Probing Substrate Binding Site of the Escherichia coli Quinol Oxidases Using Synthetic Ubiquinol Analogues*

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Substrate binding sites of the Escherichia coli bo- and bd-type quinol oxidases were probed with systematically synthesized ubiquinol analogues. The apparent \( K_m \) values of ubiquinol-2 derivatives to the bo-type enzyme were much lower than that of the corresponding 6-n-decyl derivatives. The isoprenoid structure is less hydrophobic than the saturated n-alkyl group with the same carbon number; therefore, the native isoprenoid side chain appears to play a specific role in quinol binding besides simply increasing hydrophobicity of the molecule. The \( V_{\text{max}} \) values of 2-methoxy-3-ethoxy analogues were greater than that of 2-ethoxy-3-methoxy analogues irrespective of the side chain structure. This result indicates not only that a methoxy group in the 2-position is recognized more strictly than the 3-position by the binding site but also that the side chain structure does not affect binding of the quinol ring moiety. Systematic analysis of the electron-donating activities of the analogues with different substituents in the 5-position revealed that the 5-methyl group is important for the activity. In the parallel studies with the bd-type enzyme, we obtained similar observations except that almost all quinol analogues, but not ubiquinol-1, elicited a remarkable substrate inhibition at higher concentrations. These results indicate that the two structurally unrelated terminal oxidases share common structural properties for the quinol-oxidation site.

In the aerobic respiratory chain of Escherichia coli, bo-type quinol oxidase is predominantly expressed under high oxygen tension while bd-type quinol oxidase is the primary terminal enzyme under microaerobic conditions (1). Both enzymes catalyze the 2-electron oxidation of ubiquinol-8 (\( Q_8-H_2 \)) at the periplasmic side of the cytoplasmic membrane and the 4-electron reduction of dioxygen at the cytoplasmic side and establish a transmembrane electrochemical gradient of protons via scolar protolytic reactions (2, 3). The former enzyme belongs to the heme-copper terminal oxidase superfamily and functions as a redox-coupled proton pump (3–5). Site-directed mutagenesis studies on the E. coli bo-type oxidase have identified the axial ligands of three redox metal centers in subunit I, low spin heme \( b \), high spin heme \( o \), and CuB, and have provided a clue for the understanding of energy coupling and proton translocation (6, 7). Recent x-ray crystallographic studies on cytochrome \( c \) oxidase of Paracoccus denitrificans (8) and bovine (9) confirmed a structure model for the metal centers of the heme-copper terminal oxidases based on molecular biological studies.

In contrast, the substrate oxidation site of bacterial quinol oxidases is poorly characterized. Photoaffinity cross-linking studies on the bo-type enzyme showed that a Q-binding site resides in subunit II (10). Therefore, electrons seem to transfer from subunit II to the metal centers in subunit I as in cytochrome \( c \) oxidase. We have carried out systematic screening of the potent \( Q-H_2 \)-oxidation site inhibitors among benzoquinones and substituted phenols and have identified the new compounds such as 2,6-dimethyl-1,4-benzoquinone and 2,6-dichloro-4-dicyanovinylphenol (11). Similarly, Meunier et al. (12) identified aurachin C as the potent inhibitor for both the bo- and bd-type enzymes, aurachin D and a tridecyl derivative of stigmatellin as the specific inhibitors for the bo- and bd-type enzymes, respectively. Subsequently, we demonstrated the presence and functional importance of a novel high affinity Q-binding site (\( Q_{\text{b}} \)) in the bo-type enzyme that is distinct from a low affinity \( Q-H_2 \)-oxidation site (\( Q_{\text{b}} \)) (13). Potentiometric studies showed that the bound \( Q_{\text{b}} \) at the \( Q_{\text{b}} \) site can be reduced to \( Q_{\text{b}}-H_2 \) through a ubisemiquinone radical (14, 15). These results indicate that the \( Q_{\text{b}} \) site mediates the intramolecular electron transfer from \( Q_{\text{b}} \)-H2 to the \( Q_{\text{b}} \) site to low spin heme \( b \) (13, 14). Determination of the mutation points for the analogue resistance will facilitate the elucidation of the Q-binding site in terminal quinol oxidases (16).

