

## Chapter 3

# The respiratory electron transport chain

In this chapter, I will describe function and location of the native cytochrome b (Cb) in the mitochondrial electron transport chain. In the frame of this doctoral work, we were interested to model an artificial protein, which mimics the central part of the Cb structure consisting of a four helix-bundle motif (Rau and Haehnel, 1998). The artificial protein exhibits the electron transfer between its two hemes, similar as in the native Cb. Secondly, our aim was to evaluate the redox potentials of the hemes in the artificial and native Cb. Therefore, to better understand the role of the two hemes (high  $H_h$  and low  $H_l$  potential) in the Cb, it is useful to explain the structure, function and role of native Cb in mitochondrial electron transfer chain.

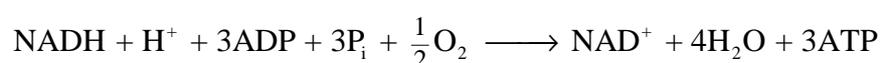
The cytochrome  $bc_1$  ( $Cbc_1$ ) complex is one of the four major respiratory protein membrane complexes residing in the inner mitochondrial membrane. The  $Cbc_1$  complex transfers the electrons from ubiquinol to cytochrome  $c$  and uses the energy thus released to form an electrochemical gradient across the inner membrane.

Cytochrome b (Cb) appears as a membrane spanning subunit of the mitochondrial  $Cbc_1$  complex (Xia et al., 1997; Iwata et al., 1998) and the photosynthetic cytochrome  $b_6f$  ( $Cb_6f$ ) (Martinez et al., 1994) protein complexes. The central part of the Cb subunit is a four-helix bundle with two hemes each axially ligated by two histidines.

### 3.1 Coupling of oxidative phosphorylation to electron transport

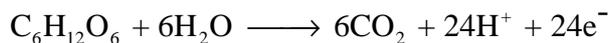
Energy conversion in the biosphere occurs mainly through respiration and photosynthesis, and represents a flux several orders of magnitude greater than all anthropogenic energy usage. The underlying mechanism involves coupling electron transfer, along a chain of redox or photoredox enzymes, to proton translocation across an organellar membrane in which those redox components are embedded. This gives rise to a transmembrane electrochemical proton gradient, which can be coupled to energy-consuming processes, including synthesis of ATP – a principle first proposed by Mitchell in his chemiosmotic hypothesis (Mitchell, 1961).

In 1951 Lehninger provided experimental proof that electron transport from NADH to oxygen is the direct source of energy used for the coupled phosphorylation of ADP. In his experiment, the NADH was rapidly oxidized to  $NAD^+$  at the expense of molecular oxygen, and simultaneously up to three molecules of ATP were formed from ADP and phosphate. The overall equation for the respiratory-chain phosphorylation can be written as:



This reaction consists of an exergonic part (oxidation of NADH by oxygen), which is coupled with an endergonic part (phosphorylation of ADP). However, in living systems, the electron transfer process occurs through a multistep pathway that harnesses the liberated free energy to form ATP.

Thus, the complete oxidation of glucose by molecular oxygen is given by two half-redox reactions, as:



The 12 electron pairs involved in the glucose oxidation are not transferred directly to  $\text{O}_2$ , but rather they are transferred to the coenzymes  $\text{NAD}^+$  and  $\text{FAD}$  to form  $10 \text{NADH} + 2 \text{FADH}_2$  in the reactions catalysed by the glycolytic enzymes. The electrons then pass into the electron-transport chain where, through reoxidation of  $\text{NADH}$  and  $\text{FADH}_2$ , they participate in the sequential redox reactions of more than 10 redox centers before finally reducing  $\text{O}_2$  to  $\text{H}_2\text{O}$ . In this process, protons are simultaneously expelled from the mitochondrion. The free energy stored in the resulting pH gradient drives the synthesis of ATP from ADP and  $\text{P}_i$  through oxidative phosphorylation.

