J. Biochem. 2009;145(3)395–401
doi:10.1093/jb/mvn179

Properties of Cytochrome *bd* Plastoquinol Oxidase from the Cyanobacterium *Synechocystis* sp. PCC 6803

Tatsushi Mogi[1,2,*] and Hideto Miyoshi[3]

[1]Department of Biomedical Chemistry, Graduate School of Medicine, the University of Tokyo, Bunkyo-ku, Tokyo 113-0033; [2]ATP System Project, ERATO, JST, Nagatsuta, Yokohama, 226-0026; and [3]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

Received October 9, 2008; accepted December 15, 2008; published online January 2, 2009

In the aerobic respiratory chain of the cyanobacterium *Synechocystis* sp. PCC 6803, cytochrome *c* oxidase serves as a major terminal oxidase while cyanide-resistant cytochrome *bd* serves as an alternative oxidase and evades the over-reduction of the plastoquinone pool under stress conditions. Here we expressed *Synechocystis* cytochrome *bd* in *Escherichia coli* and characterized enzymatic and spectroscopic properties. Cyanobacterial cytochrome *bd* showed the higher activity with ubiquinols than with decyl-plastoquinol and K_m values for quinols were 2-fold smaller than those of *E. coli* cytochrome *bd* (CydAB). The dioxygen reduction site was resistant to cyanide as in *E. coli* oxidase while the quinol oxidation site was more sensitive to antimycin A and quinolone inhibitors. Spectroscopic analysis showed the presence of the haem b_{595}-*d* binuclear centre but the sequence analysis indicates that cyanobacterial cytochrome *bd* is structurally related to cyanide-insensitive oxidase (CioAB), which does not show typical spectral changes upon reduction and ligand binding. Our data indicate that cyanobacterial cytochrome *bd* has unique enzymatic and structural properties and we hope that our findings will help our understanding the role and properties of CydAB and CioAB quinol oxidases in other bacterial species.

Key words: absorption spectra, cyanobacteria, cytochrome *bd*, kinetics, plastoquinol oxidase.

Abbreviations: HQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide; IC_{50}, the 50% inhibitory concentration.

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes but in the dark they carry out aerobic respiration. Respiratory enzymes are present in both the cytoplasmic and thylakoid membranes and use plastoquinone (2,3-dimethyl-6-*n*-prenyl-1,4-benzoquinone) as a mobile electron carrier within the membrane (*1*, *2*). For respiration, the cyanobacterium *Synechocystis* sp. PCC 6803 uses two terminal oxidases, cytochrome aa_3-type cytochrome *c* oxidase (CoxBAC) and cytochrome *bd* quinol oxidase (CydAB) (*3–6*). Cytochrome *c* oxidase is more sensitive to cyanide and serves as a major terminal oxidase (*3*, *6*). Cytochrome *bd* is sensitive to 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) and can oxidize plastoquinol in thylakoid membranes under conditions where cytochrome b_6f complex is insufficient for preventing the over-reduction of the plastoquinone pool (*5*). The role of alternative cytochrome *c* oxidase (ARTO) lacking the Cu_A centre and its *in vitro* oxidase activity are still in dispute (*5–8*). In some cyanobacteria (e.g. *Nostoc* sp. PCC 7120, *Gloeobacter violaceus* PCC 7421), the plastid-type single-subunit quinol oxidase (PTOX) serves as an alternative terminal oxidase (*9*).

In the aerobic respiratory chain of *Escherichia coli*, which lacks quinol:cytochrome *c* reductase (cytochrome bc_1 complex), cytochrome *c* and cytochrome *c* oxidase, cytochrome *bo* and *bd* quinol oxidases reoxidize quinols reduced by membrane-bound dehydrogenases [see (*10*) for a review]. Cytochrome *bo* (CyoABCD) is a predominant terminal ubiquinol oxidase under aerobic growth conditions while cytochrome *bd* (*bd*-I, CydAB) is predominantly expressed under microaerobic growth conditions. Cytochrome *bd* has an extremely high affinity for dioxygen [$K_m = 4.5$ nM (*11*)] and is resistant to cyanide [the 50% inhibitory concentration (IC_{50}) = 2 mM (*12*)]. Subunit I (CydA) binds all three redox metal centres, low-spin haem b_{558} and high-spin haem b_{595} and *d* (*10*). Haem b_{558} is ligated by His186 (transmembrane helix V) and Met393 (helix VII) and exhibits absorption peaks at 428, 531 and 561 nm in the reduced form (*10*, *13*, *14*). Haem b_{595} is bound to His19 (helix I) and shows peaks at 440, 560 and 595 nm in the reduced form (*10*, *13*). Haem b_{595} mediates electron transfer from haem b_{558} to haem *d*, where dioxygen is reduced to water. Haem *d* is a chlorin, which is bound to Glu99 or nearby residue (*15*), and exhibits the α peak at 630 nm in the fully reduced form and at 645 nm in the air-oxidized oxygenated form (d^{2+}-O_2) (*10*). Topological analysis suggests that all of the haems are located at the periplasmic end of transmembrane helices in subunit I (*16*). Suppression of ubiquinol oxidase activity by proteolytic cleavage with trypsin at

*To whom correspondence addressed. Tel: +81-3-5841-8202, Fax: +81-3-5841-3444, E-mail: tmogi@m.u-tokyo.ac.jp

Tyr290 or with chymotrypsin at Arg298 (*17*, *18*) and by binding of monoclonal antibodies to K252-LAAIEAEWE-T262 (*19*, *20*) indicates the presence of the quinol oxidation site in loop VI/VII (Q loop) of subunit I. Furthermore, photoaffinity labelling studies with azido-quinols demonstrated that Glu280 is present in a binding pocket for methoxy groups on the quinone ring (*21*). Site-directed mutagenesis studies showed that conserved Lys252 and Glu257 in the *N*-terminal region of Q-loop are involved in the quinol oxidation (*22*). These findings suggest that the ubiquinol oxidation site is located in Q loop and should be proximal to the electron-accepting haem b_{558}.