Gennis and co-workers (17–21) studied the \( Q-H_2 \)-oxidation site of the E. coli bd-type enzyme using protein chemical and immunochemical techniques. Photoaffinity cross-linking with an azidoubiquinone derivative identified subunit I as the substrate binding site (17). Anti-subunits I and II antibodies effectively inhibited the oxidation of quinols and \( N,N,N',N' \)-tetramethyl-p-phenylenediamine, respectively (18). The epitope of monoclonal antibodies which binds to subunit I and specifically blocks the quinol oxidation was mapped to a region having a single 11-residue stretch of the large periplasmic loop V || VI (Q-loop) of subunit I (19). Alternatively, limited proteolysis demonstrated that the cleavage of the Q-loop caused a loss of the quinol oxidase activity (20, 21). These results suggest that the \( Q-H_2 \)-oxidation site is localized in subunit I of the bd-type enzyme. Structure-function studies with quinone analogues

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1 The abbreviations used are: \( Q_8-H_2 \), reduced form of ubiquinone-8; DB, 2,3-dimethoxy-5-methyl-6-n-decyl-1,4-benzoquinone; PB, 2,3-dimethoxy-5-methyl-6-n-pentyl-1,4-benzoquinone; \( Q_8 \), ubiquinone; \( Q_8-H_2 \), reduced form of ubiquinone; \( Q_{\text{b}} \), high affinity Q-binding site; \( Q_{\text{b}} \), low affinity \( Q-H_2 \)-oxidation site; \( Q_{\text{b}}-H_2 \), reduced form of ubiquinone-1; \( Q_{\text{b}} \), reduced form of ubiquinone-2; \( Q_{\text{b}} \), reduced form of PB; \( Q_{\text{b}} \), reduced form of DB; \( Q_{\text{b}} \), primary quinone binding site; \( Q_{\text{b}} \), secondary quinone binding site.
have demonstrated that the E. coli bo- and bd-type quinol oxidases share a similar Q-binding site although they are structurally unrelated (11). Further, the bd-type enzyme was also shown to be able to stabilize ubisemiquinone (22).

The structural requirements for Q-H₂ in the oxidation reaction should be tightly related to the structural features of the Q-H₂-oxidation site and the reaction mechanism. Thus, a systematic set of ubiquinone analogues was used to probe the Q-mediated electron transfer systems such as mitochondrial succinate-cytochrome c (23, 24) and NADH-Q (25, 26) oxidoreductases. In contrast to mammalian mitochondrial respiratory enzymes (23-28), bacterial terminal quinol oxidases were poorly characterized in this respect. Yu and co-workers (10) synthesized the 6-alkyl derivatives of 2,3-dimethoxy-5-methyl-1,4-benzoquinone and examined the effects of a length of the n-alkyl side chain on the oxidase activity of the E. coli bo-type enzyme. The substitution pattern of the quinol ring was fixed as for that of native Q₄-H₂. Therefore, the effects of substituents at all positions in the quinol ring upon the electron transfer efficiency remain obscure. On the other hand, saturated alkyl chains such as n-pentyl and n-decyl groups have been claimed to mimic the native isoprenoid side chain in mammalian mitochondrial enzymes (26-28). Further, the electron-accepting and -donating activities of Q₆-H₂ and DB-H₂ were found to be identical in mitochondrial succinate-cytochrome c oxidoreductase (27). These results indicate that the side chain contributes primarily to an increase of hydrophobicity of the Q molecule to fit into the hydrophobic Q-binding site. To the contrary, the native isoprenoid tail structure was claimed to play a specific role in Q-binding at the QA and QB sites of the reaction center of Rhodobacter sphaeroides (29) although it is unclear whether or not the above premise holds in general for the Q-mediated electron transfer systems. Thus, it is worthwhile to examine the role of the side chains of Q-H₂ in the oxidation by bacterial quinol oxidases.

To probe the structural features of the substrate binding site based upon structure-activity studies, we examined the effects on the enzyme activity of replacement of one of the alkyl groups (or moieties) on the quinol ring by another alkyl group. Such a structural modification inevitably alters the molecular shape while minimizing changes in the redox property of the molecule because the electronic nature of the alkyl groups is almost identical (23, 24). In this study, we synthesized a wide variety of alkyl analogues of Q₆-H₂, including the compounds containing an isoprenoid side chain, and attempted to examine the effects of the side chain structures on recognition of the substituents at all positions in the quinol ring by the Q₆-H₂-oxidation site. In addition, the structural properties of the substrate oxidation site of the E. coli bo- and bd-type quinol oxidases were compared in terms of the kinetic and potentiometric properties of ubiquinol analogues.