The site, where many important biochemical processes, as the tricarboxylic acid cycle reactions, oxidation of the fatty acids, electron transport and oxidative phosphorylation occur is the mitochondrion. It is a site of eukaryotic oxidative metabolism, which is so to say the cell's "power plant". Mitochondria contain a smooth outer membrane, an inner membrane invaginated to form folds called cristae, and an internal matrix rich in protein. The two membranes differ in chemical composition, permeability and enzyme content. The enzymes of electron transport and oxidative phosphorylation are located in the inner membrane while enzymes of the tricarboxylic acid cycle are largely in the matrix. The matrix is the inner mitochondrial compartment consisting of a gel-like substance of about 50% water content. It contains remarkably high concentrations of the soluble enzymes of oxidative metabolism, as well as substrates, nucleotide cofactors and inorganic ions. The matrix also contains DNA, RNA and ribosomes. The outer mitochondrial membrane permits free diffusion of up to 10 kD molecules through nonspecific pores. The inner membrane that is considerably richer in proteins and contains the respiratory chain and transport proteins, is freely permeable only to  $\text{O}_2$ ,  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . This controlled impermeability of the inner mitochondrial membrane to most ions, metabolites and low molecular mass compounds permits the generation of ionic gradients across this barrier and results in the compartmentalization of metabolic functions between the cytosol and mitochondria.

Three sites in the respiratory chain have been identified as furnishing the energy for the phosphorylations: site I, between  $\text{NAD}$  and ubiquinone, site II, between cytochrome *b* and cytochrome *c*, and site III, between cytochrome *a* and oxygen. The phosphate and ADP are required components for maximal rates of electron transport in intact mitochondria.

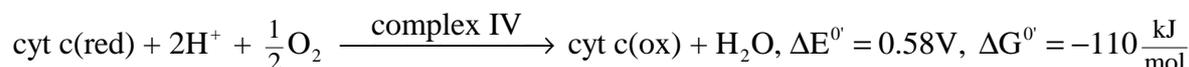
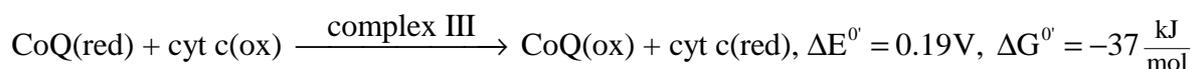
### 3.2 Electron transport

In 1961 Mitchell (Mitchell, 1961) proposed the chemiosmotic hypothesis. It postulates that the free energy of electron transport is used to pump the protons from the mitochondrial matrix (a region of low proton concentration and negative electrical potential) to the intermembrane space (which is in contact with the cytosol, a region of high proton concentration and positive electrical potential), in order to create an electrochemical proton gradient across the inner mitochondrial membrane. The energy stored by the proton gradient is subsequently used for ATP synthesis. The proton pumping across the inner mitochondrial membrane is an endergonic process. The required energy is gained by coupling with the

electron-transport chain in which electrons pass through four protein complexes containing several redox centers with progressively greater electron affinity (increasing standard redox potential). Such an electron transport is thermodynamically favourable. The large overall free energy change is broken up into three smaller energy changes, each of which is coupled with ATP synthesis. The complexes I, III and IV are sites which furnish energy for phosphorylation.

### 3.2.1 The sequence of electron transport

The free energy necessary to generate ATP is extracted from the oxidation of NADH and FADH<sub>2</sub> by the electron transport chain. It consists of four protein complexes and single protein chain of cytochrome *c* through which electrons pass from lower to higher standard redox potentials. Electrons are carried from complex I and II to complex III by the membrane (lipid) soluble coenzyme Q (CoQ or ubiquinone) and between complexes III and IV by the peripheral membrane protein cytochrome *c*. In the following, I give the overall reactions catalysed by corresponding protein complexes in the mitochondrial electron transport chain.



Complex I (NADH-coenzyme Q reductase) catalyses oxidation of NADH by CoQ(ox). It contains one flavin mononucleotide (FMN) and six to seven iron-sulfur clusters as cofactors. Complex II catalyses the oxidation of FADH<sub>2</sub> by CoQ(ox). It consists of the enzyme succinate dehydrogenase and four other small hydrophobic subunits and enables electron transfer from succinate via FADH<sub>2</sub> to CoQ. Complex III catalyses oxidation of CoQ(red) by cytochrome *c*, while complex IV catalyses oxidation of cytochrome *c* (red) by the terminal electron acceptor of the electron transport chain – molecular O<sub>2</sub>. All four processes, except the second one, supply sufficient free energy to drive ATP synthesis (which is an endergonic process, with  $\Delta G^0 = +30.5 \frac{\text{kJ}}{\text{mol}}$  ).

