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Respiratory chain complex II as general sensor for apoptosis ☆

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ABSTRACT

I review here the evidence that complex II of the respiratory chain (RC) constitutes a general sensor for apoptosis induction. This concept emerged from work on neurodegenerative diseases and from recent data on metabolic alterations in cancer cells affecting the RC and in particular on mutations of complex II subunits. It is also supported by experiments with many anticancer compounds that compared the apoptosis sensitivities of complex II-deficient versus WT cells. These results are explained by the mechanistic understanding of how complex II mediates the diverse range of apoptosis signals. This protein aggregate is specifically activated for apoptosis by pH change as a common and early feature of dying cells. This leads to the dissociation of its SDHA and SDHB subunits from the remaining membrane-anchored subunits and the consequent block of it enzymatic SQR activity, while its SDH activity, which is contained in the SDHA/SDHB subcomplex, remains intact. The uncontrolled SDH activity then generates excessive amounts of reactive oxygen species for the demise of the cell. Future studies on these mitochondrial processes will help refine this model, unravel the contribution of mutations in complex II subunits as the cause of degenerative neurological diseases and tumorigenesis, and aid in discovering novel interference options. This article is part of a Special Issue entitled: Respiratory complex II: Role in cellular physiology and disease.

1. Introduction

1.1. Complex II in healthy cells: structure and function

In order to understand the role of complex II during apoptosis it is appropriate to first revisit the composition and function of this protein aggregate in normal cells. The mitochondrial respiratory chain (mRC) consists of four multimeric protein complexes, all of which are anchored to the inner mitochondrial membrane (IMM). Together they catalyze the oxidation of reducing equivalents, mainly NADH, using molecular oxygen (O_2) as the terminal electron acceptor, which finally yields water (Fig. 1) [1]. The electron transfer within the mRC is coupled at specific points to the extrusion of protons into the mitochondrial intermembrane space. This fuels the ATP synthase complex (also known as complex V) thereby generating ATP. The coupling between mRC and ATP synthesis is called oxidative phosphorylation (OXPHOS). All mRC complexes (mRCC) are multimeric protein aggregates that are composed of factors encoded by either nuclear or mitochondrial DNA. Complex II (also known as Succinate Dehydrogenase (SDH) or Succinate Coenzyme Q Reductase (SQR)) is made up of only four subunits (SDHA, SDHB, SDHC and SDHD) and as such is the smallest mRCC. It is the only complex to be fully encoded by nuclear DNA. Apart from mRCC I, complex II is the second entry point of reducing equivalents into the mRC via FADH, which is generated by the oxidation of succinate to fumarate as part of the tricarboxylic acid ((TCA), also known as citric acid or Krebs) cycle. The electrons provided by complex II to the mRC then reduce coenzyme Q (CoQ) to ubiquinol, which is further shuffled along the mRC [1] (Fig. 1). Another particularity of complex II, and the exception among mRCCs, is that it is the only complex that does not serve to pump protons across the IMM [2]. From the crystal structure of complex II it was deduced that two transmembrane proteins, SDHC and SDHD, anchor the complex to the IMM [3,4]. Their transmembrane domains hold a redox group, a heme *b*, bound at the interface between SDHC and SDHD whose role for the transfer of electrons within complex II is so far unknown for mammals [5,6]. In eukaryotic cells the SDHB subunit is associated with the membrane anchors SDHC and SDHD and together with SDHA forms the hydrophilic head of the protein aggregate that protrudes into the mitochondrial matrix. SDHA and SDHB constitute the catalytic core of the complex that on its own can oxidize succinate (which directly binds to SDHA) to fumarate in the TCA cycle. Within the SDHA/B subcomplex the electrons are transported to the FAD cofactor contained in SDHA and finally to the three [Fe–S] clusters in SDHB [2,3]. To monitor the enzymatic activity of this part of complex II the electrons can be captured *in vitro* by artificial, exogenously added electron acceptors. The corresponding enzymatic activity of complex II is called succinate dehydrogenase activity (SDH) [7]. At the SDHC/SDHD interface two CoQ-binding sites have been found: Q_P ("p" for proximal to

