^{\bullet}(\operatorname{H}_{2}\operatorname{O})_{b}$ + $2 e^{-}$	$ \qquad \qquad$	(7)
$CoQ_0^2 - CoQ_0^2 (H_2O)_b + (c-b) H_2O$	$\leftarrow$ CoQ <sub>0</sub> <sup>2</sup> -CoQ <sub>0</sub> <sup>2</sup> (H <sub>2</sub> O) <sub>c</sub>	(8)



Figure 6. CV values of Bis-CoQ\_0 at about 0.5 mM in CH\_3CN that contained 0.15  $\rm M$  TBAP with varying concentrations of water at a scan rate of 0.1 V s^{-1}.

For the first redox process step [Eq. (9)]:<sup>[17a, 23]</sup>:

$$E_{1\underline{b}}^{(1)} = E_{1\underline{b}}^{0(1)} + RT / F \ln\left(1 + K_{eq}^{(1)}C_{H_2O}^a\right)$$
(9)

in which  $E_{\frac{1}{2}}^{0 (1)}$  is the half-wave potential of the first redox couple in the absence of water in solution, *a* is the number of water molecules that are hydrogen bonded to CoQ<sub>0</sub>-

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 $\operatorname{CoQ_0^{*-}}, K_{eq}^{(1)}$  is the equilibrium constant of Equation (4), and  $C_{H_{2O}}$  is the concentration of water. If  $K_{eq}^{(1)}C_{H_{2O}}^a \geq 1$ , a plot of  $E_{1_2}^{(1)}$  versus  $\ln C_{H_{2O}}$  gives a straight line with a slope of 2.3*aRT/F*, from which the value of *a* could be estimated. For the sake of simplicity, only one-step, two-electron processes have been considered for evaluating the degree of hydrogen bonding for tetraanions. So for the second and third redox process [Eqs. (10) and (11)], we have the reactions given in Equations (5),(6) and (7),(8):

$$E_{1\underline{b}}^{(2)} = E_{1\underline{b}}^{0} + RT / F \ln (1 + K_{eq}^{(2)}) C_{H_2O}^b / (1 + K_{eq}^{(1)}) C_{H_2O}^a$$
(10)

$$E_{\frac{1}{2}}^{(3)} = E_{\frac{1}{2}}^{0(3)} + RT / F \ln (1 + K_{eq}^{(3)}) C_{H_2O}^c / (1 + K_{eq}^{(2)}) C_{H_2O}^b$$
(11)

in which b, c, and  $K_{eq}^{(2)}$ ,  $K_{eq}^{(3)}$  are the number of water molecules that are hydrogen bonded to  $CoQ_0$ <sup>-</sup>- $CoQ_0$ <sup>-</sup>,  $CoQ_0^{2-}$ - $CoQ_0^{2-}$ , and the equilibrium constant of Equations (6) and (8), respectively. For a strong hydrogen bond, 1 in the denominator and numerator in Equations (6) and (8) could be neglected and thus the value of (b-a), (c-b) could be obtained from the plot of  $E_{l_2}^{(2 \text{ or } 3)}$  versus  $C_{H_2O}$  as shown in Figure 7. When more water is added, the equilibrium shifted



Figure 7. Plots of  $\Delta E_{\frac{1}{2}}$  of the first ( $\triangle$ ), second ( $\bigcirc$ ), and third (**•**) redox peaks of Bis-CoQ<sub>0</sub> against ln  $C_{H,O}$ .

towards the hydrogen-bonded forms, but because of the ln relationship with the equilibrium expression in the Nernst equation, the potential shifts are greatest at the lowest water concentrations. The parameters of *a*, *b*, *c*,  $K_{eq}^{(1)}$ ,  $K_{eq}^{(2)}$ , and  $K_{eq}^{(3)}$  for Bis-CoQ<sub>0</sub> are summarized in Table 1.