In cyanobacteria, spectroscopic properties of cytochrome *bd* remain to be determined. Fromwald *et al*. (*23*) demonstrated the presence of haem D in cyanobacterial membranes, but only from cells grown in the presence of thiosulfate and ammonium as sole sulfur and nitrogen sources. Based on the insensitivity of ferrous haem *d* upon dioxygen and carbon monoxide binding, the authors concluded that a functional cytochrome *bd* was absent in cyanobacteria. Pils and Schmetterer (*8*) reported that *Synechocystis* cytochrome *bd* showed a higher dioxygen binding affinity ($K_m = 0.35\,\mu M$) and a higher resistance against cyanide ($IC_{50} = 27\,\mu M$) than cytochrome *c* oxidase (1 and 7 μM, respectively). However, it is possible that these properties could be ascribable to a haem oo_3-type variant of cytochrome *c* oxidase [K_m (O_2) = 0.85 μM], which is expressed in cyanobacterial cells grown under microaerobic conditions (*23*). Furthermore, the IC_{50} value of cytochrome *bd* for cyanide (*8*) was much lower than one estimated by Büchel *et al*. (*24*) and 2 mM of the *E. coli* enzyme (*12*). Accordingly, enzymatic properties of *Synechocystis* cytochrome *bd* need to be reexamined.

Taking the advantage of the *E. coli* expression system established by Howitt and Vermaas (*6*), here we expressed *Synechocystis* cytochrome *bd* in the *E. coli* oxidase-deficient mutant ST4683 (*21*), and determined enzymatic properties of the quinol oxidation and spectroscopic properties of the partially purified enzyme, which are difficult to characterize in cyanobacterial membranes containing other electron transfer proteins and photosynthetic pigments.

MATERIALS AND METHODS

Preparation of Membrane Vesicles—The *E. coli* terminal oxidase-deficient mutant ST4683 (Δ*cyo*::Cm^R Δ*cyd*::Km^R) (*22*) was anaerobically transformed with pUC-cydAB (*6*) by using a GasPack anaerobic jar (Beckton Dickinson). Resultant transformants were aerobically grown in IM medium supplemented with trace metals (*22*) at 37°C and 150 rpm overnight in the presence of 100 μg/ml sodium ampicillin, 0.5% glucose and 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG). Cells grew slowly and the cell yield was about one-tenth of that of the *E. coli* cytochrome *bd*-overproducing strain ST4683/pNG2 (Δ*cyo* Δ*cyd*/*cyd*$^+$ Tet^R) (*22*). Cytoplasmic membranes of *E. coli* ST4683/pUC-cydAB and ST4683/pNG2 were prepared as in (*22*) and the haem content was 0.76 and 4.3 nmol/mg protein, respectively.

Enzyme Assay—Quinol oxidase assay using the *Synechocystis* or *E. coli* cytochrome *bd*-expressed membranes was performed at 25°C with a V-660 double monochromatic spectrophotometer (JASCO, Tokyo, Japan) with data acquisition at 0.05 s. The reaction mixture (1 ml) contained 50 mM potassium phosphate (pH 6.5), and 0.02% Tween 20 (protein grade, Calbiochem) (*25*). Reactions were started by addition of quinols (ubiquinol-1 or ubiquinol-2, $\varepsilon_{278} = 15\,mM^{-1}\,cm^{-1}$; decylplastoquinol, $\varepsilon_{265} = 17.3\,mM^{-1}cm^{-1}$) at a final concentration of 100 μM.

Dose–Response Analysis of the Quinol Oxidase Activity—Duplicate assays were performed with the cytoplasmic membranes at each concentration of inhibitors, and dose–response data were analysed by the non-linear regression curve-fitting with KaleidaGraph ver. 4.0 (Synergy Software, Reading, PA). Enzyme kinetics were analysed based on the modified *ping-pong bi-bi* mechanism for cytochrome *bd* by using the equation $v = (V_{max}SS)/(SS + SK_m + K_mK_m)$ (*26*). IC_{50} values were estimated as described by Cheng and Pursoff (*27*) by using the equation: relative residual activity = $1/[1 + ([Inhibitor]/IC_{50})^n]$ where *n* is the Hill coefficient. For the analysis of the biphasic data, a model for the interaction of a ligand with two binding sites, one stimulatory and one inhibitory (*28*), was used. The relative enzyme activity was expressed by the equation: relative residual activity = $1 - i/(1 + IC_{50}/[Ligand]) + e/(1 + EC_{50}/[Ligand]^n)$, where *i* and *e* are the initial amplitudes of the inhibitory and stimulatory component, respectively, IC_{50} and EC_{50} denote the 50% inhibitory and stimulatory concentration, respectively, of the ligand, and *n* is the Hill coefficient.

Spectroscopic Measurements—Synechocystis cytochrome *bd* was partially purified by anion-exchange chromatography (*22*) and absorption spectra of the air-oxidized, dithionite-reduced and reduced CO-bound forms were determined as in 50 mM Tris–HCl (pH 7.4)—0.1% sucrose monolaurate SM-1200 (Mitsubishi-Kagaku Foods Co., Tokyo, Japan) with the V-660 double monochromatic spectrophotometer (*22*).

