CELLULAR CONTROL OF MITOCHONDRIAL RESPIRATION

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THE PROBLEM

Oxygen delivery to tissues is essential for maintaining the oxygen tension at a level adequate to sustain the cellular energy supply via oxidative phosphorylation. The question is then how the cell regulates oxidative phosphorylation and, through this, the rate of oxygen consumption.

EXPERIMENTAL APPROACH

The control site(s) for any metabolic pathway must be a reaction(s) which is essentially irreversible (is accompanied by a large negative free energy change). Experimentally the search for the control site in mitochondrial oxidative phosphorylation requires three separate but sequential sets of measurements: 1) measurement of equilibrium constants for the reactions involved, including those for ATP hydrolysis and for the oxidation-reduction reactions (Em values); 2) measurement of the metabolite concentrations under cellular conditions in order to determine which of the reactions are displaced from equilibrium; 3) a study of the properties of the reaction(s) displaced from equilibrium in order to discover and evaluate the control mechanism.
Measurement of the Equilibrium Constants

With respect to the measurement of the equilibrium constants, considerable progress has been made. As shown in Table 1, the half-reduction potentials are known for components in intact mitochondria at pH 7.2 ($E_{m7.2}$). The pH dependence of many of these components has also been measured, giving a comprehensive picture of the equilibrium constants for the oxidation-reduction reactions of the respiratory chain. As can be seen from Table 1, the $E_{m7.2}$ values fall into four groups. The values for the different groups are: near -0.300 V, 0.0 V, 0.24 V and 0.36 V. The $\Delta G^o$ for ATP hydrolysis is -8.53 Kcal/mole at very low Mg$^{++}$ concentrations and -7.60 Kcal/mole at cellular Mg$^{++}$ concentrations (1 mM).

The Behavior Pattern for the Redox Reactions in Respiration

With respect to measurement of the metabolite concentrations, the steady-state reduction of several oxidation-reduction components of the respiratory chain have been measured in suspensions of isolated mitochondria. When the mitochondrial suspension was respiring in the presence of substrate, oxygen and the maximum [ATP]/[ADP][Pi] which could be formed from added ADP and Pi, the calculated $E_n$ values show the presence of isopotential groups (23), that is, groups in which the reducing equivalents are transferred at near equilibrium ($\Delta E \approx 0$). It is the transfer of reducing equivalents across the oxidation-reduction potential spans between these groups which is coupled to ATP synthesis (23,24).

Quantitative Evaluation of the Cellular Metabolite Levels and their Relationship with Respect to Equilibrium

In order to quantitatively evaluate the free energy relationship between the oxidation-reduction reactions and ATP synthesis, the mass action ratio has been measured for the reaction:

$$NADH + 2c^{+3} + 2ADP + 2Pi \rightleftharpoons NAD^+ + 2c^{+2} + 2ATP$$

in which two reducing equivalents are transferred from NADH to oxidized cytochrome $c$ ($c^{3+}$). The equilibrium constant for the reaction is expressed:

$$K = \frac{[ATP]^2}{[ADP]^2[Pi]^2} \times \frac{[NAD^+] \times [c^{2+}]^2}{[NADH] \times [c^{3+}]^2}$$

This transfer is across two of the three phosphorylation sites and is coupled to the synthesis of two moles of ATP.