

# Stabilization of a semiquinone radical at the high-affinity quinone-binding site ( $Q_H$ ) of the *Escherichia coli* *bo*-type ubiquinol oxidase\*\*

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**Abstract** Reaction of ubiquinone in the high-affinity quinone-binding site ( $Q_H$ ) in *bo*-type ubiquinol oxidase from *Escherichia coli* was revealed by EPR and optical studies. In the  $Q_H$  site, ubiquinol was shown to be oxidized to ubisemiquinone and to ubiquinone, while no semiquinone signal was detected in the oxidase isolated from mutant cells that cannot synthesize ubiquinone. The  $Q_H$  site highly stabilized ubisemiquinone radical with a stability constant of 1–4 at pH 8.5 and the stability became lower at the lower pH. Midpoint potential of  $QH_2/Q$  couple was  $-2$  mV at pH 8.5 and showed  $-60$  mV/pH dependence indicative of  $2H^+/2e^-$  reaction. The  $E_m$  was more negative than that of low-spin heme *b* above pH 7.0. We conclude that the  $Q_H$  mediates intramolecular electron transfer from ubiquinol in the low-affinity quinol oxidation site ( $Q_L$ ) to low-spin heme *b*. Unique roles of the quinone-binding sites in the bacterial ubiquinol oxidase are discussed.

**Key words:** *E. coli bo*-type ubiquinol oxidase; Quinol oxidation; Semiquinone radical; EPR; Potentiometric titration; Intramolecular electron transfer

## 1. Introduction

Cytochrome *bo*-type ubiquinol oxidase in *Escherichia coli* was first identified as cytochrome *o* in 1959 [1] and belongs to the heme-copper respiratory oxidase superfamily [2]. It catalyzes the two-electron oxidation of ubiquinol-8 ( $Q_8H_2$ ) at the periplasmic side of the cytoplasmic membrane and the four-electron reduction of dioxygen at the cytoplasmic side [3]. It has been shown that not only protolytic scalar reactions but also redox-coupled proton pumping establish an electrochemical proton gradient across the membrane [4,5].

Subunit I of the oxidase binds low-spin heme *b*, high-spin

heme *o* and  $Cu_B$  and plays a central role of dioxygen reduction and proton pumping [6,7]. Recent site-directed mutagenesis studies revealed a molecular structure of the heme *o*- $Cu_B$  binuclear center [6,7] and a possible mechanism for the facilitated heme *b*-to-heme *o* electron transfer [6,8]. Based on photoaffinity cross-linking experiments, a quinone-binding site(s) has been assigned to reside in subunit II [9]. Therefore, electrons seem to travel from the quinol oxidation site in subunit II to the metal centers in subunit I as in cytochrome *c* oxidase. Since quinols are two-electron redox components while the metal centers of terminal oxidases are one-electron transfer systems, there must be a specific mechanism for gating electron flux, which, however, is yet unknown.

Recently, we demonstrated the presence and functional importance of a novel high-affinity quinone-binding site ( $Q_H$ ) in the *E. coli bo*-type ubiquinol oxidase [10]. The  $Q_H$  site is distinct from a low-affinity quinol oxidation site ( $Q_L$  [11]) and is close to both the  $Q_L$  site and low-spin heme *b* [10]. Based on these observations, we postulated that the  $Q_H$  mediates electron transfer from the  $Q_L$  to the metal centers, like the  $Q_A$  of the photosynthetic reaction center [10]. In this article, we present evidence for involvement of the  $Q_H$  in electron transfer from the  $Q_L$  to heme *b*. It is also suggested that a mechanism of electron transfer in the  $Q_H$  site is different from that in the  $Q_A$  site that stabilizes semiquinone but destabilizes a fully reduced form, since the  $Q_H$  site can tightly bind ubiquinol and stabilize semiquinone radical.

