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Biochimica et Biophysica Acta, 1365 (1998) 71–78

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

The electron transport chain in anaerobically functioning eukaryotes

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Received 27 January 1998; received in revised form 26 February 1998; accepted 2 March 1998

Abstract

Many lower eukaryotes can survive anaerobic conditions via a fermentation pathway that involves the use of the reduction of endogenously produced fumarate as electron sink. This fumarate reduction is linked to electron transport in an especially adapted, anaerobically functioning electron-transport chain.

An aerobic energy metabolism with Krebs cycle activity is accompanied by electron transfer from succinate to ubiquinone via complex II of the respiratory chain. On the other hand, in an anaerobic metabolism, where fumarate functions as terminal electron acceptor, electrons are transferred from rhodoquinone to fumarate, which is the reversed direction. Ubiquinone cannot replace rhodoquinone in the process of fumarate reduction in vivo, as ubiquinone can only accept electrons from complex II and cannot donate them to fumarate. Rhodoquinone, with its lower redox potential than ubiquinone, is capable of donating electrons to fumarate. Eukaryotic fumarate reductases were shown to interact with rhodoquinone (a benzoquinone), whereas most prokaryotic fumarate reductases interact with the naphtoquinones menaquinone and demethylmenaquinone.

Fumarate reductase, the enzyme essential for the anaerobic functioning of many eukaryotes, is structurally very similar to succinate dehydrogenase, the Krebs cycle enzyme catalysing the reverse reaction. In prokaryotes these enzymes are differentially expressed depending on the external conditions. Evidence is now emerging that also in eukaryotes two different enzymes exist for succinate oxidation and fumarate reduction that are differentially expressed. © 1998 Elsevier Science B.V.

Keywords: Respiratory chain; Complex I; Complex II; Ubiquinone; Rhodoquinone; Parasitic helminth; Lower marine organism

1. Introduction

Living with hypoxia, or even anoxia, is an everyday experience for many organisms. Not only many prokaryotes but many eukaryotic organisms as well, can function (temporarily) without oxygen. Some species, for instance parasitic helminths, are highly adapted for prolonged survival or even continuous functioning in the absence of oxygen. Others, like lower marine animals, are adapted to alternating periods in the presence and absence of oxygen. For functioning without oxygen as terminal electron acceptor, organisms have to maintain redox balance without aerobic respiration. Therefore, the reduced co-factors produced by the catabolic pathways have to be oxidized by an alternative process. Prokaryotes

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Abbreviations: FRD, fumarate reductase; SDH, succinate dehydrogenase.

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PII: S0005-2728(98)00045-0

often use a wide variety of alternative terminal electron acceptors that are available in the environment, like nitrate, fumarate or sulphate, whereas in eukaryotes the net production of reduced co-factors is avoided when oxygen is not available. In addition to simple fermentation processes in which glucose is degraded to ethanol or lactate, many facultative anaerobic eukaryotes possess fermentation processes linked to an electron transport chain. Parasitic helminths, fresh-water snails and some lower marine organisms are known to be able to survive anaerobic conditions via such a fermentation pathway, malate dismutation, which involves the use of the reduction of endogenously produced fumarate as electron sink. This process requires adaptations in their electron transport chain. This short review is restricted to those organisms and will not include plants or higher eukaryotes and their adaptations to cope with anoxia (for reviews on those subjects see Ref. [1]). The central aspects of our discussion, that are reviewed in the respective paragraphs, are the use of succinate dehydrogenase versus fumarate reductase and the different roles for the various quinones with their different midpoint potentials: ubiquinone on the one side and rhodoquinone and menaquinone on the other.

