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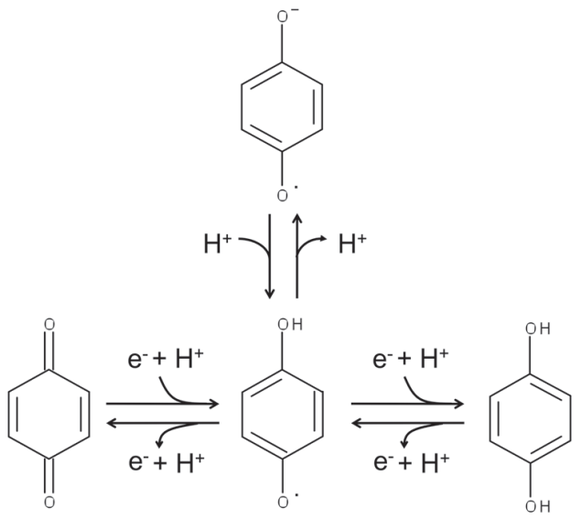
Menaquinone as Well as Ubiquinone as a Crucial Component in the *Escherichia coli* Respiratory Chain

Naoko Fujimoto[1], Tomoyuki Kosaka[2] and Mamoru Yamada[1,2]
[1]*Graduate School of Medicine, Yamaguchi University, Ube*
[2]*Faculty of Agriculture, Yamaguchi University, Yamaguchi*
Japan

1. Introduction

Isoprenoid quinones, which are found as membrane-bound compounds in almost all living organisms, generally have functions as electron carriers or antioxidants. Of the various isoprenoid quinones, ubiquinones (UQs) and menaquinones (MKs) have been extensively studied to reveal not only their physiological functions but also their biosynthesis at gene level.

In molecular structure, both UQs and MKs consist of a polar head group and a hydrophobic side chain. The latter part provides the molecules with a lipid-soluble character to allow them to perform vital functions in membrane lipid bilayers, whereas the former group enables interaction with membrane proteins. The quinone ring of the head group has a crucial activity by a two-step reversible reduction reaction to form a quinol structure (Fig. 1). There are three



Left, oxidized form; middle, semiquinone intermediate; right, reduced form; upper, semiquinone radical ion.

Fig. 1. Oxidation states of quinone ring.

oxidized states of the quinone ring. The addition of one electron and one proton to the fully oxidized form results in the semiquinone form. The addition of a second electron and proton to generates the fully reduced form. When the semiquinone loses a proton, it becomes a semiquinone radical anion. The reduced form of isoprenoid quinones becomes more polar, and thus the quinol portion may be preferentially located in the boundary region between hydrophobic and hydrophilic micro-environments (Fiorini et al., 2008; Lenaz et al., 2007).

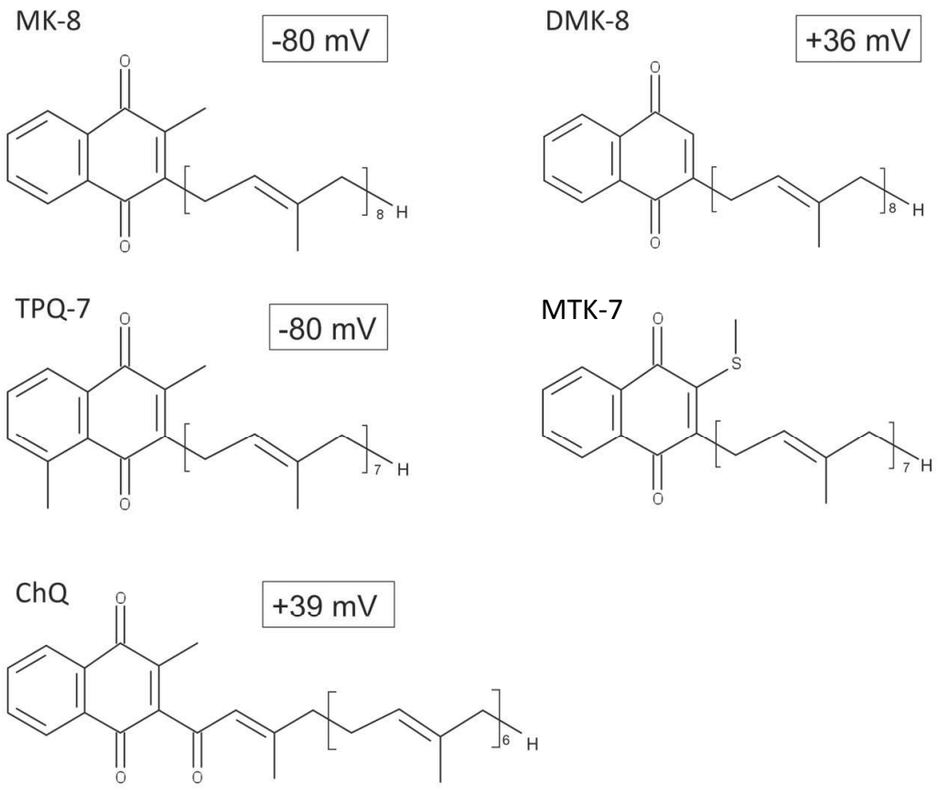
Such chemical and functional features of quinones make them suitable mediators of electron transfer between different protein complexes in biological membranes. Most of UQ or MK molecules function as mobile carriers of electrons in membranes, but some of them are tightly associated with protein molecules to function in intramolecular electron transfer (Goodwin, 1977; Lichtenthaler, 1977). In addition, UQs physiologically possess an antioxidant capacity against lipid peroxidation (Landi et al., 1984). However, little attention has been given to MKs as natural antioxidants. Phylloquinone (PhQ) and MK have been shown to prevent lipid peroxidation as dose UQ, but menadione has no such inhibitory effect (Vervoort et al., 1997).

1.1 Occurrence and structure of menaquinones

MKs, which have a low redox potential compared to that of UQs, function as the most widespread respiratory quinones. They are assumed to be evolutionarily the most ancient type of isoprenoid quinones and are found in many microorganisms such as archaea and bacteria (Goodwin, 1977; Lubben, 1995; Nitschke et al., 1995; Schoepp-Cothenet et al., 2009; Soballe & Poole, 1999).

The degree of saturated or hydrogenated polyprenyl side-chains of MKs is sometimes different depending on these microorganisms. *Archaea* contains mainly MKs, which are often dehydrogenated. The major MK in *Mycobacterium phlei* is MK-9(H_2) (Gale et al., 1963), whereas the major MK in *Corynebacterium diphtheriae* is MK-8(H_2) (Scholes & King, 1965). Such dihydromenaquinones are widespread in corynebacteria and mycobacteria, whereas even more highly saturated MKs occur in certain actinomycetes (Collins et al., 1977; Lancaster, 2003).