## 2. Materials and methods

### 2.1. Purification of *bo*-type ubiquinol oxidase

The wild-type and the bound  $Q_8$ -free oxidases were isolated from the *E. coli* strains GO103/pMFO2 (*cyo<sup>+</sup>acyd/cyo<sup>+</sup>*) and MU1227/pMFO2 (*cyo<sup>+</sup>cyd<sup>+</sup>Δ ubiA/cyo<sup>+</sup>*), which is defective in ubiquinone biosynthesis, respectively [10,12]. The purified enzymes in 50 mM Tris-HCl (pH 7.4) containing 0.1% sucrose monolaurate SM-1200 (Mitsubishi Kasei Food, Tokyo, Japan) were stored at  $-80^\circ\text{C}$  until use. By the polyethylene glycol treatment [10], any loosely bound quinones were removed from the wild-type enzyme preparation. Thus, the wild-type enzyme used in this study contains only a single tightly bound  $Q_8$  molecule [10].

### 2.2. EPR redox titration experiments

The stock enzyme was diluted to  $10\ \mu\text{M}$  with 50 mM Tris-HCl at the indicated pH containing 0.1% sucrose monolaurate and 100 mM KCl. The redox mediators used were  $40\ \mu\text{M}$  phenazine ethosulfate,  $20\ \mu\text{M}$  2,3,5,6-tetramethyl phenylenediamine,  $40\ \mu\text{M}$  *N,N,N',N'*-tetramethyl

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**Abbreviations:**  $Q_8H_2$ , ubiquinol-8;  $Q_H$  site, a high-affinity quinone-binding site;  $Q_L$  site, a low-affinity quinol oxidation site;  $Q_H$ , ubiquinone-8 bound at the  $Q_H$  site;  $Q_n$ , ubiquinone-*n*;  $Q^{\bullet-}/QH_2$ , semiquinone/quinol;  $Q/Q^{\bullet-}$ , quinone/semiquinone;  $Q/QH_2$ , quinone/quinol.

phenylenediamine and 40  $\mu\text{M}$  ferric ethylenediaminetetraacetic acid. Any quinone-like mediators were avoided for the titration. Redox titrations were performed at 25°C in oxidative direction by adding a small aliquot of the sodium ferricyanide solution to the dithionite-reduced enzyme. Redox potential of the reaction medium, monitored by a Pt electrode with an Ag/AgCl reference electrode, was expressed in mV unit referred to the normal hydrogen electrode. Samples were poised at every 25–35 mV change, transferred to EPR tubes and quickly frozen in liquid  $\text{N}_2$ . The sample and tubes were flushed with water-saturated Ar gas for 1 h before and during the titration. EPR spectra were recorded at 20 K with an X-band Bruker ER 200D spectrometer equipped with a liquid-helium cryostat (ESR-900, Oxford Instruments) in the Analytical Center of National Institute of Basic Biology as described by Hata et al. [13]. EPR conditions were as follows: temperature, 20 K; modulation frequency, 100 kHz; microwave frequency, 9.56 GHz. Microwave power and modulation amplitude were 0.2 mW and 0.5 mT, respectively, in the measurements of semiquinone and 10 mW and 2 mT, respectively, in the measurements of the high- and low-spin hemes. The spin concentration of semiquinone was determined with an oxidized P700 cation spin in the chloroplast photosystem I particles as a standard at the lower microwave power conditions.

### 2.3. Optical redox titration experiments

The enzyme was diluted to 1  $\mu\text{M}$  with 50 mM Tris-HCl (pH 7.5) containing 0.1% sucrose monolaurate and 100 mM KCl and introduced into an anaerobic glass cuvette with ~1 cm path length and 10 ml working volume. Redox titrations were carried out at room temperature with redox mediators as follows: 5  $\mu\text{M}$  pyocyanine, 50  $\mu\text{M}$  phenazine methosulfate, 20  $\mu\text{M}$  2,3,5,6-tetramethyl phenylenediamine and 50  $\mu\text{M}$  ferric ethylenediaminetetraacetic acid. The sample and cuvette were flushed with water-saturated  $\text{N}_2$  gas. Difference spectra were recorded with a Shimadzu UV-3000 spectrophotometer (Shimadzu, Kyoto, Japan) at 10–20 mV intervals upon addition of a small aliquot of the ferricyanide solution to the dithionite-reduced enzyme.

