A BIOENERGETIC RATIONALE FOR COENZYMЕ Q₁₀ SUPPLEMENTATION TO RELEAVE DEFECTIVE MITOCHONDRIAL RESPIRATION

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Highlights: There is accumulated evidence that efficient electron transfer in the mitochondrial respiratory chain is largely dependent on CoQ₁₀ associated in stoichiometric ratios within functional supercomplexes; this raises doubts on the efficacy of exogenous CoQ₁₀ supplementation in raising mitochondrial respiration and prompts the idea that the beneficial effects of CoQ₁₀ be due to other roles of the quinone. Here we discuss a possible reason why exogenous CoQ₁₀ may enhance defective respiration and we therefore provide support for the bioenergetic function of CoQ₁₀ supplementation besides its other roles.

Contrary to the random organization model of the respiratory chain, which was the prevailing view in the last decades of the past century (Hackenbrock et al. 1986), evidence has now accumulated that a large proportion of the mitochondrial respiratory complexes in a variety of organisms is arranged in supramolecular assemblies called supercomplexes or respirasomes (Schagger and Pfeiffer, 2000; Acín-Pérez et al., 2008; Lenaz and Genova, 2010) (Fig. 1).

The natural assembly of the respiratory complexes I, III, and IV into supramolecular stoichiometric entities, such as I₁III₂IV₀₄, is not just a mere structural feature but has deep functional implications on the properties of the respiratory chain (reviewed in Lenaz and Genova, 2010).

Within the mammalian supercomplexes, Complex I is almost totally associated with Complex III, and molecules of Coenzyme Q (CoQ) are present in the lipid boundary between the two complexes. This condition strongly supports the hypothesis that CoQ bound to supercomplexes participates in electron transfer by direct substrate channeling from Complex I to Complex III, providing kinetic advantage for electron transport, whereas it seems to exclude a role for the CoQ pool, present in large excess in the lipid membrane, in physiological electron transfer between the same two enzyme complexes (see Lenaz et al., 2016 for a recent review on evidence of CoQ compartmentation and respiratory rate advantage by CoQ channeling). Surprisingly, strong evidence exists that NADH-cytochrome c oxidoreductase activity still follows Michaelis-Menten (hyperbolic) kinetics with respect to CoQ in the presence of the supercomplex I₁III₂, suggesting that an intricate relation exists between electron transfer rate and CoQ concentration in the lipid membrane. Direct titration of CoQ-depleted mitochondria reconstituted with different CoQ₁₀ supplements yielded a $K_m$ (CoQ concentration yielding half-maximal velocity) of NADH oxidation for Q₁₀ (total CoQ₁₀, reduced plus oxidized form) in the range of 2-5 nmol/mg mitochondrial protein (Estornell et al., 1992), corresponding to 4-10 mM in the lipid bilayer. The $K_m$ for CoQ₁₀ of NADH-cytochrome c oxidoreductase was found to be much higher than that of succinate-cytochrome c oxidoreductase. A similar study on proteoliposomes containing mitochondrial Complex I and Complex III showed that the experimental rate of NADH-cytochrome c oxidoreductase was hyperbolically related to the content of CoQ₁₀, with an apparent $K_m$ in the same range as in mitochondria (Lenaz et al., 1999).

It is worth noting that if electron transfer in the supercomplex is operated through CoQ channeling, we expect a stoichiometric behaviour of rate vs. CoQ concentration, within the concentration limits imposed by the amounts of the two complexes, whereas the experimentally observed saturation kinetics (i.e. hyperbolic behaviour) seem to contradict this requirement (Fig. 2). Nonetheless, a careful reasoning allows us to conclude that free CoQ in the pool is still necessary for proper substrate channeling within the supercomplex I₁III₂. In fact, as a unifying model, we can assume that the CoQ molecules bound in the respiratory supercomplex must be in a dissociation equilibrium with the CoQ pool, so
that their amount, at steady state, would be dictated by the size of the pool. This equilibrium may explain the saturation kinetics exhibited by the NADH-cytochrome c oxidoreductase activity in the above mentioned studies.

Our proposition also requires that the dissociation rate constants ($k_{off}$) of bound CoQ be considerably slower than the rates of intercomplex electron transfer via the same bound quinone molecules, in order to guarantee their compartmentation within the supercomplex and the efficient channeling of electrons (Lenaz and Genova, 2010; Genova and Lenaz, 2011) (Fig. 3).

