Transient Formation of Ubisemiquinone Radical and Subsequent Electron Transfer Process in the *Escherichia coli* Cytochrome *bo*†

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ABSTRACT: To elucidate a unique mechanism for the quinol oxidation in the *Escherichia coli* cytochrome *bo*, we applied pulse radiolysis technique to the wild-type enzyme with or without a single bound ubiquinone-8 at the high-affinity quinone binding site (Q_H), using *N*-methylnicotinamide (NMA) as an electron mediator. With the ubiquinone bound enzyme, the reduction of the oxidase occurred in two phases as judged from kinetic difference spectra. In the faster phase, the transient species with an absorption maximum at 440 nm, a characteristic of the formation of ubisemiquinone anion radical, appeared within 10 μs after pulse radiolysis. In the slower phase, a decrease of absorption at 440 nm was accompanied by an increase of absorption at 428 and 561 nm, characteristic of the reduced form. In contrast, with the bound ubiquinone-8-free wild-type enzyme, NMA radicals directly reduced hemes *b* and *o*, though the reduction yield was low. These results indicate that a pathway for an intramolecular electron transfer from ubisemiquinone anion radical at the Q_H site to heme *b* exists in cytochrome *bo*. The first-order rate constant of this process was calculated to be 1.5×10^3 s^{-1} and is comparable to a turnover rate for ubiquinol-1. The rate constant for the intramolecular electron transfer decreased considerably with increasing pH, though the yields of the formation of ubisemiquinone anion radical and the subsequent reduction of the hemes were not affected. The pH profile was tightly linked to the stability of the bound ubisemiquinone in cytochrome *bo* [Ingledew, W. J., Ohnishi, T., and Salerno, J. C. (1995) *Eur. J. Biochem. 227*, 903−908], indicating that electron transfer from the bound ubisemiquinone at the Q_H site to the hemes slows down at the alkaline pH where the bound ubisemiquinone can be stabilized. These findings are consistent with our previous proposal that the bound ubiquinone at the Q_H site mediates electron transfer from the low-affinity quinol oxidation site in subunit II to low-spin heme *b* in subunit I.

Cytochrome *bo* is a four-subunit heme-copper terminal oxidase in the aerobic respiratory chain of *Escherichia coli*, and catalyzes the two-electron oxidation of ubiquinol-8 (Q_8H_2)[1] at the periplasmic side of subunit II and the four-electron reduction of dioxygen to water at the heme-copper binuclear center in subunit I (*1*−*3*). Redox reactions couple to the generation of a proton electrochemical gradient across the cytoplasmic membrane not only via scalar reactions but also via a proton pump mechanism (*4*). Subunit I binds all the redox metal centers, low-spin heme *b*, high-spin heme *o* and Cu_B, and serves as a central machinery for redox-coupled proton pumping (*1*, *2*). A low-affinity quinol oxidation site (Q_L) is located in the C-terminal hydrophilic domain of subunit II (*5*−*8*), though this subunit does not contain any redox metal centers such as Cu_A. Subunits III and IV are required for assembly of the redox metal centers in subunit I (*9*−*11*), but not involved in catalytic function.

To ensure the coupling of one-electron redox reaction to an two-electron acceptor/donor, quinone redox enzymes usually have two quinone/quinol binding sites. In the photosynthetic reaction center, Q_A acts as a tightly bound one-electron carrier between pheophytin and Q_B, whereas Q_B is a two-electron and two-proton redox component and in a dynamic equilibrium with quinone pool in the membrane (*12*−*14*). For the cytochrome bc_1 complex (complex III), two quinone/quinol redox sites, Q_o and Q_i, are both in dynamic equilibrium with the quinone pool, and the coordinated redox reactions are tightly coupled to vectorial proton translocation via the proton motive Q cycle (*15*−*19*), originally postulated by Mitchell (*20*). Site-directed mutagenesis (*21*) and X-ray crystallographic (*22*) studies on fumarate reductase (complex II) from *E. coli* demonstrated that the two menaquinone/menaquinol binding sites, Q_P and Q_D, are present at the cytoplasmic and periplasmic surfaces of the cytoplasmic membrane, respectively. Because of the absence of any redox component in the transmembrane

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[1] Abbreviations: ΔUbiA, the wild-type enzyme isolated from a ubiquinone-deficient mutant; e_{aq}^-, hydrated electron; NMA, *N*-methylnicotinamide; Q_n, ubiquinone-*n*; Q_nH_2, ubiquinol-*n*; Q_H, the high-affinity quinone binding site; Q_L, the low-affinity quinol oxidation site.