### 2.4. Miscellaneous

Protein concentration and heme content were determined as described previously [12]. The concentration of the purified enzyme was estimated from the total heme content, assuming that it contains 1 mol each of hemes B and O [12]. The syntheses of 2,6-dichloro-4-nitrophenol and 2,6-dichloro-4-dicyanovinylphenol were as described previously [11]. Other chemicals were commercial products of analytical grade.

## 3. Results

### 3.1. Stabilization of ubisemiquinone radical at $Q_H$ site

Our previous studies suggested that the  $Q_H$  site can stabilize ubisemiquinone radical during the oxidation of substrate to ensure sequential one-electron transfer to the metal centers [10]. To verify this postulate, we determined EPR spectra of the wild-type and the bound  $Q_8$ -free enzymes. The titration experiments with the wild-type enzyme were carried out at pH 8.5. At  $E_h = 0$  mV where semiquinone radical was stabilized, we observed a typical ubisemiquinone radical signal at  $g = 2.0$  with partially resolved splittings [14] (Fig. 1a). Addition of 2,6-dichloro-4-dicyanovinylphenol, a potent competitive inhibitor for the  $Q_L$  site ( $K_i = 3.1 \mu\text{M}$  [11]), to a final concentration of 100  $\mu\text{M}$  did not affect the intensity of the  $g = 2$  signal (Fig. 1b). In contrast, this signal was totally absent in the bound  $Q_8$ -free oxidase (Fig. 1c) and not observed in a free  $Q_1$  solution containing 50 mM Tris-HCl (pH 7.4) and 0.1% sucrose monolaurate (data not shown). When the reduced wild-type sample, which was anaerobically poised at -25 mV and frozen to 77 K, was subjected to several cycles of freeze-thaw under aerobic conditions to slowly elevate dioxygen level, the  $g = 2$  semiquinone signal increased its intensity until the 3rd run and became negligible after the 7th run (data not shown). This suggests electron