Materials—Synthesis of aurachin C 1–10 and D 5–10 were described as previously (*29*). Ubiquinone-1, ubiquinone-2, decylplastoquinone, HQNO and antimycin A_1 were obtained from Sigma. Ascochlorin, piericidin A and gramicind S were generous gifts from Dr Masaichi Yamamoto (aRigen Pharmaceuticals. Inc., Tokyo, Japan), Dr Shigeo Yoshida (Institute of Physical and Chemical Research, Saitama, Japan) and Meiji Seika Kaisha, Ltd (Tokyo, Japan), respectively.

RESULTS AND DISCUSSION

Kinetic Properties on Ubiquinol-1 Oxidase Activity of Synechocystis Cytochrome bd—We determined spectrophotometrically kinetic parameters for the quinol oxidation. Unexpectedly, the quinol oxidase activity was higher with ubiquinol-1 and ubiquinol-2 than with decylplastoquinol (Fig. 1, Table 1). As reported for *E. coli* cytochrome *bd* (*15*, *22*, *26*), data were analysed based on the modified *ping-pong bi-bi* mechanism (*26*).

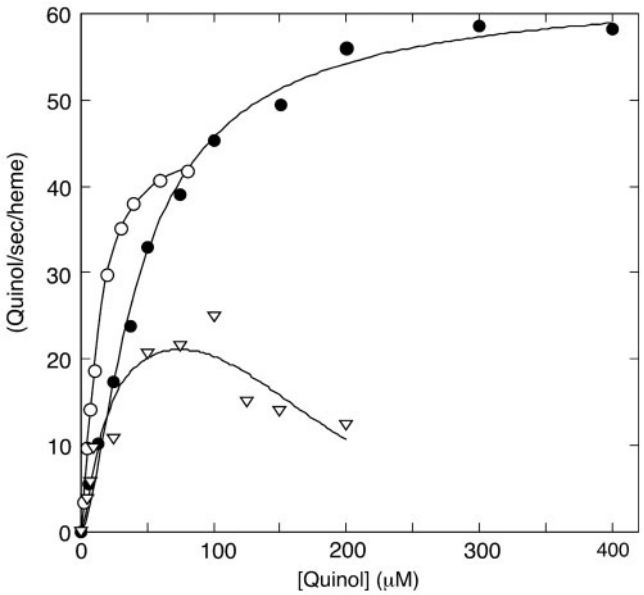

Fig. 1. **Effects of structures of quinols on kinetic parameters for the quinol oxidation by *Synechocystis* cytochrome *bd*.** The oxidation of ubiquinol-1 and ubiquinol-2 and decylplasoquinol by *Synechocystis* cytochrome *bd*-expressed membranes (32.6 μg/ml) was analysed based on the modified *ping-pong bi-bi* mechanism (*25*). The apparent V_{max} (quinol/haem/s) and K_m (μM) values were estimated to be 63.6 ± 1.4 and 30.4 ± 1.8, respectively, for ubiquinol-1 (filled circle), 46.8 ± 0.3 and 8.0 ± 0.1, respectively, for ubiquinol-2 (open circle), and 26.7 ± 5.9 and 12.4 ± 4.7, respectively, for decylplastoquinol (open inverted trialngle). The constant for substrate inhibition by decylplastoquinol was 177 ± 33 μM.

Table 1. **Properties of the quinol oxidase activity of the *E. coli* and *Synechocystis* cytochrome *bd*-expressed membranes.**

	E. coli cytochrome *bd*	*Synechocystis* cytochrome *bd*
Substrate	K_m (V_{max}, U/mg protein)	
Ubiquinol-1	56 μM (280)	30 μM (5.0)
Ubiquinol-2	14 μM (70)	8.0 μM (3.7)
Decylplastoquinol	21 μM (12)	12 μM (2.1)
Inhibitor	IC_{50}	
Aurachin C 1–10	9.0 nM	1.7 nM
Aurachin D 5–10	19 nM	3.0 nM
HQNO	670 nM	88 nM
Antimycin A_1	6.1 μM	0.11 μM
Ascochlorin	57 μM	4.2 μM
Piericidin A	7.7 μM	8.3 μM
Gramicidin S	3.3 μM	18 μM
KCN	1.3 mM	1.6 mM
NaN_3	6.4 mM	16 mM

The apparent K_m values for ubiquinol-1, ubiquinol-2 and decylplastoquinol were determined to be 30, 8.0 and 12 μM, respectively, and about 2-fold smaller than those of *E. coli* cytochrome *bd* (-I) (Fig. 1, Table 1). In contrast, *E. coli* cytochrome *bd*-II (AppBC) followed the Michaelis–Menten mechanism and showed the K_m value of 76 μM for ubiquinol-1 (data not shown). The decrease in the K_m value for ubiquinol-2 in the *Synechocystis* and *E. coli* cytochrome *bd* indicates that the 6-isoprenyl chain increased the binding affinity to the oxidases (*30*). It should be noted that the decylplastoquinol oxidase activity relative to the ubiquinol-1 oxidase activity was higher in *Synechocystis* than in *E. coli* ($V_{max}/K_m = 1.05$ and 0.114, respectively, U mg protein^{-1} μM^{-1}). This would make the *Synchoccystis* cytochrome *bd* function as plastoquinol oxidase.

