have shown that superoxide formation at the ubiquinol oxidation center of membrane-bound or purified cytochrome bc_1 complex is stimulated by the presence of oxidized ubiquinone when the ubiquinol reduction center (Q_i site) is blocked [1]. This indicated that the electron is transferred onto oxygen from reduced cytochrome b_L *via* ubiquinone in a reverse reaction rather than during the forward Q-cycle reaction. In intact rat heart mitochondria respiring on succinate, inhibitors (malonate, diazoxide, TTFA, and atpenin A5) of the succinate:ubiquinone oxidoreductase (complex II) stimulated mitochondrial ROS production at the Q_0 site of complex III under conditions of oxidant-induced reduction; this stimulation was greatly enhanced by uncoupling [2]. We conclude that cytochrome bc_1 complex linked ROS production is promoted by a partially oxidized rather than by a fully reduced ubiquinone pool. This mechanism of ROS production by complex III offers a straightforward rationale of how the redox state of the ubiquinone pool could play a central role in mitochondrial redox signaling.

References

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5P.6 Role of conformational changes in mitochondrial complex I in the hypoxic response

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Mitochondrial complex I plays a critical role in regulating cellular energy generation and the production of reactive oxygen species (ROS). Two catalytically and structurally distinct forms of mitochondrial complex I have been characterised in enzyme preparations *in vitro*: one is a fully catalytically competent, active (A)-form and the other is a dormant, silent or de-activated (D)-form. When deprived of substrate at physiological temperatures the idle enzyme undergoes conversion into the D-form. This can gradually convert back to the A-form in the presence of substrate (NADH and ubiquinone) during slow turnover(s) of the enzyme. In the D-form of complex I a critical cysteine-39 of the ND3 subunit becomes exposed to the outside of the enzyme and is susceptible to modification and inhibition by peroxynitrite, nitrosothiols or ROS. This cysteine group is not accessible to any covalent modification in the A-form. Using a cultured cell line we have shown that A-to-D transition occurred during anaerobic incubation, when the respiratory chain was reduced. Accumulation of the D-form of complex I may be protective because slow re-activation of the D-form may reduce the burst of damaging ROS that occurs after reoxygenation. We also demonstrated that re-activation of the D-form could be prevented by prolonged incubation with endogenously-generated nitric oxide (NO). It is possible, therefore, that in some circumstances NO-dependent formation of S-nitrosothiols or peroxynitrite may lead to modification of complex I when it is in its D-form and so impede its return to the active state. Indeed, accumulation of the covalently modified D-form is likely to be responsible for the so-called persistent inhibition of cellular respiration that occurs in the presence of NO. The detrimental effect of such irreversible locking of complex I in the D-form could be due to the fact that the modified D-form of the enzyme generates ROS at a higher rate than the A-form. Thus a combination of changes in mitochondrial ROS production, a change in NAD/NADH ratio and a decline in the rate of oxidative phosphorylation could lead to cellular death and might be responsible for ischaemic damage as well as for the early stages of neurodegeneration.

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5P.7 The mechanism of metformin action in 3T3-L1 adipocytes

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Metformin (dimethylbiguanidine) is the most commonly used drug for the treatment of type 2 non-insulin dependent diabetes mellitus. The beneficial effects of the drug include a decrease in blood glucose, without stimulating insulin secretion and a general improvement in peripheral insulin sensitivity. Additionally, treatment of type 2 diabetes with metformin is associated with an overall reduction in circulating lipids and body weight both leading to a lower cardiovascular risk. Metformin action involves AMPK activation although the metabolic consequences will vary in the different target tissues. In white adipose tissue metformin-induced AMPK activation stimulates catabolic pathways that results in the reduction of triglyceride stores as reflected by the smaller size of the adipocytes. Despite fifty years of research, the early steps of metformin action are far from being elucidated. It is known that biguanidine derivatives inhibit mitochondrial respiration and this effect is probably at the basis of their antidiabetic action. There are data suggesting an indirect effect of metformin and, thus, the existence of a cell-signalling pathway targeted to the respiratory chain has been proposed. The inhibition of respiration would be responsible of two important events that would signal or initiate a shift in the cellular metabolism: (1) a decrease in the ATP levels that could lead to AMPK activation and (2) an increase in the production of superoxide by the respiratory chain with the concomitant raise in the levels of reactive oxygen and nitrogen species. We have investigated the early effects of metformin on 3T3-L1 adipocytes. We have observed that metformin rapidly inhibits cellular respiration that leads to an increase in reactive oxygen species levels and to AMPK activation. UCP2 levels raise as part of the antioxidant response of the cell, since the presence of a superoxide scavenger blocks its induction. UCP2 mRNA levels are unchanged and therefore the raise in protein levels must reflect an increased mRNA translation. AMPK activation is rapid, is associated with the expected decrease in fatty acid synthesis and does not require either the induction of UCP2 or a drop in ATP levels. Interestingly, metformin inhibits pyruvate oxidation but does not prevent the oxidation of added fatty acids.

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5P.8 Mitochondria-targeted antioxidants prevented ischemic injury of kidney

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