The proteins embedded in the inner mitochondrial membrane are organized into the four respiratory complexes. Each complex consists of several associated protein subunits, which contain a variety of redox-active prosthetic groups (Table 3.1). The redox potentials of prosthetic groups increase through the electron-transport chain, making the electron transfer from one to another redox group energetically favourable. The complexes are relatively mobile within the inner mitochondrial membrane and do not form any stable highly organized structures. They are even not present in equimolar ratios.

**Table 3.1:** Components of the mitochondrial electron transport chain

	<b>enzyme complex / name</b>	<b>mass (kD)</b>	<b>number of subunits</b>	<b>prosthetic groups</b>	<b>binding partners</b>
I	NADH-Q reductase	850	26	FMN Fe-S	NADH <sup>a)</sup> Q <sup>b)</sup>
II	succinate-Q reductase	127	5	FAD Fe-S	succinate <sup>a)</sup> Q <sup>b)</sup>
III	ubiquinol- cytochrome c oxidoreductase	240	11	heme b <sub>H</sub> -562 heme b <sub>L</sub> -566 heme c <sub>1</sub> Fe-S	Q <sup>b)</sup> cyt c <sup>c)</sup>
	cytochrome c	13	1	heme c	cyt c <sub>1</sub> <sup>c)</sup> cyt a <sup>c)</sup>
IV	cytochrome oxidase	160	8	heme a heme a <sub>3</sub> Cu <sub>A</sub> & Cu <sub>B</sub>	cyt c <sup>c)</sup>

<sup>a)</sup> location of a binding site is on the matrix side of the membrane

<sup>b)</sup> location of a binding site is within the hydrocarbone core (in membrane)

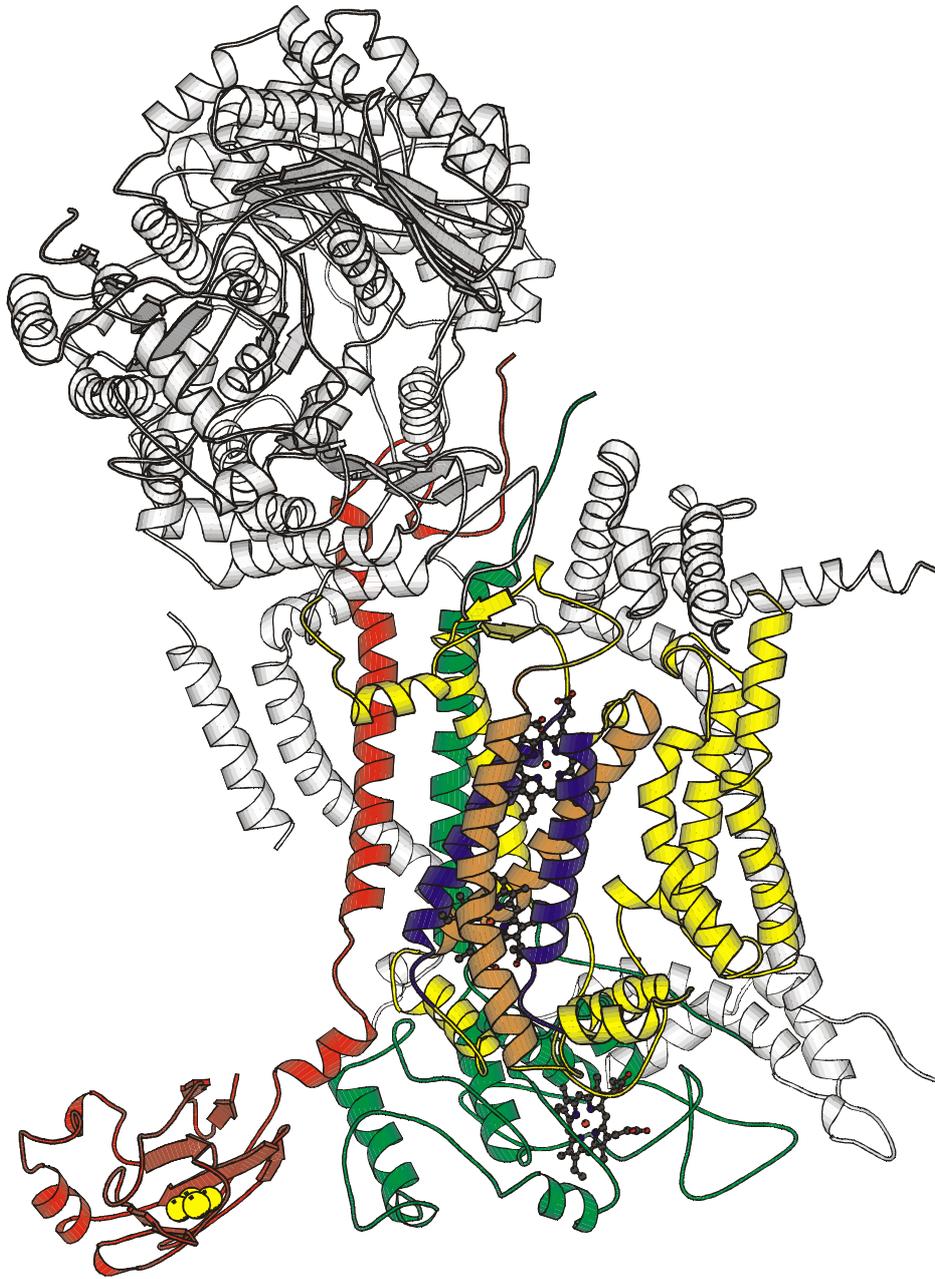
<sup>c)</sup> location of a binding site is on the cytosolic side of the membrane