To further investigate hydrogen bonding in the monoradicals, diamagnetic dianions, and tetraanions, in situ UV/Vis spectroelectrochemical experiments were conducted on Bis-CoQ<sub>0</sub> in CH<sub>3</sub>CN in the presence of three different concentrations of water (0.22, 1.11, and 2.65 M). All the band assignments of the UV/Vis spectra of Bis-CoQ<sub>0</sub> and its reduced intermediate species at varying concentrations of water are listed in Table 2.

As the concentration of water was increased to 0.22 M, a relatively small change in the UV/Vis spectrum of the singly reduced species was observed, as shown in Figure 8A. Compared with Bis-CoQ<sub>0</sub> in CH<sub>3</sub>CN (as shown in Figure 3A), the absorption band at 625 nm, which was given by the n– $\pi$ \* electronic transition of monoradicals and diamagnetic dianions, decreased with water in CH<sub>3</sub>CN but with no shifts. Absorption bands of Bis-CoQ<sub>0</sub> and its tetraanions at 274, 400, and 284 nm had no significant changes. This is indicated that the n– $\pi$ \* electronic transition of benzoquinonyl rings is sensitive to hydrogen-bonding interactions.

It is interesting that, at a high water concentration of 1.11 M, only two reduction peaks were observed, as shown in Figure 8B. The UV/Vis spectra of the species responsible for the three-step, four-electron-transfer process could not be controlled by adjusting the applied potential.

As shown in the CV results in Figure 8B, when the water concentration was increased to 1.11 M, the third reduction peak shifted to more positive potentials and finally merged with the second one. The new second peak currents increased with loss of reversibility. The first redox peaks shift positively, with no loss of reversibility. The  $n-\pi^*$  electronic transition band at 625 nm almost disappeared with an applied potential at the first reduction peak potential. With an applied potential at the second reduction peak potential, the characteristic band (at about 314 nm) of the diamagnetic dianions exhibited a slight redshift and the hyperfine band at about 350-450 nm was more clear compared to that in CH<sub>3</sub>CN. This redshift is mainly caused by hyperconjugation interactions.<sup>[24]</sup> The conformation of diamagnetic dianions changed and is more conjugative. With an increase in the time of electroanalysis, all absorption of the diamagnetic dianions decayed and the tetraanion characteristic absorption band at 285 nm occurred. It is clearly a three-step reduction process.

When the concentration of water was increased to 2.65 M, as shown in Figure 8C, only one reduction peak was observed. The spectra of the reduced forms change dramatically. With an applied potential at -1.15 V, the absorption band at 275 nm decreased and then disappeared; the new bands clearly appeared at 314, 365, 420, and 440 nm, whereas the absorption band at 625 nm completely disappeared.

Table 1. Electrochemical parameters: reduction of  $Bis-CoQ_0$  in the presence of water in  $CH_3CN$ .

	п	$K^{(n)}_{ea}$	Linear regression	R
Bis-CoQ <sub>0</sub> <sup>-</sup> (H <sub>2</sub> O) <sub>a</sub>	0.610	30.7	$\Delta E_{V}^{(1)} = 0.016 \ln C_{\text{H-O}} + 0.054$	0.997
Bis-CoQ $_0^{2-}$ (H <sub>2</sub> O) <sub>b</sub>	1.57	953	$\Delta E_{1_{L}}^{(2)} = 0.025 \ln C_{\text{H},0} + 0.084$	0.998
Bis-CoQ <sub>0</sub> <sup>4</sup> (H <sub>2</sub> O) <sub>c</sub>	4.15	$6.48 \times 10^{4}$	$\Delta E_{1_0}^{(3)} = 0.066 \ln C_{\rm H_2O} + 0.280$	0.999

with varying concentrations of water.

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(line b),  $CoQ_0^{-}-CoQ_0^{-}$  (line c), and  $CoQ_0^{2-}-CoQ_0^{2-}$  (line d) in CH<sub>3</sub>CN

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 Table 2. Band assignment of UV/Vis spectra of Bis-CoQ<sub>0</sub> and its reduced intermediates in CH<sub>3</sub>CN with varying concentrations of water.

