



Review

Succinate dehydrogenase and fumarate reductase from *Escherichia coli*Gary Cecchini^{a,b,*}, Imke Schröder^c, Robert P. Gunsalus^c, Elena Maklashina^{a,b}^a Molecular Biology Division, VA Medical Center, San Francisco, CA 94121, USA^b Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA 94143, USA^c Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA 90095, USA

Received 18 May 2001; received in revised form 16 August 2001; accepted 12 October 2001

Abstract

Succinate-ubiquinone oxidoreductase (SQR) as part of the trichloroacetic acid cycle and menaquinol-fumarate oxidoreductase (QFR) used for anaerobic respiration by *Escherichia coli* are structurally and functionally related membrane-bound enzyme complexes. Each enzyme complex is composed of four distinct subunits. The recent solution of the X-ray structure of QFR has provided new insights into the function of these enzymes. Both enzyme complexes contain a catalytic domain composed of a subunit with a covalently bound flavin cofactor, the dicarboxylate binding site, and an iron–sulfur subunit which contains three distinct iron–sulfur clusters. The catalytic domain is bound to the cytoplasmic membrane by two hydrophobic membrane anchor subunits that also form the site(s) for interaction with quinones. The membrane domain of *E. coli* SQR is also the site where the heme *b*₅₅₆ is located. The structure and function of SQR and QFR are briefly summarized in this communication and the similarities and differences in the membrane domain of the two enzymes are discussed. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Succinate dehydrogenase; Fumarate reductase; Ubiquinone reductase; Menaquinol oxidase; Flavoprotein; Iron–sulfur protein

1. Introduction

Many laboratories have contributed to our knowledge of complex II over the past 50 years and this

body of work has come from many sources, including a wide variety of prokaryotes as well as eukaryotes. Several comprehensive reviews on complex II have been published during the past decade and the reader is encouraged to peruse these for a more detailed examination of the multitude of interesting facets of this enzyme [1–4]. In this review we will focus on the properties of *Escherichia coli* succinate-ubiquinone oxidoreductase (SQR) and menaquinol-fumarate oxidoreductase (QFR) which have proved to be two excellent models for the study of complex II. Surprisingly, although *E. coli* has been a pre-eminent model organism in research laboratories for the past century, it has only been for the past 20 years that significant strides have been made in our

Abbreviations: SQR, succinate-ubiquinone oxidoreductase; QFR, menaquinol-fumarate oxidoreductase; carboxin, 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide; FAD, flavin adenine dinucleotide; HQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide; PCP, pentachlorophenol; Q₁/MQ₁ and Q₂/MQ₂, homologs of ubiquinone/menaquinone having one or two isoprenoid units at position 6 of the quinone ring, respectively; TTFA, 4,4,4-trifluoro-1-(2-thienyl)-butane-1,3-dione

* Corresponding author. Fax: +1-415-750-6959.

E-mail address: cecchini@itsa.ucsf.edu (G. Cecchini).

understanding of complex II from this organism. These advances have resulted from a combination of molecular genetic, biochemical, and biophysical approaches where the ease of working with *E. coli* can be taken of full advantage.

The ability of whole cells of *E. coli* to reversibly oxidize succinate to fumarate has been recognized for more than 75 years [5]. Some three decades later it was shown that purified preparations of mammalian succinate dehydrogenase, in addition to catalyzing succinate oxidation, are also capable of fumarate reduction [6]. Nevertheless, the kinetic properties of the mammalian enzyme suggested that it was poised to oxidize succinate rather than reduce fumarate in vivo, whereas other studies showed that enzyme preparations from obligate anaerobic bacteria catalyzed fumarate reduction more efficiently than succinate oxidation [7]. Thus, the question arose of whether or not facultative anaerobic bacteria such as *E. coli* had evolved a single enzyme that could efficiently catalyze both fumarate reduction and succinate oxidation or if two distinct enzymes were present. Hirsch et al. [8], some 38 years ago, using classical genetic approaches helped resolve this question. These workers used ethyl methanesulfonate mutagenesis of an *E. coli* K12 strain to select mutants that were defective in growth on succinate. Subsequent analysis showed there were two distinct enzymes catalyzing succinate oxidation and fumarate reduction depending on whether *E. coli* was grown aerobically or anaerobically on selective media. In agreement with the earlier studies [6,7] the aerobically expressed succinate dehydrogenase was shown to have a succinate-oxidizing to fumarate-reduction activity ratio of 25:1 [8]. By contrast, the *E. coli* fumarate reductase was found to be relatively more able to catalyze succinate oxidation with a ratio of fumarate reduction to succinate oxidation of only 1.5:1 [8].

Partially purified preparations of eukaryotic and prokaryotic succinate dehydrogenases had been available since the 1950s [6,7,9] the first characterized preparations of *E. coli* succinate dehydrogenase and fumarate reductase did not become available until the 1970s [10–12]. Both enzymes were found in the membrane fraction of cells, however, the initial purifications resulted in isolation of a soluble two-subunit form of the enzyme of about 100 kDa [10,12]. A

deoxycholate solubilized fraction of *E. coli* succinate dehydrogenase of 150 kDa was also reported [11] indicating that this was the intact complex. Isolation of highly purified four-subunit QFR and SQR complexes was finally accomplished in the 1980s using a combination of detergent extraction and anion and hydrophobic chromatography [13–15].

An advantage of studying *E. coli* QFR and SQR is that the enzymes can be overexpressed to high levels. A 20-fold overproduction of QFR can be achieved in *E. coli* cells harboring a QFR-encoding plasmid [16]. This amplification of QFR results in a significant increase in the level of membrane-associated protein and the formation of a tubular network within the cell composed of the four-subunit QFR protein and cardiolipin [16,17]. Although the major phospholipids of *E. coli* are phosphatidyl ethanolamine and phosphatidyl glycerol the increased levels of cardiolipin associated with QFR suggest that lipid biosynthesis of this phospholipid is specifically increased along with the synthesis of QFR [18]. Even though the membrane fraction becomes highly enriched in QFR, the protein complex apparently does not displace other membrane protein constituents and the cells grow normally [16]. Similar results have been obtained in *E. coli* transformed with SQR-encoding plasmids. Both the formation of tubules and an increase in membrane vesicle formation are observed when SQR is expressed to high levels [19]. The amplified SQR and QFR membrane fraction and tubules appear to contain enzyme that is fully functional and contains all the redox cofactors present in complex II. The very high levels of protein that can be obtained from *E. coli* have proved quite useful during purification and characterization of the enzyme and make these enzymes one of the best models for understanding the structure and function of complex II.

Hägerhäll [4] has grouped SQRs and QFRs from different organisms into three functional classes, based on their in vivo function and the particular quinone substrate used by the enzyme. Using her classification system *E. coli* and mammalian SQRs are examples of type 1 enzymes, in that they use ubiquinone as a substrate and are poised to oxidize succinate in vivo. *E. coli* QFR is a type 2 enzyme since it catalyzes oxidation of the lower potential menaquinol and more readily reduces fumarate in

vivo. Other classification schemes have been developed for SQRs and QFRs based on differences in the *b* heme composition [20,21], the membrane binding domain polypeptides [22], or their fumarate reduction activity [23]. However, in all of these classification schemes *E. coli* SQR is grouped with mammalian SQR indicative of the high degree of structural and functional similarity. Based on these schemes and available structures of complex II evolutionary models have been proposed. It is suggested that the present day membrane-bound enzymes have evolved from a soluble fumarate reductase that contained non-covalently bound flavin adenine dinucleotide (FAD) and was thus incapable of succinate oxidation [2,24]. Subsequently the enzyme acquired the appropriate iron–sulfur clusters and became associated with the membrane through a core four-helix bundle polypeptide(s) that spans the membrane and provides site(s) for interactions with quinones. Ultimately, as in the case of *E. coli* QFR and SQR, the FAD cofactor became covalently attached to the enzyme to enable succinate oxidation.

2. Molecular genetics of expression of FRD and SDH

2.1. Gene identification and organization

The QFR and SQR enzymes of *E. coli* are encoded by the *frdABCD* and *sdhCDAB* gene clusters located at 94 min and at 17 min on the chromosome, respectively [25,26]. At the start of the *sdhCDAB* gene cluster, the *sdhC* and *sdhD* genes encode the two membrane embedded polypeptides that interact with ubiquinone, and anchor the catalytic domain at the membrane surface. The following genes encode the flavoprotein (*sdhA*) and iron–sulfur protein (*sdhB*) subunits that catalyze succinate reduction to fumarate. For QFR, the gene order differs whereby the catalytic (*frdAB*) and membrane anchor polypeptide genes (*frdCD*) are reversed. The QFR and SQR catalytic subunit genes are highly conserved and reflect the biochemical and structural similarity of the two enzymes. It is thus not surprising that the ‘*A*’ and ‘*B*’ genes that specify the catalytic polypeptides in each complex exhibit extensive homology with respect to the primary and secondary amino acid sequences. The ‘*A*’ and ‘*B*’ gene designations are nearly univer-

sally used in bacteria to name the two catalytic polypeptides. Gene and amino acid sequences for the ‘*C*’ and ‘*D*’ membrane anchor polypeptides of *E. coli* QFR and SQR are poorly conserved. However, each gene specifies for a polypeptide, having three transmembrane-spanning α -helical regions (see below). Discovery and subsequent genetic characterizations of the *E. coli* *frd* and *sdh* genes has been reviewed elsewhere [2,27,28].

Recent advances in bacterial genome sequencing have revealed numerous members of the *frd/sdh* gene family. As of early 2001, approximately 80 *frdA/sdhA* genes were deposited in various public DNA/protein sequence repositories. Interestingly, without prior knowledge of the physiological role of a particular enzyme (i.e., that encoded by a homologous set of *frd/sdh*-like genes) in the metabolism of a given bacterium, it is not yet possible to predict whether the enzyme product functions as a QFR or as a SQR enzyme in vivo! Many enteric bacteria such as *E. coli* contain both membrane-bound enzymes due to their facultative metabolism. When microbes are either obligate aerobes or obligate anaerobes, the homologous genes are assumed to encode an SQR or QFR, respectively. Genes encoding the subunits of the catalytic domain, composed of the flavoprotein and the iron–sulfur protein, are readily predicted from the DNA sequences, albeit without inference to catalytic parameters. The genes for the membrane-spanning anchor polypeptides are identified due to their adjacency directly upstream or downstream of the catalytic subunit genes. Finally, the number of genes encoding the membrane anchor polypeptides may be either two (as in the case of *E. coli* *frdCD* and *sdhCD*) or one (as in the example of *Bacillus subtilis* *sdhC* and *Wolinella succinogenes* *frdC* [1–4]). When there are two anchor polypeptides, the number of transmembrane-spanning helices per polypeptide is always three. When there is only one polypeptide, it contains five transmembrane-spanning elements. Thus, some diversity exists among bacteria with respect to the number and the order of genes encoding the membrane associated polypeptides.