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region (*22*), a functional role of Q_D remains uncertain. In the *E. coli* cytochrome *bo*, besides the low-affinity quinol oxidation site (Q_L) in subunit II (*5*−*8*), biochemical analysis of bound quinones (*23*) and structure−function studies on quinone-related inhibitors (*24*, *25*) established the presence of a high-affinity quinone binding site (Q_H), which is close to both the Q_L site and heme *b* (*23*, *26*) and seems to be present in the periplasmic regions of helices I and II of subunit I (*27*). On the basis of steady-state kinetic analysis (*23*, *25*), potentiometric (*26*, *28*), and stopped-flow studies (*29*), we postulated that the Q_H site serves as an electron gate and mediates the sequential one-electron transfer from the Q_L site in subunit II to the hemes in subunit I.

A powerful approach for investigating electron transfer within proteins is that of pulse radiolysis through which an electron can be introduced rapidly and selectively into one redox center of enzymes (*30*−*37*). To dissect intramolecular electron-transfer processes in the heme-copper terminal quinol oxidases, we applied pulse radiolysis to the *E. coli* cytochrome *bo* with or without the bound Q_8 at the Q_H site. Here, we report the transient generation of ubisemiquinone anion radical and subsequent intramolecular electron transfer to the redox metal centers in subunit I. These findings are consitent with the proposed role of the Q_H site in intramolecular electron transfer between the Q_L site in subunit II and heme *b* in subunit I (*23*, *25*−*29*).

MATERIALS AND METHODS

Purification of Cytochrome bo. The wild-type enzyme with a single bound Q_8 was isolated from the cytochrome *bo*-overproducing strain GO103/pHN3795-1 (*cyo*$^+$ Δ*cyd ubiA*$^+$/*cyo*$^+$), as described previously (*38*), and was stored in 50 mM Tris-HCl (pH 7.4) containing 0.1% sucrose monolaurate (Mitsubishi-Kagaku Foods Co., Tokyo). The wild-type enzyme without any bound ubiquinones (ΔUbiA) was purified from the ubiquinone biosynthesis mutant MU1227/pMFO4 (*cyo*$^+$ *cyd*$^+$ Δ*ubiA*/*cyo*$^+$) (*23*). The concentration of the enzymes was calculated from the heme content determined by pyridine ferrohemochromogen method using an extinction coefficient of 20 700 (cm^{-1} M^{-1}) for heme B (*39*).

Pulse Radiolysis. Pulse radiolysis experiments were performed under anaerobic conditions with an linear accelerator at the Institute of Scientific and Industrial Research, Osaka University (*30*−*37*). The pulse width and energy were 8 ns and 27 MeV, respectively. The source for monitoring light was a 150 W halogen lamp or a 1 kW xenon lamp. After passing through an optical path, the transmitted light intensity was analyzed and monitored by a fast spectrophotometric system composed of a Nikon monochromator, an R-928 photomultiplier, and a Unisoku data analyzing system. The concentration of the *N*-methylnicotinamide (NMA) radicals generated by pulse radiolysis was determined by absorbance change at 420 nm using an extinction coefficient of 3.2 mM^{-1} cm^{-1} (*40*), and was adjusted by varying the dose of the electron beam.

Samples for pulse radiolysis were prepared as follows. The enzyme solutions, which contain 10 mM potassium phosphate (pH 6−8) or 10 mM sodium borate (pH 8−9), 0.1% sucrose monolaurate, and 5 mM NMA, were subjected to repeated deaeration followed by flushing with Ar gas. Sucrose monolaurate acts as a scavenger for OH radicals (*37*), therefore, a radical scavenger such as *tert*-butyl alcohol was not added to the solution. The quartz cells had a light path of 0.3 or 1 cm. For each pulse, a fresh sample was used, even though pulse radiolysis did not give any damage to the enzyme as judged by its visible absorption spectrum. Static absorption spectra were recorded with a Hitachi U-3000 spectrophotometer.

