ON THE RELATION BETWEEN THE ACTIVATION OF SUCCINATE OXIDATION AND THE ACTIVATION OF DPN REDUCTION IN MITOCHONDRIA

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It has been reported that the oxidation of succinate by tissue homogenates or mitochondria can be inhibited under various conditions, such as under the influence of oxaloacetate (Das, 1937), DPN (Swingle et al. 1942, Pardee and Potter, 1948) dinitrophenol (Stanbury and Mudge 1954), ethylenediaminetetraacetate (Kunz et al. 1950), arsenate and dicoumarol (Azzone and Ernster 1960), ADP (Chance and Hagihara 1960 a). ATP was found to be able to reactivate the succinate oxidation inhibited by oxaloacetate (Pardee and Potter 1948, Tyler 1955) or by arsenate (Azzone and Ernster 1960). Further studies showed, that in the arsenate plus dicoumarol treated liver mitochondria an activation of succinate oxidation by ATP is accompanied by a small increase in the reduction of the mitochondrial DPN and TPN (Azzone, Ernster, Klingenberg 1960). In other studies only on the succinate linked DPN reduction, ATP was found to initiate an extensive reduction of DPN in rat skeletal muscle mitochondria (Klingenberg 1960) and in pigeon heart mitochondria (Chance and Hagihara 1960 b).

In the course of comparative studies mitochondria from pigeon breast muscle were found to be a very favourable system for studying inhibition and activation of succinate oxidation and its relation to the DPN reduction. In these mitochondria succinate oxidation is nearly completely inhibited by 15 min. pre-incubation without the addition of further agents.

Experiments and Results. The pigeon breast muscle mitochondria were prepared in a medium containing 0.13 M KCl, 3 mM EDTA, 10 mM triethanolamine-HCl, pH 7,2 and were incubated in the

same medium at 25°C. The oxygen uptake was measured by the platinum electrode. The reduction of the DPN and TPN systems were followed by spectrophotometric recording and measured by enzymatic analysis. The results are assembled in table 1.

The pigeon breast mitochondria, when incubated immediately after preparation, respire at an appreciable rate due to the endogenous substrates. When succinate is added shortly after incubation of the mitochondria the respiration increases strongly. At the same time DPN and TPN become largely reduced. About 15 min. after incubation without added substrates the respiration of the mitochondria has decreased nearly to zero. When succinate is added at that time its oxidation is largely inhibited and the pyridine nucleotides remain oxidized. The subsequent addition of ATP activates the respiration almost immediately about 20 fold. Now DPN becomes about 10 % reduced and TPN about 50 %.

Further substances, such as phosphate and serum albumin, which may facilitate the endogenous formation of ATP, were found to activate the succinate oxidation. After addition of these substances, succinate oxidation is activated only after a certain time until the full steady state velocity has been reached. The pyridine nucleotide reduction increases closely parallel with the respiration.

The succinate oxidation can also be reactivated by the addition of DPN linked substrates, such as malate and ketoglutarate. Also substrates such as glutamate, cysteinesulfinate and pyruvate, which possibly remove oxaloacetate by condensation or transamination function as activators. The respiration with these substrates alone, as measured in control experiments not shown here, amounts only to about 20 to 30 % of the activated respiration with succinate.

In all these cases the respiration is activated to about the same extent as by ATP. On the other hand the reduction of the DPN is activated to a much higher degree by most of the substrates than by ATP.

It appears conceivable that the activating substrates, by way of their oxidation, effect the phosphorylation of endoge-

Succinate linked respiration and pyridine nucleotide reduction in pigeon breast muscle mitochondria