2. Maintenance of redox balance via fumarate reduction

In organisms that are adapted to anoxic functioning via malate dismutation, carbohydrates are degraded by the usual glycolytic pathway to phosphoenolpyruvate, which is then converted to malate. This malate, produced in the cytosol, is transported into the mitochondria for further degradation. In a split pathway one portion of this malate is oxidized via pyruvate to acetate and another portion is reduced to succinate [2]. Although several variations of malate dismutation with various end products occur, the use of the production of succinate as an electron sink is universal. The reduction of malate to succinate occurs in two reactions that reverse part of the Krebs cycle, and the reduction of fumarate is the essential NADH-consuming reaction to maintain redox balance. Fumarate reduction is linked to electron transport via electron-transferring enzyme complexes in an anaerobically functioning electron transport chain (Fig. 1). Malate dismutation occurs in many adult parasitic helminths [2], which are constantly dependent on this process, as well as in lower marine animals like mussels [3], oysters [4] and lugworms [5], which are intermittently dependent on this process when the

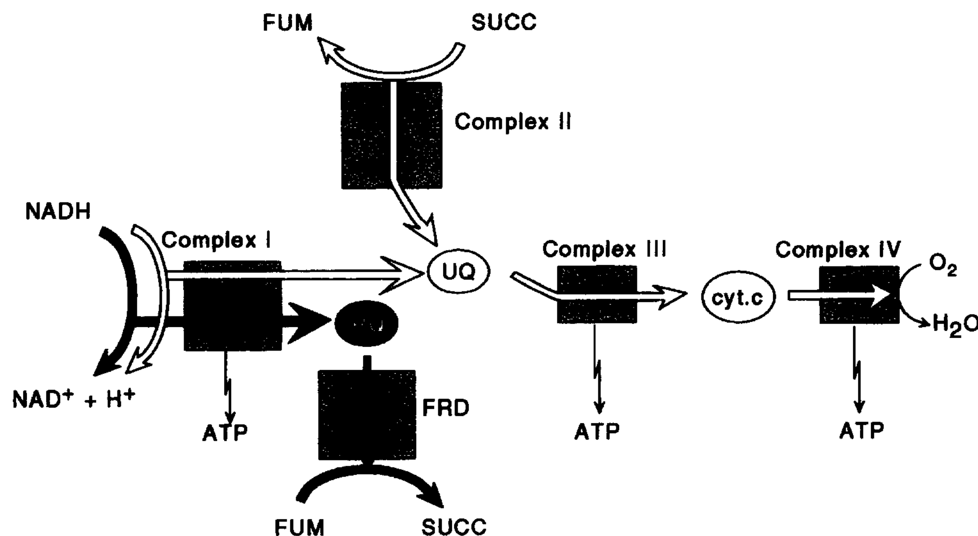

Fig. 1. Schematic representation of the electron transport chain in facultative anaerobic functioning eukaryotes. Electron flow to oxygen during aerobic respiration shown with open arrows, whereas the electron flow during fumarate reduction is indicated by solid arrows. Abbreviations: cyt. c, cytochrome *c*; FRD, fumarate reductase; FUM, fumarate; RQ, rhodoquinone; SUCC, succinate; UQ, ubiquinone.

tides of the sea force them to function anaerobically [6].

3. Electron transfer chain adaptations in fumarate reduction

In anaerobically functioning invertebrates like parasitic helminths and lower marine animals, the pathway of electron transport is altered because oxygen is not available. Endogenously produced fumarate functions as terminal electron acceptor instead. The reduction of fumarate is the reverse reaction of succinate oxidation in the Krebs cycle, catalyzed by succinate dehydrogenase, also known as complex II of the electron transport chain (Fig. 1). However, in prokaryotes these opposite reactions are catalyzed by two different but homologous enzyme complexes which are expressed under different conditions: succinate dehydrogenase (SDH) for succinate oxidation under aerobic conditions, and fumarate reductase (FRD) for the reduction of fumarate under anaerobic conditions [7,8]. Distinct enzyme complexes for these processes are also present in parasitic helminths [9,10]. This replacement of succinate oxidation by fumarate reduction has major consequences for the electron transport chain (Fig. 1). In aerobically functioning organisms, electrons are transferred from NADH and succinate to ubiquinone (UQ) via complex I and II of the respiratory chain, respectively. Subsequently, these electrons are transferred from the formed ubiquinol to oxygen via complexes III and IV of the respiratory chain (Fig. 1, open arrows). In an anaerobic metabolism complexes III and IV are no longer active as oxygen can no longer function as final electron acceptor. The reduction of fumarate by NADH is, however, also coupled to an electron transport-linked phosphorylation of ADP at site 1 of the respiratory chain. In this case electrons are transferred from NADH to fumarate via complex I, rhodoquinone and fumarate reductase (Fig. 1, closed arrows).