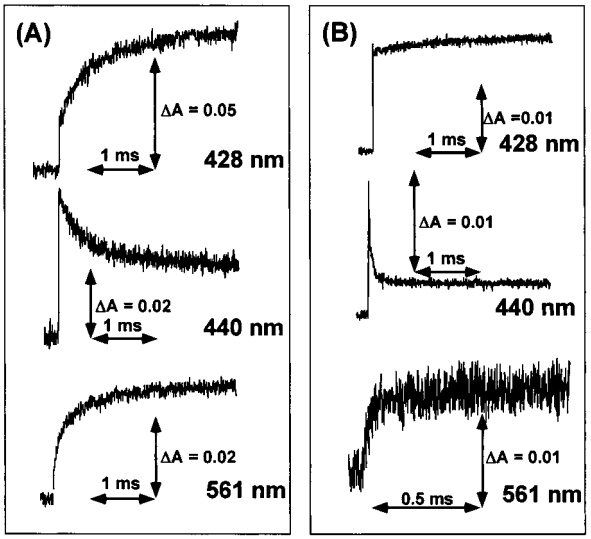

FIGURE 1: Absorption changes after pulse radiolysis of the Q_8-bound (A) and ΔUbiA (B) enzymes monitored at 428, 440, and 561 nm. The concentrations of the enzymes with or without the bound Q_8 are 205 and 210 μM, respectively. All samples contained 5 mM NMA, 0.1% sucrose monolaurate, and 10 mM potassium phosphate (pH 7.4).

RESULTS

One-Electron Reduction of the Q_8-Bound Enzyme. Pulse radiolysis experiments involve the almost instantaneous generation of the NMA radical with hydrated electrons (e_{aq}^-), and the NMA radical in turn can reduce redox center(s) within a protein. In the Q_8-bound form of the wild-type cytochrome *bo*, upon pulse radiolysis, an initial transient increase of the NMA radical was observed at 440 nm and subsequent simultaneous increase of the reduced hemes *b* and *o* at 428 and 561 nm (Figure 1A). The latter slower changes on the millisecond time scale were accompanied by the decrease at 440 nm.

The kinetic difference spectrum at 10 μs after pulse radiolysis (Figure 2A), corresponding to the faster phase, shows that the reaction intermediate has absorption maxima at around 402 and 440 nm and a tail in the range from 480 to 580 nm, and these properties are similar to those of ubisemiquinone anion radical (*41*).

Since the concentration of NMA (5 mM) is sufficiently higher than that of the enzyme (50 μM), a direct electron transfer from the e_{aq}^- to the bound Q_8 within the enzyme is unlikely. Thus, the formation of ubisemiquinone anion radical is attributable to intermolecular electron transfer from the NMA radical to the Q_8 bound at the Q_H site of cytochrome *bo*. Unfortunately, the formation and decay of the NMA radical and the subsequent formation of ubisemiquinone radical in the Q_8-bound enzyme were not resolved, due to the spectral overlapping between two species. The reduction of the bound Q_8 was almost stoichiometric with the concentration of the NMA radical consumed, using the values

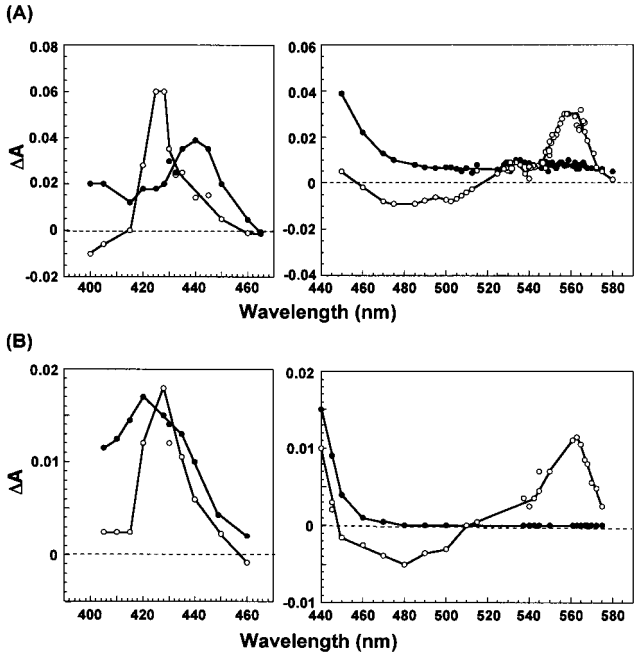

FIGURE 2: Kinetic difference spectra at 10 μs (○) and 2 ms (●) after pulse radiolysis of the Q_8-bound (A) and ΔUbiA (B) enzymes. Pulse radiolysis was carried out as described in the legend to Figure 1.