MKs mostly possess methylnaphthoquinone as a head group, but different modifications of the head group are present in some prokaryotes, which provide them with different redox potentials (Fig. 2). Demethylmenaquinones (DMKs) in some bacteria (Baum & Dolin, 1965; Collins & Jones, 1979; Lester et al., 1964) lack the ring methyl substitutant (C-2). DMKs with different sizes of polyprenyl side chains from one to nine isoprene units have been reported (Hammond & White, 1969). Methylmenaquinone (MMK) with an additional methylation has a redox potential similar to that of MK. Thermoplasmaquinone (TPQ) is one kind of MMK. Methionaquinone (MTK) contains a methylthio group instead of a methyl group in the naphtoquinone (Hiraishi et al., 1999; Ishii et al., 1987). Both MTK and TPQ occur in Thermoplasma (Lubben, 1995; Shimada et al., 2001). DMKs and MMK are often found in proteobacteria and Gram-positive bacteria (Biel et al., 2002; Collins & Jones, 1981). Chlorobiumquinone (ChQ), which is found in photosynthetic green sulfur bacteria, is an isoprenoid naphthoquinone containing a carbonyl group in its side chain (Collins & Jones, 1981).



Menaquinone-8 (MK-8) and demethylmenaquinone-8 (DMK-8) occur in *E. coli*. Thermoplasmaquinone-7 (TPQ-7) and methionaquinone-7 (MTK-7) occur in *Thermoplasma acidphilum*. ChQ, chlorobiumquinone (1'-oxo-menaquinone-7) is present in *Chlorobium tepidum*. Adapted from work of Nowicka and Kruk (Nowicka & Kruk, 2010). The redox potential of MTK-7 is not available.

Fig. 2. Structures and redox potentials of isoprenoid naphthoquinones.

Most Gram-positive bacteria and anaerobic Gram-negative bacteria contain MK as a sole isoprenoid quinone (Collins et al., 1981). Most *Bacteroides* and *Bacillus spp.* produce MK as a major isoprenoid quinone. *Thermus thermophilus, Actinobacillus actinoides*, *Thermoplasma acidphilum*, *Lactobacillus mali*, *Lactobacillus yamanashiensis, Streptococcus cremoris, Planococcus*, *Staphylococcus, Corynebacterium*, *Brevibacterium, Arthrobacter, Chlorobium thiosulfatophium* and *Chloropseudomonas ethylicum* produce MKs as their sole isoprenoid quinones. *Halobacterium* and *Halococcus* have an unsaturated MK (MK-8) and a dihydrogenerated MK (MK-8(H_2)) (Kushwaha et al., 1974). Typical strains of species within the genera *Actinobacillus* and *Pasteurella* produce DMKs as major isoprenoids. *Actinobacillus actinomycetemcomitans, Actinobacillus suis, Pasteurella bettii, Pasteurella pneumotropica*, *Streptococcus faecalis, Haemophilus influenza* and *Haemophilus aergyptilus* produce only DMKs. Gram-negative facultatively anaerobic rods and several genera within the family *Enterobacteriaceae* such as *Escherichia, Klebsiella* and *Promonas* contain mixtures of MK and DMK. Certain species of the genera *Aeromonas* and *Erwinia* also contain a mixture of MKs.

The appearance of MKs on Earth is assumed to be associated with the reducing character of the atmosphere before the occurrence of oxygenic photosynthesis and dramatic increase in environmental oxygen concentration (Schoepp-Cothenet et al., 2009). It is thought that

various quinones with higher redox potentials had diverged independently from naphthoquinones in a few groups of prokaryota along with acquisition of aerobic metabolism. The reduced form of MKs is highly reactive with molecular oxygen and is subjected to non-catalytic oxidation, and it is therefore thought to function inefficiently in an oxygen-containing atmosphere (Schoepp-Cothenet et al., 2009). The isoprenoid side chain of most MKs consists of 6-10 prenyl units, but MKs bearing 1-5 or 11-14 prenyl units are found in some species (Collins & Jones, 1981). The side chain is generally unsaturated, but it is also partially or fully saturated in some organisms (Collins & Jones, 1981). The degree of saturation of the side chain appears to be dependent on growth temperature.

1.2 Biosynthesis of menaquinones

1.2.1 Biosynthesis of the isoprenoid side chain of menaquinones

The head group and the isoprenoid side chain of MK are separately synthesized. Both parts are then combined together by an enzyme in the prenyltransferase family and then subjected to further modifications. The isoprenoid side chain is synthesized from dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP). There are two distinct pathways to build IPP precursors: the mevalonate (MVA) pathway and the deoxyxylulose 5-phosphate (DXP) pathway (Lange et al., 2000). *Archaea* are thought to have acquired the MVA pathway, based on evidence that species in this group possess genes homologous to those encoding enzymes in this pathway but not in the DXP pathway. On the other hand, all bacteria except for *Myxococcus fulvus*, *Chloroflexus aurantiacus*, *Streptomycetes*, *Rickettsia prowazekii* and *Mycoplasma genitalium* have acquired the DXP pathway (Lange et al., 2000). All animals and fungi utilize the MVA pathway. Higher plants utilize both pathways in different organelles, the DXP pathway for plastids and the MVA pathway for the cytosolic compartment. In higher plants, the biosynthesis of prenyl chains of phylloquinone, plastoquinone, tocopherolquinone, chlorophyll and tocopherol thus proceed by the DXP pathway, whereas UQ synthesis is carried out by the MVA pathway (Lichtenthaler, 1999). Algae and *Protista* also show distinctive utilization of both pathways. *Euglena gracilis* utilizes only the MVA pathway, *Chlamydomonas reinhardtii* utilizes only the DXP pathway, and the rhodophyte *Cyanidium caldarum* uses both pathways (Lange et al., 2000). This complication in utilization of the pathways might be due to horizontal gene transfer and/or endosymbiotic origin of organelles. The isoprenoid side chain is synthesized by a specific prenyl diphosphate synthase through a series of condensation reactions of precursors.

1.2.2 Biosynthesis of the head group of menaquinones

Two pathways are known to be involved in biosynthesis of the head group of MK (Fig. 3a and b). In both pathways, the precursor of the head group is 1,4-dihydroxy-2-naphthoate (DHNA), which is derived from chorismate in the shikimate pathway. In one of the two pathways, there are 7 enzymes required for formation of a quinone portion, DHNA, in which 6 *men* genes are involved (Widhalm et al., 2009) (Table 1). The quinone portion synthesized by the successive reactions is combined with the isoprenoid side chain by the condensation reaction of DHNA prenyltransferase. The number of prenyl units composing the isoprenoid side chain, which differs from species to species, may be determined by the length in the tertiary structure of isoprenoid diphosphatease. For example, *E. coli* and

Geobacter metallireducens produce MK-8, whereas, *Bacillus firmus* produces MK-7 (Hedrick et al., 2009; Soballe & Poole, 1999). Following the condensation reaction, the naphthoate group is methylated by C-metyltransferase (Lee et al., 1997).