4. Complex I

Complex I of the electron transport chain of anaerobically functioning eukaryotes transfers electrons from NADH to rhodoquinone. In a classical mammalian-type respiratory chain, however, complex I transfers electrons from NADH to ubiquinone. These quinones, rhodoquinone and ubiquinone, differ not only in structure (Fig. 2), but they also differ in their standard electron potential.

In *Escherichia coli* a similar situation exists. During aerobic respiration NADH dehydrogenase transfers electrons from NADH to ubiquinone, whereas during fumarate respiration electrons are transferred from NADH to menaquinone [11,12]. The standard electron potential of menaquinone ($E^{0\prime}=-80$ mV) is comparable to that of rhodoquinone ($E^{0\prime}=-63$ mV). *E. coli* is known to have two distinct NADH dehydrogenases, I and II, which are encoded by the *nuo* and *ndh* gene, respectively [13]. NADH dehydrogenase I (*nuo*) consists of 14 subunits and translocates four protons per NADH oxidized, whereas NADH dehydrogenase II (*ndh*) is a single-subunit enzyme which does not translocate protons upon oxidation of NADH. During aerobic respiration *E. coli* uses predominantly NADH dehydrogenase II, and to a lesser extent NADH dehydrogenase I, whereas only NADH dehydrogenase I is used for NADH oxidation during respiration with fumarate [11,12]. Apparently, the use of the dehydrogenase is usually not selected for energy conservation in *E. coli*, unless the dehydrogenase provides the only coupling site in the respiratory chain, as in fumarate

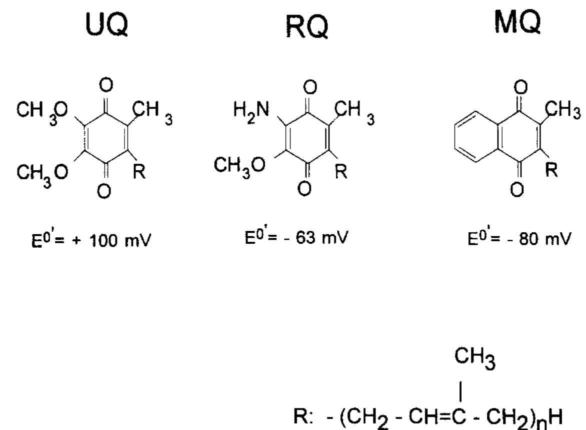

Fig. 2. Structures of quinones: ubiquinone (UQ), rhodoquinone (RQ) and menaquinone (MQ). R indicates a poly-isoprenoic side chain.

reduction. It is unknown yet whether distinct NADH dehydrogenases are present in anaerobically functioning eukaryotes as well.

5. Quinone composition

Under aerobic conditions the reducing equivalents of complexes I and II are transferred to ubiquinone (Fig. 1). During fumarate reduction, however, prokaryotes are known to use different quinones, which have a lower redox potential (menaquinone and demethylmenaquinone) [14]. Menaquinone and demethylmenaquinone are not present in eukaryotes, but in parasitic helminths the presence of rhodoquinone besides ubiquinone is known already for a long time [15]. Since rhodoquinone is present mainly in the anaerobic, fumarate-reducing stages of parasitic helminths, it was suggested that rhodoquinol functions as electron donor in fumarate reduction, similar to menaquinol in other organisms. Recently it was shown that rhodoquinone is an essential component for the electron transport associated with fumarate reduction in eukaryotes in general [16]. Rhodoquinone is present not only in all investigated parasitic helminths, but also in all examined eukaryotes that reduce fumarate under anaerobic conditions in vivo, like the sea mussel *Mytilus edulis*, the oyster *Crassostrea angulata*, the lugworm *Arenicola marina* and the fresh-water snail *Lymnea stagnalis* [16]. In lower unicellular eukaryotes that reduce fumarate during anoxia, like *Euglena gracilis*, rhodoquinone is also present, whereas those unicellular eukaryotes that do not reduce fumarate during anoxia, do not possess rhodoquinone [16,17].

Rhodoquinone is also an indispensable component of another pathway that functions as electron sink in the parasitic helminth *Ascaris suum*: the production of branched chain fatty acids via enoyl-CoA reductase [18].