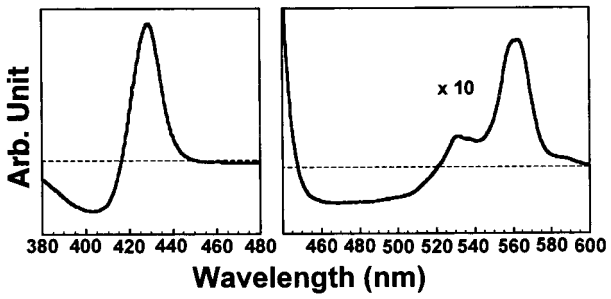

FIGURE 3: Dithionite-reduced *minus* air-oxidized redox difference spectrum of the Q_8-bound cytochrome *bo*. The fully reduced form was obtained upon addition of excess sodium dithionite to the air-oxidized enzyme (12.6 μM) in 10 mM potassium phosphate (pH 7.4) containing 0.1% sucrose monolaurate.

of extinction coefficients of the NMA radical (ϵ_{420nm} = 3.2 mM^{-1} cm^{-1}) (*40*) and semiquinone anion radical (ϵ_{445nm} = 8.6 mM^{-1} cm^{-1}) (*41*).

In the slower phase, the kinetic difference spectrum of the Q_8-bound enzyme at 2 ms after pulse radiolysis (Figure 2A) is essentially identical to the fully reduced *minus* air-oxidized redox difference spectrum (Figure 3), indicating the simultaneous reduction of the hemes *b* and *o*.

A simultaneous increase in the absorbance at 428 and 561 nm and a decay at 440 nm obeyed first-order kinetics. The rate constant of 1.5 × 10^3 s^{-1} for the increase of the 561-nm absorbance was independent, within experimental error, of the enzyme concentration (Figure 4). Therefore, we conclude that the slower process is the intramolecular electron transfer from the ubisemiquinone radical to heme *b* in the enzyme.

From these findings, we conclude that the bound Q_8 at the Q_H site was first reduced with the NMA radical produced by pulse radiolysis and that the resultant ubisemiquinone anion radical reduces hemes *b* and *o* in subuit I via the intramolecular electron transfer.

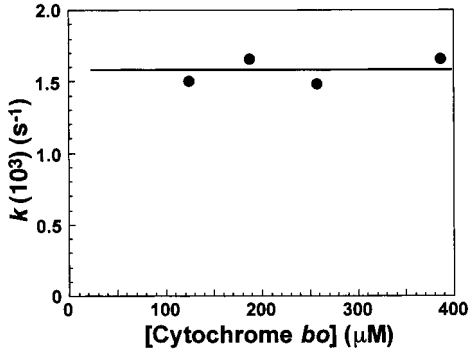

FIGURE 4: Concentration dependence of apparent first-order rate constants of the reduction of heme *b* monitored at 561 nm. Pulse radiolysis was carried out as described in the legend to Figure 1, except the enzyme concentration.

One-Electron Reduction of the Bound Q_8-Free Enzyme. In the bound Q_8-free wild-type enzyme (ΔUbiA), on the other hand, the absorption changes at 428 and 561 nm corresponding to the reduced hemes *b* and *o* were considerably small, and took place rapidly, as compared with the Q_8-bound enzyme (Figure 1). The absorbance increase at 561 nm was observed with a half time of 50 μs (Figure 1B), and the absorbance at 428 nm increased with the decay of the NMA radical at 440 nm.

The kinetic difference spectra at 10 μs after pulse radiolysis showed a large difference between the Q_8-bound and ΔUbiA enzymes (Figure 2). The bound Q_8-free enzyme showed an absorption maximum at 420 nm (Figure 2B, left) is consistent with the formation of the NMA radical (*40*). In contrast, the kinetic difference spectrum of the slower phase (Figure 2B) was essentially idential to that of the Q_8-bound enzyme (Figure 2A). A yield of the heme *b* reduction was estimated to be ca. 10−30% of the NMA radical formed, whereas the reduction of heme *b* in the Q_8-bound enzyme was nearly quantitative. Accordingly, though the NMA radical can directly reduce heme *b* in subunit I, a competition between the dimerization of the NMA radical and electron transfer from the NMA radical to heme *b* of the enzyme occurs. For attempts to determine the second-order rate constant of the reaction, we performed the experiments under various conditions (radiolytic low doses, enzyme concentration) such that the dimerization reaction can be minimized. However, due to the reaction of the NMA radical with the traces of residual dioxygen in the reaction mixture, the rate constants carry a large error and only estimates to be the order of 10^7 M^{-1} s^{-1}.