Additions	Respiration[1]	$\frac{DPNH}{DPNH+DPN}$ [2]	$\frac{TPNH}{TPNH+TPN}$ [3]
After 2 min. Preincubation			
—	0.27	0.05	—
4 mM Succinate	0.95	0.55	0.90
After 15 min. Preincubation			
—	0.05	0	0.10
4 mM Succinate	0.06	0	0.10
" + 2mM ATP	1.3	0.10	0.55
" + 4mM Phosphate	1.1	0.13	0.60
" + 0.2% Albumin	1.05	0.09	0.50
" + 2mM Malate	1.05	0.50	0.75
" + 2mM Ketoglutarate	1.0	0.12	0.60
" + 2mM Pyruvate	1.2	0.45	0.75
" + 2mM Glutamate	1.3	0.33	0.65
" + 2mM Cysteinesulfinate	1.15	0.48	0.65
After 5 min. Preincubation with 0.1 mM Dinitrophenol			
4 mM Succinate	0.04	0.01	0.12
" + 2mM ATP	0.45	0.01	0.12
" + 4mM Phosphate	0.10	0	0.07
" + " + 2mM Malate	0.35	0.04	0.15
" + " + 2mM Pyruvate	0.25	—	—
" + " + 2mM Ketoglut.	1.20	0.05	0.15
" + " + 2mM Glutamate	0.90	—	—
" + " + 2mM Cysteine-sulfinate	0.35	0	0.12

Conditions: 5.5 mg mitochondrial protein per ml., 0.13 M KCl, 3mM EDTA, 10mM triethanolamine-HCl, pH 7,2, 25°.

1) $\frac{\text{uatom O /2}}{\text{sec g protein}}$ 2) DPNH+DPN = 5.5 - 7.5 uMol/g protein 3) TPNH+TPH = 0.9 - 1.25 uMol/g protein

nous adenosine phosphate which then activates the succinate oxidation. We therefore conducted experiments where oxidative phosphorylation was uncoupled by dinitrophenol. After 5 min. pre-incubation with dinitrophenol respiration with succinate

is nearly completely inhibited. On the addition of ATP respiration increases, however only to about 1/3 of the rate in the absence of dinitrophenol. An activation of respiration by phosphate is nearly completely inhibited by dinitrophenol. The subsequent additions of malate and pyruvate increase the respiration only about threefold. As seen in control experiments, this does not result from an activated succinate oxidation, but from an oxidation of the added substrates. The threefold increase of respiration with cysteine sulfinate presumably is also not due to an activated succinate oxidation but to the further oxidation of malate and pyruvate, the latter being formed from the cysteine sulfinate. Thus it is to be concluded that dinitrophenol also inhibits largely the activation by these substrates. Only on addition of ketoglutarate or glutamate can the respiration be fully reactivated. In all these experiments the reduction of DPN is completely or largely suppressed by the presence of dinitrophenol.

Discussion. These results show clearly in agreement with previous studies that a rapid succinate oxidation is a provision for the succinate linked DPN reduction but that the succinate DPN linked reduction does not necessarily result from a rapid succinate oxidation. It may be recalled from earlier research on the succinate and also glycerol-phosphate linked DPN reduction that the DPN reduction is abolished by phosphate plus ADP or uncouplers. On this basis we can understand that in our system, after activation by ATP, DPN is reduced to a lower degree than by the activation with DPN-linked substrates, since ATP is rapidly split to ADP and phosphate under these conditions. Thus also in the presence of dinitrophenol no extensive reduction of DPN can be expected even in the instances of a complete reactivation of succinate oxidation by ketoglutarate and glutamate. These results may be compared with previous studies on arsenate plus dicoumarol treated liver mitochondria (Azzone, Ernster and Klingenberg 1960). In this system in the presence of uncouplers, the partial reactivation of succinate oxidation by ATP ensued with a small reduction of DPN and a larger reduction of TPN.

We conclude that there is no basic difference between both systems and that dinitrophenol has the tendency to suppress an ATP effect on the DPN reduction.

It is possible that with succinate the activation of the overall electron transport and of the DPN reduction result from the same primary reaction of ATP. Both phenomena are intimately linked as far as a "controlled state" of electron transport can be established. An ATP effect on the DPN reduction might be regarded only as resulting from an activation of succinate oxidation and not as demonstrating an energy requirement of the succinate linked DPN reduction. However, as previously shown (Klingenberg and Schollmeyer 1960) with glycerolphosphate, the effect of ATP on the DPN reduction is not linked to an activation of the glycerolphosphate oxidation. This demonstrates, that ATP can also interact with the respiratory chain in a manner different from an activation of substrate utilization.

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