Rhodoquinone, like menaquinone, has a lower redox potential than ubiquinone, and can therefore function as electron donor to fumarate (Fig. 3). Apparently, eukaryotic fumarate reductases interact with rhodoquinone (a benzoquinone), whereas most prokaryotic FRDs interact with the naphthoquinones, menaquinone and demethylmenaquinone [19].

Interestingly, fumarate-reducing members of the

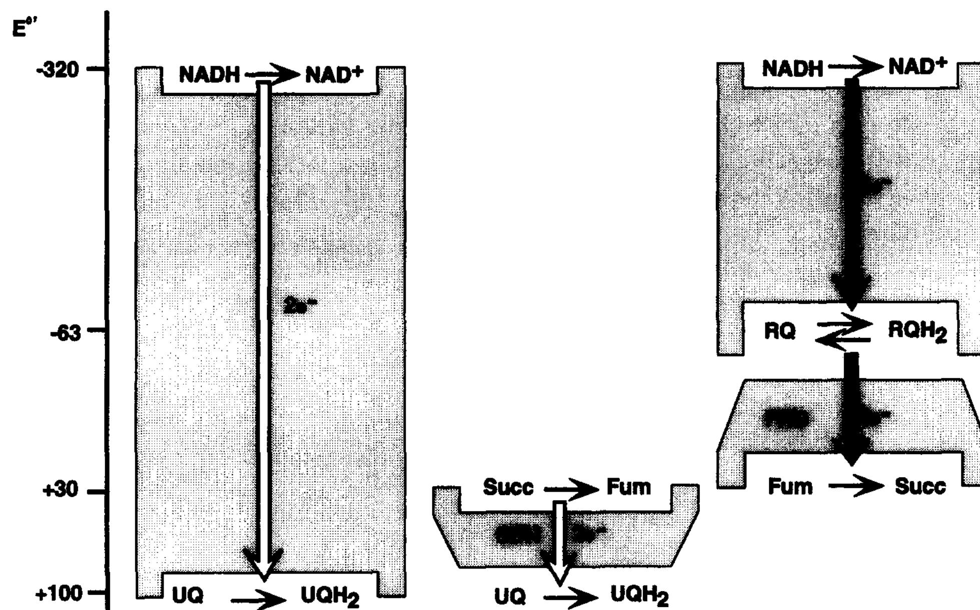

Fig. 3. Function of complex I and II in aerobic respiration and fumarate reduction. Complex I (left) and complex II (succinate dehydrogenase, SDH) of the aerobic respiratory chain accept two electrons from NADH and succinate, respectively. Subsequently these electrons are transferred to ubiquinone (UQ) (open arrow). However, during fumarate reduction, complex I (upper right) transfers two electrons to rhodoquinone (RQ) instead of ubiquinone (solid arrow). Subsequently, rhodoquinol is re-oxidized by complex II (fumarate reductase, FRD). Hence, the direction of electron transfer through the complex is in the opposite direction in FRD compared to SDH. The vertical bar represents a scale for the standard redox potentials in mV.

family Rhodospirillaceae, which are purple non-sulphur bacteria, contain rhodoquinone as well. The origin of this difference in these Rhodospirillaceae compared with all other known fumarate-reducing prokaryotes is still unknown, but might be linked to a putative close phylogenetic relation between these particular prokaryotes and eukaryotes [20]. However, it should be noted that only those Rhodospirillaceae that contain rhodoquinone or menaquinone possessed fumarate reductase, whereas the species containing exclusively ubiquinone possessed only succinate dehydrogenase [21].