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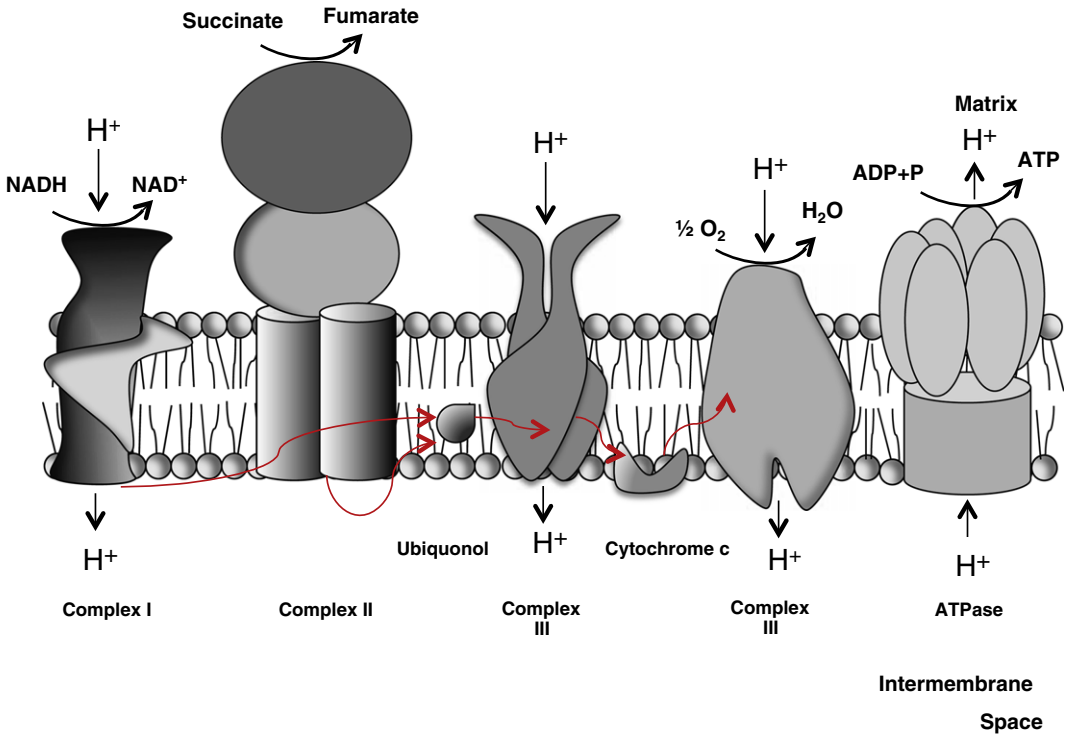

Fig. 1. Schematic representation of the mitochondrial respiratory chain complexes and the OXPHOS system. The four complexes of the respiratory chain and the ATP synthase and the electron/proton movements along these complexes are depicted. RCC I and II are the two main entries of reducing equivalents into the RC. RCC I receives electrons from NADH and transfers them via a flavin mononucleotide (FMN) cofactor to iron–sulfur (Fe–S) clusters to eventually reduce ubiquinone (coenzymeQ, CoQ, Q) to ubiquinol, which is coupled to the translocation of protons from the matrix into the intermembrane space (IMS). RCC II (succinate:ubiquinone oxidoreductase) also contributes to the ubiquinol pool via the transfer of electrons from succinate, a tricarboxylic acid (TCA) cycle intermediate, to the complex II-embedded cofactor FAD (flavin-adenin dinucleotide) and then to several Fe–S clusters. The ubiquinol pool delivers its electrons to RCC III (ubiquinol:cytochrome c oxidoreductase) through two heme centers (cytochromes b and c1) and one Fe–S cluster. This is likewise coupled to a translocation of protons across the IMM. Finally, RCC III delivers its electrons to cytochrome c (Cyt C), which transfers them to RCC IV (cytochrome c oxidase). This terminal RCC possesses uses the energy generated by the electron transfer to translocate protons. The final complex, ATP synthase (complex V), uses the protomotive force to generate ATP. This coupling between RC and ATP synthesis is called oxidative phosphorylation (OXPHOS).