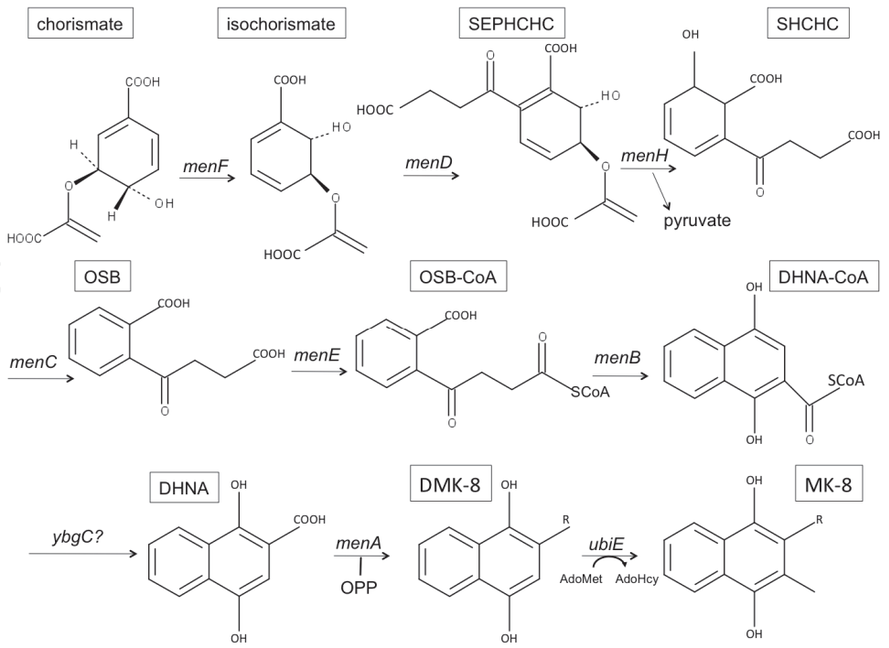
In the other pathway called the futalosine pathway, which has recently been discovered in some microorganisms, orthologues of certain *men* genes are absent (Hiratsuka et al., 2008) (Fig. 3B). Four enzymes specific for this pathway are involved at the beginning of DHNA biosynthesis followed by the same reactions as those in the former pathway (Hiratsuka et al., 2009) (Table 2).

Gene	Product
menF	Isochorismate synthase
menD	2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase
menH	(1R, 6R)-2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylic acid synthase
menC	*o*-succinyl benzoate synthase
menE	*o*-succinyl benzoate-CoA synthase
menB	1,4-dihydroxy-2-naphthoyl-CoA synthase
ybgC	1,4-dihydroxy-2-naphthoate thioesterase
menA	1,4-dihydroxy-2-naphthoate octaprenyltransferase
ubiE	2-DMK methyltransferase

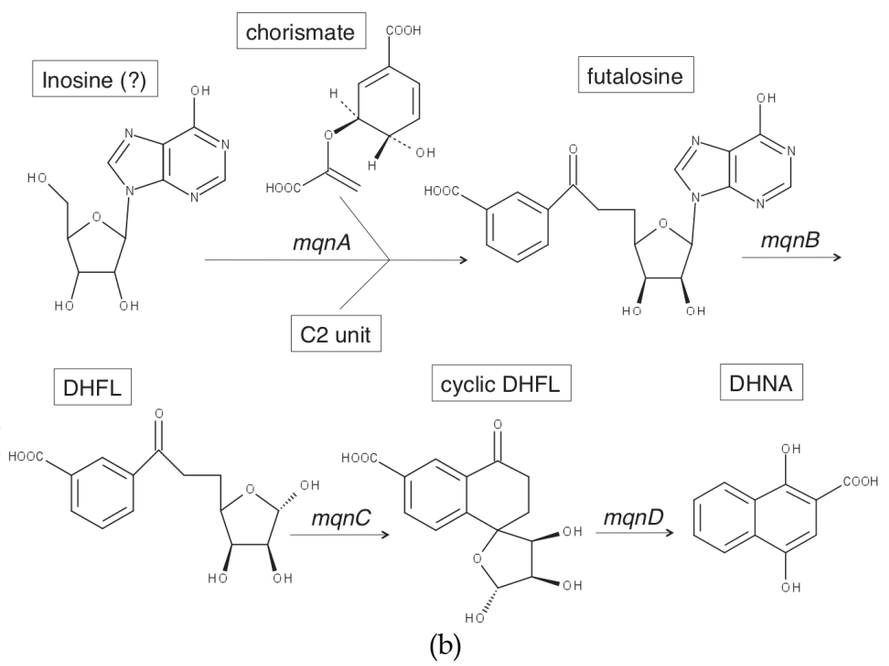
Table 1. Genes and their products related to the biosynthetic pathway of menaquinone-8 in *E. coli*.

Gene	Function
mqnA	Condensation of chrismate, inosine and C2 unit to form futalosine
mqnB	Futalosin hydrolase
mqnC	Cyclization of dehypoxanthinylfutalosine to form cyclic dehypoxanthinylfutalosine
mqnD	Cleavage of cyclic dehypoxanthinylfutalosine to form 1,4-dihydroxy-2-naphthoate

Table 2. Genes and their functions related to the futalosine pathway.



(a)



(b)

(a) The pathway to form menaquinone-8 in *E. coli*. DHNA, 1,4-dihydroxy-2-naphthoate; R, octaprenyl side chain; OSB, *o*-succinylbenzoate; SEPHCHC, 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylic acid; SHCHC, (1R, 6R)-2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylic acid; OPP, octaprenyl diphosphate; AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine.
(b) The alternative pathway to form DHNA. DHFL, dehypoxanthinylfutalosine. Adapted from works of Hiratsuka et al. (Hiratsuka et al., 2008) and Nowicka and Kruk (Nowicka & Kruk, 2010).

Fig. 3. Biosynthetic pathways of menaquinone.

1.3 Functions of menaquinones

1.3.1 Menaquinones in the bacterial photosynthetic electron transport chain

MKs or UQs are known to engage in photosynthetic electron transport of several photosynthetic microorganisms (Nowicka & Kruk, 2010). MKs are found in photosystem II (PSII)-type reaction centers (RCs) of purple bacteria and green filamentous bacteria and in photosystem I (PSI)-type RCs of green sulfur bacteria and heliobacteria (Ke, 2001). On the other hand, UQs are involved in photosynthesis of many purple bacterial species. *Halorhodospira halophila* seems to distinctively utilize the two quinones, MK-8 for the photosynthetic electron transfer reaction and UQ-8 for the respiratory reaction (Schoepp-Cothenet et al., 2009). Green filamentous bacteria dominantly utilize MK-10 both for photosynthetic and respiratory electron transport reactions (Hale et al., 1983). MK-4 is present in the PSI of the cyanobacterium *Gloeobacter violaceus* or *Synechococcus* PCC 7002 diatoms and primitive red alga *C. caldarum* (Ikeda et al., 2008; Mimuro et al., 2005; Sakuragi et al., 2005). Green sulfur bacteria contain MK-7 in their PSI-type RCs (Hauska et al., 2001), similar to that of PSI of green filamentous bacteria. The involvement of MK in the reaction of their RCs is not obvious because the electron transfer activity in their RCs was not hampered without MK. MK-9 is found at the A1 site of the RC of *Helicobacterium chlorum*, but its physiological function remains to be clarified (Neerken & Amesz, 2001; Oh-oka, 2007).