### 3.2.2 Complex III

Since the focus of our interest is the cytochrome *b* unit, here I will provide some more information about the complex III of the respiratory chain (Figure 3.1) and the Cb itself.

The central component of the electron-transfer chain in mitochondrial and in many aerobic or photosynthetic bacteria is the membrane protein complex known as the cytochrome bc<sub>1</sub> complex, or ubiquinol-cytochrome c oxidoreductase (E.C.1.10.2.2). This enzyme complex catalyses electron transfer from ubiquinol to a soluble cytochrome *c* with the generation of a proton gradient across the mitochondrial membrane. Electron transfer is coupled to translocation of two protons across the inner mitochondrial membrane per oxidized ubiquinol (Mitchell, 1976; Crofts, 1985; Hinkle, et al., 1991). The cytochrome bc<sub>1</sub> complex contains three subunits with active redox centers – cytochrome *b*, cytochrome *c*<sub>1</sub> and the Rieske protein (ISP) (Robertson, et al., 1993). The mitochondrial system of eukaryotes contains additional subunits not present in bacteria (Crofts, 1985; Schaeffer, et al., 1986).

The Cbc<sub>1</sub> complex isolated from beef heart consists of 11 different polypeptide chains and has a total molecular mass of 240kD (Schaeffer, et al., 1995) (see Table 3.1). There are four redox centers, namely, two heme groups, b<sub>H</sub> and b<sub>L</sub>, of cytochrome *b*, one heme group in cytochrome *c*<sub>1</sub>, and one iron-sulfur cluster [2Fe-2S] of the Rieske protein. A mechanism describing quantitatively the proton translocation coupled to electron transport by this enzyme is a version of the "proton-motive Q cycle" of Mitchell (Mitchell, 1976; Crofts, 1985). The mechanism also explains the pattern of inhibition by the ubiquinone analogues antimycin, stigmatellin, undecylhydroxydiazo-benzothiazole, myxothiazole and methoxy-acrylo-stilbene, which bind specifically at one of the two catalytic sites at which quinone is processed (Mitchell, 1976; Crofts, 1985).



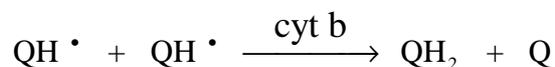
**Figure 3.1:** The X-ray structure of the complex III (cytochrome  $bc_1$  complex or ubiquinol-cytochrome  $c$  oxidoreductase) in the respiratory electron transport chain from bovine heart (solved by Iwata et al., 1998). The four-helix bundle of cytochrome  $b$  (Cb) is displayed in blue and orange colour. The rest of the Cb is shown in yellow. The Reiski iron-sulfur protein (ISP) is displayed in red and cytochrome  $c_1$  in green. All other subunits are shown in grey. The whole complex III consists of eleven polypeptide chains and contains three hemes and one 2Fe-2S cluster as prosthetic groups.