pH-Dependence of One-Electron-Transfer Reaction in Cytochrome bo. The rate constants for the intramolecular electron transfer from ubisemiquinone to hemes *b* and *o*, determined from the absorbance change at 440 and 561 nm, decreased considerably with increasing the external pH from 6.5 to 9.0 (Figures5 and 6). In contrast, the yield of the formation of ubisemiquinone and the kinetic difference spectra of the Q_8-bound enzyme corresponding to the faster and slower phases were not significantly affected by the external pH in the range 6.0−9.0 (data not shown). This indicates that the yield of the subsequent reduction of hemes *b* and *o* was independent of pH. The pH dependence of the intramolecular electron transfer from ubisemiquinone to the hemes (Figure 6) appears to be well correlated with the pH dependence of the steady-state level of the bound ubisemi-

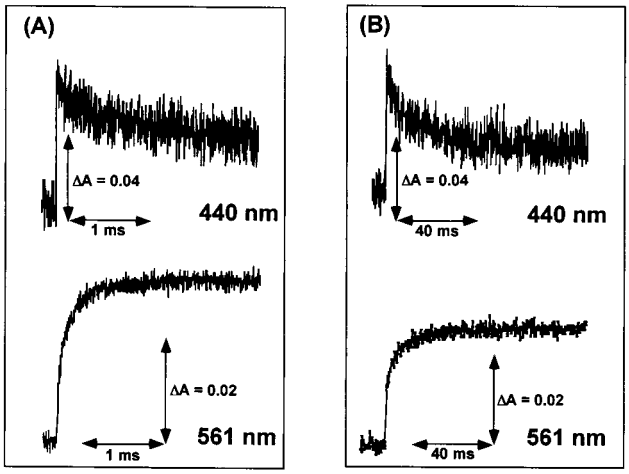

FIGURE 5: Absorption changes after pulse radiolysis of the Q_8-bound enzyme at pH 6.0 (A) and 9.0 (B). The concentration of the enzyme was 30 μM. All samples contained 5 mM NMA, 0.1% sucrose monolaurate, and 10 mM potassium phosphate (pH 6.0), or 10 mM sodium borate (pH 9.0).

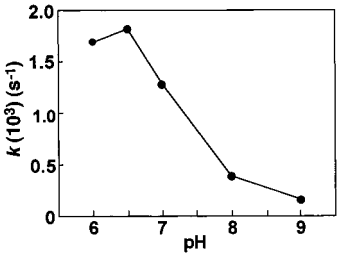

FIGURE 6: pH dependence of the rate constants of the reduction of heme *b* monitored at 561 nm. Pulse radiolysis was carried out as described in the legend to Figure 1.

quinone determined by EPR (*28*). Below neutral pH ubisemiquinone is unstable and the intramolecular electron transfer to the hemes dominates over the reverse reaction, whereas at alkaline pH ubisemiquinone can be stabilzed and the intramolecular electron transfer to the hemes is strongly inhibited.

DISCUSSION

Bacterial quinol oxidases except cytochrome *bd* are members of the heme-copper terminal oxidases, and have evolved from cytochrome *c* oxidase of a Gram-positive bacterium (*1*−*3*, *42*). Structure−function studies on the quinone/quinol redox sites in the *E. coli* cytochrome *bo* revealed the presence and properties of the Q_L and Q_H sites in bacterial quinol oxidases (*5*−*8*, *23*−*29*, *43*−*51*), instead of Cu_A, an electron accepting site in subunit II of cytochrome *c* oxidases (*2*, *52*−*55*). During the catalytic cycle, the bound Q_8 at the Q_H site can be stabilized as ubisemiquinone radical and could undergo the two-electron reduction followed by protonation to yield Q_8H_2 (*26*, *28*). However, the bound Q_8 or Q_8H_2 remains kept in the Q_H site (*23*), in contrast to the Q_L site of cytochrome *bo* (*24*, *43*, *44*), the Q_B site of the photosynthetic reaction center (*12*−*14*), and the Q_o and Q_i sites of cytochrome bc_1 complex (*16*−*19*), which are in dynamic equilibrium with the membrane quinone pool. These findings indicate that a unique mechanism for the quinol oxidation is operative in cytochrome *bo* (*23*−*26*, *29*).