MK occurs in chlorosomes of green photosynthetic bacteria (Frigaard et al., 1997; Frigaard et al., 1998; Kim et al., 2007). A small amount of MK-7 with a large amount of chlorobiumquinone is found in chlorosomes of the green sulfur bacterium *Chlorobium tepidum* (Frigaard et al., 1997). On the other hand, MK is present as a major quinone in chlorosomes of the thermophilic green bacterium *Chloroflexus auranticus*, which lacks chlorobiumquinones. It has been reported that chlorosomes of *C. tepidum* exhibited high fluorescence, which rapidly decreased under aerobic conditions (Frigaard et al., 1997). The authors speculated that chlorobiumquinone senses a redox state and inhibits electron transfer to the RC under aerobic conditions to avoid possible oxidative stress (Frigaard et al., 1997).

1.3.2 Menaquinone in the prokaryotic respiratory chain

MK mediates electron transport reactions as does UQ in respiratory chains of prokaryotes. Both quinones contribute to the formation of transmembrane potential via electron-accepting or -donating reactions with other respiratory components. The transmembrane potential that consists of a proton gradient and an electron gradient across membranes is used for ATP synthesis and for transport of many materials between the inside and outside of cells. There are two mechanisms for the formation of the proton gradient: proton pump mechanism and redox loop mechanism. In the latter mechanism, during electron transfer reactions, oxidation of one substrate and proton(s) release simultaneously occur on the inside of membranes and then reduction of the other substrate and proton(s) binding simultaneously occur on the outside of the membrane (Richardson & Sawers, 2002). MK as well as UQ is thus crucial components in the respiratory chain of prokaryotic cells for energy production and cell maintenance.

Reduction of MK is coupled with oxidation of reductants, such as NADH, succinate, sulfide, thiocyanate, ammonium, formate and hydrogen, and oxidation of reduced MK (MKH_2) is coupled with reduction of oxidants such as oxygen, nitrogen dioxide, nitrite, sulfate, sulfide,

thiosulfate, polysulfide, elemental sulfur and fumarate. These reduction/oxidation reactions are carried out by specific enzymes, reductases and oxidases that participate in the reaction of MK and oxidation of MKH_2, respectively. A number of MK reductases called as oxidoreductases or dehydrogenases for NADH, succinate, formate, hydrogen, malate, pyruvate and glycerol-3-phosphate have been studied (Lancaster & Simon, 2002; Richardson, 2000).

NDH-1-type dehydrogenases (NDH-1) functioning as an NADH:MK oxidoreductase in many microorganisms are able to pump protons across the membrane (Yagi et al., 1998). They are complexes consisting of 13–14 subunits and analogous to mitochondrial complex I. Their detailed structure and the relationship between their structure and function, however, are still poorly understood. NDH-2-type dehydrogenases also acting as an NADH:MK oxidoreductase are also ubiquitous in bacteria but are simpler and smaller in structure than NDH-1. The NDH-2-type dehydrogenases lack proton pumping activity (Nantapong et al., 2005). This type of dehydrogenase might be crucial for substrate oxidation under relatively transmembrane potential-rich conditions.

Succinate dehydrogenase as a succinate:MK oxidoreductase, which is also widely spread in microorganisms, is analogous to mitochondrial complex II but has no proton-pumping activity (Azarkina & Konstantinov, 2010; Fernandes et al., 2005; Kurokawa & Sakamoto, 2005; Madej et al., 2006; Xin et al., 2009). The tertiary structure of succinate dehydrogenase in *E. coli*, which consists of 4 subunits, has been resolved (Iverson et al., 1999). However, there are controversial opinions regarding the site for quinone reduction in the dehydrogenase molecule (Fernandes et al., 2005). The dehydrogenase utilizes both quinones to perform coupled reaction of oxidation of succinate to fumarate and reduction of quinone and also catalyzes the coupled reaction of reduction of fumarate to succinate and oxidation of the reduced form of quinone. Since MK has a redox potential lower that that of UQ, the reduction of MK is endergonic and that of UQ is exergonic under standard conditions (Madej et al., 2006). Therefore, no succinate oxidation coupled with MK reduction occurs without energy supply from outside. In Gram-positive aerobic bacteria having only MK, such as *Bacillus subtilis* and *Bacillus licheniformis*, energization of the cellular membrane is necessary for succinate:MK oxidoreductase activity (Azarkina & Konstantinov, 2010; Madej et al., 2006). Therefore, MK reduction reaction of the enzyme is proposed to be proton-driven.

Formate dehydrogenase, which oxidizes formate to carbon dioxide, can generate a proton gradient by a redox loop mechanism. As described above for the redox loop mechanism, the formate oxidation site of the enzyme is orientated towards the periplasm, whereas the MK reduction site is located close to the cytoplasmic side of the membrane (Jormakka et al., 2002). Similarly, hydrogenase that catalyzes hydrogen oxidation coupled with MK reduction is assumed to form a proton gradient by the redox loop mechanism (Kröer et al., 2002).

$F_{420}H_2$:quinone oxidoreductase as an MK reductase in the strictly anaerobic, sulfate-reducing archeon *Archaeoglobus fulgidus* is thought to be a functional equivalent of NADH:quinone oxidoreductase (Bruggemann et al., 2000). This enzyme is a multi-subunit complex similar in structure to bacterial NDH-1 and mitochondrial complex I and may be composed of three subcomplexes. The significant homology of archaebacterial enzyme subunits to those of bacterial NDH-1 and complex I (Bruggemann et al., 2000) allows us to consider the evolutionary relationship as a biochemical unity.

On the other hand, MKH_2 oxidation reactions are known to be performed by several MKH_2:oxidant oxidoreductases. Coupling oxidants for the reactions are nitrate (nitrite), sulfate, fumarate, and oxygen as an terminal electron acceptor. Membrane-bound nitrate reductase (Nir) in *E. coli*, which is expressed under anaerobic or microaerobic conditions, catalyzes the reduction reaction of nitrate to nitrite coupled with oxidation of MKH_2 and concomitantly generates a proton gradient (Pinho et al., 2005). *E. coli* Nir, which is expressed at a high concentration of nitrate, can utilize both MKH_2 and UQH_2 as electron donors (Brondijk et al., 2002; Giordani & Buc, 2004). Enzymes similar in structure to nitrate reductase have been postulated to participate in sulfate and selenate reduction in some bacteria (Ma et al., 2009; Pires et al., 2003). Fumarate respiration in anaerobic bacteria is achieved by using fumarate as a terminal electron acceptor and formate and hydrogen as electron donors into the chain (Lancaster, 2003). Since the reaction of MKH_2:fumarate oxidoredactase is exergonic, the enzyme is assumed to be able to generate a proton gradient by a redox loop mechanism (Madej et al., 2006).