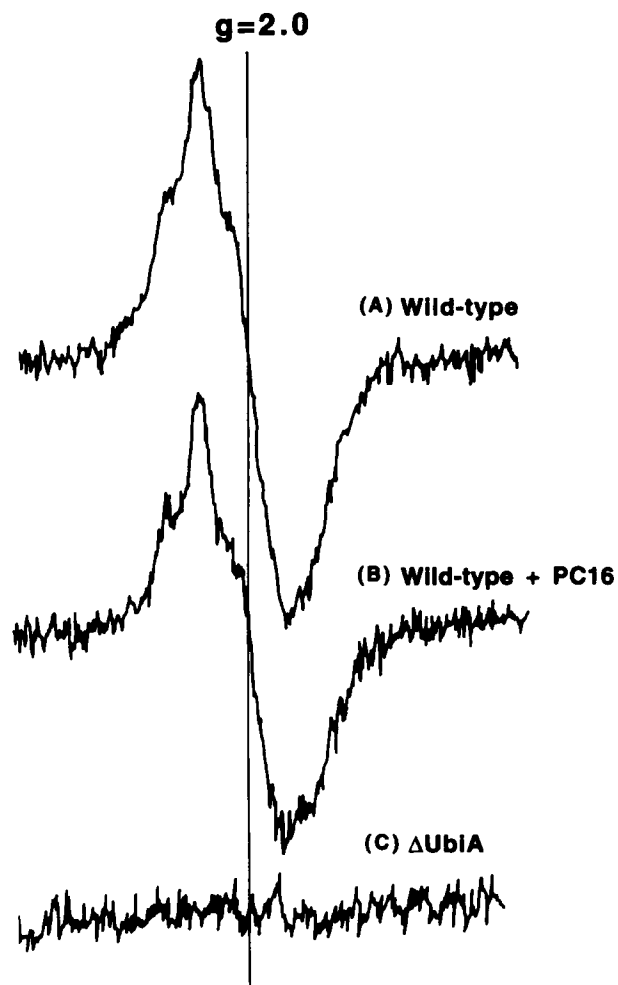


Fig. 1. EPR spectra of the wild-type oxidase in the absence (A) and presence (B) of 2,6-dichloro-4-dicyanovinylphenol (PC16) and of the bound  $Q_8$ -free oxidase (C). Measurements were done at pH 8.5 with or without preincubation with 100  $\mu\text{M}$  2,6-dichloro-4-dicyanovinylphenol for 15 min on ice.

transfer from the  $Q_H$  site to dioxygen via the metal centers. These observations strongly suggest that the  $Q_H$  site can stabilize ubisemiquinone radical during the oxidation of  $Q_8\text{H}_2$  supplied from a quinol pool in the cytoplasmic membrane.

### 3.2. Potentiometric EPR analysis of bound $Q_8$ and metal centers

The  $g = 2$  semiquinone radical was further analysed potentiometrically with the wild-type enzyme. The redox titrations revealed that  $Q_8$  bound at the  $Q_H$  site gives a bell-shaped curve with a peak at -2 mV. This suggests that  $Q_8$  can accept two electrons to form  $Q_8\text{H}_2$  (Fig. 2). The titration curve was analysed using the Nernst's equation as described in figure legend. The midpoint potential values for the  $Q^{\bullet-}/Q\text{H}_2$  and  $Q/Q^{\bullet-}$  couples ( $E_2$  and  $E_1$ ) were determined to be +10 and -13 mV, respectively. The value for a two-electron couple,  $Q/Q\text{H}_2$  ( $E_m$ ), was obtained as an average of those of one-electron couples (-1.5 mV). The concentration of  $g = 2$  semiquinone spin at  $E_h = 0$  mV peak was estimated to be  $0.4 \pm 0.1$ /oxidase from the

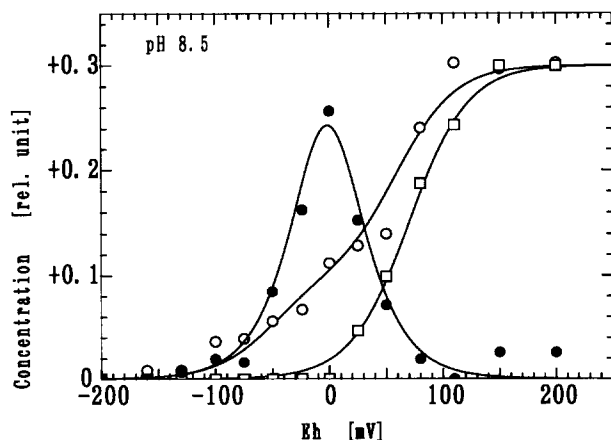


Fig. 2. Potentiometric titrations of the  $g = 2$  semiquinone signal (closed circles), the  $g = 3$  low-spin signal (open squares) and the  $g = 6$  high-spin signal (open circles) at pH 8.5. The titration curve of semiquinone was fitted by a function; [signal intensity] =  $C / (1 + 10^{(E_h - E_1)/59} + 10^{(E_2 - E_h)/59})$  with  $E_1$  and  $E_2$  values of  $-13$  and  $+10$  mV, respectively, where  $C$  is a optimal concentration of semiquinone at the  $Q_H$  site. The curve of heme titration was fitted by a function; [signal intensity] =  $C / (1 + 10^{(E_m - E_h)/59})$  with  $E_m = 70$  mV in the case of  $g = 3$  low-spin heme and by a sum of two set of this function with  $E_m$  value of  $-50$  and  $60$  mV, respectively, in the case of  $g = 6$  high-spin heme. The constant  $59$  (mV) comes from  $RT/F$  where  $R$  is the gas constant,  $F$  is the Faraday constant and  $T$  is absolute temperature.

measurement of spin density. The stability constant of the semiquinone ( $= [Q^{\cdot-}]/[Q][QH_2]$ ) is, then, calculated to be 1–4 at pH 8.5 indicative of the high stabilization of semiquinone in the  $Q_H$  site.

