1. Introduction

1.1. Mitochondria and energy metabolism

Life on earth requires energy for its growth, proliferation, and for any expression of liveliness. To earn more of the cellular energy ATP, aerobic cells have developed a specific mechanism, oxidative phosphorylation. Most aerobic bacteria and all eukaryotic cells generate about 15 times more ATP by oxidative phosphorylation compared to glycolysis, which produces lactic acid or alcohol as the end product. In eukaryotes oxidative phosphorylation takes place in mitochondria, cellular organelles which contain their own genome (e.g. human mtDNA = 16569 bp) which is inherited maternally. Only 13 polypeptides are encoded by mitochondrial DNA which all represent subunits of the enzyme complexes of oxidative phosphorylation. Three enzyme complexes of the mitochondrial respiratory chain (NADH:ubiquinone oxidoreductase = complex I, cytochrome c reductase = complex III, and cytochrome c oxidase = complex IV) are electron transport-driven proton pumps which transfer reducing equivalents from nutrition to dioxygen accompanied by transfer of protons across the inner mitochondrial membrane, and generate a proton motive force. The proton motive force consists of an electrochemical gradient and a pH gradient.

$$\Delta p = \Delta\psi - Z \cdot \Delta pH, \quad Z = 2.303 \cdot RT / F, \Delta p \cdot F = \Delta\mu H^+$$

The ATP synthase in mitochondria uses the proton motive force to produce ATP from ADP and inorganic phosphate [Abrahams et al., 1994; Junge et al., 1997]. Cytochrome c oxidase is the terminal enzyme of the mitochondrial respiratory chain which transfers electrons from cytochrome c to molecular oxygen, coupled with the uptake of protons from the matrix forming water, and with the translocation of protons across the mitochondrial inner membrane.

1.2. Cytochrome c oxidase

1.2.1. Structure of cytochrome c oxidase

The mammalian enzyme is composed of 13 subunits which are encoded on the mitochondrial (subunits I, II, and III) and nuclear genome (subunits IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc, VIII). The bovine heart enzyme crystallizes as a dimer [Tsukihara et al., 1996],

as shown in Fig. 1. In contrast, the bacterial enzyme is monomeric, consisting of only 2-4 subunits [Iwata et al., 1995].

Subunit I contains the redox center heme a and the oxygen binding binuclear center heme a_3-Cu_B, and subunit II contains Cu_A and the cytochrome c binding site. Heme a and heme a_3 are chemically identical, but differ in their spectral properties, due to the different environment in subunit I. The properties of subunits I and II are nearly identical from bacterial to mammalian cytochrome c oxidase and have been shown to resemble each other in the crystal structures of the bovine heart enzyme [Tsukihara et al., 1995] and *Paracoccus denitrificans* enzyme [Iwata et al., 1995]. Additionally 1 Zn, 1 Mg, and 1 Na (or Ca) are observed in the mammalian enzyme [Yoshikawa et al., 1998], but their functions are unknown.