**EXPERIMENTAL PROCEDURES**

**Materials—**Compounds 8 to 11 and 14 to 17 (Fig. 1) were the same samples as described previously (25). Ubiquinone-1 (Q₁) and -2 (Q₂) were generous gifts from Eisai Co., Ltd., Tokyo. Other chemicals were commercial products of analytical grade and used as received.

**Synthesis of Compound 2**—The quinone form of compound 2 (see Fig. 1) was synthesized from 2-methoxy-3-ethoxy-5-methyl-1,4-benzoquinone, which was prepared by a previous method (25), through Diels-Alder-type adduct (30), as shown in Scheme 1. To a solution of 2-methoxy-3-ethoxy-5-methyl-1,4-benzoquinone (1.5 g, 7.6 mmol) in 25 ml of CH₂Cl₂, freshly distilled cyclopentadiene (2.1 g, 30.3 mmol) was added, and the mixture was kept at room temperature until the orange color had disappeared. The solvent and excess reagent were removed in vacuo, and the product (compound 2b) was isolated by silica gel column chromatography (ethyl acetate:hexane, 1:9) in quantitative yield. To a solution of compound 2b (1.4 g, 5.0 mmol) in 20 ml of dry Et₂O at -70°C under N₂, freshly sublimed t-KOBU (0.7 g, 5.5 mmol) was added. After 30 min at -40°C, the mixture was again cooled to -70°C, and geranyl bromide, -70°C to room temperature; and iii) 80°C, 3 h.

![Scheme 1](image)

**Experimental Procedures**

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The quinone form of compound 6 (Fig. 1) was prepared from 2,3-dimethoxy-5-ethyl-1,4-benzoquinone and 2,3-dimethoxy-5-ethyl-1,4-benzoquinone, respectively, by an ordinary radical coupling reaction with diundecanoylperoxide (25). Preparations of 2,3-dimethoxy-1,4-benzoquinone and 2,3-dimethoxy-5-ethyl-1,4-benzoquinone, respectively, were used as the standard compounds. For compound 15, the side chain structure is n-hexyl but not n-pentyl.

Synthesis of Compounds 12 and 13—The quinone forms of compounds 12 and 13 (Fig. 1) were prepared from 2,3-dimethoxy-1,4-benzoquinone and 2,3-dimethoxy-5-ethyl-1,4-benzoquinone, respectively, by an ordinary radical coupling reaction with diundecanoylperoxide (25). Preparations of 2,3-dimethoxy-1,4-benzoquinone and 2,3-dimethoxy-5-ethyl-1,4-benzoquinone were as described above. Compound 12, 1H NMR (CDCl3, 300 MHz) δ 0.88 (t, J = 6.7 Hz, 3H, CH3), 1.26 (m, 14H, –CH2–), 1.50 (m, 2H, ArCH2CH3), 2.40 (t, J = 7.6 Hz, 2H, ArCH2), 3.99 (s, 3H, OCH3), 4.02 (s, 3H, OCH3), 6.37 (t, J = 1.4 Hz, 1H). Anal. Caled for C18H28O4: C, 70.10; H, 9.15. Found: C, 69.87; H, 9.13. Compound 13, 1H NMR (CDCl3, 300 MHz) δ 0.85 (t, J = 6.9 Hz, 3H, CH3), 1.06 (t, J = 7.5 Hz, 3H, CH3), 1.26–1.36 (m, 16H, –CH2–), 2.46 (2q, 4H, ArCH2), 3.98, 3.99 (2 s, 6H, OCH3). Anal. Caled for C20H32O4: C, 71.39; H, 9.59. Found: C, 71.14; H, 9.82.

Synthesis of Compound 7—The quinone form of compound 7 (Fig. 1) was prepared by reducing Q2 with 10% palladium on carbon in methanol with a catalytic amount of HCl under hydrogen gas at room temperature. 1H NMR (CDCl3, 300 MHz) δ 1.06 (t, J = 7.5 Hz, CH3), 1.65 (s, 3H, CH3), 1.74 (s, 3H, CH3), 1.90–2.09 (m, 4H, –CH2–), 2.48 (q, J = 7.5 Hz, 2H, ArCH2CH3), 3.19 (d, J = 7.0 Hz, 2H, ArCH2–), 3.99 (s, 6H, OCH3), 4.94 (t, J = 6.9 Hz, 1H), 5.04 (t, J = 6.7 Hz, 1H). Anal. Caled for C18H28O4: C, 72.26; H, 8.49. Found: C, 72.16; H, 8.76.