The  $g = 3$  low-spin heme signal was titrated as a single  $n = 1$  component with a midpoint potential of  $+70$  mV. This value is compatible with the idea that ubisemiquinone radical in the  $Q_H$  site donates an electron to ferric low-spin heme  $b$ . The  $g = 6$  high-spin signal of heme  $o$ , on the other hand, showed a titration curve that can be fitted by a 2:1 mixture of two components with midpoint potential values of  $60$  and  $-50$  mV, respectively, although the data quality was insufficient to determine these values correctly. The amount of the  $-50$  mV fraction roughly corresponds to the amount of semiquinone radical in the oxidase and suggests their interaction.

### 3.3. Effect of bound $Q_8$ on redox potentials of metal centers

Previously, we found that the absence of  $Q_8$  at the  $Q_H$  site results in a 3-nm red shift of the Soret peak of the air-oxidized enzyme to 412 nm [10]. This shift is likely to be associated with a change in the redox potential of low-spin heme  $b$  [10]. To examine the effect of the bound  $Q_8$  on the metal centers, optical redox titration analyses were carried out with the wild-type and bound  $Q_8$ -free oxidases by monitoring the  $\alpha$  and Soret peaks in redox difference spectra.

It is known that low-spin heme  $b$  contributes to most of the  $\alpha$  absorption while the Soret absorption is a sum of equal contributions of low- and high-spin hemes [15]. The titration curves for both bands at pH 7.5, therefore, can be fitted by a two-component model with similar  $E_m$  values, as shown in Fig. 3. The composite curves can be attributed to heme  $b$ -heme  $o$  interactions in the oxidase molecule as has been suggested by [16]. The  $E_m$  values for the wild-type and bound  $Q_8$ -free oxi-

dases were calculated to be 67 and 123 mV and 92 and 143 mV, respectively. This indicates that binding of a  $Q_8$  molecule to the  $Q_H$  site resulted in 20–25 mV reduction of the  $E_m$  value for low-spin heme  $b$  and possibly for heme  $o$  through the heme  $b$ -heme  $o$  interactions. As discussed previously [10], the  $E_m$  shift could occur via direct electrostatic interactions between the  $Q_H$  and heme  $b$  or conformational change of the enzyme upon binding of  $Q_8$  to the  $Q_H$  site. Similar findings were reported for interactions between the  $Q_i$  site and cytochrome  $b_{562}$  of cytochrome  $bc_1$  complex [17]. In addition to the main components, the titration curve in the Soret region of the wild-type enzyme indicated a presence of a small fraction with a midpoint potential of about  $-50$  mV. This fraction seems to correspond to the fraction of high-spin heme  $o$  titrated with an estimated midpoint potential of  $-50$  mV in Fig. 2. The titration in the bound  $Q_8$ -free oxidase does not show such a low potential fraction (this was confirmed by EPR) so that the high-spin heme with this negative potential may reflect some kind of interaction between  $Q^{\cdot-}$  and the heme.

### 3.4. pH dependence of midpoint potentials of ubisemiquinone and of metal centers

We examined the pH dependence of the midpoint potentials of the  $g = 2$  semiquinone signal and the  $g = 3$  low-spin heme signal. The peak intensities of the semiquinone signal at pH 7.6 and 6.2 in the titrations were  $\sim 20\%$  that at pH 8.5 (not shown)

