# A NOVEL PROPERTY OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION*

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## SUMMARY

The reaction of cyanide with the oxidized form of cytochrome c oxidase in mitochondria is strongly inhibited by adenosine triphosphate (ATP). This inhibition is strictly dependent on the ATP concentration and is insensitive to changes in the concentrations of adenosine diphosphate (ADP) and orthophosphate. It is completely prevented by oligomycin or uncouplers of oxidative phosphorylation. This ATP effect is proposed to result from a structural interaction of ATP synthetase with cytochrome c oxidase, such that the formation of an ATP complex of the synthetase results in a decrease in the affinity of the oxidized form of cytochrome c oxidase for cyanide in the formation of an intermediate in the overall measured cyanide reaction.

## INTRODUCTION

It has been reported that there exists a coupling between the chemical properties of cytochrome c oxidase in intact mitochondria and the phosphorylation state $\left(\frac{[ATP]}{[ADP][Pi]}\right)$ of the extramitochondrial medium (see, for example 1,2) This coupling is such that the spectral properties of both the oxidized and reduced forms of cytochrome c oxidase (2,3,4), as well as the half-reduction potentials of both cytochromes a (2,5) and $a_3$ (1,2) are dependent on the $\frac{[ATP]}{[ADP][Pi]}$ ratio. As expected for reactions associated with mitochondrial oxidative phosphorylation, the antibiotic oligomycin and uncouplers of oxida-

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tive phosphorylation, prevent the interaction of the phosphorylation state and these chemical properties of the cytochrome c oxidase.

It has also been reported that, in mitochondria, cyanide reacts with the oxidized form of cytochrome c oxidase in a reaction which can be readily measured by the accompanying spectral change (6,7). The presence of ATP strongly inhibits the rate at which the cyanide reaction occurs (8) and this inhibition by ATP is prevented by oligomycin and/or uncouplers. On the basis of this data, the inhibition was assumed to share a dependence on the phosphorylation state with the other measured interactions.

In the present communication, it is demonstrated that this assumption is incorrect and that the inhibition of the cyanide reaction is strictly dependent on the ATP concentration, but is prevented by oligomycin and uncouplers. This ATP effect is an expression of a novel aspect of the mechanism of oxidative phosphorylation: the specific modification of a chemical property of cytochrome c oxidase by ATP in an oligomycin and uncoupler sensitive reaction.

## MATERIALS AND METHODS

Pigeon heart mitochondria were isolated using a 0.225 M mannitol, 0.075 M sucrose and 0.2 mM ethylene dimitrilotetraacetate(EDTA) medium, pH 7.0. The rate of reaction of cyanide with the mitochondria was measured at room temperature as previously described (6,8). The chemicals used were all reagent grade Adenosine triphosphate (ATP) and adenosine diphosphate (ADP) were obtained from Sigma Chemical Company.

## RESULTS

The ATP dependence of the rate of reaction of cyanide with the oxidized form of cytochrome c oxidase. Isolated pigeon heart mitochondria have very little endogenous substrate. When these mitochondria are suspended in an aerobic medium in the absence of added substrates, the respiratory chain compon-

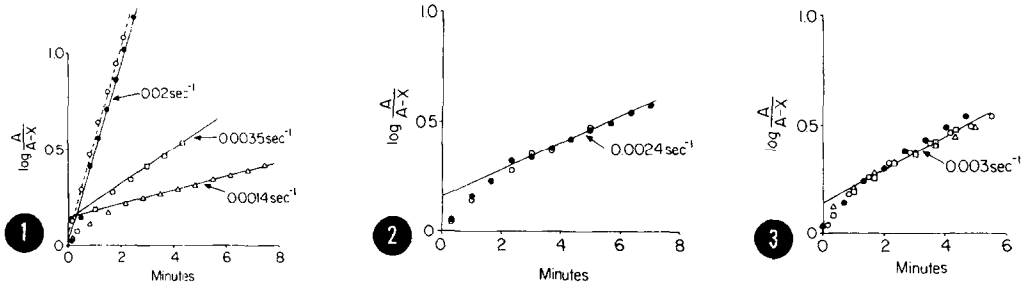


Figure 1: The effect of ATP on the reaction of cyanide with the oxidized form of cytochrome c oxidase. Pigeon heart mitochondria were suspended at 0.6 mg/ml in a 0.2 M mannitol, 0.05 M sucrose, 20 mM morpholinopropane sulfonate, and 1.0 mM EDTA medium, pH 7.08. 800 μM potassium ferricyanide, 1.0 mM ADP and 4 mM orthophosphate were then added. The reaction was started by adding the indicated ATP concentration and, 30 seconds later, 40 μM potassium cyanide. The ATP concentrations were 0 μM (●); 100 μM (□); 200 μM (Δ) or 400 μM plus 2 μg oligomycin/mg protein (o). A is the absorbance change at infinite time and x is the absorbance change from t = 0 to t = x.

Figure 2: The effect of ADP on the reaction of cyanide with the oxidized form of cytochrome c oxidase. The experimental conditions were the same as for Figure 1, except that the ATP and Pi concentrations were held constant at 200 μM and 4 mM, respectively, while the ADP concentrations were 1 mM (o) and 4 mM (●), respectively.