2.2. Promoter location and structure

The transcriptional regulation of the *E. coli* *frdABCD* and *sdhCDAB* genes has been studied ex-

tensively over the past 15 years as a model for understanding the control of bacterial QFR and SQR enzyme synthesis. The *frdABCD* and *sdhCDAB* gene clusters each contain a single promoter that allows for their coordinated expression in an operon unit. The site of *frdABCD* transcription is located 93 bp upstream of the first gene, *frdA* [29]. The *sdhCDAB* operon is expressed from single promoter located 219 bp before the start of *sdhC* transcription [30,31]. Expression of the two operons in *E. coli* occurs under quite different conditions and reflects the physiological roles of the two enzymes in cellular metabolism.

2.3. Control of gene expression

The physiological roles of the SQR and QFR complexes are to provide for electron transport during aerobic and anaerobic cell growth conditions, respectively [1–4,32]. Thus, the transcription of the *frdABCD* and *sdhCDAB* operons responds to environmental as well as internal cell signals to modulate gene expression to meet cellular needs for energy and/or carbon intermediates. The regulation of each operon is briefly described below.

The *frdABCD* operon is optimally expressed during anaerobic cell growth conditions [29]. This reflects the role of the QFR enzyme in the terminal step of anaerobic respiration with fumarate as an electron acceptor. The anaerobic control is provided by the anaerobic global regulator Fnr, that serves to positively regulate *frdABCD* transcription (i.e., an activator). When cells are shifted to anaerobic conditions, Fnr becomes activated through the formation of a iron–sulfur (4Fe–4S) cluster that allows it to dimerize [33,34]. It then binds to a 14 bp Fnr recognition site centered approximately 41 nucleotides before the start of *frdA* transcription [35]. Similar Fnr activator sites are positioned at other Fnr-activated operons in *E. coli* [34,36].

Overriding the Fnr-dependent anaerobic control is a second layer of transcriptional control that occurs in response to the presence of nitrate in the cell environment [29,35]. This anaerobic respiratory substrate suppresses *frdABCD* gene expression via the Nar two-component regulatory system [37,38]. As nitrate respiration provides a higher potential for energy generation than does fumarate, the cell pre-

fers to respire using nitrate when oxygen is absent. NarX and NarQ along with NarL and NarP form this unusual two-component regulatory system that regulates the expression of a number of genes involved in anaerobic respiration and fermentation [37–39]. NarX and NarQ are typical sensor transmitter proteins that detect the presence of the two anions, nitrate and nitrite. Nitrate is the superior signal and functions at two to three orders of magnitude lower concentration than does nitrite [40,41]. Signal reception results in ATP-dependent phosphorylation of NarX and/or NarQ to give NarX-phosphate and NarQ-phosphate. Either sensor transmitter can function independently to activate NarL to give NarL-phosphate [42]. NarL-phosphate then binds in the *frd* promoter region to suppress FRD synthesis. The mechanism for the nitrate-dependent control is by repression of *frdABCD* transcription. Recognition and/or binding of RNA polymerase at the *frdA* promoter is apparently prevented when NarL-phosphate is bound [35,38].

Thus, when cells are growing anaerobically with nitrate present, fermentation pathway carbon flow is suppressed, and anaerobic respiration is directed to nitrate as the terminal electron acceptor. When nitrate is absent, respiration can then occur with dimethyl sulfoxide, trimethylamine *N*-oxide, or fumarate as the electron acceptor [37,43]. The anaerobic induction of *frd* expression is about 10–15-fold via Fnr, while the nitrate repression is about 25-fold [35]. Finally, the addition of fumarate to glucose grown cells results in a two-fold induction of *frdABCD* gene expression via a distinct two-component regulatory system called DcuR/DcuS [44]. This control element also modulates the uptake and/or export of dicarboxylates from the cell via specific inducible membrane transporter.

The *sdhCDAB* operon is optimally expressed during aerobic cell growth [30,43]. This expression reflects the role of the SQR enzyme in the trichloroacetic acid (TCA) cycle where it catalyzes the initial step of aerobic respiration to oxygen with succinate as an electron donor. Expression of the *sdhCDAB* operon is lowered by about 10-fold when cells are shifted to anaerobic growth with glucose as the carbon supply [30]. Under the latter condition, the cell has an excess of reducing equivalents and cannot deal with the production of additional NADH/

FADH, or reduced quinones generated by the TCA cycle. The negative control of *sdh* gene expression occurs by a repression mechanism due to the binding of the ArcA regulatory protein at the *sdhC* promoter [31]. ArcA is in an inactive state during aerobic cell growth and only when cells are shifted to anaerobic conditions does ArcA become activated by ArcB. These two proteins compose an anaerobic responsive two-component regulatory system [45,46] distinct from Nar. The environmental signal(s) that ArcB, the sensor transmitter, responds to is unknown. However, once ArcB detects the signal(s), it is phosphorylated in an ATP-dependent fashion and in turn, phosphorylates ArcA to give ArcA-phosphate. Of the four ArcA binding sites in the *sdhA* promoter region, only ArcA site II appears to be essential for the repression of *sdhCDAB* gene expression [31]. The *sdh* operon is also catabolite controlled, presumably by CRP/cAMP. Gene expression varies by 10-fold when cells are grown aerobically with different carbon compounds [30].

2.4. Translational coupling

The translation of the genes on the *frdABCD* and *sdhCDAB* mRNAs are most likely coupled although this has not been experimentally tested. By this translationally coupled process, translation initiates at a ribosome binding site (RBS) located upstream of a given gene, and then extends downstream into the following gene(s) without being released from the mRNA. This readthrough process is often mandatory when the downstream gene(s) lack a recognizable RBS [47,48]. This mechanism of translational coupling thus provides a means to ensure that a multi-subunit containing enzyme complex contains one molecule each of the newly synthesized polypeptide (e.g., FrdA, FrdB, FrdC, and FrdD in the mature QFR complex). Of the four genes in the *frdABCD* operon, three physically overlap by 4 and 14 bp (*frdB*/*frdC*/*frdD*) [49]. This overlap is typical of some bacterial genes that are transcribed in a coupled fashion [47]. Likewise, in the *sdhCDAB* operon, the two membrane anchor *sdhCD* genes overlap by 4 bp [50]. The contribution of translational coupling between *sdhDA* and between *sdhAB* is less clear. Alternatively, ribosomes may reinitiate at unique RBSs upstream of *sdhA* and *sdhB* as a means

to ensure equimolar amounts of the needed polypeptides.

2.5. Enzyme assembly and membrane localization signals

Little is known about either the primary or secondary polypeptide sequence determinants that specify for the ordered assembly and insertion of the QFR and SQR enzymes into the bacterial cytoplasmic membrane. From inspection of the gene order of the *frdABCD* and *sdhCDAB* genes, it is apparent that the catalytic or the membrane subunits may be synthesized before the other (e.g., as the order of genes are reversed in the respective operons). Studies have been done on the assembly of the QFR holoenzyme using a T7-promoter-conditional expression system [51]. It was found that even though the gene order is *frdABCD*, the FrdCD polypeptides first assemble together in the membrane and provide a site for subsequent attachment of the catalytic FrdAB subunits. Similar studies have not been done with *sdhCDAB*, however, there the gene order shows that the membrane anchor polypeptides SdhCD are synthesized before the catalytic subunits and thus once assembled within the membrane would also provide attachment sites for the catalytic subunits SdhAB.

3. Structure–function of QFR and SQR

3.1. Overview of *E. coli* QFR structure

Over the past 2 years there has been a wealth of structural information that has become available for our understanding of complex II. Structures for the complete *E. coli* QFR complex [52], as well as, one from *W. succinogenes* [53] have been described. In addition to these membrane-bound structures, four soluble homologs of the flavoprotein subunit of fumarate reductase have been described [54–57], thus allowing a detailed comparison of the structure of this subunit and the mechanism of catalysis by the enzyme. These available structures have also resulted in several insightful review articles discussing the catalytic mechanism [20,58–61] and thus in this communication we will focus only on the structure of the *E. coli* enzymes.

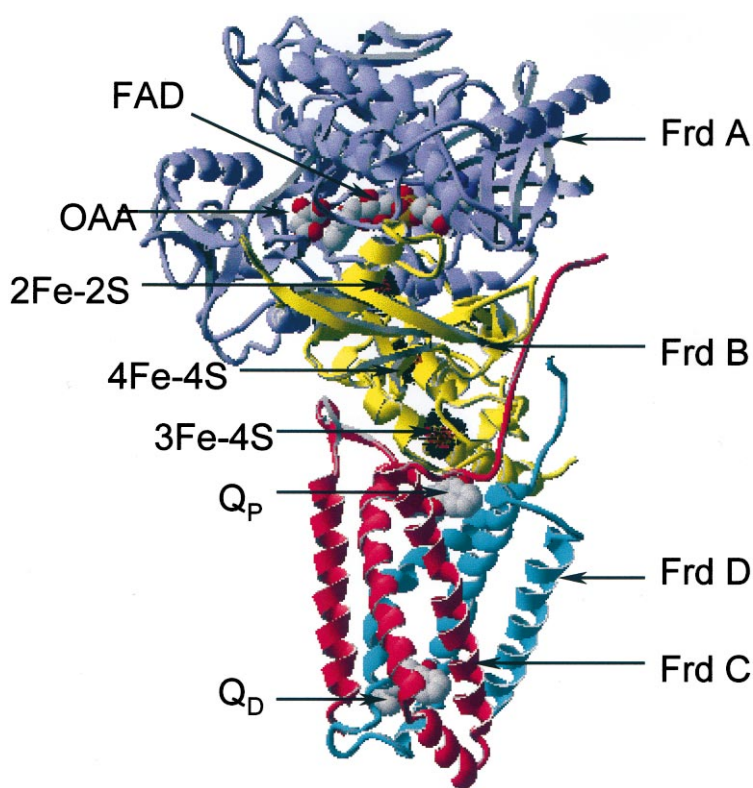


Fig. 1. Structure of *E. coli* menaquinol-fumarate oxidoreductase (PDB code 1FUM). The QFR monomer is shown. The flavoprotein (FrdA) is colored lavender, the physiological inhibitor oxaloacetate (OAA), and the covalently bound FAD cofactor are shown in gray. The iron-sulfur protein subunit (FrdB) is shown in yellow and the [2Fe-2S], [4Fe-4S], and [3Fe-4S], clusters are shown in black and red. The membrane-spanning subunit FrdC is shown in red and FrdD is shown in cyan. The two menaquinone molecules, Q_p and Q_d are shown in gray within the FrdC and FrdD helices.