Effects of Dioxygen Reduction Site Inhibitors on Ubiquinol-1 Oxidase Activity of Synechocystis Cytochrome bd—Previously, Howitt and Vermass (*6*) reported that *Synechocystis* cytochrome *bd* plastoquinol oxidase complemented a defect of the aerobic growth of the *E. coli* terminal oxidase-deficient mutant GO105 (*cyo* Δ*cyd recA*), suggesting that it can utilize ubiquinol (a reduced form of 2,3-dimethoxy-5-methyl-6-*n*-prenyl-1, 4-benzoquinone) in the *E. coli* membrane. We extended this work and determined properties of the quinol oxidase activity of *Synechocystis* cytochrome *bd*. The IC_{50} value for cyanide so far reported for *Synechocystis* cytochrome *bd* has been likely determined in the presence of a haem oo_3-type variant of cytochrome *c* oxidase, which is produced by replacement of haem A by haem O under microaerobic growth conditions (*23*). Thus, it is not clear whether this parameter represents properties of cyanobacterial cytochrome *bd* or the oo_3-type cytochrome *c* oxidase. By using the *E. coli* ST4683/pUC-cydAB membranes, where *Synechocystis* cytochrome *bd* has been expressed as a sole terminal oxidase, we determined IC_{50} values for potassium cyanide and sodium azide to be 1.6 and 16 mM, respectively, which are comparable to 1.3 and 6.4 mM, respectively, of *E. coli* cytochrome *bd* (Table 1).

Effects of Quinol Oxidation Site Inhibitors on the Ubiquinol-1 Oxidase Activity of Synechocystis Cytochrome bd—Recently, we screened the Kitasato Institute for Life Sciences Chemical Library with *E. coli* cytochorme *bd*, and identified gramicidin S (*25*) and five prenylphenols [LL-Z1272β, γ (aschochlorin), δ, ε and ζ] (*31*) as new inhibitors of cytochrome *bd*. To probe structural differences in the quinol oxidation site between cyanobacterial and *E. coli* cytochrome *bd*, we determined IC_{50} values for gramicidin S, aschochlorin and known inhibitors for cytochrome *bd* (*12*, *29*, *32*, *33*). The IC_{50} values determined for aurachin C 1–10, aurachin D 5–10, HQNO, antimycin A, piericidin A and gramicidin S were 1.7 nM, 3.0 nM, 88 nM, 0.11 μM, 8.3 μM and 17.6 μM, respectively (Fig. 2, Table 1). *Synechocystis* cytochrome *bd* showed the inhibitor resistance spectrum similar to that of the *E. coli* enzyme but more sensitive to quinolone inhibitors, antimycin A and ascochlorin.

Spectroscopic Properties of Synechocystis Cytochrome bd—The *Synechocystis* cytochrome *bd*-expressed membranes showed peaks at 411 nm in the air-oxidized state and at 427, 530 and 559 nm in the fully reduced state, but peaks originated for haems b_{595} and *d* were difficult to identify (data not shown). The partially purified *Synechocystis* oxidase showed peaks at 411 nm and 640 nm in the air-oxidized state and

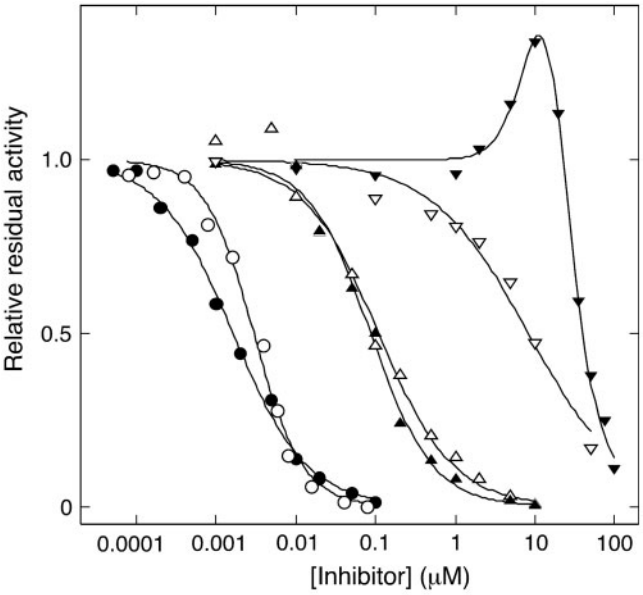

Fig. 2. **Effects of quinol oxidation site inhibitors on ubiquinol-1 oxidase activity of *Synechocystis* cytochrome *bd*.** Reactions by ST4683/pUC-cydAB membranes were measured at 100 μM ubiquinol-1 and data points were average values from duplicate assay. The IC_{50} values were estimated to be 1.7 ± 0.1 nM (aurachin C 1–10, filled circle), 3.0 ± 0.2 nM (aurachin D 5–10, open circle), 88 ± 7 nM (HQNO, filled triangle), 0.11 ± 0.01 μM (antimycin A, open triangle), 8.3 ± 1.2 μM (piericidin A, open inverted triangle) and 17.7 ± 0.4 μM (gramicidin S, filled inverted triangle).

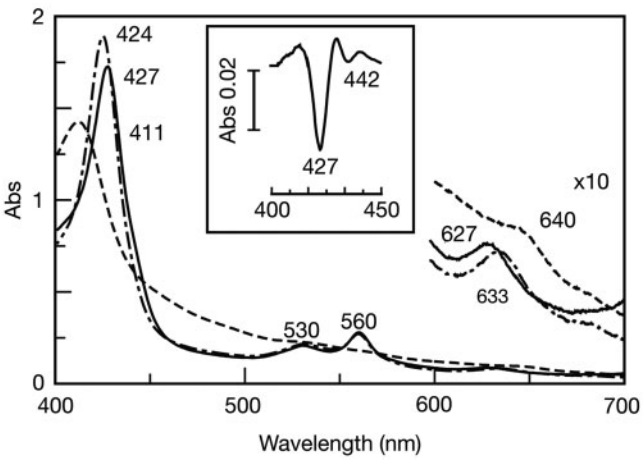

Fig. 3. **Absorption spectra of *Synechocystis* cytochrome *bd*.** The air-oxidized (broken line), dithionite-reduced (solid line) and reduced CO-bound (chain line) forms of partially purified *Synechocystis* cytochrome *bd* (10 μM protohaem IX) were recorded at 25°C. Inset: the second-order finite difference spectrum of the Soret region of the dithionite-reduced form.

