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Superoxide production by cytochrome bc_1 complex: A mathematical model

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ABSTRACT

Reactive oxygen species (ROS) are involved in the pathophysiology of several diseases (*e.g.* Alzheimer or atherosclerosis) and also in the aging process. The main source of ROS in aerobic organisms is the electron transport chain (ETC) in the inner mitochondrial membrane. Superoxide is produced at complexes I and III of the ETC, starting a complex network of ROS reactions. To achieve a deeper mechanistic understanding of how ROS are generated by complex III, we developed a mathematical model that successfully describes experimental data of complex III activity in various rat tissues, the production of ROS with and without antimycin and ROS generation depending on different values of the membrane potential $\Delta\Psi$. The model also reinforces the idea of ubiquinone acting as a redox mediator between heme b_L and oxygen, as proposed earlier.

1. Introduction

In 1956, Harman [1] proposed that reactive oxygen species (ROS) are at the center of the aging process. ROS, such as the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) or the hydroxyl radical (HO•), are highly reactive byproducts of oxygen metabolism, and are able to damage all biologically relevant molecules (proteins, lipids, DNA) of the cell. The role of ROS for the aging process has been investigated in numerous studies [2–8], but nevertheless the exact mechanisms of ROS production and the reactions involved remain poorly understood [9,10]. Furthermore, ROS are involved in many diseases and play an important role as cell signaling molecules for various processes [11].

The majority of ROS are produced by the mitochondrial electron transfer chain, more precisely by complex I (NADH:ubiquinone oxidoreductase) and complex III (cytochrome bc_1 complex) [12–14]. The catalytic mechanism of complex III is the protonmotive Q-cycle [15]. For a long time, a semiquinone formed as an intermediate during the oxidation of ubiquinol by complex III has been postulated as the reductant for oxygen converting it to superoxide [16–18]. However, mechanistic studies revealed that this intermediate, if at all is only formed at very low occupancy [19–22] rendering this mechanism very unlikely. An alternative mechanism was deduced from the observation that the production of ROS by antimycin A inhibited complex III increases with the fraction of oxidized quinone reaching a maximum at about 70% reduction of the pool, suggesting that superoxide is primarily formed in a reverse reaction involving transient reduction of ubiquinone by heme b_L [9]. This hypothesis was supported by independent results suggesting that reduced heme b_L was required for superoxide formation by complex III [23,24]. These two opposing mechanisms are in the focus of an ongoing discussion (overview in [25]).

To investigate these ideas we developed a mathematical model for complex III that describes in detail the reactions of the protonmotive Q-cycle, the influence of the membrane potential and the production of superoxide. The aim was to design a model that was (i) detailed enough to reproduce the effect of the addition of various inhibitors, changes of pH or membrane potential $\Delta\Psi$, as well as different metabolic states (*e.g.* variations of protein quantities occurring in different tissues or young *vs.* old organisms), and (ii) simple enough to be extended by and incorporated into more general models.

1.1. The protonmotive Q-cycle

At complex III, the electrons are transferred from QH_2 to cytochrome *c* in a sophisticated process, called protonmotive Q-cycle [15,26]. In summary (see Fig. 1) two QH_2s pass through complex III, one is oxidized to Q while the other is formally first oxidized to Q and then reduced

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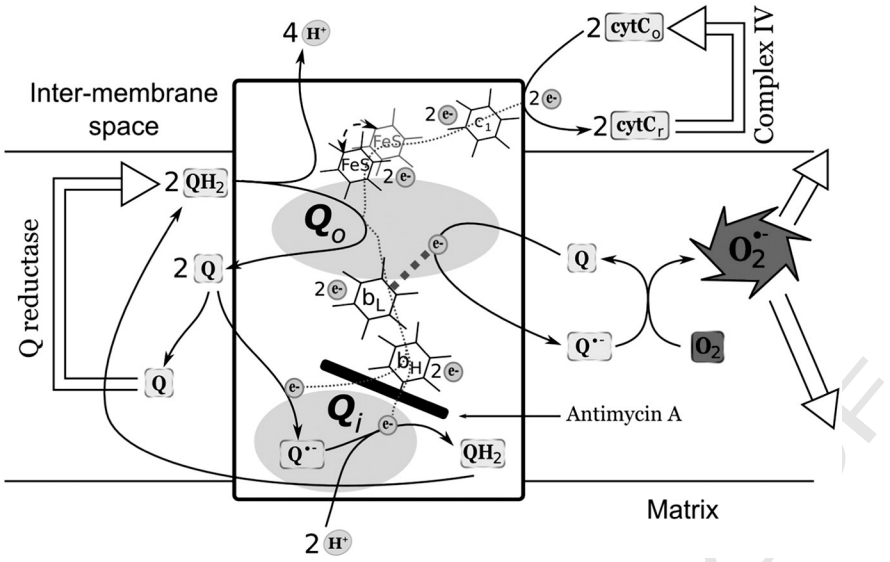