Other MK/MKH_2-involved reactions are found in cytochrome bc_1 complexes (Schutz et al., 2000; Trumpower, 1990) and quinol oxidases (Ingledew & Poole, 1984) in respiratory chains. Quinone like UQ may form a Q cycle in the cytochrome bc_1 complex, which has been proposed to form a proton gradient by a mechanism similar to a redox loop mechanism. A model of the Q cycle allows us to speculate that there are at least two quinone-binding sites in the complex. The tertiary structure of the ubiquinol oxidase from *E. coli* has been resolved and the binding sites of quinone have been predicted (Abramson et al., 2000).

One of quinol-oxidizing enzymes is ubiquinol oxidase as a terminal oxidase in bacteria. Terminal oxidases utilizing oxygen as an electron acceptor in bacterial respiratory chains can be classified into two families: heme:copper oxidases and cytochrome *bd* oxidases. The former family catalyzes quinol oxidation reaction coupled with the reduction of oxygen as a terminal electron acceptor and generates a proton gradient partially by a proton pump mechanism (Kusumoto et al., 2000; Unden & Bongaerts, 1997), whereas the latter family transfers electrons from quinol to oxygen and generates a proton gradient by a redox loop mechanism (Junemann, 1997).

Another family of MKH_2-oxidizing enzymes is the NapC/NirT family. Each enzyme in this family occurs as a membrane-bound complex of tetraheme or pentaheme *c*-type cytochromes and catalyzes quinol oxidation reaction coupled with reduction of periplasmic proteins in cytoplasmic membranes of Gram-negative bacteria. The membrane-bound NrfH in *Wolinella succinogenes* reduces the periplasmic complex of nitrite reductase NrfA (Simon et al., 2001). NapC in *E. coli* may participate in electron transfer to the periplasmic complex of nitrate reductase NapA-NapB (Brondijk et al., 2002). CymA in *Shewanella oneidensis* is assumed to transfer electrons to a wide range of reductases (Schwalb et al., 2003).

Facultative anaerobic bacteria are known to be able to utilize both MK and UQ in respiratory chains. *E. coli* possesses two distinct but structurally similar enzymes, succinate dehydrogenase and fumarate reductase. The former is involved in oxidation reaction of succinate coupled with the reduction of UQ under aerobic conditions, and the latter functions in reducing fumarate and oxidizing reduced MK under anaerobic conditions. The cells might perform an efficient reaction by a combination of these enzymes and types of isoprenoid quinones, which are differently synthesized or produced under the two different conditions (Maklashina et al., 2006).

Strictly aerobic bacteria and strictly anaerobic bacteria possess only UQ and MK, respectively (Soballe & Poole, 1999). Facultative anaerobic proteobacteria including *E. coli* have both UQ and MK (Soballe & Poole, 1999; Wallace & Young, 1977). Such bacteria that are able to synthesize both quinones seem to control the relative production ratio of the two quinones depending on growth conditions. The oxygen level appears to affect relative amounts of these two kinds of quinones. When *E. coli* grows under aerobic conditions, UQ level is 4–5-times higher than the sum of MK and DMK levels, whereas under anaerobic conditions, the amount of UQ is three-times smaller than the sum of the amounts of both MKs (Wallace & Young, 1977; Wissenbach et al., 1990). Since the redox potential of MKs is lower than that of UQs, the former isoprenoid quinones are more suitable for respiratory chains with lower-potential electron acceptors (Nitschke et al., 1995; Soballe & Poole, 1999). When *E. coli* lacks one of the two quinones, the remaining one can functionally replace the missing one, but this is not the case for all respiratory pathways (Wallace & Young, 1977). Dependence of the quinone chosen at different oxygen concentrations has been also reported in Archaea. The facultative anaerobic, thermophilic archaeon *Thermoplasma acidophilum* has three isoprenoid naphthoquinones of MK-7, TPQ-7 and MTK-7 with different redox potentials. Under anaerobic conditions, TPQ-7 constitutes 97% of the total quinone pool, whereas under aerobic conditions, all the three quinones exist in nearly equal amounts Shimada et al., 2001).

In addition to the function in electron transfer chains, MK may have a crucial function in gene or cellular regulation of some microbes. MK has been shown to participate in the regulation of nitrogen fixation in *Klebsiella pneumoniae*. NifL, which acts as a co-repressor in the regulation of expression of nitrogen fixation genes, is reduced by the reduced form of MK under anaerobic conditions and becomes incompetent by its sequestration on the membrane (Thummer et al., 2007). A derivative of MK-9(H_2) with modification of a sulfate group at its isoprenoid chain has recently been identified in *Mycobacterium tuberculosis* and has been shown to act as a negative regulator of virulence in mice, suggesting its involvement in the regulation of host-pathogen interactions (Holsclaw et al., 2008).

2. Role of menaquinone as an electron acceptor and as a prosthetic group for dehydrogenases in *E. coli*

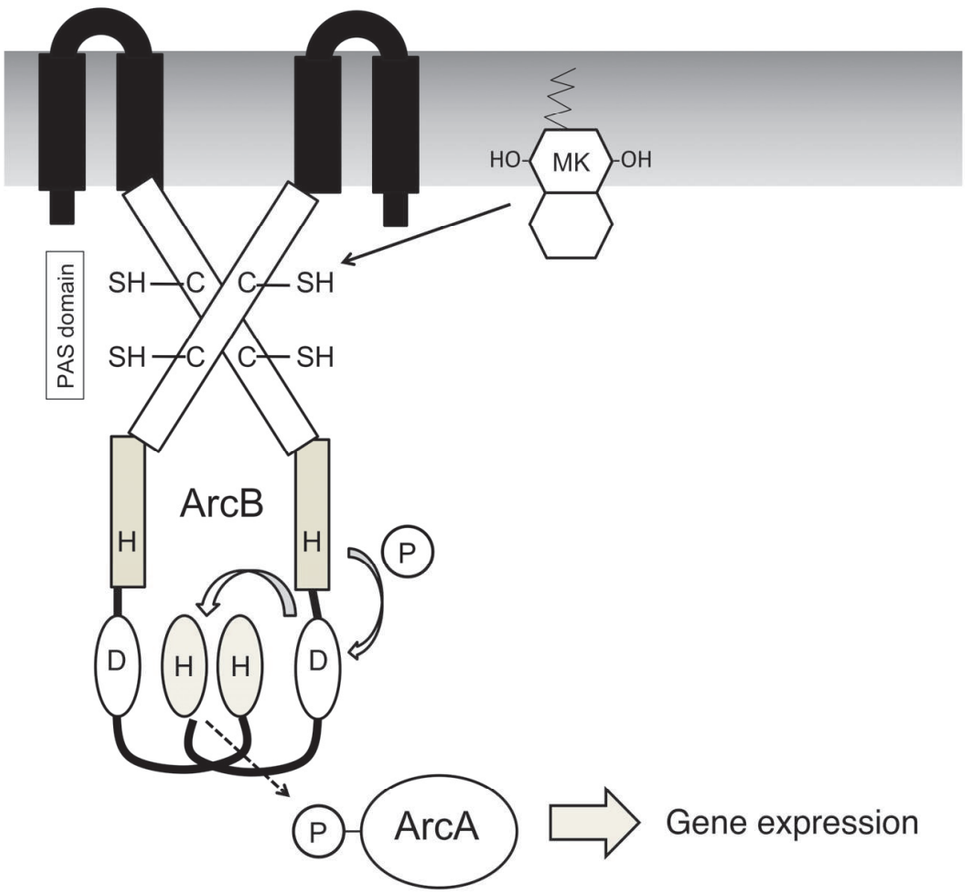
2.1 Menaquinone has a crucial role as an electron acceptor like ubiquinone in *E. coli*