Previous studies provided supporting evidence for our proposal that the Q_H site mediates intramolecular elecron transfer from the Q_L site in subunit II to heme *b* in subunit I (*23*, *25*, *26*, *29*). However, due to a fast turnover rate (i.e., 10^3 e^- s^{-1}) of cytochrome *bo* (*24*, *56*), stopped-flow technique is not sufficient to monitor the redox reactions at the Q_H site in the oxidation of Q_1H_2 at the Q_L site (*29*). Thus, we applied pulse radiolysis technique to cytochrome *bo* to demonstrate directly the involvement of the Q_H site in intramolecular electron-transfer processes. By using NMA as the electron mediator, we can time-resolve one-electron-transfer processes in the redox proteins in the forward direction (*30*−*37*).

Upon pulse radiolysis, a transiently generated NMA radical transferred one electron to the bound Q_8 at the Q_H site to yield a ubisemiquinone anione radical, which in turn reduced hemes *b* and *o* in the millisecond time range (Figures 1 and 2). Such changes were not observed in the ΔUbiA enzyme lacking the bound Q_8, supporting this assignment. In addition, we found a tight correlation between the pH dependence of the rate for the ubisemiquinone-to-heme *b* electron transfer (Figure 6) and that of the stability of the bound ubisemiquinone in cytochrome *bo* revealed by EPR spectroscopy (*28*). The pK_a value of 7.25 for the ubisemiquinone (*28*) was similar to that of the pH dependence of the intramolecular electron transfer (Figure 6). The yield of the ubisemiquinone formed remains constant in the pH range examined, thus the reoxidation of the ubisemiquinone by ferric heme *b* is the rate-limitting step in the intramolecular electron-transfer processes. These results are consistent with our proposal that the bound Q_8 in the Q_H site is an intrinsic electron-transfer component in the heme-copper terminal quinol oxidase, but do not support the Q cycle model proposed by Chan and his colleagues (*57*, *58*).

The first-order rate constant for the intramolecular electron transfer from the bound Q_8 to heme *b* (1.5×10^3 s^{-1}; Figure 4) is comparable to the turnover rate for Q_1H_2 (1.0×10^3 e^- s^{-1}) (*24*, *56*). In addition, the rate is comparable to that of electron transfer from the bound Q_8H_2 to heme *b* (1.4×10^3 s^{-1}), accompanied by a net release of 1.1 H^+, in flow-flash studies (*59*). These data suggest that the reoxidation of the bound quinol at the Q_H site by heme *b* is the rate-limiting step in the steady-state turnover. The other processes, the two-electron transfer from Q_8H_2 at the Q_L site to the Q_H site and the one-electron transfer from the Q_H site to heme *b* observed here take place more rapidly ($>10^3$ s^{-1}). Accordingly, the Q_H site appears to function as an gate for electron flow to heme *b* through the sequential one-electron transfer (*23*, *25*, *26*, *29*). To complete the four-electron reduction of dioxygen, two quinol molecules must be oxidized consecutively at the Q_L site. Rate limiting the Q_H-to-heme *b* electron transfer could provide enough time for binding of the second quinol molecule to the Q_L site.

Present experiments showed that hemes *b* and *o* were reduced simultaneously from the one-electron reduced state of the bound Q_8 (Figures 2 and 3). However, the reduction of the hemes must take place through heme *b*, which subsequently transfers one electron to heme *o* untill equilibrium is reached. The rate constant for the heme *b*-to-heme *o* electron transfer in flow-flash studies on the fully reduced enzyme with dioxygen (*37*, *59*, *60*) and in reverse electron-transfer studies with the mixed-valence enzyme (*61*) were estimated to be $(2-5) \times 10^4$ s^{-1} and $>1 \times 10^4$ s^{-1}, respectively, and much faster than the Q_H-to-heme *b* electron transfer (1.5×10^3 s^{-1}). A similar result was recently

obtained in the pulse radiolysis studies with cytochrome *bd*-type ubiquinol oxidase from *E. coli*, where both hemes b_{558} and b_{595} were reduced simultaneously (*37*).