Fig. 1. Structure of Q8-H2 and quinon analogues synthesized in this study. Q8-H2 (compound 1), DB-H2 (compound 8), and PB-H2 (compound 14) were used as the standard compounds. For compound 8, the side chain structure is n-hexyl but not n-pentyl.
DB by means of different synthetic routes. The introduction of a bulky monoethoxy or diethoxy group was shown to be useful in discriminating these two positions by the mitochondrial Q-mediated electron transfer system (24, 25). Here we applied the synthetic methods to preparations of the monoethoxy and diethoxy analogues that have a native isoprenoid side chain (i.e. compounds 2, 3, and 4) (Fig. 1).

Taking Q2-H2 as a reference compound, we first compared the electron-donating activity of compounds 2, 3, and 4 (Table I). The activity of compound 2, in terms of apparent Km and Vmax values, was almost identical to that of Q2-H2. In contrast, the Vmax value of compound 3 decreased to 18% of the reference while Km was doubled. A change in the electron-donating activity of the diethoxy analogue (compound 4) relative to Q2-H2 was the same as that of compound 3. Our synthetic strategy now revealed that a methoxy group at the 2-position is recognized more strictly than the 3-methoxy group by the QL site.

**Role of Isoprenoid Side Chain in Reaction with the bo-type Enzyme—**Subsequently, we examined the effects of side chain structures on the oxidase activity using the ethoxy analogues with two isoprenoid units (compounds 1–4) or an n-decyl group (compounds 8–11) and found that the effects of 2- and 3-substituents on the electron-donating activity were similar irrespective of the side chain structures (Table I). The order of the Vmax values were 2,3-dimethoxy > 3-methoxy-3-ethoxy > 2-ethoxy-3-methoxy for the diethoxy derivatives. This finding indicates that binding of the quinol ring moiety to the QL site is not significantly affected by the side chain structure.

It is, however, notable that the apparent Km values of Q2-H2 and its 3-monoethoxy analogues (compound 2) were 10-fold lower than that of corresponding n-decyl analogues (DB-H2 and compound 9, respectively) while the total number of carbon atoms in these side chains is identical. In general, the hydrophobicity of the hydrocarbon tail differs significantly depending upon its structure. Based on water to hexane solvent-transfer free energies calculated from the partition coefficient in the hexane-water system, Warncke et al. (29) estimated the hydrophobicity of Q analogues with various side chains. Values for Q2 and DB are found to be -7.34 and -9.79 kcal/mol, respectively (29). Therefore, the isoprenoid structure is rather less hydrophobic than the saturated n-alkyl group of the same number of carbon atoms (also see Ref. 26). Accordingly, a lower Km value of Q2-H2 (or compound 2) cannot be attributed to its hydrophobicity. The native isoprenoid tail structure (two isoprenoid units in this case) may increase the binding affinity through a specific interaction, as claimed for Q-binding at the QA and QB sites of the reaction center of R. sphaeroides (29).

Our observation that saturation of the isoprenoid side chain of Q2-H2 resulted in a marked increase in the Km value (Q2-H2 versus compound 7) supports this possibility.

It is still unclear whether the increase in the Km value of saturated alkyl analogues is related to a lack of π-electron systems or due to unfavorable conformational energy in the protein-bound state. Warncke et al. (29) examined the native isoprenoid tail structure of the bound quinones at the QA and QB sites of the R. sphaeroides reaction center (36) and ruled out the contribution of favorable enthalpic interactions of the π-electron systems in the isoprenoid double bonds with the protein. An x-ray crystallographic study of the Q-mediated respiratory enzymes will provide a clue for understanding this effect (37).

To examine the role of isoprenoid tail structure in the electron transfer reaction, we also compared the kinetic parameters between Q2-H2 and PB-H2 (5-carbon atom analogues). In contrast to 10-carbon atom analogues (Q2-H2 and DB-H2), activities of Q2-H2 and PB-H2 may be taken to be almost identical (Table I). This finding suggests that the second isoprenoid unit of Q2-H2 plays a specific role in the binding interactions. Fato et al. (26) reported a similar observation in the reaction of Q analogues with mitochondrial NADH-Q oxidoreductase: Q2 is not only a much poorer electron acceptor than DB but also acts...
as an inhibitor at higher concentrations, whereas Q₃ and PB are comparable electron acceptors. However, electron transfer reactions of mitochondrial succinate-cytochrome c oxidoreductase with Q₂ and DB are identical (27), suggesting that the molecular recognition of the 6-isoprenyl group by the Q-binding site differs in the respiratory redox proteins. To elucidate the roles of the isoprenoid side chain, Q₂-H₂ analogues in which a double bond of the first or second isoprenoid unit is selectively saturated should be useful. Synthetic strategy that enables the selective saturation of the two double bonds is now being examined.