2.1.1 Menaquinone is involved in activation of ArcB in a two-component system

Two-component systems that are widely spread in prokaryotes and plants are crucial for adaptation to changes in environmental and intracellular conditions. The two-component system of ArcB and ArcA as a transmembrane sensor kinase and a cognate response regulator, respectively, allows *E. coli* to sense aerobiosis to control over 30 operons (Fig. 4) (Iuchi & Lin, 1988; Iuchi et al., 1989; Iuchi et al., 1990). ArcBA is involved in sensing oxygen availability (Rolfe et al., 2011) or the redox state of the quinone pool (Bekker et al., 2010) and the concomitant transcriptional regulation of oxidative and fermentative catabolism. ArcB possesses an elaborate cytosolic structure that comprises three catalytic domains, each of which has a specific function of a primary transmitter with a conserved His292, a receiver with a conserved Asp576, or a secondary transmitter with a conserved His717. ArcA consists of an N-terminal receiver domain with a conserved Asp54 and a C-terminal helix-turn-helix DNA-binding domain (Kwon et al., 2000).

Two ArcB molecules form two reversible disulfide bridges at Cys180 and Cys241, which are located in the so-called PAS domain of the protein at the cytoplasmic side of *E. coli*. The PAS domain is the site to interact with UQ-UQH_2 couple or MK-MKH_2 couple (Malpica et al., 2004). The kinase activity of ArcB is highly dependent on the covalent linkage. Under signal perception, ArcB undergoes autophosphorylation at His292, which is enhanced by several fermentation metabolites such as D-lactate, pyruvate and acetate. The phosphoryl group is then sequentially transferred to ArcA via a His292 to Asp576 to His717 to Asp54 phosphorely (Kwon et al., 2000).

Bekker et al. (2010) have demonstrated that the ArcBA two-component system is regulated by the redox state of both the UQ and the MK pool in membranes (Fig. 4). The ArcBA



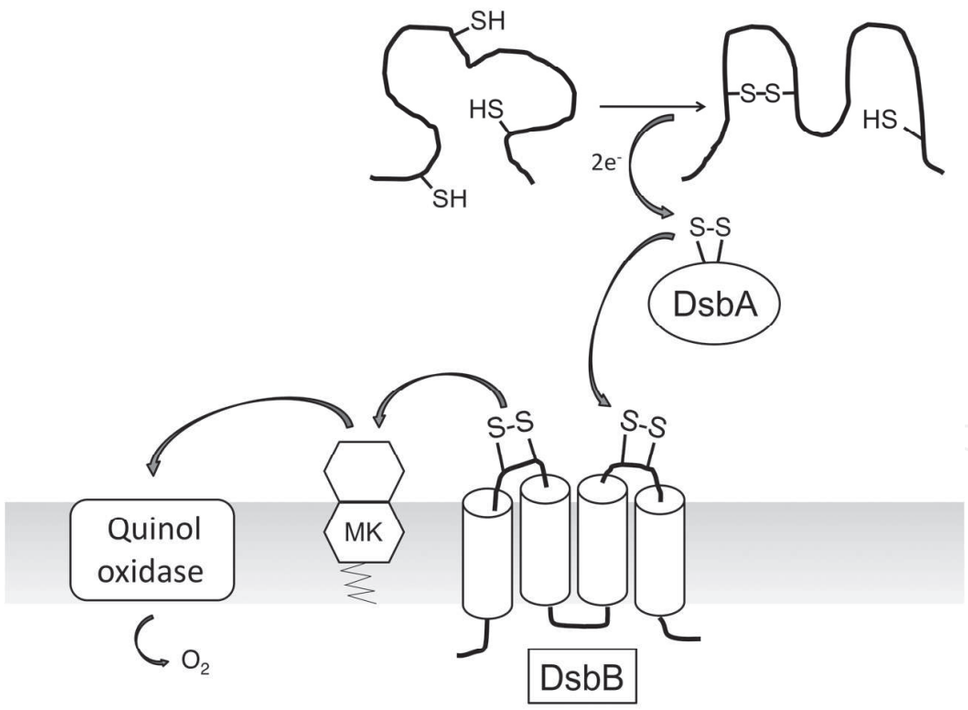
Two ArcB molecules form reversible disulfide bridges via two cysteine residues at the PAS domain of the protein. ArcB is activated by phosphorylation, depending on the reduction level of the disulfide bridges, which causes an intramolecular relay of the phosphoryl group and finally the phosphoryl group is transferred to ArcA, which in turn expresses its regulon. Recently, Bekker et al. (Bekker et al., 2010) indicate that the MK pool plays an important role in ArcB activation. Adapted from works of Bekker et al. (Bekker et al., 2010) and Malpica et al. (Malpica et al., 2004). H, histidine kinase domain; D, receiver domain.

Fig. 4. Involvement of MK in the signal transduction pathway via ArcBA two-component system.

system exhibits high and low activities under anaerobic and aerobic conditions, respectively. *In vitro* experiments have revealed that the residues in disulfide bonds of the ArcB complex can be oxidized by UQ (Malpica et al., 2004). Bekker et al. (2010) has shown that the deletion of *ubiC,* encoding an enzyme for the UQ biosynthesis, causes no effect on regulation of ArcB in the anaerobic-aerobic transition, but the deletion of *menB*, encoding an enzyme for the MK biosynthesis, leads to inactivation of ArcB. Therefore, MKs seems to play a major role in ArcB activation.