the [3Fe-4S] cluster) and Q_D ("D" for distal to the [3Fe-4S] cluster) [2,3]. CoQ reduction at the Q_P site, which has a high affinity for CoQ [8], is assumed to be a two-step process. A partially reduced semiquinone is formed by the first electron transferred. This semiquinone radical appears to be stabilized for complete reduction by the second electron that then produces ubiquinol [2,9]. This mechanism is thought to safeguard complex II against excessive electron leakage under normal physiological conditions [2,9]. The entire electron transfer within RCCII, from succinate at the catalytic site of SDHA to CoQ at the Q_P site at the SDHC/D interface, constitutes the succinate CoQ oxidoreductase (SQR) activity of RCC II, which can be measured, similar to the SDH activity, with an appropriate enzymatic assay *in vitro* [7].

2. A general role of complex II in apoptosis

The concept that complex II contributes to apoptosis evolved only relatively recently based on its role in Leigh syndrome, also known as Subacute Necrotizing Encephalomyelopathy (SNEM), a neurodegenerative disease that affects the central nervous system and is associated with neuronal cell death, which eventually leads to impaired motor functions [10]. The connection was discovered through SDHA mutations that were linked to this disease [11], an observation that was later corroborated by various studies in neuronal cells showing the pro-apoptotic effects of specific complex II inhibitors, in particular the irreversible complex II inhibitor 3-nitropropionic acid (3-NP) and the competitive inhibitor methylmalonate [12,13]. Both reagents target the succinate-binding site in SDHA and both, as a consequence, inhibit the SDH as well as the downstream SQR activity [7,14]. The most recent, and most prominent, indication that complex II is involved in apoptosis regulation was made when the tumor-suppressor gene function of the SDHD, SDHC and SDHB subunits were discovered [15–17] (see below). Three mechanisms were proposed to account for these results: Firstly, the accumulation of succinate in mitochondria as a consequence of complex II inhibition and its subsequent transport to the cytosol where it inhibits HIF1α prolyl hydroxylase (PHD), leads to HIF1 stabilization and the establishes a pseudo-hypoxic state. This favours glycolysis and promotes tumor formation possibly through the Warburg effect and its growth-promoting consequences, among them, notably, apoptosis inhibition [18–20].

A second possible scenario of complex II inhibition, whose connection to apoptosis might be indirect, is that sublethal levels of superoxides are formed, which can contribute to either genomic instability or tumorigenesis. In fact, it has been acknowledged for some time that modest oxidative stress is furthering the proliferation of cells [21–25]. The third explanation of the defects of complex II subunits in cancer cells assumes a direct role of this protein aggregate in apoptosis, *i.e.* that it acts as a sensor for cell death. If this sensor is not properly functioning anymore, resistance to apoptosis signals builds up that otherwise curtail tumor formation. Indeed, various studies brought to light the role of complex II as a pro-apoptotic sensor. The first study suggesting than complex II is a transmitter of apoptosis signals observed that after mitochondrial outer membrane (MOM) permeabilization, a crucial step in apoptosis signalling, complex II (and complex I) are inhibited in a caspase-dependent manner and contribute to apoptotic cell death via reactive oxygen species (ROS) production and $\Delta\Psi\mu$ collapse [26]. ROS formation is a common theme when complexes of the RC are integrated into the apoptosis signalling process. This is achieved through the production of high,