at 427 nm, 530 nm, 560 nm and 627 nm in the fully reduced state (Fig. 3). These peak positions are slightly blue-shifted from those of *E. coli* cytochrome *bd* (414 nm, 646 nm, 429 nm, 531 nm, 561 nm, 596 and 630 nm, respectively) (*22*). Spectroscopic properties are similar to those of *E. coli* cytochrome *bd*-II (AppBC or CbdAB; 411 nm, 648 nm, 428 nm, 530 nm, 559 nm, 589 nm and 628 nm, respectively) (*34*) and *Azotobacter vinelandii* cytochrome *bd* (410 nm, 646 nm, 428 nm, 530 nm, 559 nm, 595 nm and 628 nm, respectively) (*35*), although the peak intensity of ferrous haem *d* relative to that of ferrous haem b_{558} was much lower in the *Synechocystis* oxidase (Fig. 3). The Soret peak for ferrous haem b_{595} can be identified as a small trough at 442 nm in the second-order finite difference spectrum (cf., 440 nm in *E. coli* cytochrome *bd*), while the α peak was insignificant due to its low extinction coefficient and/or a low content of the haem b_{595}-*d* centre (i.e. haem *d*/haem *b* ratio of ~0.05). Accordingly, cyanobacterial cytochrome *bd* would be difficult to be identified in the cyanobacterial membranes, which contain a large amount of photosynthetic pigments and other electron transfer proteins. Upon CO-binding, the ferrous haem *d* peak shifted to 633 nm, which was accompanied by the Soret peak shift to 424 nm from 427 nm, indicating that, on the contrary to (*23*), haem *d* in cyanobacterial cytochrome *bd* was functional, in terms of ligand binding.

Conclusion and Perspectives—Here we report for the first time the enzymatic properties and spectroscopic properties of cyanobacterial cytochrome *bd* plastoquinol oxidase and showed the similarities to those of the *E. coli* cytochrome *bd*. Sequence analysis on cytochrome *bd* (CydAB) and cyanide-insensitive oxidase (CioAB) revealed the presence of the characteristic features around the haem b_{595}-*d* binding sites in subunit I (CydA/CioA) (Fig. 4). The haem b_{595} ligand, H19 (the *E. coli* CydA numbering) in transmembrane helix I, is followed by 'x_3VP' in CydA and by 'x_3PA/V' in CioA while a putative haem *d* ligand, Glu99 in transmembrane helix III, is preceded by 'Px_3' in CydA and by 'P(/T or S)x_4' in CioA. In contrast to the enzymatic and spectroscopic properties, the locations of helix breakers in helices I and III suggest that cyanobacterial quinol oxidase is rather related to the CioAB oxidase, which does not show spectroscopic properties of cytochrome *bd* in the wild-type strains (*37*, *38*). These features may be related to the low content of the haem b_{595}-*d* binuclear centre in cyanobacterial cytochrome *bd*. In Q loop, as expected from kinetic parameters, there is no characteristic feature for cyanobacterial oxidases. Finally, phylogenetic analysis placed cyanobacterial oxidases between cytochrome *bd* and cyanide-insensitive oxidase (Fig. 5), being consistent with their unique functional and structural properties. We hope that our findings will help our understanding the role and properties of CydAB and CioAB quinol oxidases in other bacterial species.

ACKNOWLEDGEMENTS

We would like to thank Drs W.F.J. Vermaas (Arizona State University), M. Yamamoto (aRigen Pharmaceuticals. Inc.), S. Yoshida (Institute of Physical and Chemical Research) and Meiji Seika Kaisha, Ltd for plasmid pUC-cydAB, ascochlorin, piericidin A and gramicind S, respectively.

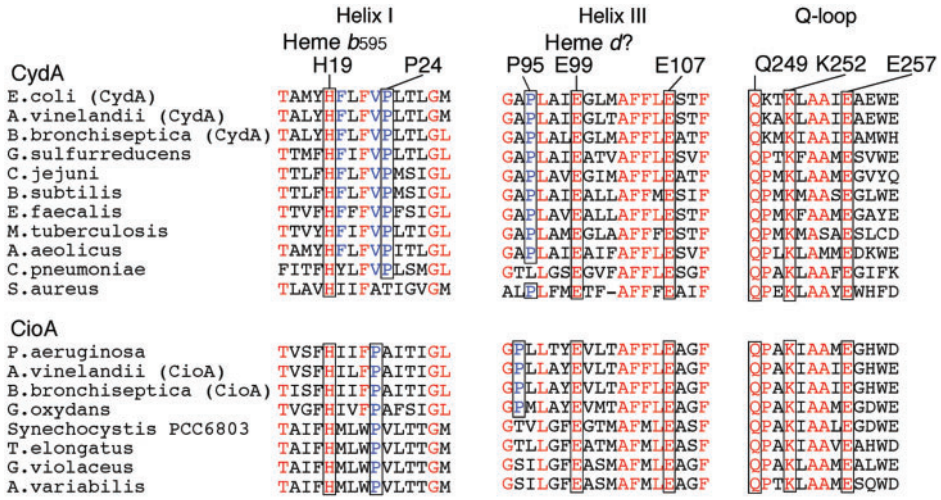

Fig. 4. **Sequence alignments of the haem b595 and d binding sites and of Q loop in the CydA.CioA family proteins.** Alignment of a part of transmembrane helices I and III, and the *N*-terminal region of loop VI/VII (Q loop) of the CydA/CioA family proteins. Sequence alignments were done with ClustalX 2.0 (*36*). Conserved amino acid residues are shown in *red* and amino-acid residues which characterize each subfamily in *blue* (e.g. P24 and P95 in the *E. coli* CydA). H19 is the axial ligand of haem b_{595} (*13*) and protons are taken up from the cytoplasm and transferred to E99 in the putative haem *d*-binding site through E107 (*15*). K252 and E257 are essential for the quinol binding (*22*).