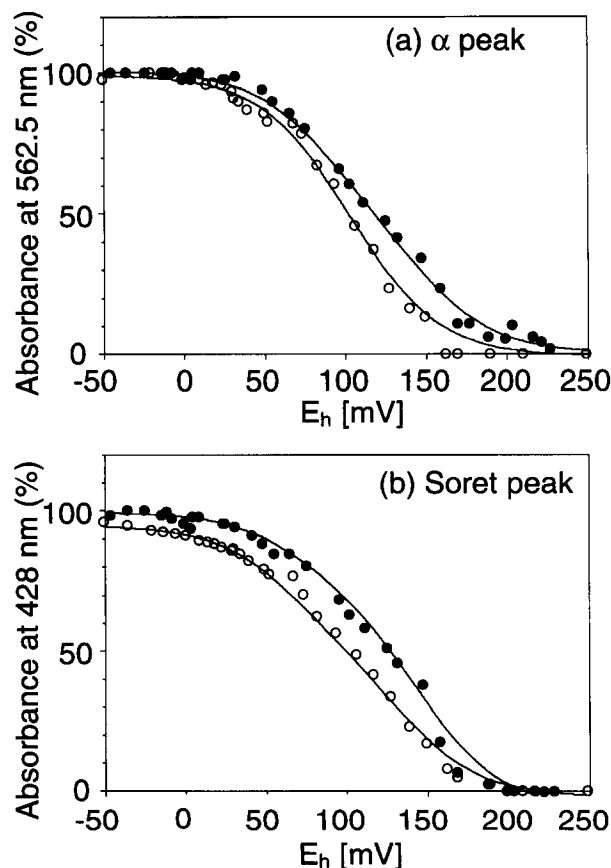


Fig. 3. Potentiometric titrations of the  $\alpha$  (A) and Soret (B) peaks of the wild-type (open circles) and the bound  $Q_8$ -free (closed circles) oxidases at pH 7.5. The data for the  $\alpha$  and Soret bands are best fitted by a two-component model with similar  $E_m$  values.

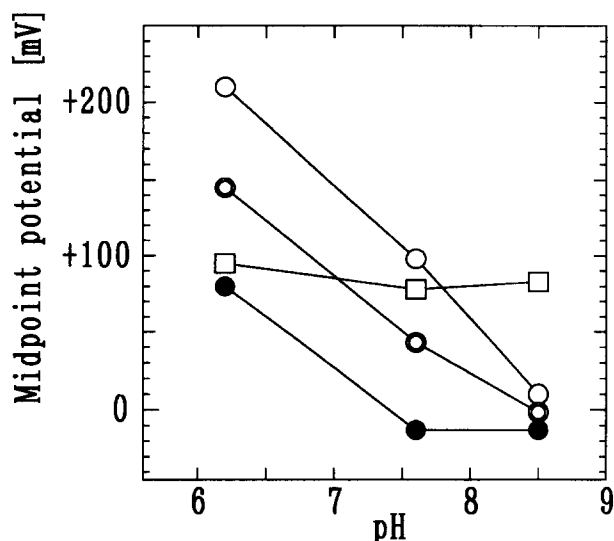


Fig. 4. pH dependence of the midpoint potential of the  $Q^{\bullet -}/QH_2$  ( $E_2$ , ○),  $Q/Q^{\bullet -}$  ( $E_1$ , ●),  $Q/QH_2$  ( $E_m$ , ⊙) and the  $g = 3$  low-spin signal (□). Midpoint potential values were calculated as described in Fig. 2.

indicating the destabilization of semiquinone at the lower pH. With these peak heights, and the positions and shapes of titration curves, the values of  $E_1$ ,  $E_2$  and  $E_m = (E_1 + E_2)/2$  were estimated and plotted in Fig. 4. The  $E_m$  value decreased sharply with increasing the medium pH with a slope of approximately  $-60$  mV/pH unit indicative of  $2H^+/2e^-$  ratio of the quinol oxidation at the  $Q_H$  site.  $E_2$  decreased with a slope of approximately  $-120$  mV/pH unit around pH 8.  $E_1$  showed a pK at around pH 7.5 and increased below this pH with a dependency of  $-60$  mV/pH unit.  $E_2$  decreased with a steeper slope. Above pH 7.5, the deprotonation seems to occur mainly in the oxidation step from  $QH_2$  to  $Q^{\bullet -}$ .

