CONTROL OF MITOCHONDRIAL RESPIRATION BY THE PHOSPHATE POTENTIAL*

David F. Wilson, Charles Owen
Department of Biophysics and Physical Biochemistry
Johnson Research Foundation
University of Pennsylvania
Philadelphia, Pennsylvania 19174

Leena Mela and Louis Weiner
Harrison Department of Surgical Research
University of Pennsylvania
Philadelphia, Pennsylvania 19174

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SUMMARY: The rate of respiration of suspensions of mitochondria in the presence of excess oxygen and substrate is shown to be dependent on the ratio of the concentration of adenosine triphosphate (ATP) to the product of the concentrations of adenosine diphosphate and orthophosphate. The mitochondrial respiratory chain is essentially in equilibrium with the reactions for ATP synthesis. The rate of mitochondrial respiration is controlled by the free energy requirement for ATP synthesis and this control is expressed on the rates of the reactions for reduction of the dehydrogenases by substrate and the oxidation of cytochrome $\underline{a}_3$ by molecular oxygen.

The control of mitochondrial respiration is essential to the regulation of cellular metabolism in general and the cellular supply of adenosine triphosphate (ATP) in particular. It has been generally considered that the control results from a cellular regulation of the concentration of adenosine diphosphate (ADP) available to the phosphorylation mechanism of the mitochondria to values less than the K_m for ADP (1,2). This was proposed to control the respiratory rate because an increase in the ADP concentration would result in increased respiration and a decrease in the ADP concentration would result in an inhibition of respiration. Klingenberg and coworkers (3,4) observed an ATP dependent increase in the K_m for ADP in respiration and interpreted this as indicating control by the ratio of the concentration of ATP to the product of the concentrations of ADP and orthophosphate ($\frac{[ATP]}{[ADP]\ [Pi]}$), the "phosphate potential". However, the ATP and ADP are kinetically competitive in both the ATPase reaction (5)

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and in the adenylate exchange reaction (6). Inasmuch as the orthophosphate concentration dependence was not measured the data were equally well interpreted by the kinetic control mechanism. Recently, however, strong evidence has been presented for the mitochondrial respiratory chain being essentially in equilibrium with the external phosphate potential under conditions of controlled respiration (3,7-9). If equilibrium is approached then the kinetic explanation of respiratory control is incorrect and the respiratory control is an expression of a combination of the free energy of hydrolysis of ATP and its effect on the oxidation-reduction potentials of the substrates and of the component reacting with molecular oxygen (10). In this communication we present evidence that the control of respiration is dependent on the ratio of the concentration of ATP to the product of the concentrations of ADP and orthophosphate. This observation is supporting evidence for the equilibrium treatment of mitochondrial oxidative phosphorylation and appears to eliminate the ADP kinetic mechanism for the control of mitochondrial respiration.

MATERIALS AND METHODS

Dog heart mitochondria were prepared from the left ventricular muscle at least three weeks after the surgical induction of chronic hypoxia (11). The respiratory activity of the mitochondrial suspensions at room temperature (23°) was measured by the polarographic technique using a sample chamber especially designed to eliminate any oxygen entry from the atmosphere. The adenosine diphosphate (Grade 1) and adenosine triphosphate (Sigma Grade) were obtained from Sigma Chemical Co.

RESULTS

One of the problems encountered in attempting to determine the nature of respiratory control has been the relatively high rate of respiration in the inhibited state (a mitochondrial suspension with excess substrate and oxygen

after the maximum phosphorylation of an addition of ADP and orthophosphate). The mitochondria used in this study eliminate much of this problem. As may be seen in Figure 1, trace A, these mitochondria become very strongly inhibited