 Water
 Characteristic absorption band [nm]

 concentration in CH<sub>3</sub>CN [M]
 Bis-CoQ<sub>0</sub>
 Bis-CoQ<sub>0</sub><sup>2</sup>
 Bis-Co

concentration in CH <sub>3</sub> CN [M]	Bis-CoQ <sub>0</sub>	Bis-CoQ <sub>0</sub>	Bis-CoQ <sub>0</sub> <sup>2-</sup>	Bis-CoQ <sub>0</sub> <sup>4-</sup>
0	275, 400	625	306, 625	284
			350, 450	
0.22	275, 400	625	306, 400	284
			625	
1.11	275, 400	625	314, 365	285
			420, 440	
2.65	275, 400		313, 365	306
			420, 440	



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The absorption bands of monoradicals were not observed,

or perhaps the absorption bands of monoradicals and diamagnetic dianions are too similar to separate them. It may

be caused by the fact that the first two electron-transfer pro-

cesses were too close to be distinguished separately. With an

increase in the time of electroanalysis, all absorption of the

radicals had decayed and the characteristic  $\pi$ - $\pi$ \* electronic transition absorption band for tetraanions at 310 nm occurred. The absorption band of the tetraanion experienced a

redshift of about 20 nm relative to that in CH<sub>3</sub>CN with low

water concentration. The obvious redshift cannot be due to

the change in bulk polarity, since even in water the absorbance band of tetraanions of  $Bis-CoQ_0$  is at 288 nm. To our

knowledge, electron-transfer reactions and the electrochemical behavior of compounds that contain several identical redox centers without electronic communications should be

the same as those of monomeric ones. The absorption bands of diamagnetic dianions and tetraanions of  $Bis-CoQ_0$  and

their electrochemical behavior are very similar to the absorption bands of radical anions and dianions of  $CoQ_0$ 

under the same solution conditions. The three-step reduc-

tion process with a total transfer of four electrons gradually converts into a two-step, two-electron reduction process for

Bis-CoQ<sub>0</sub> with increasing water concentrations in water/

 $CH_3CN$  solutions. The process is that the two quinonyl groups of Bis-CoQ<sub>0</sub> accept one electron respectively, and then the diamagnetic dianions of Bis-CoQ<sub>0</sub> convert in tet-

raanions by accepting the other two electrons. The absorption bands of  $Bis-CoQ_0$  intermediates exhibit redshifts. The electronic communications gradually decreased with en-

hanced hyperconjugative interactions. When only one reduction peak was observed, the electronic communications of

Assessment of Antioxidant Capacity To estimate the antioxidation efficacy of Bis-CoQ<sub>0</sub>, the protective effect of Bis-CoQ<sub>0</sub> against oxidative damage induced

by hydrogen peroxide to HeLa cells was investigated. The capability of  $Bis-CoQ_0$  to attenuate the damage induced by hydrogen peroxide toward HeLa cells was much improved

compared to that of coenzyme  $Q_{10}$  (Co $Q_{10}$ ). Co $Q_{10}$  is an es-

sential natural cofactor found in the mitochondria of every

cell in the body and has an antioxidation biological function.

Therefore, Bis-CoQ<sub>0</sub> may have potential as a therapeutic

Bis-CoQ<sub>0</sub> may have been blocked.

agent in the treatment of oxidative stress-related tissue injury and thereby minimize the oxidative damage suffered by mitochondrial DNA.

### Conclusion

In summary, we have synthesized a methylene-bridged biscoenzyme Q<sub>0</sub> and investigated its electrochemical, UV/Vis, and EPR spectroelectrochemical properties under different redox states in organic solvents. The chemical reaction in the third redox step process was confirmed by variable temperature cyclic voltammetry. Strong intramolecular electronic communications between two methylene-bridged quinonyl moieties of Bis-CoQ<sub>0</sub> were found. Hydrogen-bonding interactions between water and the electrochemically reduced intermediates of Bis-CoQ<sub>0</sub> in an aqueous organic solvent have also been studied. The electronic communications of Bis-CoQ<sub>0</sub> may have been blocked when one reduction peak was observed with proper quantities of water in water/ CH<sub>3</sub>CN solution. The presence of anion radicals and diamagnetic dianions during the redox processes of Bis-CoQ<sub>0</sub> may strengthen its capacity as an antioxidant. We are currently investigating this capability. In addition, this study gave us an indication that it might be possible to modify and control the electrochemical properties of Bis-CoQ<sub>0</sub> by tuning the coupling groups and therefore widen the potential application window of CoQs.