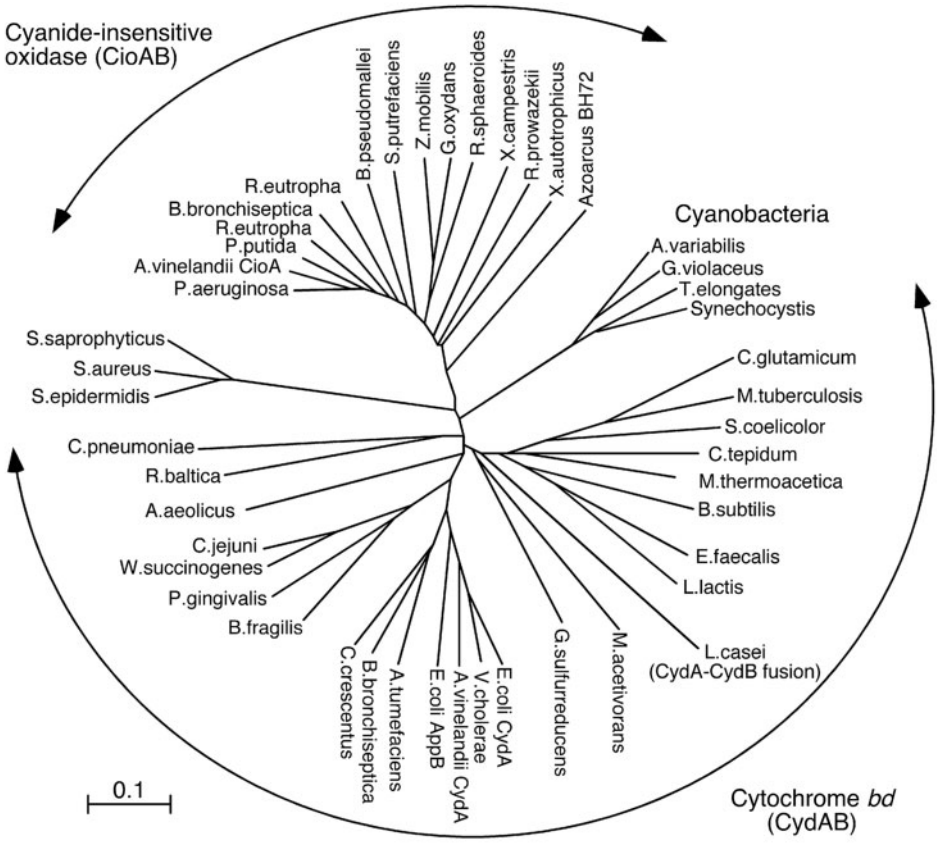

Fig. 5. **Unrooted phylogenetic tree for the CydA/CioA family proteins.** CydA sequences (GenBank accession no) used are *Corynebacterium glutamicum* (NP_600377), *Mycobacterium tuberculosis* (NP_336115), *Streptomyces coelicolor* (NP_628129), *Chlorobium tepidum* (NP_662697), *Moorella thermoacetica* (YP_430930), *Bacillus subtilis* (NP_391755), *Enterococcus faecalis* (NP_815724), *Streptococcus agalactiae* (NP_736221), *Lactococcus lactis* (NP_266860), *Lactobacillus casei* (YP_805320, a CydA-CydB fusion), *Methanosarcina acetivorans* (NP_615957), *Geobacter sulfurreducens* (NP_952691), *E. coli* (NP_415261 [CydA], NP_415497 [AppB]), *Vibrio cholerae* (NP_231478), *A. vinelandii* (ZP_00418656), *Agrobacterium tumefaciens* (NP_356555), *B. bronchiseptica* (NP_891032), *Caulobacter crescentus* (NP_419579), *Bacteroides fragilis* (YP_099157), *Porphyromonas gingivalis* (NP_905143), *Wolinella succinogenes* (NP_907966), *Campylobacter jejuni* (NP_281294), *Aquifex aeolicus*