Figure 3: The effect of orthophosphate on the reaction of cyanide with the oxidized form of cytochrome c oxidase. The experimental conditions were the same as for Figure 1, except that the ATP and ADP concentrations were held constant at 200 μM and 2 mM and Pi concentrations of 2 mM (●), 4 mM (□), 8 mM (Δ), or 16 mM (o) were added.

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ents are highly oxidized. If 800 μM ferricyanide is added, the complete oxidation is assured and addition of cyanide is followed by the spectral change which is consistent with a high-spin ferric heme reacting with cyanide to form a low spin cyanide compound (6,7). For a single cyanide addition, the cyanide reaction is first order with respect to the unreacted cytochrome c oxidase, as indicated in Figure 1.

The rate of reaction is dramatically inhibited when ATP is added; the inhibition increases with increasing ATP concentration. Under the indicated experimental conditions, 200 μM ATP inhibits the rate of reaction 14-fold. The presence of oligomycin in the reaction mixture completely prevents the ATP effect, returning the reaction rate to that observed in the absence of added ATP. A similar complete reversal of the ATP inhibition is observed on addition of uncouplers.

The cyanide reaction is biphasic in the presence of ATP, indicating that part of the reaction is not affected by ATP. The relative portions of sensitive and insensitive reaction vary with the mitochondrial preparation. In the experiments shown in Figures 1-3, the ATP-insensitive portion was 30% or less of the total change. This appears to represent a population of poorly-coupled mitochondria combined with any contamination of the preparation by myoglobin and hemoglobin.

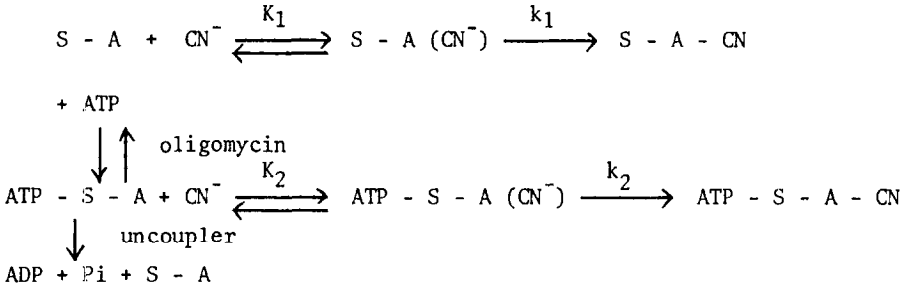
The ADP and Pi dependence of the rate of reaction of cyanide with the oxidized form of cytochrome c oxidase. In the experiment shown in Figure 1, the cyanide reaction was measured at various ATP concentrations while the ADP and Pi concentrations were held constant. When the ATP and Pi concentrations are constant, with an ATP concentration which inhibits the cyanide reaction from a half-time of 50 sec to a half-time of 300 sec, a change in the ADP concentration of 1 mM to 4 mM has no effect on the reaction rate (Figure 2). Similarly, when the ADP and ATP concentrations are held constant and the Pi concentration is changed from 2 mM to 16 mM, no change is observed in the rate of cyanide reaction (Figure 3).

## DISCUSSION

The reaction of cyanide with a heme in the oxidized cytochrome c oxidase in intact mitochondria is clearly an expression of the chemical properties of the electron transport system of the respiratory chain. All previously measured interactions between the respiratory chain components and ATP have been dependent on the phosphorylation state (a.e. free energy of ATP hydrolysis) and all current hypotheses for energy transduction incorporate reaction intermediates (non-phosphorylated high energy intermediates, or a membrane potential) between the ATP synthetase and the respiratory chain components. It is, therefore, somewhat surprising that an interaction exists which is dependent only

on the concentration of ATP (and independent of the concentrations of ADP and Pi), but which retains the property of being sensitive to both oligomycin and uncouplers.

In as much as the effect of ATP on the cyanide reaction cannot be readily rationalized within the existing hypothesis, this reaction is potentially capable of contributing to our knowledge of the coupling reactions. A speculative schematic description of the currently available data is given as follows:




It is suggested that the ATP synthetase (S) interacts physically with the cytochrome c oxidase (A). It has been shown that the oxidized form of cytochrome c oxidase binds cyanide in a rapid and reversible reaction, to form a cyanide complex spectrally indistinguishable from the unreacted oxidase (dissociation constant $K_1$). This cyanide complex then undergoes a strongly exergonic conversion to a more stable cyanide complex, the latter conversion being accompanied by the measured spectral change (6). The exergonic conversion is first order with respect to the concentration of the intermediate cyanide complex (and, therefore, to the total unreacted oxidase). Binding of ATP to the synthetase gives a structurally different form of the synthetase which, in turn, modifies the cytochrome c oxidase. The experimental data presented in this paper, as well as the more complete analysis which will be presented elsewhere (Wilson and Fairs, manuscript in preparation) are consistent with the dissociation constant, $K_2$, being much greater than the dissociation constant, $K_1$, and with the rate constant $k_2$ being less than or equal to $k_1$.

It should be noted that the presence of such an interaction between the ATP synthetase and cytochrome c oxidase is also supported by the reported stoichiometry for the release of azide and hydroxylamine inhibition by uncouplers (9,10) and is consistent with the properties of the Pi $\rightleftharpoons$ $H_2O$ oxygen exchange reported by Boyer and co-workers (11).

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