The crystal structure of *E. coli* QFR shows two complexes that are related by a two-fold axis that are approximately parallel to the membrane normal and these complexes associate through their trans-membrane regions [52]. The crystal contacts are mediated by two ordered detergent molecules of Thesit ($C_{12}E_9$, polyoxyethylene(9)dodecyl ether). The contact region between the fumarate reductase molecules is, however, relatively small ($\sim 325 \text{ \AA}^2$) and there is no suggestion that the functional fumarate reductase exists as a dimer in the cell. The QFR monomer is approximately 70 \AA in diameter at its widest point (the FrdAB catalytic domain) and the molecule is 110 \AA in length. The overall structure of *E. coli* QFR is consistent with what had been proposed by numerous workers using biochemical and molecular genetics methodologies (see [1–4] and references therein) (Fig. 1). The structure clearly shows two structurally distinct domains. A membrane-extrinsic catalytic domain composed of the

FrdA flavoprotein subunit and the iron-sulfur protein domain (FrdB). Although an X-ray structure is so far unavailable for any succinate dehydrogenase it can be anticipated that the general structure of the catalytic domain (SdhAB) will be similar to that seen for FrdAB based on the high degree of sequence similarity [49,50,62,63] and cofactor composition [64] (Table 1). The transmembrane domain of *E. coli* QFR (FrdCD) is organized around a central four-helix bundle, with two additional helices outside this central core. The four-helix bundle arrangement for the FrdCD subunits is also consistent with a general model for the transmembrane domain of complex IIs that predates the X-ray structures [22]. A difference between *E. coli* QFR and SQR is the lack of a heme moiety in the former complex. The crystal structure of the *W. succinogenes* QFR, which contains two hemes, has both heme molecules approximately perpendicular to the membrane surface [53,59]. Based on the structural models for complex

II anchor domains [4,22] and site-directed mutagenesis studies of the *E. coli* SQR heme binding domain [65,66] the single *E. coli* b_{556} heme may reside in an environment similar to that of the heme b_p from *W. succinogenes* on the cytoplasmic side of the membrane and proximal to the [3Fe–4S] cluster of SQR.

3.2. Flavoprotein (FrdA) of *E. coli* QFR

The FrdA subunit contains two major domains. The first is the FAD binding domain based on a Rossmann-type fold which is further associated with the FrdA subunit through a covalent bond between the flavin C8 α methyl group and the Ne atom of the side chain of His44. This type of covalent flavin linkage had first been experimentally demonstrated in beef succinate dehydrogenase [67] and later for QFR from *W. succinogenes* [68] and *E. coli* [69]. The reason the FAD is bound covalently in complex II is still not completely understood, however, it is apparent that the covalent linkage and protein environment raise the redox potential of the flavin some 140–170 mV (Table 1) as compared to free FAD ($E_m = -219$ mV). This is in agreement with the observation that in soluble fumarate reductases which contain a non-covalently bound FAD cofactor the E_m is much lower (–152 mV) [70] and the enzymes are unable to oxidize succinate. Site-directed mutants of *E. coli* QFR where His44 was replaced also contain stoichiometric amounts of non-covalently bound FAD and also lose the ability to oxidize succinate [71] a result also observed in *Saccharomyces cerevisiae* succinate dehydrogenase [72]. This led to the suggestion that evolution of the covalent flavin linkage was a necessary requirement during the evolution of complex II to raise the redox potential of the flavin with the result that this class of enzymes could then func-

tion as a succino-oxidase [71]. The mechanism of how flavins become covalently attached to complex II is still not completely understood, however, it has been proposed that attack by a nucleophilic amino acid side chain on a quinone methide form of the isoalloxazine ring at the 8 α -carbon may result in covalent attachment of the flavin ([73], see [74] for general review of this subject). In this regard, it has been shown that flavinylation is stimulated in fumarate reductase and succinate dehydrogenase by the presence of citric acid cycle intermediates, such as succinate, fumarate, malate, and OAA, [75,76] all of which may bind with different degrees of affinity to the dicarboxylate binding site of complex II. The binding of substrate or inhibitor to the flavoprotein subunit thus may induce a conformational change that properly aligns the flavin and peptide backbone of the protein to allow the covalent flavin bond to form. The same flavin linkage is present in *E. coli* SQR and the covalent linkage has been used to advantage by using [14 C]riboflavin to identify immunoreactive components of complex II [77].

The second major domain of the FrdA flavoprotein subunit is a capping domain which is a general feature of the flavoprotein containing subunit of all the structurally analyzed fumarate reductases and related flavoproteins [52–60]. The capping domain and flavin domain are connected by a small hinge region consisting of two β strands and the dicarboxylate binding site is located at the interface of these two domains. The hinge connecting these two domains can bend by more than 30° [60] consistent with an opening and closing of the active site region during catalysis. It has been suggested that flavoenzymes often have flexible domains that may have a role in controlling access to the active site of the enzyme [78].

Table 1
Midpoint potentials (mV) of the redox cofactors of *E. coli* QFR and SQR^a

Enzyme	FAD	[2Fe–2S]	[4Fe–4S]	[3Fe–4S]	Quinone (acceptor/donor)	<i>b</i> heme	Reference
QFR	–48 to –55	–20 to –79	–285 to –320	–50 to –70	MQ ^b –74	absent	[79,82–87]
SQR	n.d. ^c	+10	–175	+65	UQ +113	+36	[87,88]
Beef SQR ^a	–79	0	–260	+60	UQ +113	–185	[87,89–92]

^aData from beef heart SQR are included for comparison to the *E. coli* enzymes.

^bMQ, menaquinone; UQ, ubiquinone.

^cNot determined.

3.3. Iron–sulfur protein (FrdB) of *E. coli* QFR

The FrdB subunit appears to be an example of a modularly constructed iron–sulfur protein. In agreement with sequence [49,63] and electron paramagnetic resonance (EPR) analysis [1,2,79–81] the FrdB subunit is organized into two domains. The N-terminal domain (FrdB residues 1–91) contains the [2Fe–2S] cluster and has a fold similar to plant-type ferredoxins [52]. The C-terminal domain of the subunit (FrdB residues 145–221) exhibits a fold reminiscent to that of bacterial ferredoxins and contains cysteine ligands for the [4Fe–4S] and [3Fe–4S] clusters of the enzyme. The iron–sulfur clusters are arranged in a linear chain and their close physical organization (edge-to-edge distances less than ~ 12 Å) suggests they all participate in electron transfer between the quinol pool and the flavin at the active site [20,52,60]. This had been an area of controversy for a number of years as the low potential of the [4Fe–4S] cluster (Table 1) had led to speculation that this cluster did not participate directly in electron transfer [2,79]. The low potential of the [4Fe–4S] cluster is not, however, a thermodynamic barrier to electron transfer in *E. coli* QFR because of the close spatial proximity as detailed by others [20,93]. The structure and theoretical calculations are also consistent with observation that catalytic electron transfer in FrdAB involves the [4Fe–4S] cluster. Protein film voltammetry has shown that at higher pH and elevated fumarate levels the [4Fe–4S] cluster makes an important contribution to catalytic electron transfer in *E. coli* FrdAB [94].

The arrangement of the iron–sulfur clusters in SQR are undoubtedly the same as that found for QFR as the iron–sulfur protein subunits have a high degree of sequence similarity including the arrangement of the cysteine residues. The *E. coli* SdhB subunit does have one anomaly with respect to the arrangement of cysteine residues for the [2Fe–2S] cluster. The third cysteine in SdhB is replaced by an aspartate residue (CxxxxCxxD...C) [63], however, it was shown that replacing the third cysteine in the equivalent *E. coli* FrdB sequence with an aspartate allowed retention of the [2Fe–2S] cluster although with a slightly higher redox potential [95]. The mutant QFR enzyme retained normal catalytic activity and supports the interpretation that aspartate is a

normal ligand for the [2Fe–2S] cluster in *E. coli* SQR.

3.4. Catalytic activity of fumarate reductase and succinate dehydrogenase

The soluble catalytic domain of fumarate reductase (FrdAB) and succinate dehydrogenase (SdhAB) as described above are often used to characterize enzymatic activity of the enzymes. In fact many of the early studies with the *E. coli* enzymes used partially purified protein which consisted of only the soluble domain of the enzymes [10,12]. Studies of fumarate reduction by membrane-bound SQR and QFR using the benzylviologen radical as electron donor have revealed two types of kinetic behavior [23]. *E. coli* QFR demonstrates simple kinetic behavior in that the rate of the reaction is decreased as the donor is oxidized during the reaction. By contrast *E. coli* SQR, and all enzymes classified as SQRs [23], show a negative order kinetics in that the rate of the reaction increases as the concentration of the electron donor benzylviologen decreases during the reaction. This phenomenon has been elegantly studied using protein film voltammetry with the soluble forms FrdAB and SdhAB absorbed on to a graphite electrode [94,96,97]. In the case of soluble *E. coli* FrdAB the rate of electron transfer between the electrode and succinate/fumarate is a function of electrode potential (driving force) and the resulting current is proportional to the rate of succinate oxidation (higher potential) or fumarate reduction (low potential) [94]. By contrast, soluble succinate dehydrogenase from either *E. coli* (SdhAB) or beef heart demonstrates the effect which has been termed the ‘diode effect’, i.e., a sharp drop in catalytic activity when the critical redox potential of lower than -85 mV is exceeded [96,97]. It was proposed, therefore, that the reduction of FAD at the active site could alter the rate of catalysis and function as a switch that shuts down fumarate reduction at low potentials. These studies further show that *E. coli* SdhAB in addition to functioning as a succinate dehydrogenase can also function as an excellent fumarate reductase, however, its activity in the latter direction is limited to narrow range of redox potential [97]. These results are consistent with in vivo studies showing that *E. coli* SQR can function as a fumarate

reductase if allowed to express [19]. The electrochemical assay used in the study of SdhAB/FrdAB seems to be a powerful tool to investigate redox enzymes. Non-catalytic voltammetry allows one to identify redox centers of the enzymes and determine their thermodynamic properties [94,96–98]; and the electrical current during steady-state catalysis correlate very well with data obtained by traditional kinetic methods [99].