The observation by Schneider et al. (1982) that dilution of the inner membrane proteins with phospholipids lower electron transfer and that the effect is reversed by CoQ addition is in line with our unifying model. Earlier studies by Heron et al. (1978) also reported that endogenous CoQ$_{10}$ leaks out of the supercomplex I$_1$II$_2$ when extra phospholipid is present, causing a decrease in redox activity that could be alleviated by adding more ubiquinone. It is likely that the function of the large amount of ubiquinone in the natural membrane may be, therefore, to maintain the proper CoQ$_{10}$ content in the supercomplex unit when it is formed.

Analysis of the literature shows that the physiological CoQ content of several types of mitochondria is in the range of 2-4 nmol/mg protein and, therefore, only half-saturating for NADH oxidation activity (Battino et al., 1990). This implies that we would enhance the respiration rate if we further increase the content of CoQ in the inner mitochondrial membrane.

It is clear nowadays that CoQ$_{10}$ supplementation is able to restore mitochondrial respiration in primary CoQ$_{10}$ deficiencies and is
Figure 2 – Stoichiometric behaviour vs. pool behaviour. In a fixed assembly composed by Complex I and Complex III (i.e. supercomplex I\textsubscript{1}III\textsubscript{2}) containing bound CoQ\textsubscript{10} in dissociation equilibrium with the CoQ\textsubscript{10} pool of the lipid bilayer, the amount of bound CoQ\textsubscript{10} is dictated by the size of the pool and the rate of NADH-cytochrome c oxidoreductase follows a hyperbolic curve (black curve) that increases monotonically up to the saturation plateau (Michaelis-Menten kinetics). This resembles the curve of a two-enzyme model where Complex I and Complex III are randomly distributed in the membrane, and the rate of NADH-cytochrome c oxidoreductase depends on the number of useful collisions of free CoQ\textsubscript{10} molecules with their partner enzymes (pool behaviour). On the contrary, in a theoretical model (red curve) of a supercomplex I\textsubscript{1}III\textsubscript{2} with extremely high affinity for CoQ\textsubscript{10}, the integrated activity of NADH-cytochrome c oxidoreductase is quasi-proportional (linear kinetics) to the content of bound CoQ\textsubscript{10} up to the saturation plateau, which would be obtained with a concentration of CoQ\textsubscript{10} stoichiometric to the content of the redox components in the supercomplex and therefore very much lower than the physiological CoQ\textsubscript{10} concentration. In this theoretical case, the excess of CoQ\textsubscript{10} pool would exert no function (cf. text for details).

beneficial in several diseases in which a secondary CoQ\textsubscript{10} deficiency is usually postulated. In this latter case, a bioenergetic deficit is not always apparent, and the health improvements are often ascribed to other properties of the quinone, such as its antioxidant activity.

Is the existence and role of supercomplexes compatible with the interpretation on bioenergetic grounds of the beneficial effects of orally administered exogenous CoQ\textsubscript{10}? A major problem of CoQ\textsubscript{10} administration is its low bioavailability due to its extreme hydrophobicity (Beg et al., 2010). A water-soluble formulation has recently been shown to be easily incorporated into cultured cells and their mitochondria, enhancing respiration and antioxidant properties (Bergamini et al., 2012). The same formulation was found to improve grip strength and to inhibit apoptosis in aged rats (Xu et al., 2010).

Ignoring the above mentioned notion that bound CoQ is in a chemical equilibrium with CoQ in the membrane pool, would make the existence of respiratory supercomplexes, where only bound CoQ is active, apparently incompatible with a dose-dependent effect of added CoQ\textsubscript{10}. On the contrary, our interpretation supports the idea that even a slight decrease of endogenous CoQ\textsubscript{10} content in the membrane is sufficient to dissociate
Figure 3 – Possible mechanism for CoQ_{10} molecules bound to the supercomplex I\_I\_II\_II in dissociation equilibrium with the CoQ_{10} pool. Kinetic evidence for substrate channeling within the supercomplex I\_I\_II\_II requires the dissociation rate constants of ubiquinone (Q) and ubiquinol (QH\_2) to be considerably slower than the rates of electron transfer via the same quinone molecules bound to the supercomplex.

Indeed, a direct study by Estornell et al. (1997) demonstrated that a Complex I failure in vitamin A-deficient rats could be counteracted by an increase of CoQ\textsubscript{10} after exogenous supplementation. We think that the unifying model of bound/pool CoQ described in this paper is the most likely explanation why CoQ\textsubscript{10} supplementation was found to ameliorate several disease states even if a CoQ deficiency was not demonstrated.

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Figure 4 – Coenzyme Q₁₀ supplementation restores impaired NADH oxidation. Being the physiological content of CoQ₁₀ in the first order arm of the curve, an increase of CoQ₁₀ concentration is bound to enhance NADH oxidation both in normal mitochondria (black) and in mitochondria with defective respiration impaired by causes different from CoQ deficiency (blue).

References


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