**Role of the 5-Methyl Group of Quinol Ring in Reaction with the bo-type Enzyme**—Effects of a methyl group at the 5-position on the physicochemical properties of the Q molecule are complicated. Substitutions of the 5-methyl group alter not only the shape and redox potential of the molecule but also the conformational property of the vicinal (6-position) alkyl side chain by steric hindrance. Molecular orbital calculation suggests that the Q molecule is stable when the side chain nearby the Q ring extends almost perpendicular to a plane of the Q ring due to a neighboring 5-methyl group (25, 38). Therefore, the effects of the 5-methyl group on the electron-donating activity should be considered separately from the steric effect against conformational property of the vicinal side chain.

We found that the \( V_{\text{max}} \) value of compound 5, which lacks the 5-methyl group, markedly decreases with respect to Q₂-H₂ (Table 1). Similar observations were obtained with a pair of the analogues with different side chain structures (DB-H₂ versus compound 12 and PB-H₂ versus compound 15). However, these results are not enough to conclude whether the change in the activity is due to a lack of the 5-methyl group itself or due to the secondary effect on enhanced flexibility of the alkyl side chain as discussed above.

The conformational properties of the side chain of compounds 16 and 17 lacking the 5-methyl group are similar to that of PB-H₂ because of steric congestion arising from a branched structure at the \( \alpha \)-position of the side chain (25). Therefore, a comparison of the electron-donating efficiencies between PB-H₂ and compound 16 or 17 is useful for probing a specific role of the 5-methyl group. As shown in Table 1, the electron-donating activities of these two compounds are remarkably poorer than that of PB-H₂, indicating that the 5-methyl group itself is important for the electron donation. In addition, we found that replacement of the 5-methyl group by an ethyl group results in a slight but significant decrease in the activity for Q₂-H₂ and DB-H₂ derivatives (i.e., Q₂-H₂ versus compound 6 and DB-H₂ versus compound 13, respectively). This indicates that the 5-alkyl substituents larger than the methyl group are unfavorable to fit into the Q₁ site due to steric restriction arising from the protein environment. It is noteworthy that similar changes in the electron-donating activity against the structural modifications at the 5-position were again observed irrespective of the side chain structure. This result also supports the above notion that a binding manner of the quinol ring moiety into the Q₁ site is not significantly affected by the side chain structure.

**Electron-donating Activity of Q-H₂ Analogues in Reaction with the bd-type Enzyme**—The above set of Q-H₂ analogues were used to probe the Q₃-H₂-oxidation site of the bd-type quinol oxidase. Recent kinetic analysis showed that the reaction with Q₃-H₂ proceeds by a mixed ping-pong/sequential reaction involving a ternary complex (39). Fig. 2 shows the concentration dependence of the electron-donating activity of four reference compounds (Q₁-H₂, Q₂-H₂, DB-H₂, and PB-H₂). A remarkable decrease in the efficiency was observed at higher concentrations of Q₂-H₂, DB-H₂, and PB-H₂ but not with Q₁-H₂. The apparent \( K_m \) and \( V_{\text{max}} \) values for Q₁-H₂ were 205 \( \mu \)M and 32 \( \mu \)mol/min/nmol of enzyme, respectively, comparable with those previously reported (2, 39). Similar unusual kinetics have been reported for the electron-donating activity of compound 12 with isolated mitochondrial ubiquinol-cytochrome c oxidoreductase (23).

To characterize the unusual kinetics for Q₂-H₂, DB-H₂, and PB-H₂, in the presence or absence of 200 \( \mu \)M Q₁-H₂, the concentration dependence of the electron-donating activity of Q₂-H₂ was further examined as a representative case (Fig. 3). The Q₂-H₂ oxidase activity in the presence of Q₁-H₂ increased slightly to a plateau level and then decreased gradually. The presence of Q₁-H₂ shifted maximal activity to a lower concentration of Q₂-H₂. Above ~60 \( \mu \)M Q₂-H₂, the activity was about the same with or without Q₁-H₂. These observations indicate that Q₂-H₂ works as both an electron donor and an inhibitor. Since Q₂-H₂ (or Q₁-H₂) oxidase activity was not completely reduced in the presence of various concentrations of the oxidized form of Q₂ (data not shown), the substrate inhibition cannot be attributed to competition for the Q₂-H₂-oxidation site between the substrate (Q₂-H₂) and the product (Q₁) molecules. The precise mechanism of the inhibition remains to be elucidated.