FUNDING

This study was supported by a grant-in-aid for scientific research (20570124) and Creative Scientific Research (18GS0314) from the Japan Society for the Promotion of Science and for scientific research on Priority Areas (18073004) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Schmetterer, G. (1994) Cyanobacterial respiration in *The Molecular Biology of Cyanobacteria* (Bryant, D.A., ed.) pp. 409–435, Kluwer Academic, Dordrecht
2. Paumann, M., Regelsberger, G., Obinger, C., and Peschek, G.A. (2005) The bioenergetic role of dioxygen and the terminal oxidase(s) in cyanobacteria. *Biochim. Biophys. Acta* **1707**, 231–253
3. Schmetterer, G., Alge, D., and Gregor, W. (1994) Deletion of cytochrome *c* oxidase genes from the cyanobacterium *Synechocystis* sp. PCC 6803—evidence for alternative respiratory pathways. *Photosyn. Res.* **42**, 43–50
4. Büchel, C., Zsiros, O., and Garab, G. (1998) Alternative cyanide-sensitive oxidase interacting with photosynthesis in *Synechocystis* PCC 6803. Ancestor of the terminal oxidase of chlororespiration? *Photosynthetica* **35**, 223–231
5. Berry, S., Schneider, D., Vermaas, W.F.J., and Rögner, M. (2002) Electron tranport route in whole cells of *Synechocystis* sp. strain PCC 6803: the route of the cytochrome *bd*-type oxidase. *Biochemistry* **41**, 3422–3429
6. Howitt, C.A. and Vermaas, W.F.J. (1998) Quinol and cytochrome oxidases in the cyanobacterium Synechocystis sp. PCC 6803. *Biochemistry* **37**, 17944–17951
7. Pils, D., Gregor, W., and Schmetterer, G. (1997) Evidence for *in vivo* activity of three distinct respiratory terminal oxidases in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *FEMS Microbiol. Lett.* **152**, 83–88
8. Pils, D. and Schmetterer, G. (2001) Characterization of three bioenergetically active respiratory terminal oxidases in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *FEMS Microbiol. Lett.* **203**, 217–222
9. McDonald, A.E. and Vanlerberghe, G.C. (2006) Origins, evolutionary history, and taxonomic distribution of alternative oxidase and plastoquinol terminal oxidase. *Comparative Biochem. Physiol. Part D* **1**, 357–364
10. Mogi, T., Tsubaki, M., Hori, H., Miyoshi, H., Nakamura, H., and Anraku, Y. (1998) Two terminal quinol oxidase families in *Escherichia coli*: Variations on molecular machinery for dioxygen reduction. *J. Biochem. Mol. Biol. Bioiphys.* **2**, 79–110
11. D'mello, R., Hill, S., and Poole, R.K. (1996) The cytochrome *bd* quinol oxidase in *Escherichia coli* has an extremely high oxygen affinity and two oxygenbinding haems: implications for regulation of activity *in vivo* by oxygen inhibition. *Microbiology* **142**, 755–763
12. Kita, K., Konishi, K., and Anraku, Y. (1984) Terminal oxidases of *Escherichia coli* aerobic respiratory chain. II. Purification and properties of cytochrome b_{562}-*o* complex from cells grown with limited oxygen and evidence of branched electron-carrying systems. *J. Biol. Chem.* **259**, 3375–3381
13. Fang, G.H., Lin, R.J., and Gennis, R.B. (1989) Location of heme axial ligands in the cytochrome *d* terminal oxidase complex of *Escherichia coli* determined by site-directed mutagenesis. *J. Biol. Chem.* **264**, 8026–8032
14. Kaysser, T. M., Ghaim, J. B., Georgiou, C., and Gennis, R. B. (1995) Methionine-393 is an axial ligand of the heme b_{558} component of the cytochrome *bd* ubiquinol oxidase from *Escherichia coli*. *Biochemistry* **34**, 13491–13501
15. Mogi, T., Endo, S., Akimoto, S., Morimoto-Tadokoro, M., and Miyoshi, H. (2006) Glutamates 99 and 107 in transmembrane helix III of subunit I of cytochrome *bd* are critical for binding of the heme b_{595}-*d* binuclear center and enzyme activity. *Biochemistry* **45**, 15785–15792
16. Zhang, J., Barquera, B., and Gennis, R.B. (2004) Gene fusions with β-lactamase show that subunit I of the cytochrome *bd* quinol oxidase from *E. coli* has nine transmembrane helices with the O_2 reactive site near the periplasmic surface. *FEBS Lett.* **561**, 58–62
17. Lorence, R.M., Carter, K., Gennis, R.B., Matsushita, K., and Kaback, H.R. (1988) Trypsin proteolysis of the cytochrome *d* complex of *Escherichia coli* selectively inhibits ubiquinol oxidase activity while not affecting *N*, *N*, *N*′, *N*′-tetramethyl-*p*-phenylenediamine oxidase activity. *J. Biol. Chem.* **263**, 5271–5276
18. Dueweke, T.J. and Gennis, R.B. (1991) Proteolysis of the cytochrome *d* complex with trypsin and chymotrypsin localizes a quinol oxidase domain. *Biochemistry* **30**, 3401–3406
19. Kranz, R.G. and Gennis, R.B. (1984) Characterization of the cytochrome *d* terminal oxidase complex of *Escherichia coli* using polyclonal and monoclonal antibodies. *J. Biol. Chem.* **259**, 7998–8003
20. Dueweke, T.J. and Gennis, R.B. (1990) Epitopes of monoclonalantibodies which inhibit ubiquinol oxidase activity of *Escherichia coli* cytochrome *d* complex localize functional domain. *J. Biol. Chem.* **265**, 4273–4277
21. Matsumoto, Y., Murai, M., Fujita, D., Sakamoto, K., Miyoshi, H., Yoshida, M., and Mogi, T. (2006) Mass spectrometric analysis of the ubiquinol-binding site in cytochrome *bd* from *Escherichia coli*. *J. Biol. Chem.* **281**, 1905–1912
22. Mogi, T., Akimoto, S., Endou, S., Watanabe-Nakayama, T., Mizuochi-Asai, E., and Miyoshi, H. (2006) Probing the ubiquinol-binding site in cytochrome *bd* by site-directed mutagenesis. *Biochemistry* **45**, 7924–7930
23. Fromwald, S., Zoder, R., Watsyn, M., Lübben, M., and Peschek, G. (1999) Extended heme promiscuity in the cyanobacterial cytochrome *c* oxidase: characterization of native complexes containing hemes A, O and D, respectively. *Arch. Biochem. Biophys.* **367**, 122–128
24. Büchel, C., Zsíros, O., and Garab, G. (1998) Alternative cyanide-sensitive oxidase interacting with photosynthesis in *Synechocystis* PCC6803. Ancestor of the terminal oxidase of chlororespiration. *Photosynthetica* **35**, 223–231

