ATP CONTROLLED REDOX STATES OF RESPIRATORY CARRIERS UNDER THE INFLUENCE OF DPNH-HYDROGEN ACCEPTING SUBSTRATES

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A redox equilibrium between two respiratory carriers under the influence of the system of oxidative phosphorylation had been suggested as an explanation for the energy dependent reduction of DPN and flavoprotein with non-DPN-linked substrates, such as succinate, glycerol-phosphate and fatty acids (Klingenberg 1960a, Klingenberg and Bücher 1961). According to this mechanism reduced flavoprotein under the influence of ATP forms an energy-rich intermediate which is in a redox equilibrium with the DPN system and favours an extensive reduction of the DPN. A reversibility of oxidative phosphorylation, or in other terms, a controlling influence of ATP on this redox equilibrium had been directly demonstrated with the ATP dependent reduction both of DPN and flavoprotein in the presence of succinate or glycerol-phosphate in rat skeletal muscle mitochondria (Klingenberg 1960a, Klingenberg and Schollmeyer 1960) and independently with succinate in pigeon heart muscle mitochondria (Chance and Hagihara 1960).

In the studies with skeletal muscle mitochondria an influence of ATP on the stationary redox state of the cytochromes was also observed. Thus ATP could effect a decrease of the steady state reduction of cytochromes c and a. Simultaneously overall electron transport, i.e. respiration, approached the state of respiratory control (Klingenberg and Schollmeyer 1960). Furthermore it was shown that on partial inhibition of electron transport by azide the oxidizing effect of ATP addition on cytochromes c and a was considerably increased (Klingenberg 1960b). In other studies with sulfide treated pigeon heart muscle mitochondria an ATP induced oxidation of cytochromes c

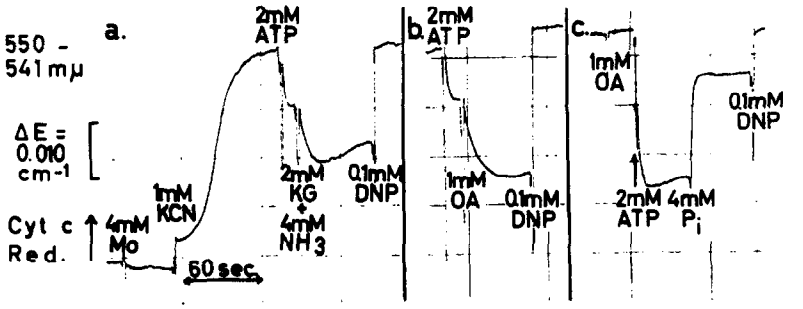

Fig 1. ATP dependent oxidation of cytochrome c with hydrogen accepting substrates in cyanide treated mitochondria. Spectrophotometric recordings of rat liver mitochondria, incubated in a medium containing 0.25 M sucrose, 1 mM ethylenediamine tetraacetate, 10 mM triethanolamine-HCl, 2 mM β-Hydroxybutyrate, pH 7,2, 25°. In experiments b and c malonate and cyanide had been previously added.
Abbreviations: Mo = malonate; KG = α-ketoglutarate; DNP = dinitrophenol; OA = oxaloacetate.

and a was described and interpreted as a reversal of electron transfer from these carriers to the DPN (Chance 1960).

In this communication new evidence will be reported in support of the concept of an ATP controlled redox equilibrium which may extend from cytochrome a to DPNH-hydrogen accepting substrates, such as oxaloacetate or ketoglutarate plus NH_3. The following findings were observed during studies of an ATP-dependent hydrogen transfer from succinate or glycerol-phosphate to the DPNH-hydrogen accepting substrates, when electron transport towards oxygen was excluded by cyanide or anaerobiosis. It was found that these substrates, besides causing a partial oxidation of pyridine nucleotides, also promote an oxidation of flavoprotein and the cytochromes if ATP is provided.

The studies to be described were conducted with rat liver mitochondria, isolated and twice washed in 0.25 M sucrose. Experiments with results similar to those reported here have also been performed with mitochondria from other organs. A spectrophotometric recording at the wavelength specific for cytochrome c shows (Fig 1a), that the addition of cyanide to the mitochondria respiring with β-hydroxybutyrate effects only a slow reduction of cytochrome c. On addition of ATP a partial

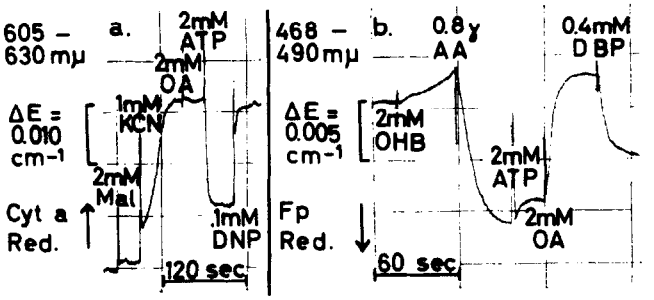

Fig 2. The ATP dependent oxidation of cytochrome a and flavoprotein with oxaloacetate. Conditions: cf legend of Fig 1. Abbreviations: Mal = malate; OHB = β-hydroxybutyrate; AA = antimycin A; DBP = dibromophenol.