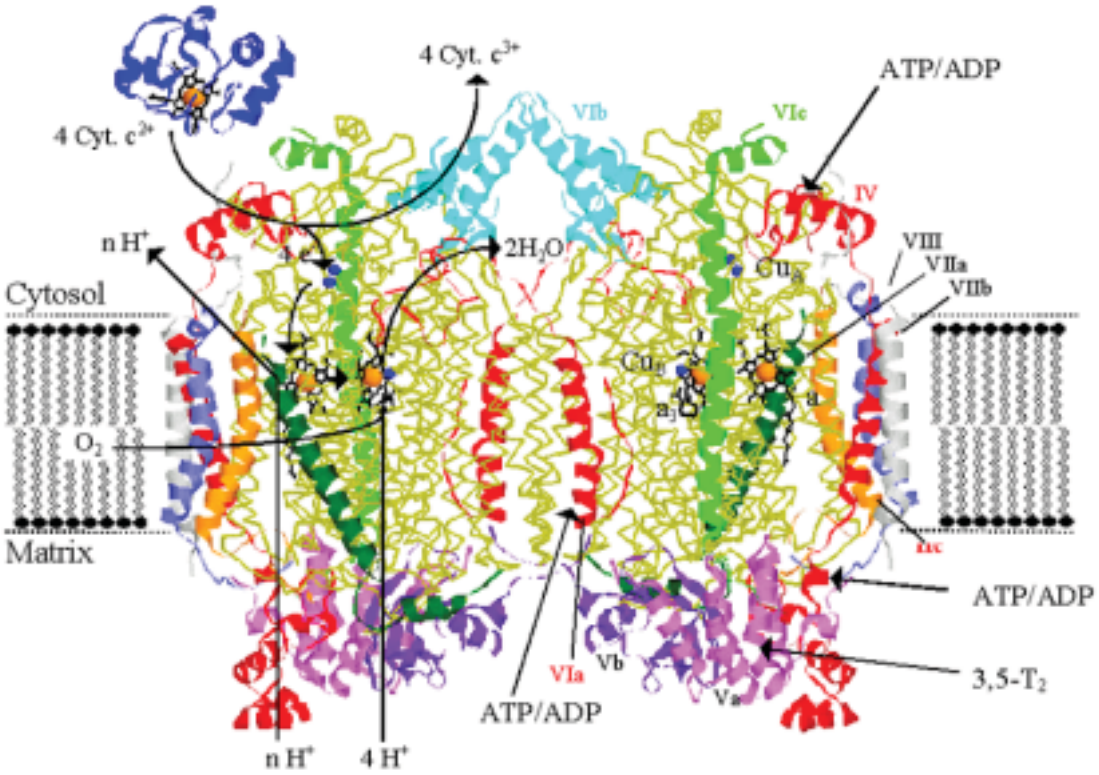

Fig. 1. Crystal structure of cytochrome c oxidase and cytochrome c from bovine heart. Each monomer of the dimeric enzyme contains 3 mitochondrial coded subunits (I, II, and III, yellow backbone), containing the cytochrome c binding site and two copper atoms (Cu_A) at subunit II, and heme a and the dioxygen binding site at the binuclear center heme a_3/Cu_B in subunit I. The copper atoms are presented in blue, heme groups in black and the iron atoms in the heme groups in orange. The 10 nuclear coded regulatory subunits (ribbons) are indicated in different colors (subunits IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc, and VIII). Left monomer: indicates the catalytic reaction. Right monomer: shows the binding sites for ATP or ADP and 3,5-diiodothyronine. Data source for cytochrome c oxidase: protein data bank Brookhaven, Tsukihara et al. [1996]. This modeling was prepared with the program "RasMol 2.6".

In Fig. 1 the nuclear coded subunits are presented in different colors. Seven of them are located as transmembraneous polypeptides and the other three are located outside of the inner membrane: subunit VIb at the cytosolic side and subunits Va and Vb at the matrix side.

The subunits of cytochrome c oxidase from turkey [Hüttemann et al., 2000] and tuna [Arnold et al., 1997] contain also thirteen subunits, similar to the enzyme from mammals. On the other hand, 11 subunits were found in the enzyme from yeast [Geier et al., 1995], seven from *Dictiostelium discoideum* [Bisson et al., 1986], but only four subunits occur in cytochrome c oxidase from the bacteria, *P. denitrificans* [Iwata et al., 1995].

For a long time, the regulatory function of the 10 nuclear coded subunits in mammalian cytochrome c oxidase [Kadenbach and Merle, 1981; Kadenbach et al., 1983; Tsukihara et al., 1996], which do not occur in the bacterial enzyme [Iwata et al., 1995], was questioned [Saraste, 1983]. The identification of tissue-, species-, and developmental-specific isoforms of nuclear coded subunits, however, suggested specific regulatory functions. The heart type subunits of VIa, VIIa, and VIII (VIaH, VIIaH, and VIIIH) are expressed in heart and skeletal muscle, whereas the liver type subunits (VIaL, VIIaL, and VIIIL) are ubiquitously expressed [Schlerf et al., 1988; Lightowlers et al., 1990; Kennaway et al., 1990; Seelan and Grossman, 1991; Linder et al., 1995]. Recently the cDNA of an isoform of subunit IV (IV-2) was found and its transcript was observed in the lungs of adult and fetal human and adult rat, as well as in the muscle of fetal human by Northern Blot analysis. In contrast, subunit IV-1 was ubiquitously transcribed in all tissues, including lung [Hüttemann et al., 2001]. Only one isoform was found for subunit VIII in human, for subunit VIIa in rat and for subunit VIa in fish [Grossman and Lomax, 1997; Linder et al., 1995; Hüttemann et al., 1997]. In the adult rat heart, the cytochrome c oxidase subunit VIa consists of two thirds of the heart type isoform (VIaH) and one third of the liver type isoform (VIaL) [Kadenbach et al., 1990], whereas in the skeletal muscle almost 100 % of subunit VIaH is expressed [Anthony et al., 1990]. In fetal heart and skeletal muscle mostly the liver type of subunits VIa and VIIa (VIaL and VIIaL) are expressed, but switch to the heart type isoforms after birth [Taanman et al., 1992; Bonne et al., 1993; Grossman et al., 1995; Parsons et al., 1996]. Interestingly, a different subunit composition of cytochrome c oxidase from wheat germ and wheat seedling suggests the existence of developmental- or tissue-specific isoforms also in the plant enzyme [Peiffer et al., 1990]. In yeast, the expression of subunit V isoforms (corresponding to subunit IV in mammals), Va and Vb, are dependent on the oxygen concentration in the growth medium [Burke and Poyton, 1998]. Different compositions of subunits and isoforms of cytochrome oxidases from various species are listed in Table 1.