In addition, to the elegant use of protein film voltammetry to measure catalytic activity the most common spectrophotometric method used for determining the succinate dehydrogenase activity of the soluble enzyme forms, as well as for SQR and QFR, is to use phenazine ethosulfate as primary electron acceptor coupled to the reduction of 2,6-dichlorophenolindophenol ([2] and references therein, [100,101]). In the case of *E. coli* QFR the succinate dehydrogenase activity can be measured by the former method or the same turnover number is found with $K_3Fe(CN)_6$. The ferricyanide reductase activity of QFR is catalyzed equally well by the enzyme in the membrane, as an isolated four-subunit complex, and by the soluble FrdAB domain. This is different than that found for soluble bovine heart succinate dehydrogenase where both a high and low K_m ferricyanide reductase activity is observed [2,102] or for *E. coli* SQR whose properties are like the mammalian enzyme [100]. The presence of the ferricyanide reductase activity in *E. coli* QFR indicates that one of the redox centers is more exposed to solvent and directly able to reduce the acceptor as compared to SQR where this redox center is not apparently accessible. The most likely redox center is the $[3Fe-4S]$ cluster based on studies with bovine heart succinate dehydrogenase [2,102]. These data would also predict that the $[3Fe-4S]$ cluster in succinate dehydrogenase is in a more hydrophobic (thus impermeant to $Fe(CN)_6$) environment than the same cluster in *E. coli* fumarate reductase.

3.5. Transmembrane anchor domain of *E. coli* QFR and SQR

The transmembrane domain of *E. coli* SQR and QFR each encompasses two hydrophobic subunits (SdhC/FrdC and SdhD/FrdD) with each subunit having three transmembrane helices connected by ex-

tra-membrane loops [4,22,52]. As shown from the structure of *E. coli* QFR [25], and anticipated from models of the hydrophobic domain of SQR [4,22], the amino-terminus is on the cytoplasmic side of the membrane and the carboxy-terminus of each subunit is located on the periplasmic side of the membrane. A major difference between *E. coli* SQR and QFR is the composition of the redox cofactors present in the membrane-spanning domain. *E. coli* SQR and QFR operate in an environment where both ubiquinone and menaquinone are present. *E. coli* QFR when expressed under aerobic growth conditions effectively supports aerobic respiration [103]. In a similar fashion, if SQR is expressed under anoxic conditions, the SQR complex is able to support a low rate of cell growth by operating as a menaquinol-fumarate reductase [19]. Thus, in vivo both enzymes can complement each other if the organism is genetically manipulated to express only one or the other. As shown in the structure for QFR [52] (Fig. 1) two menaquinone molecules are present and these are located on opposite sides of the membrane. The presence of menaquinone is consistent with the anaerobic expression of QFR which is the predominant quinone synthesized by *E. coli* under anoxic conditions [104].

SQR, in addition to differences in the type of quinone it uses as compared to QFR, is known to contain a single b_{556} heme moiety placing it in the type I class of complex IIs [4]. The heme in *E. coli* SQR has been shown to have bis-histidine axial ligation [105], as is the case for the heme(s) for other SQR and QFR complexes that have been studied [53,106,107]. It is known that the heme in SQR is important for proper assembly of complex II. It was first shown in *B. subtilis* SQR that di-heme-deficient mutants accumulate the flavoprotein and iron-sulfur protein catalytic domain subunits in the cytoplasm [107]. Similar results have been reported for the single b heme containing *E. coli* SQR [108]. As predicted by models for the membrane anchors of SQR [22] the axial ligands for the heme in *E. coli* SQR bridge between the SdhC and SdhD subunits. This was confirmed by site-directed mutagenesis studies where His84 of SdhC and His71 of SdhD were shown to be the heme ligands in the *E. coli* enzyme [65,66]. As evidenced by sequence alignments of the *E. coli* SQR and QFR membrane domain sub-

Table 2

Kinetic parameters of the succinate-quinone and quinol-fumarate reductase reactions of SQR and QFR^a

	Succinate oxidation			Fumarate reduction		
	<i>E. coli</i> QFR ^b	<i>E. coli</i> SQR ^b	Bovine heart SQR ^c	<i>E. coli</i> QFR ^b	<i>E. coli</i> SQR ^b	Bovine heart SQR ^c
Reaction with Q ₂ /Q ₂ H ₂						
<i>k</i> _{cat} (s ⁻¹)	28	85	111		1.7	2.7
<i>K</i> _m (μM)	1.3	2	0.3		5	1.5
Reactions with MQ ₁ /MQ ₁ H ₂						
<i>k</i> _{cat} (s ⁻¹)	14			177	3.4	
<i>K</i> _m (μM)	1.5			5.4	3	
<i>K</i> _i ^{PCP} (μM)	23	13		37	17	1
<i>K</i> _i ^{carboxin} (μM)		30	2		35	3
<i>K</i> _i ^{HQNO} (μM)	0.075			0.2		

^aTurnover number is defined as mol substrate reduced/oxidized per s per mol of enzyme.^bpH 7.8, 30°C (data from [100]).^cpH 7.8, 38°C (data from [110]); turnover number calculated based on 6 nmol FAD mg⁻¹.

units [4]ahistidyl residue equivalent to His71 is lacking in FrdD. This may be one reason this QFR complex lacks heme although the packing of the trans-membrane helices also might preclude the ligation of the heme.

3.6. Activity of QFR and SQR with quinones

The succinate quinone reductase and menaquinol fumarate oxidase reactions of purified *E. coli* SQR and QFR may be assayed with analogues of ubiquinone and menaquinone as has been described in the references noted [100,109]. The large variety of donors/acceptors used by different workers complicates a direct comparison of the activity of the enzymes under similar conditions. Therefore, in Table 2 are shown kinetic parameters for *E. coli* SQR and QFR assayed under the same conditions and for comparative purposes data is shown for bovine SQR [110,111] where the same assays have been used (although at 38°C vs. 30°C for the *E. coli* enzymes). The efficiencies of the enzymes as a functional succinate-quinone reductase or quinol-fumarate oxidase depends on the type of quinone used for assay. QFR is very potent in fumarate reduction while operating with menaquinol, however, ubiquinol-fumarate reductase activity was not detected. QFR does function well as a succinate-quinone reductase with the ubiquinone analogue Q₂. Similar results are seen with SQR where with ubiquinone/ubiquinol the enzyme is proficient in succinate oxidation and interaction with menaquinone/menaquinol transforms the

enzyme into a fumarate reductase. *E. coli* SQR and QFR show similar *K*_m values with more hydrophobic quinone analogues such as Q₂/QH₂ and MQ₁/MQ₁H₂. Using more soluble quinone analogues, however, does reveal significant differences in the affinity of SQR and QFR for quinones. SQR seems indifferent to the number of isoprenoid groups attached to quinone head group, while QFR shows more than a one order of magnitude lower affinity for the soluble quinone analogues (Q₁ and 2,3-dimethyl-1,4-naphthoquinone) [100], lapachol (*K*_m = 85 μM) and plumbagin (*K*_m = 155 μM) for fumarate reduction [109].

Comparison of different quinone site inhibitors reveals common inhibitors and those specific for only QFR or mammalian enzymes. Pentachlorophenol (PCP) [100] and a series of 2-alkyl-4,6-dinitrophenols are potent inhibitors of both *E. coli* enzymes as well as mammalian SQR [112] (Table 2). For example, 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide (carboxin), which for many years has been known as a potent inhibitor of mammalian SQR ([2] and references therein), and *Paracoccus denitrificans* SQR [113] has a one order magnitude higher *K*_i in *E. coli* SQR than it does in mammalian SQR; and carboxin does not inhibit *E. coli* QFR even at mM levels (Table 2). 4,4,4-Trifluoro-1-(2-thienyl)-butane-1,3-dione (TTFA) another strong and often used inhibitor for mammalian SQR, thought to inhibit at a binding site similar to the carboxin site, does not inhibit either *E. coli* SQR or QFR. By contrast, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) is

a very potent inhibitor of *E. coli* QFR, but *E. coli* and mammalian SQR are not sensitive to this inhibitor. The fact that the structure of HQNO resembles a semi(naphtho)quinone and thus menaquinone more than ubiquinone may be significant in this regard. The differences in sensitivity to the different inhibitors is not surprising in agreement with the poor degree of sequence similarity of the membrane anchoring subunits of complex II. It has been shown that single amino acid substitutions can have major effects on the sensitivity of complex II to quinone site inhibitors [113,114]. Subtle changes in the site where quinones interact can thus have major effects on the binding of quinone site inhibitors.

3.7. Activity of enzymes as a function of pH

A similar pH profile in assays of succinate-quinone reductase and quinol-fumarate reductase activities has also been shown for *E. coli* QFR and SQR [100]. The similarity of pH profiles exhibited by the two enzymes suggests that similar amino acid residues may be involved in quinol deprotonation and

oxidation in the *E. coli* enzymes. The pH profile observed for quinone reactions of the two bacterial enzymes is similar to bovine SQR where it was suggested that ionization of a group with a pK_a near 7.0 was important for enzyme activity. By contrast, a mirror-image pH profile was observed for bovine SQR succino-oxidase and fumarate reductase activities and it was suggested that the imidazole moiety of a histidyl residue was responsible [115]. A conserved histidyl residue near the dicarboxylate binding site of *E. coli* QFR was subsequently shown to be important for catalytic activity in support of the previous observations [116]. The availability of structures of both membrane-bound and soluble fumarate reductases has greatly expanded our knowledge of the detailed mechanism of catalysis at the dicarboxylate binding site. Detailed kinetic and crystallographic studies support a common mechanism for fumarate reduction and show the importance of conserved arginine and histidine residues at the active site of this class of enzymes [117,118]. The reader is referred to these excellent articles for a complete description of the proposed mechanism for fumarate reduction.