2.1.2 Menaquinone works as an electron acceptor in the DsbA-DsbB system

Formation of a disulfide bridges is crucial for the maturation process for envelope and secretary proteins, which intrinsically depends on distinct functions of several Dsb proteins (Collet & Bardwell, 2002; Kadokura et al., 2003). DsbA is involved in the oxidative folding of proteins newly synthesized in the periplasm of *E. coli* using its own disulfide bridge (Cys30-Cys33), and then re-oxidized by DsbB, an inner membrane protein to recycle the catalytic activity of DsbA (Bardwell et al., 1993; Missiakas et al., 1993; Guilhot et al., 1995; Kishigami et al., 1995). Oxygen is a ultimate electron acceptor for the DsbA/DsbB system under aerobic conditions (Bader et al., 1999; Kobayashi & Ito, 1999), where UQ receives electrons from DsbB (Bader et al., 1999; Bader et al., 2000) and the further electron transfer process to oxygen is mediated by the respiratory chain (Fig. 5) (Kobayashi et al., 1997).



A disulfide bridge is introduced by DsbA, and the extracted electrons are transferred from DsbA to DsbB. The electrons are further transferred to oxygen via the respiratory chain, in which MK or UQ are involved. The arrows indicate the flow of electrons in the DsbB-DsbA oxidative pathway. Adapted from work of Inaba and Ito (Inaba & Ito, 2008).

Fig. 5. Involvement of MK in the DsbB-DsbA oxidative pathway.

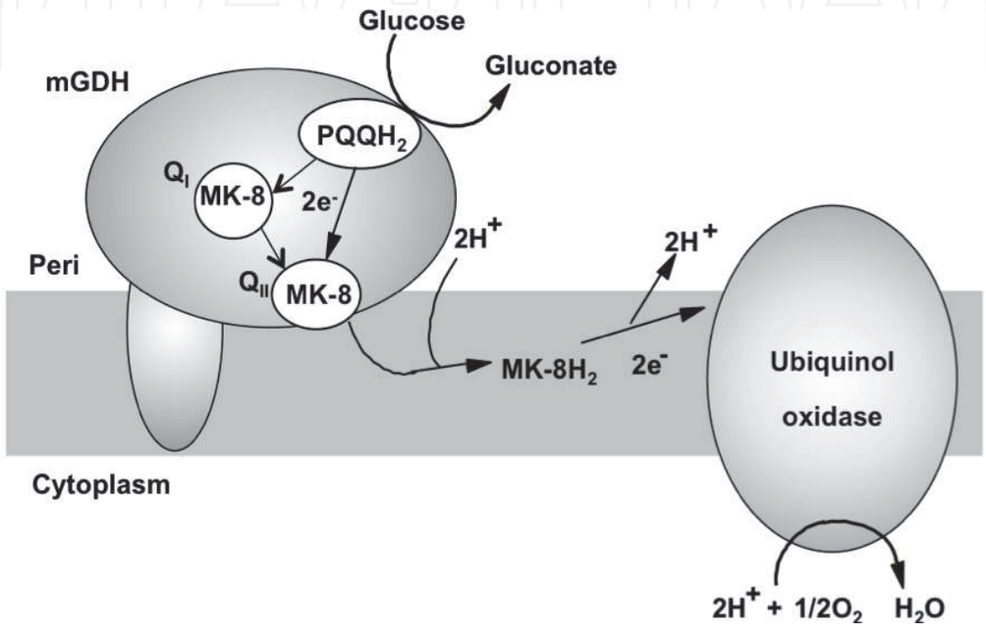
In the inner membrane, UQ receives electrons from DsbB to restore the DsbA-oxidizing activity of DsbB (Bader et al., 2000). DsbB possesses two pairs of essential cysteines in its periplasmic domains as shown in Fig. 5, which are Cys41 and Cys44 in the N-terminal loop and Cys104 and Cys130 in the C-terminal loop (Jander et al., 1994). The former pair is oxidized by respiratory components (Kobayashi & Ito, 1999). The interaction of DsbB at the region around its Cys44 with UQ has been suggested, though the UQ-binding site on DsbB has not been directly determined (Xie et al., 2002). Mutations of residues 42 and 43 affect the redox potential and reactivity of DsbB, and those of the short segment between Cys44 and the second transmembrane region impair UQ-8-dependent DsbB oxidation *in vivo* (Inaba & Ito, 2002; Kadokura et al., 2000; Kobayashi et al., 2001). The DsbA/DsbB system is also functioning in disulfide bridge formation under anaerobic conditions in *E. coli* (Bader et al., 1999; Bader et al., 2000; Kadokura et al., 2000), where MK occurs the major quinone species (Wallace & Young, 1977). According to the assumption that MK plays an electron acceptor like UQ in the DsbA/DsbB system (Kadokura et al., 2000; Kishigami et al., 1995), mutational analysis was performed, which revealed that impairment of both the UQ and MK biosynthetic pathways results in dysfunction of the DsbA/DsbB system (Kobayashi et al., 1997). Arg48 substitutions in DsbB result in a low-activity enzyme that can no longer utilize the MK analog menadione as an *in vitro* electron-accepting substrate (Kadokura et al., 2000). MK-8 has been shown to associate with DsbB, similar to that of UQ, by spectroscopic analysis (Takahashi et al., 2004). The *in vitro* reaction of DsbA oxidation with MK-8 has been shown to be slower than the UQ-dependent reaction.

2.2 Menaquinone as well as UQ as a bound quinone is crucial for catalytic activity and intramolecular electron transfer in *E. coli* membrane-bound glucose dehydrogenase

Membrane-bound glucose dehydrogenase (mGDH) is a good model for primary dehydrogenases in terms of its occurrence as a single protein and as an apo-protein (Yamada et al., 1993a; Matsushita et al., 1997; Elias & Yamada, 2003), which allows study with both forms of apo- and holo-enzymes (Ameyama et al., 1985). It has been demonstrated that mGDH has two quinone (Q)-binding sites, one (Q_I) for bound Q and the other (Q_{II}) for bulk Q (Elias et al., 2004), which is near the membrane surface rather than in the hydrophobic interior (Miyoshi et al., 1999), and that intramolecular electron transfer following the catalytic reaction occurs from $PQQH_2$ directly to Q in the Q_{II} site or via bound Q (Fig. 6). Pulse radiolysis analysis revealed that the two redox centers are closely located at a distance of 11-13 Å (Kobayashi et al., 2005) and that Asp466 and Lys493 are involved in proton donation to the semiquinone anion radical of bound Q and in electron transfer from bound UQ to PQQ, respectively (Elias et al., 2000; Mustafa et al., 2008a). Recent mGDH analysis provided the first evidence that the primary dehydrogenase in respiratory chains utilizes both MK and UQ as a bound Q and suggest that bound MK occurs in a fashion similar to that of bound UQ in the mGDH molecule and functions as an electron acceptor from PQQ (Mustafa et al., 2008b). We also presented the data for the first time that suggest the requirement of bound Q for catalytic reaction in quinoprotein dehydrogenases (Mustafa et al., 2008b).