The mitochondrial cytochrome  $bc_1$  complex performs two functions (bifunctional enzyme). It is a respiratory multienzyme complex and it recognizes a mitochondrial targeting presequence (Braun, et al., 1992; Braun & Schmitz, 1995). The X-ray structures of the complex III reveal two different positions for the Rieske iron-sulfur subunit (ISP) (Zhang, et al., 1998; Iwata, et al., 1998). One location is close to the low potential heme of Cb, and the other is close enough to cytochrome  $c_1$ . It was suggested that ISP may possibly be moving around a pivot joint during the catalytic cycle of the functioning  $bc_1$  complex. The reaction mechanism involves movement of the extrinsic domain of the ISP in order to shuttle electrons from ubiquinol to cytochrome  $c_1$  (Zhang, et al., 1998; Iwata, et al., 1998).

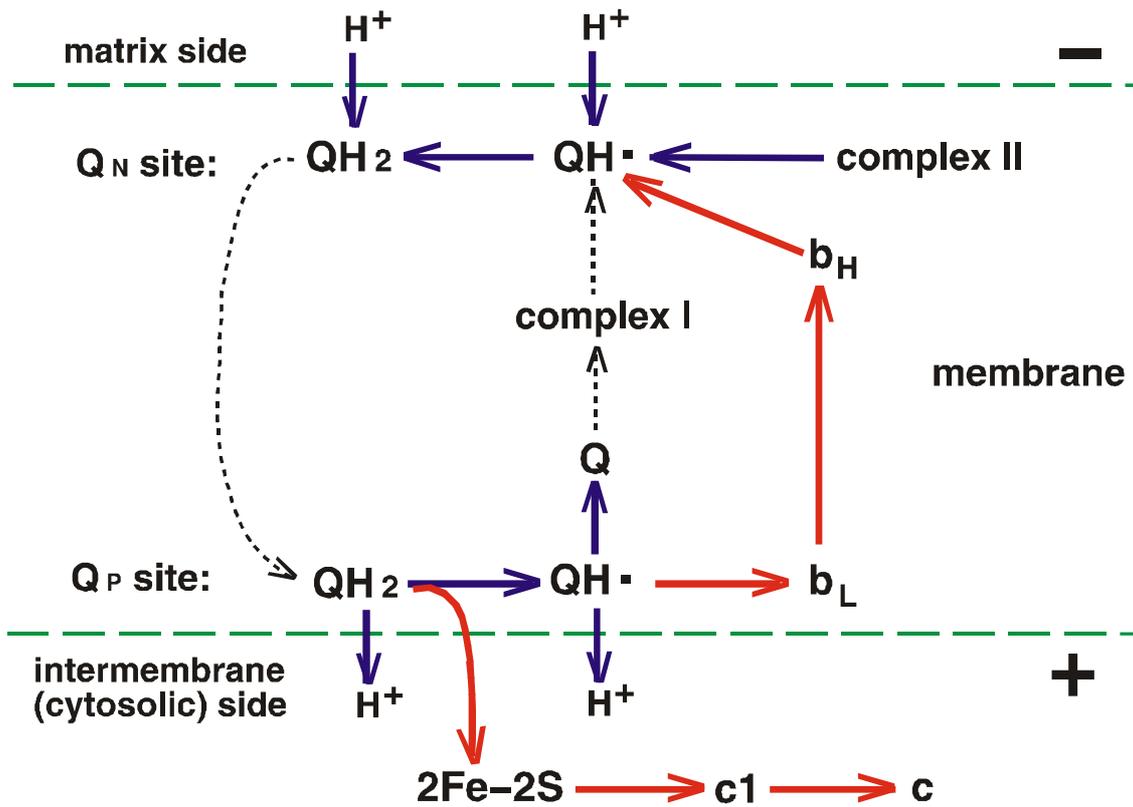
Cytochrome  $b$  is a transmembrane protein. Its polypeptide chains from various species have 379 to 385 residues and exhibit considerable sequence homology. Cytochrome  $b$  possesses eight transmembrane helices ( $\alpha A$  to  $\alpha H$ ) and four horizontal helices on the intermembrane side ( $\alpha ab$ ,  $\alpha cd1$ ,  $\alpha cd2$  and  $\alpha ef$ ). All eight stretched transmembrane helices are more than 20 residues long and contain predominantly hydrophobic residues. They span the membrane bilayer. The two hemes,  $b_L$  and  $b_H$  coordinated to four invariant His residues, are in the center of a four- $\alpha$ -helical bundle formed by helices  $\alpha A$ ,  $\alpha B$ ,  $\alpha C$  and  $\alpha D$ . Hemes  $b_L$  and  $b_H$  are close to the intermembrane (cytoplasmic) and matrix side, respectively. There are two possible binding sites,  $Q_P$  and  $Q_N$ , where the semiquinones or corresponding inhibitors can be bound. The  $Q_P$  site is in contact with the positive (intermembrane) side of the membrane, while the  $Q_N$  site is in contact with the negative (matrix) side of the membrane. For example, the binding sites of myxothiazol and antimycin A are observed close to hemes  $b_L$  and  $b_H$ , respectively. Myxothiazol binds in the semiquinone oxidation ( $Q_P$ ) site of the  $Cbc_1$  complex, blocking the electron transfer to both the ISP and heme  $b_L$ , while antimycin A binds in the semiquinone reduction ( $Q_N$ ) site, preventing the reduction of the semiquinone by the  $b_H$  heme.