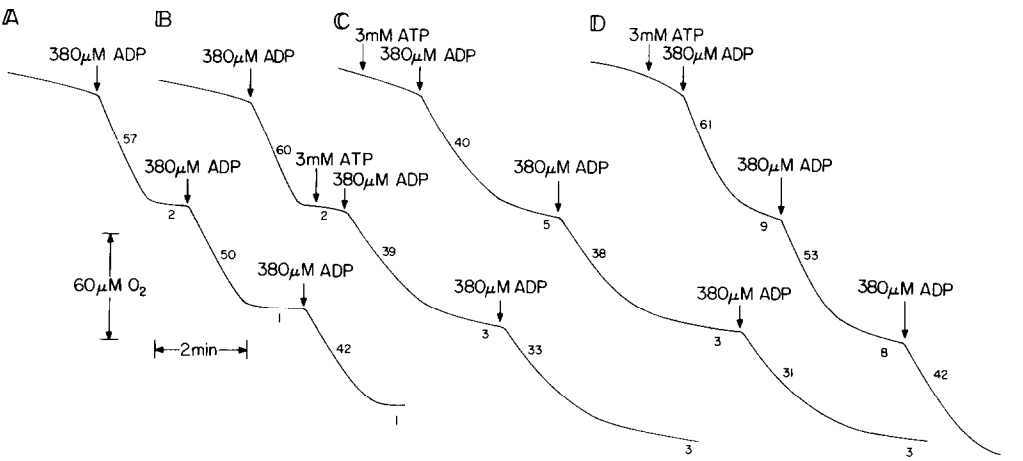

Figure 1. The dependence of the respiratory rate of a mitochondrial suspension on the concentrations of ADP, ATP and orthophosphate. The dog heart muscle mitochondria were suspended at 1.1 mg protein per ml in a medium containing 0.22 M mannitol, 0.05 M sucrose, 1 mM ethylenedinitrillotetraacetate and 30 mM morpholinopropane sulfonate (pH 7.0). Respiration was initiated by adding 7.6 mM glutamate and 7.6 mM malate. In the mitochondrial suspensions for the oxygen cathode recorder traces A, B and C, an orthophosphate concentration of 3 mM was added while for the recorder trace D, an orthophosphate concentration of 20 mM was added. The numbers on the recorder traces give the rates of oxygen consumption in μMO_2/min.

when the ADP is phosphorylated. The respiratory control as calculated by the conventional methods (12) is greater than 25. Thus the respiratory rate after the ADP is phosphorylated is negligible and essentially all of the respiration is associated with ADP phosphorylation. When the added orthophosphate concentration is 3 mM (Fig. 1A) the addition of 380 μM ADP causes an increase in respiration which then ceases as the ADP is phosphorylated. In sequential additions of two additional aliquots of ADP, each elicits a typical cycle of increased respiration and then inhibition. In trace B, 3 mM ATP was added after the first ADP cycle, resulting in a decrease in the rate of respiration measured

immediately after the ADP addition (50 μMO_2/min to 39 μMO_2/min) and a greatly increased curvature of the oxygen consumption rate. A similar effect is present when the ATP is added before the first ADP addition (trace C). Thus the presence of 3 mM ATP causes an increase in the apparent K_m value for ADP. This increase in the apparent K_m for ADP is the same as that observed by Klingenberg (3,4) and could be attributed to a competitive inhibition by ATP, except that, as may be seen in trace D, increasing the orthophosphate concentration from approximately 3 mM to 20 mM reverses the ATP effect. The presence of high orthophosphate and ATP concentrations also induces some ATPase activity in the mitochondrial suspension, depressing the respiratory control ratios to approximately 7.

The phosphorylation efficiency of the mitochondrial suspensions. In order to relate the oxygen consumption rate to the concentration of ADP it is essential to know the number of moles of ADP phosphorylated per mole of oxygen atoms consumed by respiration (ADP/O ratio). As may be seen in Table 1, when malate and glutamate are used as substrates the ADP/O ratio is essentially constant at a value of 3.2 even when the concentration of ATP is increased by 10 fold or more. Therefore we may assume that the oxygen consumption is proportional to ADP phosphorylation under all of our experimental conditions and can directly calculate the ADP concentration for each point on the oxygen electrode tracing.

The dependence of the respiratory rate on the concentrations of ADP, ATP and orthophosphate. It may be seen from Figure 1 that the respiratory rate of a mitochondrial suspension is dependent on the ADP concentration but this dependence is different if the ATP concentration or the Pi concentrations are altered. The concentrations of ADP required to give a respiratory rate of approximately 40% of the maximum (25 μMO_2/min whereas excess ADP and Pi give a rate of 65 μMO_2/min) are presented in Table 2. Various ATP and Pi concentrations were added at the beginning of the experiment and the ADP concentration required for a respiratory rate of 25 μMO_2/min determined by assuming a stoichiometry between the oxygen consumed and ADP phosphorylated. The resulting data prove that the respiratory rate does not correlate with the concentration of ADP because the same respiratory

TABLE 1

The Dependence of the Mitochondrial ADP Phosphorylation Efficiency on the Concentrations of ATP and Orthophosphate.

Addition	ATP*(mM)	Pi*(mM)	ATP/Pi	ADP/O
A 1	.38	2.62	0.14	3.16
A 2	.76	2.24	0.34	3.27
A 3	1.14	1.86	0.61	3.35
A‡	-----	-----	-----	3.27
B 1	.38	2.62	0.14	3.18
B 2	3.76	2.24	1.7	3.24
B 3	4.14	1.86	2.2	3.24
B‡	----	----	----	3.07
C 1	3.38	2.62	1.2	3.13
C 2	3.76	2.24	1.7	3.20
C 3	4.14	1.86	2.2	3.39
C‡	----	----	----	3.07

The respiration of the mitochondrial suspension was measured as described in Figure 1 and its legend. A1, A2, A3 represent sequential additions of three aliquots of 380 μM ADP. The ADP/O ratios were calculated by the usual method (12).

* The ATP and Pi concentrations are calculated by summing the concentrations added and the concentrations generated or used by phosphorylation.