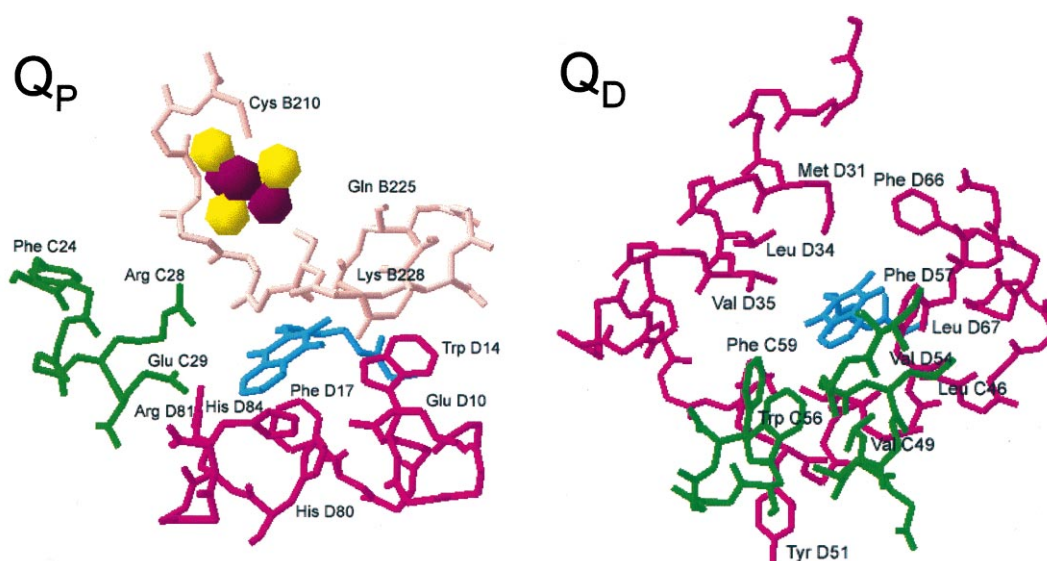


Fig. 2. Quinone binding sites in *E. coli* QFR. The Q_P binding pocket is shown on the left of the figure. The Q_P menaquinone (shown in blue) is in a relatively polar pocket surrounded by amino acid residues from the FrdB (pink), FrdC (green), and FrdD (magenta) subunits. The [3Fe-4S] cluster is shown as a space filling model. The Q_P binding pocket suggests hydrogen bonds from the menaquinone to the sidechains of FrdB Lys228 and FrdC Glu29. The edge-to-edge distance from Q_P to the [3Fe-4S] cluster is 8.2 Å. On the right of the figure is shown the binding pocket for menaquinone Q_D which is on the opposite side of the membrane from Q_P and the two quinones are separated by 25 Å. Q_D is situated in a relatively apolar pocket within the membrane bilayer. The amino acid side chains are colored the same as for quinone Q_P .

3.8. Binding sites for quinone in *E. coli* enzymes

Since a structure is now available for the *E. coli* QFR complex we can for the first time obtain an actual picture of the residues at the quinone binding sites. In the *E. coli* QFR complex one of the menaquinone molecules (Q_P) is positioned proximal (8 Å distance) to the [3Fe–4S] cluster of the FrdB subunit (Fig. 2) in a relatively polar pocket containing protonatable amino acid residues similar to the exchangeable Q_B site of the photosynthetic reaction center [119]. Amino acids from the FrdB, FrdC, and FrdD subunits appear to stabilize the quinone at the Q_P site. At this site the menaquinone is within hydrogen bonding distance of FrdC Glu29 and FrdB Lys228. This lysine residue is conserved in the iron–sulfur protein subunits of SQR and QFR. Approximately 25 Å away on the opposite side of the membrane a second menaquinone (Q_D) distal to the [3Fe–4S] cluster resides (Fig. 2). Q_D on the periplasmic side of the membrane is found in a hydrophobic pocket that resembles the Q_A site of photosynthetic reaction centers.

The *E. coli* QFR system has allowed the direct detection of a very fast relaxing semiquinone free

radical species ($P_{1/2} \gg 500$ mW) which shows spatial proximity to the [3Fe–4S] cluster similar to the semiquinone species found in beef heart SQR [120]. This was accomplished by EPR analysis of an FrdC Glu29 to Leu substitution which gives a much more intense semiquinone EPR spectrum because of the enhanced stability of the semiquinone ($K_{\text{stab}} = 1.2 \times 10^{-2}$ at pH 7.2, some four orders of magnitude more stable than the semiquinone in wild-type QFR [120]). It is apparent in wild-type QFR that Glu29 destabilizes the anionic semiquinone at the Q_P site as compared to the Leu substitution. Previous to these studies with the mutant forms of QFR it had not been possible to directly detect stabilized semiquinones associated with complex II in bacteria. It has also been shown that quinone binding site inhibitors such as carboxin and TTFA perturb a quinone binding site in bovine SQR which is proximal to the [3Fe–4S] cluster [121–123]. HQNO has a K_i in the nM range for quinone reactions with *E. coli* QFR [94,124,125] and *B. subtilis* SQR [126]. The location of the binding site for HQNO in *E. coli* QFR is clearly near the Q_P site based on inhibition properties and perturbation of the EPR signal of the [3Fe–4S] cluster [127]. In the

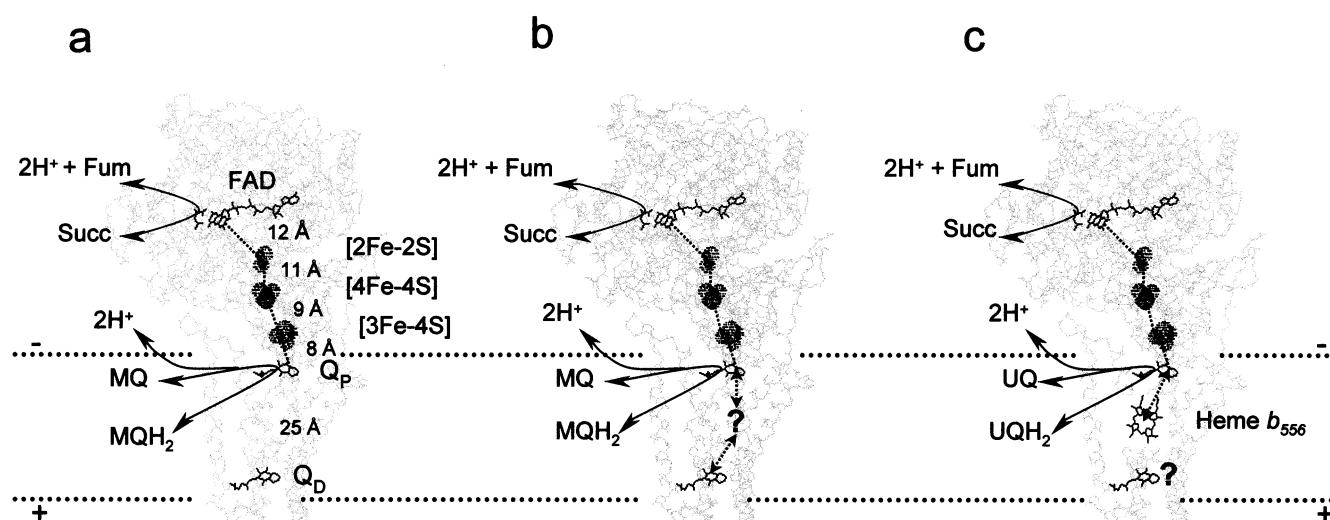


Fig. 3. Electron pathways in *E. coli* QFR (a, b) and SQR (c). Scheme (a) represents the electron pathways from succinate/fumarate to menaquinone/menaquinol through FAD, the three iron–sulfur centers to a single quinone exchangeable Q_P site. In scheme (b) an additional redox mediator (?) is suggested to mediate the electron transfer between the Q_P site and the hydrophobic non-exchangeable Q_D site (dashed arrows). In scheme (c) *E. coli* SQR accepts the single Q_P site electrons directly from the [3Fe–4S] center. The dashed arrow shows the fast electron exchange between the Q_P site and heme b_{556} . The presence of a Q_D site in SQR is as yet unknown and marked with a (?).

case of *E. coli* QFR the 25 Å distance between Q_P and Q_D suggests that these quinones are not the interacting anionic semiquinone pair seen in the mammalian enzyme. Thus, the simplest mechanism for quinone-reduction involves a single quinone Q_P where electron transfer between the [3Fe–4S] cluster and quinone occurs. In such a model the quinone found at Q_D would play a structural role rather than participate in electron transport in *E. coli* QFR (Fig. 3a).

The existence of two quinone binding sites has been suggested by site-directed mutagenesis studies of *E. coli* QFR [128], *S. cerevisiae* SQR [129,130] and by photoaffinity labeling studies of mitochondrial and *E. coli* SQR [131,132]. The large distance between Q_P and Q_D would seem to preclude direct electron transfer between the two quinones as discussed above and by Dutton and co-workers [20,93]. Nevertheless for many years it has been known in mammalian SQR that a stabilized semiquinone pair exists in mammalian complex II [133]. EPR simulations suggested that the interacting semiquinone pair were some 8 Å apart [133] and that the quinone rings were oriented perpendicular to the membrane plane [134]. The stability constant for the ubisemiquinone radical was found to be 10 which is many orders of magnitude greater than for unbound radical (10^{-10}) [121]. Spin-coupled $Q^{\bullet-}Q^{\bullet-}$ split signals as seen in mammalian complex II were, however, not detected in *E. coli* QFR. EPR signals from an interacting semiquinone pair are much more sensitive to perturbation than those from a single semiquinone and thus it can be very difficult to observe spin-coupled semiquinones [121]. As indicated above in *E. coli* QFR only a single stabilized anionic semiquinone species is observed rather than an interacting pair. Thus if both Q_P and Q_D are involved in electron transfer reactions and additional redox active cofactor must intervene between the two quinones because of their large spatial separation. As diagrammed in Fig. 3b such a factor (?) could close the gap between the two quinones. The additional redox active cofactor could be another quinone which is not represented in the structure and not detected by EPR or another redox active factor which is not seen in the X-ray structure. As indicated above a stabilized semiquinone pair is often very

difficult to detect and thus its presence cannot be ruled out.