Ubiquinone cannot replace rhodoquinone in the process of fumarate reduction in vivo, as ubiquinone can only accept electrons from complex II and cannot donate them to fumarate (Fig. 3). Rhodoquinone, with its lower redox potential than ubiquinone, is capable of donating electrons to fumarate, which occurs via a very comparable enzyme complex (see below). In this respect it should be stressed that in all investigated systems, including eukaryotic ones, fumarate reduction is coupled to a dedicated quinone with a lower redox potential [7,8,16,19,22,23]. Therefore, during the development of parasitic helminths, changes in quinone content occur, parallel to the changes in energy metabolism [16,24–26]. Stages with an aerobic energy metabolism with Krebs cycle activity, and hence succinate dehydrogenase activity, possess mainly ubiquinone, whereas stages that are dependent on fumarate reduction have predominantly rhodoquinone. Facultative anaerobic lower marine organisms, on the other hand, contain substantial amounts of both ubiquinone and rhodoquinone [16]. This correlates with their energy metabolism, which changes every 6 h with the tides of the sea. When immersed in water, they have an aerobic energy metabolism and rely on Krebs cycle activity, including succinate oxidation, whereas part of the time, during low tide when they are not covered by water, they function anaerobically and reduce fumarate [6]. Hence, both ubiquinone and rhodoquinone are required within the limited time span of a few hours, and therefore, both substances should be continuously present in substantial amounts as the half-life of quinones is in the order of days [25,27]. The absolute amount of rhodoquinone in these lower marine organisms is lower than in parasitic helminths [16]. However, unlike most parasitic helminths, these marine organisms are not solely dependent on fumarate reduction, but also use other fermentative pathways, producing specific end products like octopine, alanopine and strombine [6].

6. Complex II: succinate dehydrogenase and fumarate reductase

An aerobic energy metabolism with Krebs cycle activity is accompanied by electron transfer from succinate to ubiquinone via complex II of the respiratory chain, whereas in an anaerobic metabolism where fumarate functions as terminal electron acceptor, electrons are transferred from a quinone to fumarate, which is the reversed direction (Fig. 1). In *E. coli*, which can also change between an aerobic and an anaerobic metabolism, two different enzymes are expressed for these reactions: succinate dehydrogenase (SDH, succinate-ubiquinone oxidoreductase, also called Complex II) for succinate oxidation under aerobic conditions, and fumarate reductase (FRD, menaquinol-fumarate oxidoreductase) for the reduction of fumarate when oxygen is absent but fumarate present to function as terminal electron acceptor [7,8,19]. The interconversion of succinate and fumarate is readily reversible in both enzymes. However, under standard conditions in the cell, oxidation and reduction reactions preferentially occur when electrons are transferred to an acceptor with a higher standard redox potential (Fig. 3). Therefore, as explained above, FRD complexes interact with quinones having a lower redox potential, whereas SDH complexes interact with quinones with a higher redox potential.

FRD and SDH complexes are structurally very similar and each comprises usually four non-identical subunits: a flavin-containing A subunit (F_p subunit), a B subunit that contains three iron–sulphur clusters (I_p subunit), and the two hydrophobic, cytochrome *b*-containing subunits C and D that are essential for the attachment of the catalytic subunits A and B to the membrane and for the interaction of the catalytic subunits with the quinones [7,8,19,24,28,29]. Comparing SDH and FRD, the direction of electron flow through these two enzyme complexes is reversed and this implies differences in the affinity for electrons

(standard redox potential) of the electron-binding domains of these enzyme complexes [7,19].

The F_p and I_p subunits of SDH are highly conserved in different species and are also closely related to the F_p and I_p subunits of FRD. Nevertheless, these two enzymes are clearly distinct and are differentially expressed in prokaryotes: depending on external conditions, either SDH or FRD is expressed. It has been shown that *Haemonchus contortus* possesses two different genes for the I_p subunit that are differentially expressed during the development of this parasitic helminth [9]. Differential expression during development also occurs in another parasitic helminth, *A. suum*, in which the existence of two different, stage-specific forms of complex II was also demonstrated [10]. Analyses of enzyme kinetics, as well as the known differences in primary structures of prokaryotic and eukaryotic complexes that reduce fumarate, led to the suggestion that fumarate-reducing eukaryotes possess an enzyme complex for the reduction of fumarate that is structurally related to SDH-type complex II, but has the functional characteristics of the FRD complexes of prokaryotes [16].

Apparently also in parasitic helminths, these enzyme complexes are differentially expressed to suit the conditions, i.e. the presence or absence of oxygen. Studies on complex II of anaerobically functioning eukaryotes have mostly been restricted to the parasitic helminth *Ascaris* (for reviews on these extensive studies see Refs. [24,30]). The enzyme complexes responsible for fumarate reduction in eukaryotes other than parasitic helminths have not yet been studied. However, the presence of a specialized quinone, rhodoquinone, in lower marine organisms indicates that these facultative anaerobic organisms possess fumarate reductase complexes comparable to those in parasitic helminths [16].