‡ These ADP/O values were calculated as the ratio of the total added ADP (1.14 mM) to the total oxygen consumed. The total respiration time involved was 6.5 min, 10 min and 11 min for A, B, and C respectively.

rate is observed at ADP concentrations from approximately 6 μM to 250 μM. In the last column of Table 2, however, the ratio of the concentration of ATP to the product of the concentrations of ADP and Pi ("phosphate potential") is presented. Quite clearly this ratio is a constant, providing strong evidence that the respiratory rate is dependent on the "phosphate potential" and not on the concentrations of the individual reactants or on the [ADP]/[ATP] ratio as has been recently reported by Slater and coworkers (13). A similar analysis for other

TABLE 2

The Dependence of the Respiratory Rate on the Concentrations of ADP, ATP and orthophosphate.

Respiratory rate* (μMO_2/min)	ATP (mM)	Pi (mM)	$\frac{[ATP]}{[Pi]}$	ADP (μM)*	$\frac{[ATP]}{[ADP][Pi]}$
25	0.4	12	0.03	< 6	> $5 \times 10^3 M^{-1}$
25	0.4	2.6	0.14	16	$9 \times 10^3 M^{-1}$
25	0.76	2.2	0.34	32	$11 \times 10^3 M^{-1}$
25	1.1	4	0.27	24	$11 \times 10^3 M^{-1}$
25	3.7	12	0.31	45	$7 \times 10^3 M^{-1}$
25	3.36	2.6	1.2	120	$10 \times 10^3 M^{-1}$
25	3.76	2.2	1.7	160	$10 \times 10^3 M^{-1}$
25	6.4	3.0	2.1	250	$8 \times 10^3 M^{-1}$

The mitochondria were suspended and assayed as described in the legend of Figure 1. The concentrations of ATP and Pi were those added after correction for concentration changes due to ADP phosphorylation.

* The ADP concentrations were calculated for the point in the oxygen cathode trace at which the slope indicated an oxygen consumption rate of 25 μMO_2/min. The maximal respiratory rates with excess ADP and Pi were 65 μMO_2/min.

respiratory rates shows that the respiration in all cases is dependent on the $\frac{[ATP]}{[ADP][Pi]}$ ratio.

DISCUSSION

Control of the rate of a reaction can occur only at a step which is associated with a large negative free energy change, i.e. a step which may to a first approximation be regarded as irreversible. The oxidation-reduction reactions of the mitochondrial respiratory chain are not only reversed by ATP addition (14,15) but are essentially in thermodynamic equilibrium with the group transfer reactions of the synthesis of ATP under conditions of controlled respiration (7-9). The control of mitochondrial respiration by the "phosphate potential" represents

a highly effective control mechanism in which the rate of reaction with molecular oxygen is the principal reaction being kinetically controlled.

With a "phosphate potential" of approximately 10^5 M^{-1} a minimum respiratory rate is observed with the substrates and oxygen concentrations used in this study (7,9,16). Using a $\Delta G'_o$ for ATP hydrolysis of -7.9 Kcal/mole (17,18) this corresponds to a ΔG of ATP hydrolysis at each phosphorylation site of 14.7 Kcal/site. Thus each energy transduction site in the respiratory chain must lie between oxidation-reduction components having a difference in their oxidation-reduction potentials of 340 mV. The substrates impose an E_h value of approximately -370 mV on the pyridine nucleotide pool and thus at equilibrium with ATP synthesis the component which reacts with oxygen will be 3 x 340 mV more positive or will have an oxidation-reduction potential of approximately 670 mV. When the "phosphate potential" is decreased to 10^4 M^{-1} as in Table 2 the difference in oxidation-reduction potential across each transduction site decreases to 310 mV. The component which reacts with oxygen will then be 3 x 310 mV more positive than the pyridine nucleotides (which can be measured or in some cases assumed unchanged because of rapid equilibration with the substrates) or 90 mV more negative than when a minimum rate was observed. Because:

$$1) \qquad E_h = E_m + \frac{0.060}{n} \log \frac{[\text{Oxidized}]}{[\text{Reduced}]}$$

where E_h is the oxidation-reduction potential and E_m is the half-reduction potential. The more negative E_h will result in a reduction of the component. As may be seen from equation 1, if the component (cytochrome $\underline{a}_3$?) has an E_m of at least 120 mV more negative than 600 mV and has an n value of 1.0, the concentration of the reduced form will increase 10 fold for each 60 mV change in E_h. A complete description of the control mechanism will be presented elsewhere (18,19).

In mitochondrial preparations in which the respiratory control is smaller (less than 10) it is not possible to make accurate measurements of the ADP/O ratios because of the high rates of nonphosphorylating respiration. It is

this respiration which may be responsible for the decrease in the measured ADP/O ratios which has been reported to occur at high "phosphate potentials" (20).

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