The flow of electrons among the four redox-active prosthetic groups of the ubiquinol-cytochrome  $c$  reductase complex, is explained in Figure 3.2. First, ubiquinol ( $QH_2$ ) transfers one of its two high-potential electrons to the Rieske 2Fe-2S cluster. This electron is further shuttled to the cytochrome  $c_1$  heme, which has a higher redox potential. Finally, a peripheral on the matrix side located cytochrome  $c$  receives that electron and transports the electron from complex III to complex IV. By this electron transfer the ubiquinol ( $QH_2$ ) is partially oxidized to the semiquinone ( $QH^\bullet$ ). Since, the 2Fe-2S cluster is a one-electron carrier, it can not accept the second electron from  $QH^\bullet$  at the same time. Therefore, the electron pathway is bifurcated (Wikstrom & Berden, 1972; Wikstrom, 1973). Namely, the second electron of the  $QH^\bullet$  species, which is bound to the semiquinone oxidation ( $Q_P$ ) site, will be transferred to heme  $b_L$ . At the same time the semiquinone ( $QH^\bullet$ ) is oxidized to quinone (Q). Then, the electron transfer goes from low ( $b_L$ ) to high ( $b_H$ ) potential heme of the Cb. More details about the bifurcation of the central reaction of the Q-cycle can be found in recent reviews (Brandt & Trumpower, 1994; Link, 1997; Brandt, 1998).

The two cytochrome  $b$  hemes, although chemically identical, have different electron affinities and redox potentials, since they are located in different protein environments. In turn, the high potential ( $b_H$ ) heme reduces the semiquinone  $QH^\bullet$  (bound to the second binding site  $Q_N$  close to  $b_H$ ) to ubiquinol ( $QH_2$ ). Thus, the net reaction catalysed by cytochrome  $b$  is:



The cytochrome  $b$  is, so to say, some kind of the "recycling device" that enables a two-electron carrier (ubiquinol,  $QH_2$ ) to interact with a one-electron carrier [2Fe-2S] iron-sulfur cluster of the Rieske protein and to pass both of its electrons in the respiratory chain.



**Figure 3.2:** Electron transfer pathway in the complex III. The cytochrome *b* hemes play a central role in the process of the complete oxidation of semiquinone (QH•) to quinone (Q) and on that way the second electron of ubiquinol will be also pushed along the electron transport chain. Red arrows designate the electron transfer, while blue arrows designate the protonation-deprotonation reactions of ubiquinones. Since different ubiquinone species come from the so-called Q-pool, the black dashed arrows designate only an possible pathway of ubiquinones. For more discussion see text.