7. Evolutionary aspects of fumarate reduction in eukaryotes

The F_p subunits of all characterized FRDs and SDHs possess a remarkable amino acid sequence similarity, and this is also the case for the I_p subunit, but to a slightly lesser extent [19]. These similarities are an indication for a common ancestor for the catalytic subunits of both FRD and SDH.

The primitive organisms present at the origin of life degraded carbohydrates to lactate via glycolysis [31]. Later in evolution this fermentation was extended with the reduction of pyruvate to succinate, a process in which NADH is reoxidized, and therefore, this process functioned as an extra electron sink to maintain redox balance in the cell [31,32]. It was suggested that these early fumarate reducing systems were soluble and relatively simple, and functioned only to ensure redox balance [33]. In the course of evolution it was energetically advantageous to couple phosphorylation with electron transport to fumarate by association of the enzyme with the membrane. This required the presence of intermediate electron carriers of suitable redox potential: iron–sulphur clusters, menaquinone and cytochrome *b* [31].

During further evolution several biosynthetic pathways evolved, such as the conversion of succinate to succinyl-CoA for the synthesis of tetrapyrroles, and the production of 2-oxoglutarate from acetyl-CoA and oxaloacetate via citrate for the synthesis of glutamate. It is assumed that these biosynthetic processes, together with the production of succinate from pyruvate via fumarate reduction and the development of 2-oxoglutarate dehydrogenase and succinate thiokinase, were linked to form the citric acid cycle when the oxygen concentration in the atmosphere increased due to photosynthetic activity [34]. The fumarate reductase present at that time, was used in the direction of succinate oxidation and during evolution this enzyme system was probably adapted to succinate oxidation by covalent binding of the flavin [35], the increased standard redox potentials of the iron–sulphur clusters of the enzyme [31], and synthesis of ubiquinone instead of menaquinone to raise the redox potential of the electron acceptor. Such a hypothetical scenario explains the existence of a succinate dehydrogenase system functioning with ubiquinone, and a fumarate reductase system functioning with menaquinone. Both systems are still present in many prokaryotes and the parallel existence of these two systems most likely evolved via gene duplication.

It is now generally believed that mitochondria evolved by an endosymbiotic event between an anaerobically functioning eubacterium or archaebacteria and an aerobic α-proteobacterium. Although this theory was postulated already in the 19th century, the investigations of Margulis [36] raised evidence in support of this theory. It has been demonstrated that

organisms in which fumarate is efficiently reduced in vivo contain rhodoquinone in addition to ubiquinone, whereas organisms that do not reduce fumarate in vivo contain only ubiquinone [16]. The evolutionary origin of the mitochondria of these facultative anaerobes is still enigmatic.

It is tempting to speculate that facultative anaerobically functioning mitochondria containing rhodoquinone, evolved from normal aerobically functioning mitochondria that contained ubiquinone and a succinate dehydrogenase, and lacked both rhodoquinone and menaquinone as well as fumarate reductase. Subsequently, these aerobically functioning mitochondria could have obtained rhodoquinone from an (ancestral) prokaryote by horizontal gene transfer, and became thus adapted to facultative anaerobic functioning. This ancestral prokaryote, from which rhodoquinone might have been obtained, was then probably also the ancestor of the Rhodospirillaceae, and contained rhodoquinone but lacked menaquinone. The hypothesis that anaerobically functioning mitochondria evolved from aerobically functioning mitochondria by acquiring rhodoquinone is supported by the notion that mitochondria have derived and lost genetic information during evolution [37]. However, future research on DNA and proteins from distinct types of mitochondria as well as from distinct eubacteria will be necessary to unravel the exact evolution of rhodoquinone and anaerobically functioning mitochondria.

Acknowledgements

Research in the authors' laboratory was supported by the Netherlands Foundation for Chemical research (SON) and the Netherlands Life Science Foundation (SLW) with financial aid from the Netherlands Organization for Scientific Research (NWO).

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