The present data showed that the second-order rate constant for the reduction of the bound Q_8 with the NMA radical in the Q_8-bound enzyme is much faster than that of the heme *b* reduction in the ΔUbiA enzyme. The difference can be explained by the accesibility of the redox site to solvent. The NMA radical can rapidly reduce Cu_A of bovine cytochrome *c* oxidase (*30*), the type-I Cu of copper-containing nitrite reductase (NIR) (*34*), and heme *c* of cytochrome cd_1 nitrite reductase (*36*), respectively. Their electron entry points are located near the surface of protein molecules, according to their X-ray structures (*54*, *55*, *62*, *63*). In cytochrome *bo*, the rapid reduction of the bound Q_8 with the NMA radical is consistent with the location proposed for the Q_H site, which is present in the periplasmic region of helice I and II of subunit I, whereas heme *b* is well buried in a hydrophobic pocket formed by helices I, II, XI, and XII of subunit I (*27*).

Recent X-ray crystallographic studies on cytochrome *bo* at 3.5 Å resolition (*27*) showed that tertiary structures of subunits I, II, and III are similar to those of SoxM-type cytochrome *c* oxidases from the soil bacterium *Paracoccus denitrificans* (*52*, *53*) and bovine heart mitochondria (*54*, *55*). In bacterial cytochrome *c* oxidase, it was shown that ferrocytochrome *c* binds to an acidic patch on subunit II (*64*) and reduces Cu_A via electron transfer through side chains of Trp121 and Met227 (Cu_{A1} ligand) (*65*, *66*). Subsequently, electrons are transferred to heme *a* in subunit I through hydrogen bonds between $N_{\epsilon 2}$ of His224 (Cu_{A2} ligand) and a peptide carbonyl group of Arg473 in loop XI−XII of subunit I and between a peptide amide group of Arg473 and a propionate group of heme *a* (*52*, *66*). The distance between Cu_A and heme *a* in cytochrome *c* oxidase is about 19−19.5 Å (*52*−*55*).

Though the X-ray structure of cytochrome *bo* lacks any bound quinone molecule (*27*), biochemical and molecular biological studies revealed that the Q_L site, like Cu_A of cytochrome *c* oxidase (*52*−*55*), is located at the proximal end of the C-terminal hydrophilic domain of subunit II (*5*−*8*), which is close to the membrane surface. It was shown also that the Q_H site is close to both the Q_L site and heme *b* (*23*, *26*). On the basis of sequence comparisons of quinol oxidases and site-directed mutagesis studies, Abramson et al. (*27*) postulated that Arg71 and Asp75 in the C-terminal half of helix I and His98 and Gln101 in the N-terminal half of helix II of subunit I, which are totally conserved in ubiquinol oxidases,[2] can form a pocket for the tightly bound ubiquinone (i.e., the Q_H site). Present pulse radiolysis studies indicate that the Q_H site is located at the periplasmic surface of the oxidase molecule, and thus accessible to the exogenous electron donors. The rate of the intramolecular electron transfer from the Q_H site to heme *b* is similar to those between two redox sites of the copper-containing nitrite reductase (*34*−*36*) and cytochrome cd_1 nitrite reductase (*32*). Thus, the distance between the Q_H site and heme *b* would be about 12−13 Å (*62*, *63*), which is good agreement with the distance from the face of the quinone ring to the heme *b* iron (13 Å) in the proposed Q_H site model (*27*). Such a spatial arrangement of the two quinone redox sites in cytochrome *bo* can facilitate dynamic equilibrium of the Q_L site with the membrane quinone pool and is compatible with electron transfer from the Q_L site at the heme *b* side of the C-terminal hydrophilic domain of subunit II (*8*) to heme *b* through the Q_H site in the periplasmic halves of transmembrane helices I and II of subunit I (*27*).

Conclusion. Here we report for the first time the transient formation of the ubisemiquinone anion radical in cytochrome *bo* at ambient temperature and subsequent quantitative electron transfer to hemes *b* and *o* in subunit I. These findings are consitent with our proposal that the bound ubiquinone at the Q_H site mediates electron transfer to heme *b* in subunit I from the Q_L site in subunit II where the two-electron oxidation of quinols takes place (*23*−*26*, *29*). Determination of the molecular structures of the Q_L and Q_H sites and the spatial organizations of the redox centers will help understanding the redox chemistry of the quinol oxidation and intramolecular electron-transfer processes in bacterial quinol oxidases.

[2] Gln101 is not conserved in menaquinol oxidases from *Bacillus subtilis* (M86548) and *B. halodurans* (AP001514), and only the Gln101Asn mutation eliminated the bound Q_8 from cytochrome *bo* (*27*).

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