It is well established that heme b_{556} in *E. coli* SQR is important for enzyme assembly [66,108]. Earlier suggestions that His71 of SdhD is one of the axial ligands of the heme [65] have been recently confirmed [66]. These later studies, however, show that substitution of His84 by Leu in SdhC result in retention of low-spin heme which became reactive with carbon monoxide when the enzyme was reduced [66]. As SdhC His84 is considered the other axial ligand to the heme [65] these results were interpreted as indicating that another histidyl residue in the membrane domain of SQR could substitute for SdhC His84 in the mutant enzyme [66]. Further studies of additional amino acid substitutions at SdhC His84 have shown that a high-spin heme is formed confirming the original interpretation that SdhC His84 is indeed the second axial ligand for heme b_{556} in *E. coli* SQR (E. Makalshina, R.A. Rothery, J.H. Weiner, G. Cecchini, in preparation). It was also shown that the quinone site inhibitor PCP perturbs the heme optical spectrum of wild-type *E. coli* SQR but not that of an SdhC His84Leu mutant enzyme [66]. There was as well a significant increase in the K_m and a decrease in the k_{cat} for ubiquinone-1 in the mutant enzyme. These results suggest that SdhC His84 may be part of a Q binding pocket or that the loss of the heme axial ligand significantly effect the conformation of the anchoring domain. In *E. coli* SQR azidoquinones have been used to label the SdhC subunit and have implicated Ser27 and Arg31 as being part of a quinone binding site in the enzyme [135]. Thus, SdhC His84 would be part of a quinone binding pocket along with Ser27 and Arg31 (Fig. 3c). The results also agree with available structures and models for the transmembrane domain of complex II [4,22,52,53]. As SdhC His84 is normally one of the axial ligands for the *b* heme of *E. coli* SQR [65] the results further suggest that this binding site for quinone is similar to Q_P of *E. coli* QFR, except the *b* heme is located nearby in *E. coli* SQR. In alignments of the C-subunits of complex II [4] FrdC His82 and SdhC His84 are in conserved positions. In QFR FrdC His82 is approximately 7–9 Å from Q_P which may supports the location of SdhC His84 as being near a quinone binding site. Therefore, in *E. coli*

SQR like QFR a Q_P site is located near the [3Fe–4S] cluster from the iron–sulfur protein subunit on the cytoplasmic side of the membrane.

There is no high resolution structural information yet available for *E. coli* SQR. Nevertheless, the structural information available for *E. coli* QFR shows that the Q_P site is nearer the [3Fe–4S] cluster (9 Å) than is the heme of *W. succinogenes* QFR to that [3Fe–4S] cluster (17 Å). Heme *b*₅₅₆ in *E. coli* SQR is completely reducible by succinate under anaerobic conditions with a rate of about two orders of magnitude slower than the turnover number of the enzyme [143], however, the heme is in fast equilibrium with UQ/UQH₂. The simplest mechanism of quinone reduction in *E. coli* SQR would thus be direct reduction of quinone via the [3Fe–4S] cluster in a site like Q_P. Here heme *b*₅₅₆ would not be an obligatory component of the electron transfer pathway. It should be noted that in *B. subtilis* SQR it has been reported that the high potential heme is reducible by succinate with the same rate as the turnover of the enzyme [136] indicating that this heme does participate directly in electron transfer. The simple Michaelis–Menten kinetics for quinone reduction/quinol oxidation seen in *E. coli* SQR support the hypothesis of a single quinone binding site being involved in the catalytic mechanism [100].

In *B. subtilis* SQR, HQNO induces a shift in the spectrum and *E*_m of heme *b*_D indicating that the inhibitor binds on the distal (outside) side of the membrane in that organism. Thus, it has been speculated that HQNO binds to topographically different, but functionally similar sites in *E. coli* QFR and *B. subtilis* SQR [120]. This is also in agreement with observations from *W. succinogenes* where the site for menaquinol oxidation is found near heme *b*_D on the outside of the membrane and where a glutamate residue has also been shown to be important in reaction with quinones [137]. Since the structural determination of *E. coli* QFR shows density for only the two menaquinone molecules [52] it has been hypothesized that a third redox active cofactor may exist to mediate electron transfer between Q_P and Q_D if in fact both quinones participate in catalysis [20,60]. For *E. coli* SQR the intervening cofactor could be the *b* heme moiety (Fig. 3c). There is no evidence to suggest that either *E. coli* SQR or QFR is involved in the generation of transmembrane $\Delta\mu_{H^+}$ [20,53] and

the role of the hydrophobic Q_D binding site seen in *E. coli* QFR [52] thus remains enigmatic. That a menaquinone molecule is part of the electron transport chain on the periplasmic face of *W. succinogenes* QFR has recently been established by site-directed mutagenesis studies [137] of that enzyme and is in agreement with proton-transfer models for that enzyme [20,53] although proton translocation by that enzyme has yet to be unequivocally established. As evidenced by the different proposed classifications schemes for QFR and SQR [4,20] the *E. coli* enzymes may retain structural evolutionary features [24] but no longer have the ability to couple electron transfer to the production of a transmembrane $\Delta\mu_{H^+}$.

3.9. Reactivity of *E. coli* QFR and SQR with oxygen

If in *E. coli*, SQR and QFR can functionally replace each other the question remains as to why the organism has evolved two enzymes. A possible answer may involve one of the significant differences between the two enzymes. *E. coli* QFR can rapidly generate superoxide anion upon reduction of the enzyme by succinate [138]. Superoxide production by *E. coli* QFR is some two orders of magnitude greater than that produced by *E. coli* SQR. The production of superoxide is not sensitive to quinone site inhibitors and apparently the reaction of FAD with oxygen is responsible for the high levels of production of superoxide. The maximum rate for superoxide production is found at low (about K_m^{succ}) concentrations of succinate and higher concentrations of succinate inhibit superoxide production by QFR. The degree of reduction of QFR, or conformational changes upon occupation of the dicarboxylate binding site of QFR, may be responsible for controlling the rate of generation of superoxide anion. Since QFR normally operates in an anaerobic environment production of superoxide would not normally be a problem for the cell [138], however, if it was normally expressed aerobically the large amount of superoxide might prove toxic to *E. coli*. It would be of interest to know if *B. subtilis* SQR also generates high levels of superoxide like *E. coli* QFR. *B. subtilis* SQR operates in an aerobic environment with menaquinone and does not show the diode effect [23] similarly to *E. coli* QFR even though operationally it is a succinate dehydrogenase.

4. Perspective on complex II

Our understanding of the structure and function of complex II has advanced significantly in the past several years particularly because of the availability of three dimensional structures of fumarate reductases. A more general picture will emerge when we also obtain structures for SQR which can be anticipated in the near future. Although QFR and SQR are highly similar some fascinating differences in their catalytic behavior make them intriguing models for study of redox enzymes. An advantage of *E. coli* for study of complex II is that one can readily investigate the biochemistry and physiology of the enzymes in their native environment and the organism allows a wealth of experimental manipulations. Recently mutations in nuclear genes encoding complex II subunits have been associated with a myopathy [139], oxidative stress and more rapid aging [140], and paraganglioma [141,142]. The structures now available suggest that these mutations causing paraganglioma syndrome and more rapid aging are affecting the heme and quinone binding domains of complex II. Understanding the function of these redox active cofactors in complex II and how alterations in the environment where they work should give us a more complete knowledge of the physiological role of complex II in various disease states. The QFR structures and the high degree of similarity of *E. coli* SQR to its mammalian counterparts will allow experimentalists to manipulate the *E. coli* enzymes to provide insights into the function of complex II in mammals. Thus 75 years after the first observation of an equilibrium between succinic and fumaric acids in *E. coli* [5] complex II from this organism continues to be an excellent model for our understanding of this fascinating membrane-bound protein complex.

Acknowledgements

The authors wish to thank the Department of Veterans Affairs, National Institutes of Health (HL16251, GM61606, and GM49694) and National Science Foundation (MCB-9726778) for their support of work from the authors' laboratories. We

would also like to thank Victoria Yankovskaya for help with the figures.

References

- [1] L. Hederstedt, T. Ohnishi, in: L. Ernster (Ed.), *Molecular Mechanism in Bioenergetics*, Elsevier Science Publishers, Amsterdam, 1992, pp. 163–198.
- [2] B.A.C. Ackrell, M.K. Johnson, R.P. Gunsalus, G. Cecchini, in: F. Müller (Ed.), *Chemistry and Biochemistry of Flavoenzymes*, Vol. III, CRC Press, Boca Raton, FL, 1992, pp. 229–297.
- [3] J.J. Van Hellemond, A.G.M. Tielens, *Biochem. J.* 304 (1994) 321–331.
- [4] C. Hägerhäll, *Biochim. Biophys. Acta* 1320 (1997) 107–141.
- [5] J.H. Quastel, M.D. Whetham, *Biochem. J.* 18 (1924) 519–534.
- [6] V. Massey, T.P. Singer, *J. Biol. Chem.* 228 (1957) 263–274.
- [7] M.G.P.J. Warringa, O.H. Smith, A. Giuditta, T.P. Singer, *J. Biol. Chem.* 230 (1958) 97–109.
- [8] C.A. Hirsch, M. Rasminsky, B.D. Davis, E.C.C. Lin, *J. Biol. Chem.* 238 (1963) 3770–3774.
- [9] D.M. Ziegler, K.A. Doeg, *Biochem. Biophys. Res. Commun.* 1 (1959) 344–349.
- [10] I.C. Kim, P.D. Bragg, *Can. J. Biochem.* 49 (1971) 1098–1104.
- [11] T.L.P. Reddy, R.W. Hendler, *J. Biol. Chem.* 253 (1978) 7972–7979.
- [12] P. Dickie, J.H. Weiner, *Can. J. Biochem.* 57 (1979) 813–821.
- [13] B.D. Lemire, J.J. Robinson, J.H. Weiner, *J. Bacteriol.* 152 (1982) 1126–1131.
- [14] B.D. Lemire, J.H. Weiner, *Methods Enzymol.* 126 (1985) 377–386.
- [15] K. Kita, C.R.T. Vibat, S. Meinhardt, J.R. Guest, R.B. Genis, *J. Biol. Chem.* 264 (1989) 2672–2677.
- [16] J.H. Weiner, B.D. Lemire, M.L. Elmes, R.D. Bradley, D.G. Scraba, *J. Bacteriol.* 158 (1984) 590–596.
- [17] M.L. Elmes, D.G. Scraba, J.H. Weiner, *J. Gen. Microbiol.* 132 (1986) 1429–1439.
- [18] S.T. Cole, C. Condon, B.D. Lemire, J.H. Weiner, *Biochim. Biophys. Acta* 811 (1985) 381–403.
- [19] E. Maklashina, D.A. Berthold, G. Cecchini, *J. Bacteriol.* 180 (1998) 5989–5996.
- [20] T. Ohnishi, C.C. Moser, C.C. Page, P.L. Dutton, T. Yano, *Structure* 8 (2000) 23–32.
- [21] J. Schirawski, G. Unden, *Eur. J. Biochem.* 257 (1998) 210–215.
- [22] C. Hägerhäll, L. Hederstedt, *FEBS Lett.* 389 (1996) 25–31.
- [23] B.A.C. Ackrell, F.A. Armstrong, B. Cochran, A. Sucheta, T. Yu, *FEBS Lett.* 326 (1993) 92–94.
- [24] L. Hederstedt, *Science* 284 (1999) 1941–1942.
- [25] B.J. Bachmann, *Microbiol. Rev.* 54 (1990) 130–197.
- [26] F.R. Blattner, G. Plunkett, C.A. Bloch, N.T. Perna, V. Ber-