(NP_213934), *Rhodopirellula baltica* (NP_869127), and *Chlamydophila pneumoniae* (NP_224310). CioA sequences (GenBank accession no.) used are *Pseudomonas aeruginosa* (NP_252619), *P. putida* (NP_746760), *Azotobacter vinelandii* (ZP_00418266), *Ralstonia eutropha* (YP_840698, YP_840979), *Bordetella bronchiseptica* (NP_887784), *Burkholderia pseudomallei* (YP_001074378), *Shewanella putrefaciens* (YP_001182458), *Zymomonas mobilis* (YP_163306), *Gluconobacter oxydans* (YP_190717), *Rhodobacter sphaeroides* (YP_354730), *Xanthomonas campestris* (NP_639037), *Rickettsia prowazekii* (NP_220602), *Xanthobacter autotrophicus* (YP_001416174), *Azoarcus* sp. BH72 (YP_933275). Cyanobacterial and *Staphylococcus* sequences used are *Anabaena variabilis* (YP_320076), *Gloeobacter violaceus* sp. PCC 7421 (NP_924143), *Thermosynechococcus elongates* (NP_682392), *Synechocystis* sp. PCC 6803 (NP_440505), *Staphylococcus epidermidis* (YP_188257), *S. aureus* (NP_371610) and *S. saprophyticus* (YP_301795). Phylogenetic tree was constructed with Njplot (*39*).

25. Mogi, T., Ui, H., Shiomi, K., Ōmura, S., and Kita, K. (2008) Gramicidin S identified as a potent inhibitor for cytochrome *bd*-type quinol oxidase. *FEBS Lett.* **582**, 2299–2302
26. Matsumoto, Y., Muneyuki, E., Fujita, D., Sakamoto, K., Miyoshi, H., Yoshida, M., and Mogi, T. (2006) Kinetic mechanism of quinol oxidation by cytochrome *bd* studied with ubiquinone-2 analogs. *J. Biochem.* **139**, 779–788
27. Cheng, Y.C. and Prusoff, W.H. (1973) Relationship between the inhibition constant (K_I) and the concentration of inhibitor which cause 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**, 3099–3108
28. Bronnikov, G.E., Zhang, S.-J., Cannon, B., and Nedergaard, J. (1999) A dual component analysis explains the distinctive kinetics of cAMP accumulation in brown adipocytes. *J. Biol. Chem.* **274**, 37770–37780
29. Miyoshi, H., Takegami, K., Sakamoto, K., Mogi, T., and Iwamura, H. (1999) Characterization of the ubiquinol oxidation sites in cytochromes *bo* and *bd* from *Escherichia coli* using aurachin C analogues. *J. Biochem.* **125**, 138–142
30. Sakamoto, K., Miyoshi, H., Ohshima, M., Kuwabara, K., Kano, K., Akagi, T., Mogi, T., and Iwamura, H. (1998) Role of isoprenyl tail of ubiquinone in reaction with respiratory enzymes: Studies with bovine heart mitochondrial complex I and *Escherichia coli bo*-type ubiquinol oxidase. *Biochemistry* **37**, 15106–15113
31. Mogi, T., Ui, H., Shiomi, K., Ōmura, S., Miyoshi, H., and Kita, K. (2008) Antibiotics LL-Z1272 identified as novel inhibitors discriminating bacterial and mitochondrial quinol oxidases. *Biochim. Biophys. Acta* in press
32. Meunier, B., Madgwick, S.A., Reil, E., Ottemeier, W., and Rich, P.R. (1995) New inhibitors of the quinol oxidation sites of bacrterial cytochrome *bo* and *bd*. *Biochemistry* **34**, 1076–1083
33. Jünemann, S. (1997) Cytochrome *bd* terminal oxidase. *Biochim. Biophys. Acta* **1321**, 107–127
34. Sturr, M.G., Krulwich, T.A., and Hicks, D.B. (1996) Purification of a cytochrome *bd* terminal oxidase encoded by the *Escherichia coli app* locus from a Δ*cyo* Δ*cyd* strain complemented by genes from *Bacillus firmus* OF4. *J. Bacteriol.* **176**, 1742–1749
35. Kolonay, J.F. Jr., Moshiri, F., Gennis, R.B., Kaysser, T.M., and Maier, R.J. (1994) Purification and characterization of the cytochrome *bd* complex from *Azotobacter vinelandii*: Comparison to the complex from *Escherichia coli*. *J. Bacteriol.* **176**, 4177–4181
36. Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., and Higgins, D.G. (2007) ClustalW2 and ClustalX version 2. *Bioinformatics* **23**, 2947–2948
37. Matsushita, K., Yamada, M., Shinagawa, E., Adachi, O., and Ameyama, M. (1983) Membrane-bound respiratory chain of *Pseudomonas aeruginosa* grown aerobically. A KCN-insensitive alternate oxidase chain and its energetics. *J. Biochem.* **93**, 1137–1144
38. Cunningham, L., Pitt, M., and Williams, H.D. (1997) The *cioAB* genes from *Pseudomonas aeruginosa* code for a novel cyanide-insensitive terminal oxidase related to the cytochrome *bd* quinol oxidases. *Mol. Microbiol.* **24**, 579–591
39. Perrière, G. and Gouy, M. (1996) WWW-Query: An on-line retrieval system for biological sequence banks. *Biochimie* **78**, 364–369