oxidation of cytochrome c is noted which subsequently increases greatly on addition of either ketoglutarate plus NH_3 (Fig 1a) or oxaloacetate (Fig 1b). If first oxaloacetate and then ATP are added, the oxidation ensues with only ATP, as shown in a third experiment (Fig 1c). The redox state of other electron carriers such as flavoprotein, cytochromes b and a, undergo similar changes in this experiment, as demonstrated also for cytochrome a (Fig 2a). In principle, the same response of the cytochromes is observed if electron transport is inhibited by anaerobiosis (oxygen being excluded by nitrogen), or in the case of flavoprotein by antimycin A (Fig 2b). In this experiment, where the incubation medium contains magnesium ions, the oxidation of flavoprotein may almost fully depend on oxaloacetate in addition to ATP.

The oxidizing effects of ATP and the DPNH hydrogen acceptors could be reversed by uncouplers, by phosphate and ADP, and by amytal. Titration studies showed that about 4 μM ATP effects a 10 % oxidation and 50 μM ATP half of the maximum (about 30 %) oxidation. On the other hand about 40 μM ketoglutarate (plus 4 mM NH_3) and about 140 μM oxaloacetate cause half maximum oxidation in the presence of a saturating concentration of ATP (2 mM).

The degree of reduction of the respiratory carriers as measured in the state of maximum oxidation after the addition of both an excess of ATP and hydrogen accepting substrates has

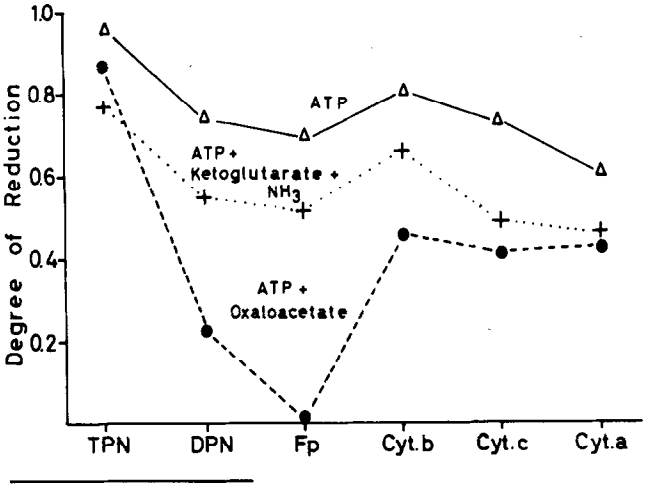

Fig 3. The redox state of the respiratory carriers under the influence of ATP and hydrogen accepting substrates. Conditions: cf legend of Fig 1.

been plotted in Fig 3. The 100 % reduction of the cytochromes refers to the state in the presence of cyanide and uncouplers when the largest increase of absorption is recorded. For flavoprotein the range can only be arbitrarily defined by cyanide addition (100 % reduction) and by the addition of ATP and oxaloacetate (0 % reduction). Thus in this case the data are only of relative value. The values for DPN and TPN have been determined by enzymatic analysis.

Fig 3 illustrates that ATP alone causes a partial oxidation of the carriers. It may be conceived that in this case endogenous substrates of the mitochondria serve as hydrogen acceptors. In the presence of both ATP and added hydrogen acceptors the cytochromes are oxidized between 40 and 60 %. With oxaloacetate a larger degree of oxidation is obtained than with ketoglutarate plus NH_3. Also DPNH is oxidized more completely by oxaloacetate. However, it should be added that the redox state of the DPN, in contrast to the other carriers, in all these cases is not influenced by the addition of ATP.

Discussion. Since electron transport towards oxygen is inhibited, the aspect of an ATP dependent redox equilibrium can be more easily applied to the experiments reported than to the previously studied ATP influence on aerobic states with an appreciable overall electron transport. In terms of this interpretation the

results reported would show that the equilibrium is controlled by the phosphorylation state of the adenosine nucleotide system as well as by the redox state of DPN coupled oxidizing and reducing substrates. The establishment of this redox equilibrium by addition of ATP may ensue with a reverse electron transfer in the whole respiratory chain onto hydrogen accepting substrates.

This aspect of an ATP controlled redox equilibrium should also be applicable to the previously described influence of ATP on the stationary redox state of the cytochromes when electron transport towards oxygen was not inhibited or only partially inhibited by azide (Klingenberg and Schollmeyer 1960, Klingenberg 1960b). In particular, the state of respiratory control may thus be interpreted as an equilibrium state rather than a steady state which is controlled only by the phosphate acceptor concentration (Chance and Williams 1956). In this sense the state of respiratory control would be the most prominent example of a reversibility of oxidative phosphorylation.

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