mGDH is expressed not only under aerobic conditions but also under anaerobic conditions, although the expression level is relatively low (Yamada et al., 1993b). mGDH expressed in the *ubiA* mutant defective in UQ biosynthesis was found to contain MK-8, and its MK content in purified mGDH protein was estimated to be 0.9 ± 0.03 mol/mol of mGDH (Mustafa et al., 2008b). Functional activities of purified bound MK-containing mGDH (MK-

mGDH) and non-bound quinone-containing mGDH (Q-free mGDH) purified from *ubiA menA* cells were compared with those of bound UQ-containing mGDH (UQ-mGDH) from *menA* mutant cells. Both PMS reductase activity as glucose dehydrogenase activity and UQ-2 reductase activity of MK-mGDH were found to be equivalent to those of UQ-mGDH. The latter activity reflects the total ability of catalytic reaction and the successive intramolecular electron transfer from PQQ to UQ-2 at the Q_{II} site. The Q-free enzyme, however, exhibited only 18% of the dehydrogenase activity and 6% of the UQ-2 reductase activity of UQ-bearing mGDHs (Mustafa et al., 2008b).



mGDH contains bound Q at the Q_I site and interacts with bulk Q at the Q_{II} site. Electrons from reduced PQQ following D-glucose oxidation are transferred to bound Q and then bulk Q or directly to bulk Q. Both MK and UQ as a Q can be incorporated into the Q_I site of mGDH molecule and interact at the Q_{II} site.

Fig. 6. Involvement of MK-8 in the intramolecular electron transfer in mGDH of *E. coli*.

It has been reported that UQ-1 incorporated into Q-free DsbB functions in a manner similar to that of bound Q (Inaba et al., 2005). External addition of UQ-1 showed UQ-1 dose-dependent increase in PMS reductase activity in Q-free mGDH. A similar level of increase in UQ-2 reductase activity was observed in the presence of UQ-1 in Q-free mGDH (Mustafa et al., 2008b). A radiolytically generated hydrated electron reacted with the bound MK to form a semiquinone anion radical with an absorption maximum at 400 nm. Subsequently, decay of the absorbance at 400 nm was accompanied by an increase in the absorbance at 380 nm with a first order rate constant of 5.7 X 10^3 s^{-1}. This indicates an intramolecular electron transfer from the bound MK to the PQQ. EPR analysis revealed that characteristics of the semiquinone radical of bound MK are similar to those of the semiquinone radical of bound UQ and indicated an electron flow from PQQ to MK as in the case of UQ. Taken together, the results suggest that MK is incorporated into the same pocket as that for UQ to perform a function almost equivalent to that of UQ and that bound quinone is involved at least partially in the catalytic reaction and primarily in the intramolecular electron transfer of mGDH (Mustafa et al., 2008b).

2.3 Possible interaction of menaquinone with ubiquinone-binding protein in *E. coli*

It is generally accepted that almost 90% of UQ is distributed freely in membranes as a UQ pool (Lenaz, 2001). Recently, Barros et al. (2005) speculated that Coq10 as a mitochondrial UQ-binding protein, which is not associated with succinate- and NADH-UQ reductase or the bc_1 complex in *Saccharomyces cerevisiae*, is involved in transport of UQ from its synthetic site to its functional site. Cui and Kawamukai (2009) reported that Coq10 binds to CoQ10 and is required for 11 proper respiration activity in Schizosaccharomyces pombe. These findings not only expand our knowledge of the regulation of UQ but also allow us to challenge the longstanding notion that UQ molecules are freely moving in the hydrophobic environment of membranes. Coq10 possesses 13 highly conserved hydrophobic amino acid residues presumably involved in the binding to CoQ_{10}. The presumption was partially proven by alanine substitution of these amino acid residues, in which L63A and W104A caused defective respiration and growth retardation on minimal medium. A human Coq10 ortholog has been shown to functionally compensate for a *coq10* null mutant. Therefore, Coq10 seems to be crucial for respiratory activity in various organisms.

Coq10 orthologs are also present in various microorganisms including *E. coli*, though their physiological functions remain to be established. Our preliminary experiments showed that an *E. coli* YfjG plays a function similar to that of Coq10 and allowed us to speculate that it interacts with UQ or MK to enhance the activity of electron transfer between dehydrogenase and ubiquinol oxidase. The alignment of YfjG orthologs in Gram-negative bacteria exhibited 11 highly conserved amino acid residues, which are partially overlapping with the conserved residues in Coq10 orthologs, some of which are involved in UQ binding (Cui & Kawamukai, 2009). A *yfjG*-disrupted mutant exhibited different properties in respiration activity of *E. coli*. Dehydrogenase activities of mGDH and NADH dehydrogenase and oxidase activity of ubiqionol oxidase in the *yfjG*-disrupted mutant were nearly the same as those in the parental strain. However, glucose and NADH oxidase activities were significantly decreased. Notably, the catalytic sites of mGDH and NADH dehydrogenase face the periplasm and cytoplasm, respectively. The mutation thus seems to affect the electron transfer ability between dehydrogenases and ubiquinol oxidase. Therefore, it is speculated that YfjG binds to UQ or MK to stimulate electron transfer in the respiratory chain.

3. Conclusion

In this chapter, first, we have shown the fundamental information on MKs including their structures, biosynthesis and physiological functions. We have also introduced several proteins intrinsically interacting not only with UQ-8 but also with MK-8 in *E. coli*. These proteins can associate with both quinones that have different ring structures and different redox potentials. In some cases, both quinones function as a mediator of electrons via the respiratory chain or as a sensor of the redox state of cells. In other cases, both quinones are incorporated into protein molecules and indispensable for enzyme activity. Such microbes that are capable of synthesizing both types of quinones might have evolved to adapt different growth conditions only by changing quinone, but not by changing the cognate protein(s). We thus notice one of ingenious strategies of microbes in utilization of the quinones.

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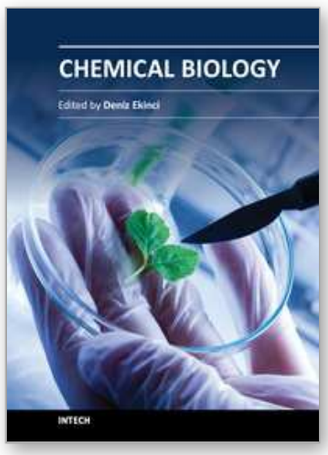

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Chemical biology utilizes chemical principles to modulate systems to either investigate the underlying biology or create new function. Over recent years, chemical biology has received particular attention of many scientists in the life sciences from botany to medicine. This book contains an overview focusing on the research area of protein purification, enzymology, vitamins, antioxidants, biotransformation, gene delivery, signaling, regulation and organization. Particular emphasis is devoted to both theoretical and experimental aspects. The textbook is written by international scientists with expertise in synthetic chemistry, protein biochemistry, enzymology, molecular biology, drug discovery and genetics many of which are active chemical, biochemical and biomedical research. The textbook is expected to enhance the knowledge of scientists in the complexities of chemical and biological approaches and stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemical biology.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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