Fig. 1. The protonmotive Q-cycle starts with two electrons entering complex III. QH_2 gives one electron to FeS (which is eventually transferred to cytochrome *c via* heme c_1) and a second electron reduces heme b_L. This electron is transferred to an oxidized quinone at the Q_i site *via* heme b_H. The semiquinone then remains there, stabilized by b_H. Next a second QH_2 gives its electrons to complex III at the Q_o site (as before, one goes to FeS, and one to b_L); the second electron reduces the semiquinone waiting at the Q_i site, which then takes up two protons from the matrix to form QH_2. The model describes superoxide production *via* an electron flow from reduced heme b_L to Q and then onto oxygen leading to the formation of superoxide [9].

back to QH_2. In the first step a fully reduced ubiquinone diffuses to the Q_o site, located at the outer side of the inner mitochondrial membrane where it is assumed to be oxidized in a concerted mechanism [20,21,27]. The first electron goes to the "Rieske" iron–sulfur cluster and the second to heme b_L. During this reaction, two protons are released into the inter-membrane space.

The hydrophilic domain of the reduced "Rieske" iron–sulfur protein moves from a position near heme b_L to a position near the heme center of cytochrome c_1. The electron is transferred to cytochrome c_1 and the oxidized FeS returns to its original position, ready to receive again an electron from another ubiquinol.

Meanwhile an oxidized ubiquinone, Q, enters the Q_i site, waiting to be reduced. This becomes possible after the reduced heme b_L transfers its electron to the oxidized heme b_H from where it moves onto an oxidized ubiquinone at the Q_i site. The resulting ubisemiquinone is stabilized by b_H [28], waiting for a second electron. This additional electron comes from a second ubiquinol entering the Q_o site, which gives its electrons to the iron–sulfur cluster and heme b_L, as in the first half-cycle. Again the electron is passed from b_L to b_H and then to the semiquinone waiting at the Q_i site. This last process involves the uptake of two protons from the matrix to form a fully reduced quinone QH_2.

Per round of Q-cycle, this results in the formation of two reduced cytochrome *c*, together with the generation of one Q. Additionally, four protons are released into the inter-membrane space, while two protons are taken up from the matrix. That means just looking at complex III only two charges cross the membrane and this is the pump stoichiometry energetically relevant for its contribution to the formation of the protonmotive force. The two additional protons released to the intermembrane space by complex III are "scalar", *i.e.* they do not contribute to the vectorial charge translocation as such, although – together with the protons taken up during reduction of ubiquinone by the dehydrogenase – they are important to balance the overall stoichiometry of chemical protons.

$$QH_2 + 2cytC_{ox} + 2H^+_{MX} \rightleftharpoons Q + 2cytC_{red} + 4H^+_{IMS}.$$

Since the initial proposal of the protonmotive Q-cycle [15], modifications have been proposed to explain the experimental data gathered over the years [29]. The double-gating theory has been introduced to avoid short-circuits in the model that are not seen experimentally [30,31]. In the model presented here, we avoid short-circuits by assuming a concerted mechanism for the quinone oxidation at complex III: QH_2 releases both electrons effectively at the same time and no transfer between bL and FeS is allowed. Reverse electron transfers (and so QH_2 production at Q_o) are only possible if b_L and FeS are reduced.

Over the last years several models of the respiratory chain have been developed [32–35], but only a few address the question of superoxide production by complex III [36–40]. Unfortunately, all models allow for the presence of a semiquinone at Q_o when at the same time b_L is in its reduced form. But this situation is quite improbable due to electrostatic considerations [19]. Additionally, there are several points that render the existing models unsuitable for us. Selivanov's model [36] comprises more than 400 state variables representing the different electronic configurations of complex III, making it quite complex and difficult to handle. Quinlan's model [39] focuses on Q_o reactions, and only addresses complex III inhibition by antimycin A. And Demin's model [41,38] not only considers the quinone oxidation at the Q_o site as a two step reaction, but also omits the existence of a semiquinone at the Q_i site.

A recent, thermodynamically consistent, simple model with only 6 state variables [40] could be nicely fitted to experimental data of turnover rates of the bc_1 complex. However, its simplicity makes it unsuitable to test our hypothesis, since the positions of electrons at the Q_o site are not determined.

Thus, we developed a new model to set a different focus and to avoid some shortcomings of previous models. Precisely, we incorporated the hypothetical mechanism of ROS production by complex III involving reverse electron transfer from heme b_L to oxygen *via* oxidized ubiquinone [9].

2. Material and methods

2.1. Units

All concentrations are expressed in mol per L. The kinetic constant units depend on the reactions considered: $mol^{1-A} \cdot L^{A-1} \cdot s^{-1}$, with *A* the sum of the orders of the species. $\Delta\Psi$ is in mV, with $\Delta\Psi = \Psi_{IMS} - \Psi_{MX}$, where *IMS* denotes inter-membrane space, and *MX* the matrix.

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