In contrast, the low-spin heme signal showed almost no pH dependence at the pH values examined. The  $E_m$  of the heme was more positive than the  $E_m$  of  $QH_2/Q$  couple above pH 7 indicative of electron transfer from  $QH_2$  to low-spin heme  $b$ .

#### 4. Discussion

Cytochrome *bo*-type ubiquinol oxidase in *E. coli* contains the high-affinity quinone-binding site ( $Q_H$  [10]) and the low-affinity quinol oxidation site ( $Q_L$  [11]). It was postulated that the  $Q_H$  site functions as a molecular gate which regulates electron flux from the two-electron redox component (i.e. quinols) to one-electron carriers (i.e. heme irons) and mediates electron transfer from the  $Q_L$  site in subunit II to low-spin heme  $b$  in subunit I [10]. Thus, the  $Q_H$  site is expected to stabilize transiently ubisemiquinone radical during the catalytic cycle, in a manner similar to the  $Q_A$  site of the photosynthetic reaction center. In the present study, we carefully prepared the wild-type oxidase with and without a single bound  $Q_8$  molecule at the  $Q_H$  site and carried out detailed potentiometric analyses of ubisemiquinone and the low-spin heme signals. We obtained clear evidence for stabilization of ubisemiquinone radical in the oxidase by monitoring the  $g = 2$  signal associated with the bound  $Q_8$  at the  $Q_H$  site. A loss of this signal in the bound  $Q_8$ -free enzyme supports this conclusion. Since the  $Q_H$  can be doubly reduced, redox

chemistry in the  $Q_H$  site (i.e. two one-electron transfer reactions and the addition of two protons) resembles that of the  $Q_B$  site rather than the  $Q_A$  site of the photosynthetic reaction center although the tight-binding of quinone in the  $Q_H$  site resembles that in the  $Q_A$  site. It seems that the  $Q_H$  site functions as a transient electron reservoir that facilitates the two-electron oxidation of  $Q_8H_2$  that is bound to the  $Q_L$  site. Subsequently, the  $Q_8$  at the  $Q_L$  site will be replaced by another  $Q_8H_2$  molecule in the membrane pool and a next redox cycle of quinone will be started. To ensure the successive four-electron reduction of molecular oxygen at the heme-copper binuclear center, the  $Q_L$  site may have a low affinity for  $Q_8$  to achieve rapid dynamic equilibrium with the  $Q_8H_2$  pool.

Very recently, Ingledew et al. [14] have reported EPR studies on the purified *E. coli bo*-type ubiquinol oxidase in the presence of 20-fold excess amount of  $Q_1$ . Based on detailed theoretical considerations, they concluded that ubisemiquinone radical can be stabilized at the quinol oxidation site although it is unclear whether this semiquinone signal was originated from either the  $Q_L$  site or the  $Q_H$  site. Their EPR spectra and titration curve quite resemble to ours. The result in the present study suggests that their signals are also originated from the  $Q_H$  site rather than the  $Q_L$  site.

We also tried to demonstrate stabilization of semiquinone radical at the  $Q_L$  site, as postulated previously [11]. The  $Q_H$  site of the bound  $Q_8$ -free oxidase was first reconstituted with 2,6-dichloro-4-nitrophenol, a high-affinity quinone analog for the  $Q_H$  site ( $K_d = 1.8 \mu M$ ) (M. Sato-Watanabe, T. Mogi, H. Miyoshi and Y. Anraku, unpubl. data), and then we observed a similar semiquinone radical signal in the EPR spectrum of the reconstituted enzyme in the presence of 1 mM  $Q_1$  (data not shown). However, due to a slow exchange of the bound 2,6-dichloro-4-nitrophenol at the  $Q_H$  site with free  $Q_1$  molecules [10], the assignment of observed semiquinone signal was difficult.