- land, M. Riley, J. Collado-Vides, J.D. Glasner, C.K. Rode, G.F. Mayhew, J. Gregor, N.W. Davis, H.A. Kirkpatrick, M.A. Goeden, D.J. Rose, B. Mau, Y. Shao, *Science* 277 (1997) 1453–1474.
- [27] J.R. Guest, *J. Gen. Microbiol.* 138 (1992) 2253–2263.
- [28] J.R. Guest, G.C. Russell, *Curr. Top. Cell Regul.* 33 (1992) 231–247.
- [29] H.M. Jones, R.P. Gunsalus, *J. Bacteriol.* 164 (1985) 1100–1109.
- [30] S.J. Park, C.P. Tseng, R.P. Gunsalus, *Mol. Microbiol.* 15 (1995) 473–482.
- [31] J. Shen, R.P. Gunsalus, *Mol. Microbiol.* 26 (1997) 223–236.
- [32] R.B. Gennis, V. Stewart, in: F.C. Neidhardt (Ed.), *Escherichia coli* and *Salmonella typhimurium*, Cellular and Molecular Biology, 2nd edn., American Society for Microbiology, Washington, DC, 1996, pp. 217–261.
- [33] P.J. Kiley, H. Beinert, *FEMS Microbiol. Lett.* 22 (1998) 341–352.
- [34] G. Unden, J. Schirawski, *Mol. Microbiol.* 25 (1997) 205–210.
- [35] H.M. Jones, R.P. Gunsalus, *J. Bacteriol.* 169 (1987) 3340–3349.
- [36] J.R. Guest, J. Green, A. Irvine, S. Spiro, in: E.C.C. Lin, A.S. Lynch (Eds.), *Regulation of Gene Expression in Escherichia coli*, R.G. Landes, Austin, TX, 1996, pp. 317–342.
- [37] R.P. Gunsalus, *J. Bacteriol.* 174 (1992) 7069–7074.
- [38] A. Darwin, V. Stewart, in: E.C.C. Lin, A.S. Lynch (Eds.), *Regulation of Gene Expression in Escherichia coli*, R.G. Landes, Austin, TX, 1996, pp. 333–337.
- [39] V. Stewart, R.S. Rabin, in: J.A. Hoch, T.J. Silhavy (Eds.), *Two-Component Signal Transduction*, American Society for Microbiology, Washington, DC, 1995, pp. 233–252.
- [40] H. Wang, C.P. Tseng, R.P. Gunsalus, *J. Bacteriol.* 181 (1999) 5303–5308.
- [41] A.I. Lee, A. Delgado, R.P. Gunsalus, *J. Bacteriol.* 181 (1999) 5309–5316.
- [42] I. Schröder, C.D. Wolin, R. Cavicchioli, R.P. Gunsalus, *J. Bacteriol.* 176 (1994) 4985–4992.
- [43] R.P. Gunsalus, S.-J. Park, *Res. Microbiol.* 145 (1994) 437–450.
- [44] E. Zeintz, J. Bongaerts, G. Unden, *J. Bacteriol.* 180 (1998) 5421–5425.
- [45] S. Iuchi, E.C.C. Lin, in: J.A. Hoch, T.J. Silhavy (Eds.), *Two-Component Signal Transduction*, American Society for Microbiology, Washington, DC, 1995, pp. 223–231.
- [46] S. Lynch, E.C.C. Lin, in: E.C.C. Lin, A.S. Lynch (Eds.), *Regulation of Gene Expression in Escherichia coli*, R.G. Landes, Austin, TX, 1996, pp. 361–373.
- [47] D.E. Draper, in: F.C. Neidhardt (Ed.), *Escherichia coli* and *Salmonella typhimurium*, Cellular and Molecular Biology, 2nd edn., American Society for Microbiology, Washington, DC, 1996, pp. 902–908.
- [48] D.S. Oppenheim, C. Yanofsky, *Genetics* 95 (1980) 785–795.
- [49] S.T. Cole, T. Grundström, B. Jaurin, J.J. Robinson, J.H. Weiner, *Eur. J. Biochem.* 126 (1982) 211–216.
- [50] D. Wood, M.G. Darlinson, R.J. Wilde, J.R. Guest, *Biochem. J.* 222 (1984) 519–534.
- [51] D.J. Latour, J.H. Weiner, *Biochem. Cell Biol.* 67 (1989) 251–259.
- [52] T.M. Iverson, C. Luna-Chavez, G. Cecchini, D.C. Rees, *Science* 284 (1999) 1961–1966.
- [53] C.R.D. Lancaster, A. Kröger, M. Auer, H. Michel, *Nature* 402 (1999) 377–385.
- [54] A. Mattevi, G. Tedeschi, L. Bacchella, A. Coda, A. Negri, S. Ronchi, *Structure* 7 (1999) 745–756.
- [55] V. Bamford, P.S. Dobbin, D.J. Richardson, A.M. Hemmings, *Nat. Struct. Biol.* 6 (1999) 1104–1107.
- [56] P. Taylor, S.L. Pealing, G.R. Reid, S.K. Chapman, M.D. Walkinshaw, *Nat. Struct. Biol.* 6 (1999) 1108–1112.
- [57] D. Leys, A.S. Tsapin, K.H. Nealson, T.E. Meyer, M.A. Cusanovich, J.J. Van Beeumen, *Nat. Struct. Biol.* 6 (1999) 1113–1117.
- [58] B.A.C. Ackrell, *FEBS Lett.* 466 (2000) 1–5.
- [59] C.R.D. Lancaster, A. Kröger, *Biochim. Biophys. Acta* 1459 (2000) 422–431.
- [60] T.M. Iverson, C. Luna-Chavez, I. Schröder, G. Cecchini, D.C. Rees, *Curr. Opin. Struct. Biol.* 10 (2000) 448–455.
- [61] G.A. Reid, C.S. Miles, R.K. Moysey, K.L. Pankhurst, S.C. Chapman, *Biochim. Biophys. Acta* 1459 (2000) 310–315.
- [62] S.T. Cole, *Eur. J. Biochem.* 122 (1982) 479–484.
- [63] M.G. Darlinson, J.R. Guest, *Biochem. J.* 223 (1984) 507–517.
- [64] T.P. Singer, M.K. Johnson, *FEBS Lett.* 190 (1985) 189–198.
- [65] C.R.T. Vibat, G. Cecchini, K. Nakamura, K. Kita, R.B. Gennis, *Biochemistry* 37 (1998) 4148–4159.
- [66] E. Maklashina, R.A. Rothery, J.H. Weiner, G. Cecchini, *J. Biol. Chem.* 276 (2001) 18968–18976.
- [67] W.H. Walker, T.P. Singer, *J. Biol. Chem.* 245 (1970) 4224–4225.
- [68] W.C. Kenney, A. Kröger, *FEBS Lett.* 73 (1977) 239–243.
- [69] J.H. Weiner, P. Dickie, *J. Biol. Chem.* 254 (1979) 8590–8593.
- [70] P.S. Dobbin, J.N. Butt, A.K. Powell, G.A. Reid, O.J. Richardson, *Biochem. J.* 342 (1999) 439–448.
- [71] M. Blaut, K. Whittaker, A. Valdovinos, B.A.C. Ackrell, R.P. Gunsalus, G. Cecchini, *J. Biol. Chem.* 264 (1989) 13599–13604.
- [72] K.M. Robinson, R.A. Rothery, J.H. Weiner, B.D. Lemire, *Eur. J. Biochem.* 222 (1994) 983–990.
- [73] C. Walsh, *Acc. Chem. Res.* 13 (1980) 148–155.
- [74] M. Mewies, W.S. McIntire, N.G. Scrutton, *Protein Sci.* 7 (1998) 7–20.
- [75] R. Brandsch, V. Bichler, *Eur. J. Biochem.* 182 (1989) 125–128.
- [76] K.M. Robinson, B.D. Lemire, *J. Biol. Chem.* 271 (1996) 4055–4060.
- [77] C. Condon, P. Owen, *FEMS Microbiol. Lett.* 14 (1982) 217–221.
- [78] A. Mattevi, M.A. Vanoni, B. Curti, *Curr. Opin. Struct. Biol.* 7 (1997) 804–810.
- [79] A.T. Kowal, M.T. Werth, A. Manodori, G. Cecchini, I. Schröder, R.P. Gunsalus, M.K. Johnson, *Biochemistry* 34 (1995) 12284–12293.