### **Experimental Section**

#### Chemicals

Tetrabutylammonium perchlorate (TBAP Fluka, >99.0%) was dried under vacuum at 358 K for 24 h and stored under vacuum. HPLC-grade CH<sub>3</sub>CN (Sigma) was initially dried by distillation over CaH<sub>2</sub> before use. All chemicals were used as received without further purification unless specified.

### Method for Nonaqueous CH<sub>3</sub>CN for Electrochemistry

HPLC-grade CH<sub>3</sub>CN (Sigma) was initially dried by distillation over CaH<sub>2</sub> before use. All solutions for electrochemistry were dried by placing the solvent, electrolyte, and Bis-COQ<sub>0</sub> inside a 25 mL vacuum syringe (Yikang Medical Instrument Group Co. Ltd, Jiangxi, China) that contained 3 Å molecular sieves (dried under vacuum at 513 K for 12 h) and storing the syringe under a dry nitrogen atmosphere for at least 48 h. During the measurement, a dry nitrogen purge maintained an oxygen and moisture-free environment. The quantitative water was added incrementally to the solution by using a microsyringe. The water content was calculated on the basis of the accurately known volume of added water.

#### Apparatus and Electrochemical Measurements

All the electrochemical experiments were conducted with a computercontrolled CHI 660 electrochemical station (Chenhua Co. Ltd, Shanghai, China). Working electrodes were 3 mm-in-diameter planar glassy carbon (GC) disks and were used in conjunction with a Pt auxiliary electrode and am Ag/AgCl wire pseudoreference electrode connected to the 0.15 M TBAP in CH<sub>3</sub>CN test solution. Accurate potentials were obtained by using ferrocenium/ferrocene as an internal standard. Variable-temperature experiments were performed with a nonisothermal electrochemical cell. The cell was jacketed in a glass sleeve and the temperature was controlled from 280 to 330 K with a Jinghong DKB-501A (Jinghong Co. Ltd, Shanghai, China) variable-temperature water-circulating bath.

#### In Situ Electron Paramagnetic Resonance (EPR) Spectroelectrochemistry.

The home-built spectroelectrochemical cell used in EPR experiments was as described earlier.<sup>[25]</sup> Silver wire was used as the working electrode and the length of it matched the height of the EPR cavity. An alloy wire electrode and a silver electrode served as the counterelectrode and the reference electrode, respectively. EPR measurements were performed in the X-band region with a Bruker EMX-8/2.7 spectrometer at room temperature. The potential during the EPR measurements was controlled with an electrochemical workstation. EPR scan parameters used the following: microwave frequency 9.87 GHz, modulated frequency 100 KHz, modulated amplitude 0.15 G, time constant 81.92 s, conversion time 163.84 s.

#### In Situ UV/Vis Spectroelectrochemistry.

In situ UV/Vis spectroelectrochemistry was carried out with a PC-controlled Oceanoptics DT-Mini-2-GS situ spectrometer at a resolution of 1 nm and with an optically transparent thin-layer electrode (OTTLE) cell at room temperature. The OTTLE cell was constructed from quartz, and the OTTLE is a platinum gauze situated in a  $(0.4\pm0.1)$  mm optical-pathlength section of the cell with a platinum wire counterelectrode, Ag/ AgCl wire pseudoreference electrode, and a Teflon tube for purging with dry nitrogen.

#### Assessment of Antioxidant Capacity

The antioxidation capability of  $Bis-CoQ_0$  against oxidative damage induced by hydrogen peroxide to HeLa cells was determined by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.^{[26]}

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