The  $Q_H$  site affected the absorption spectrum and the redox behavior of heme  $b$ . The redox potential of heme  $b$  was decreased by 20 mV by the presence of the  $Q_H$  quinone. This may facilitate electron transfer from ubiquinol to the heme by increasing the motive force (energy gap) between them. This effect might be structural or electrostatic since the absorption and Raman spectra of heme  $b$  were altered as well [10]. The interaction with high-spin heme  $o$  was also suggested since the redox midpoint potential of the latter became more negative in the presence of  $Q^{\bullet -}$  as seen in Fig. 2. The effect may be caused by the electrostatic interaction between the charges on the semiquinone and heme  $o$  or may be due to some structural effect. The details of this effect are now being analysed.

In conclusion, we have demonstrated that the  $Q_H$  site has molecular properties favorable for mediating electron transfer between the  $Q_L$  site and low-spin heme  $b$ . Recent time-resolved spectroscopic studies on *bo*-type ubiquinol oxidase provided a support for the involvement of the  $Q_H$  site in intramolecular electron transfer processes (M. Sato-Watanabe, T. Mogi, Y. Anraku and Y. Orii, unpubl. data). Identification of the  $Q_L$  and  $Q_H$  sites will provide a clue for structure and mechanism of the quinol oxidation site.

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## References

- [1] Castor, L.N. and Chance, B. (1959) *J. Biol. Chem.* 234, 1587–1592.
- [2] Saraste, M., Holm, L., Lemieux, L., Lübbers, M. and van der Oost, J. (1991) *Biochem. Soc. Trans.* 19, 608–612.
- [3] Anraku, Y. and Gennis, R.B. (1987) *Trends. Biochem. Sci.* 12, 262–266.
- [4] Kita, K., Kasahara, M. and Anraku, Y. (1982) *J. Biol. Chem.* 257, 7933–7935.
- [5] Puustinen, A., Finel, M., Virkki, M. and Wikström, M. (1989) *FEBS Lett.* 249, 163–167.
- [6] Mogi, T., Nakamura, H. and Anraku, Y. (1994) *J. Biochem. (Tokyo)* 116, 741–747.
- [7] Garcia-Horsman, J.A., Barquera, B., Rumbley, J., Ma, J. and Gennis, R.B. (1994) *J. Bacteriol.* 176, 5587–5600.
- [8] Woodruff, W.H. (1993) *J. Bioenerg. Biomembr.* 25, 177–188.
- [9] Welter, R., Gu, L.-Q., Yu, L., Yu, C.-A., Rumbley, J. and Gennis, R.B. (1994) *J. Biol. Chem.* 269, 28834–28838.
- [10] Sato-Watanabe, M., Mogi, T., Ogura, T., Kitagawa, T., Miyoshi, H., Iwamura, H. and Anraku, Y. (1994) *J. Biol. Chem.* 269, 28908–28912.
- [11] Sato-Watanabe, M., Mogi, T., Miyoshi, H., Iwamura, H., Matsushita, K., Adachi, O. and Anraku, Y. (1994) *J. Biol. Chem.* 269, 28899–28907.
- [12] Tsubaki, M., Mogi, T., Anraku, Y. and Hori, H. (1993) *Biochemistry* 32, 6065–6072.
- [13] Hata, A., Kirino, Y., Matsuura, K., Itoh, S., Hiyama, T., Konishi, K., Kita, K. and Anraku, Y. (1985) *Biochim. Biophys. Acta* 810, 62–72.
- [14] Ingledew, W.J., Ohnishi, T. and Salerno, J.C. (1995) *Eur. J. Biochem.* 227, 903–908.
- [15] Puustinen, A., Finel, M., Haltia, T., Gennis, R.B. and Wikstrom, M. (1991) *Biochemistry* 30, 3936–3942.
- [16] Salerno, J.C., Bolgiano, B., Poole, R.K., Gennis, R.B. and Ingledew, W.J. (1990) *J. Biol. Chem.* 265, 4364–4368.
- [17] Salerno, J.C., Xu, Y., Osgood, M.P., Kim, C.H. and King, T.E. (1989) *J. Biol. Chem.* 264, 15398–15403.