- [80] L. Aevarsson, L. Hederstedt, *FEBS Lett.* 232 (1988) 298–302.
- [81] M.K. Johnson, A.T. Kowal, J.E. Morningstar, J.E. Oliver, K. Whittaker, R.P. Gunsalus, B.A.C. Ackrell, G. Cecchini, *J. Biol. Chem.* 263 (1988) 14732–14738.
- [82] B.A.C. Ackrell, B. Cochran, G. Cecchini, *Arch. Biochem. Biophys.* 268 (1989) 26–34.
- [83] D. Simpkin, W.J. Ingledew, *Biochem. Soc. Trans.* 13 (1985) 603–607.
- [84] R. Cammack, D.S. Patil, J.H. Weiner, *Biochim. Biophys. Acta* 870 (1986) 545–551.
- [85] M.T. Werth, G. Cecchini, A. Manodori, B.A.C. Ackrell, I. Schröder, R.P. Gunsalus, M.K. Johnson, *Proc. Natl. Acad. Sci. USA* 87 (1990) 8965–8969.
- [86] A. Manodori, G. Cecchini, I. Schröder, R.P. Gunsalus, M.T. Werth, M.K. Johnson, *Biochemistry* 31 (1992) 2703–2712.
- [87] R.K. Thauer, K. Jungermann, K. Decker, *Microbiol. Rev.* 41 (1977) 100–180.
- [88] C. Condon, R. Cammack, D.S. Patil, P. Owen, *J. Biol. Chem.* 260 (1985) 9427–9434.
- [89] T. Ohnishi, T.E. King, J.C. Salerno, H. Blum, J.R. Bowyer, T. Maida, *J. Biol. Chem.* 256 (1981) 5577–5582.
- [90] T. Ohnishi, J.C. Salerno, D.B. Winter, J. Lim, C.-A. Yu, L. Yu, T.E. King, *J. Biol. Chem.* 251 (1976) 2094–2104.
- [91] T. Ohnishi, J. Lim, D.B. Winter, T.E. King, *J. Biol. Chem.* 251 (1976) 2105–2109.
- [92] L. Yu, J.-X. Xu, P.E. Haley, C.-A. Yu, *J. Biol. Chem.* 262 (1987) 1137–1143.
- [93] P.L. Dutton, X. Chen, C.C. Page, S. Huang, T. Ohnishi, C.C. Moser, in: G.W. Canters, E. Vigenboom (Eds.), *Biological Electron Transfer Chains: Genetics, Composition and Mode of Operation*, Kluwer Academic Publishers, Dordrecht, 1998, pp. 3–8.
- [94] H.A. Heering, J.H. Weiner, F.A. Armstrong, *J. Am. Chem. Soc.* 119 (1997) 11628–11638.
- [95] M.T. Werth, H. Sices, G. Cecchini, I. Schröder, S. Lasage, R.P. Gunsalus, M.K. Johnson, *FEBS Lett.* 299 (1992) 1–4.
- [96] A. Sucheta, B.A.C. Ackrell, B. Cochran, F.A. Armstrong, *Nature* 356 (1992) 361–362.
- [97] H.R. Pershad, J. Hirst, B. Cochran, B.A.C. Ackrell, F.A. Armstrong, *Biochim. Biophys. Acta* 1412 (1999) 262–272.
- [98] F.A. Armstrong, H.A. Heering, J. Hirst, *Chem. Soc. Rev.* 26 (1997) 169–179.
- [99] A.K. Jones, R. Camba, G.A. Reid, S.K. Chapman, F.A. Armstrong, *J. Am. Chem. Soc.* 122 (2000) 6494–6495.
- [100] E. Maklashina, G. Cecchini, *Arch. Biochem. Biophys.* 369 (1999) 223–232.
- [101] B.A.C. Ackrell, E.B. Kearney, T.P. Singer, *Methods Enzymol.* 53 (1978) 466–483.
- [102] A.D. Vinogradov, E.V. Gavrikova, V.G. Goloveshkina, *Biochem. Biophys. Res. Commun.* 65 (1975) 1264–1269.
- [103] J.R. Guest, *J. Gen. Microbiol.* 122 (1981) 171–179.
- [104] A. Shestopalov, A.V. Bogachev, R.A. Murtazina, M.B. Viryasov, V.P. Skulachev, *FEBS Lett.* 404 (1997) 272–274.
- [105] J. Peterson, C.R.T. Vibat, R.B. Gennis, *FEBS Lett.* 355 (1994) 155–156.
- [106] B.R. Crouse, C.-A. Yu, L. Yu, M.K. Johnson, *FEBS Lett.* 367 (1995) 1–4.
- [107] H. Fridén, M.R. Cheesman, L. Hederstedt, K.K. Andersson, A.J. Thomson, *Biochim. Biophys. Acta* 1041 (1990) 207–215.
- [108] K. Nakamura, M. Yamaki, M. Sarada, S. Natayama, C.R.T. Vibat, R.B. Gennis, T. Nakayashiki, H. Inokuchi, S. Kojima, K. Kita, *J. Biol. Chem.* 271 (1996) 521–527.
- [109] R.A. Rothery, I. Chatterjee, G. Kiema, M.T. McDermott, J.H. Weiner, *Biochem. J.* 332 (1998) 35–41.
- [110] V.G. Grivennikova, E.V. Gavrikova, A.A. Timoshin, A.D. Vinogradov, *Biochim. Biophys. Acta* 1140 (1993) 282–292.
- [111] P.R. Tushurashvili, E.V. Gavrikova, A.N. Ledenev, A.D. Vinogradov, *Biochim. Biophys. Acta* 809 (1985) 145–159.
- [112] V. Yankovskaya, S.O. Sablin, R.R. Ramsay, T.P. Singer, B.A.C. Ackrell, G. Cecchini, H. Miyoshi, *J. Biol. Chem.* 271 (1996) 21020–21024.
- [113] M. Matsson, L. Hederstedt, *J. Bioenerg. Biomembr.* 33 (2001) 99–105.
- [114] P.L.E. Broomfield, J.A. Hargreaves, *Curr. Genet.* 22 (1992) 117–121.
- [115] S.B. Vik, Y. Hatefi, *Proc. Natl. Acad. Sci. USA* 78 (1981) 6749–6753.
- [116] I. Schröder, R.P. Gunsalus, B.A.C. Ackrell, B. Cochran, G. Cecchini, *J. Biol. Chem.* 266 (1991) 13572–13579.
- [117] M.K. Doherty, S.L. Pealing, C.S. Miles, R. Moysey, P. Taylor, M.D. Walkinshaw, G.A. Reid, S.K. Chapman, *Biochemistry* 39 (2000) 10695–10701.
- [118] C.R.D. Lancaster, R. Groß, J. Simon, *Eur. J. Biochem.* 268 (2001) 1820–1827.
- [119] M.L. Paddock, P.H. McPherson, G. Fehr, Y. Okamura, *Proc. Natl. Acad. Sci. USA* 86 (1989) 6602–6606.
- [120] C. Hägerhäll, S. Magnitsky, V.D. Sled, I. Schröder, R.P. Gunsalus, G. Cecchini, T. Ohnishi, *J. Biol. Chem.* 274 (1999) 26157–26164.
- [121] J.C. Salerno, T. Ohnishi, *Biochem. J.* 192 (1980) 769–781.
- [122] P.C. Mowery, D.J. Steenkamp, B.A.C. Ackrell, T.P. Singer, G.A. White, *Arch. Biochem. Biophys.* 178 (1977) 495–506.
- [123] W.J. Ingledew, T. Ohnishi, *Biochem. J.* 164 (1977) 617–620.
- [124] J.H. Weiner, R. Cammack, S.T. Cole, C. Condon, N. Honore, B.D. Lemire, G. Shaw, *Proc. Natl. Acad. Sci. USA* 83 (1986) 2056–2060.
- [125] G. Cecchini, H. Sices, I. Schröder, R.P. Gunsalus, *J. Bacteriol.* 177 (1995) 4587–4592.
- [126] I. Smirnova, C. Hägerhäll, A. Konstantinov, L. Hederstedt, *FEBS Lett.* 359 (1995) 23–26.
- [127] R.A. Rothery, J.H. Weiner, *Eur. J. Biochem.* 254 (1998) 588–595.
- [128] D.J. Westenberg, R.P. Gunsalus, B.A.C. Ackrell, H. Sices, G. Cecchini, *J. Biol. Chem.* 268 (1993) 815–822.
- [129] K.S. Oyedotun, B.D. Lemire, *J. Biol. Chem.* 272 (1997) 31382–31388.
- [130] K.S. Oyedotun, B.D. Lemire, *J. Biol. Chem.* 274 (1999) 23956–23962.
- [131] G.Y. Lee, D.Y. He, L. Yu, C.-A. Yu, *J. Biol. Chem.* 270 (1995) 6193–6198.

- [132] S.K. Shenoy, L. Yu, C.-A. Yu, *J. Biol. Chem.* 272 (1997) 17867–17872.
- [133] F.J. Ruzicka, H. Beinert, K.L. Schepler, W.R. Dunham, R.H. Sands, *Proc. Natl. Acad. Sci. USA* 72 (1975) 2886–2890.
- [134] J.C. Salerno, H.J. Harmon, H. Blum, J.S. Leigh, T. Ohnishi, *FEBS Lett.* 82 (1977) 179–182.
- [135] X. Yang, L. Yu, D. He, C.-A. Yu, *J. Biol. Chem.* 273 (1998) 31916–31923.
- [136] M. Matsson, D. Tolstoy, R. Aasa, L. Hederstedt, *Biochemistry* 39 (2000) 8617–8621.
- [137] C.R.D. Lancaster, R. Groß, A. Haas, M. Ritter, W. Mantele, J. Simon, A. Kröger, *Proc. Natl. Acad. Sci. USA* 97 (2000) 13051–13056.
- [138] J.A. Imlay, *J. Biol. Chem.* 270 (1995) 19767–19777.
- [139] M.A. Birch-Machin, R.W. Taylor, B. Cochran, B.A.C. Ackrell, D.M. Turnbull, *Ann. Neurol.* 48 (2000) 330–335.
- [140] N. Ishii, M. Fujii, P.S. Hartman, M. Tsuda, K. Yasuda, N. Senoo-Matsuda, S. Yanase, D. Ayusawa, K. Suzuki, *Nature* 394 (1998) 694–697.
- [141] B.E. Baysal, R.E. Ferrell, J.E. Willett-Brozick, E.C. Lawrence, D. Myssiorek, A. Bosch, A. van der Mey, P.E.M. Taschner, W.S. Rubinstein, E.N. Myers, C.W. Richard, C.J. Cornelisse, P. Devilee, B. Devlin, *Science* 287 (2000) 848–851.
- [142] S. Niemann, U. Müller, *Nat. Genet.* 26 (2000) 268–270.
- [143] E. Maklashina, G. Cecchini, *Biochim. Biophys. Acta EBEC* 10 (1998) 216–216.