Besides the above reference compounds, almost all Q-H₂ analogues studied here elicited the substrate inhibition while the extent of inhibition varied depending upon the structures. Therefore, their kinetic parameters (\( K_m \) and \( V_{\text{max}} \)) were unable to be deduced from a double reciprocal plot analysis. In order to qualitatively analyze the substituent effects on the activity between the bo- and bd-type terminal oxidases, we focused on examining a concentration-dependent electron-donating activity in low concentration ranges.

Fig. 4 shows the concentration-dependent activities of Q₂-H₂ and its monoethoxy derivatives (compounds 2 and 3). The

![Fig. 4. Oxidation of Q₂-H₂ and its ethoxy analogues by bd-type quinol oxidase. The assay conditions with Q₂-H₂, compound 2 (△), and compound 3 (○) were the same as in the legend to Fig. 2.](image-url)

![Fig. 5. Oxidation of DB-H₂ and its analogues by bd-type quinol oxidase. The assay conditions with DB-H₂ (λ), compound 12 (●), and compound 13 (○) were the same as in the legend to Fig. 2.](image-url)
DB-H$_2$ and compound 2 elicited similar activities while the activity of compound 3 was significantly lower than the others. Unexpectedly, a diethoxy derivative (compound 4) showed greater activity compared with compound 3 (data not shown). This may be due to weaker substrate inhibition by compound 4 since the residual enzyme activity at its higher concentrations was significantly higher than that of the other three compounds. The structural factors of the Q-H$_2$ molecule required for electron donation or substrate inhibition appear to be specific for the bd-type quinol oxidase.

To examine the effects of side chain structure on binding properties of the quinol ring moiety, the electron-donating activity was compared among the monoethoxy derivatives with the n-decyl group (compounds 8–10). The order of the activity of the n-decyl analogues (i.e. 2,3-dimethoxy ≥ 2-methoxy-3-ethoxy > 2-ethoxy-3-methoxy) was similar to that of Q$_2$-H$_2$ analogues (data not shown). Thus, the effects of the substituents at the 2- and 3-positions on the electron transfer activity appear to be identical irrespective of the side chain structure. These findings indicate that a methoxy group at the 2-position of the ubiquinol ring is recognized more strictly than that at the 3-position, as observed for the bo-type enzyme. Furthermore, although a quantitative analysis is quite difficult for the bd-type enzyme, Q$_2$-H$_2$ gives an apparent maximal activity at a significantly lower concentration than DB-H$_2$ (Fig. 2). This tendency is also shared with the bo- and bd-type quinol oxidases.

Next, the electron-donating activities of DB-H$_2$ and compounds 12 and 13 to the bd-type enzyme were compared to examine the role of the 5-methyl group on the quinol ring (Fig. 5). Compound 12 gave the maximal activity at a lower concentration than DB-H$_2$, indicating that the binding affinity of the former is higher than that of the latter. The electron-donating activity of compound 13 was significantly weaker than that of DB-H$_2$ (Fig. 2). This may be due to weaker substrate inhibition by compound 4 since the residual enzyme activity at its higher concentrations was significantly higher than that of the other three compounds. The structural factors of the Q-H$_2$ molecule required for electron donation or substrate inhibition appear to be specific for the Q-H$_2$-oxidation site.

**Conclusion**—In the present study, we synthesized a systematic set of short-chain Q-H$_2$ analogues. The native isoopenoid tail structure increases the binding affinity of Q-H$_2$ molecules for the bo-type quinol oxidase. The effects of substituents at all positions of the quinol ring upon the electron-donating activity are similar between the bo- and bd-type enzymes. In addition, recognition of the quinol ring moiety by the two enzymes is not affected by the side chain structures, indicating that the quinol ring and the side chain moieties contribute independently to the Q-H$_2$ oxidation reaction. Our results indicate that the *E. coli* quinol oxidases, though they are structurally unrelated, share common structural properties for the Q-H$_2$-oxidation site.

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