KINETICS OF SUCCINATE LINKED PYRIDINE NUCLEOTIDE REDUCTION IN SONIC PARTICLES FROM BEEF HEART MITOCHONDRIA

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The energy linked reduction of pyridine nucleotides by succinate has been extensively studied, not only in intact mitochondria (1) but also in submitochondrial particles derived from pigeon heart mitochondria (2) as well as beef heart mitochondria (3). The last has the advantage of employing externally added DPN, in a system in which no functional tricarboxylic acid cycle is operative.

A series of experiments have been carried out using sonic particles derived from beef heart mitochondria, employing sulfide as a terminal inhibitor of the respiratory chain. In following the succinate linked reduction of DPN initiated by ATP, some marked differences were observed from the results reported by Low et al (3). In addition, this type of reaction system shows two striking differences from that reported for the succinate linked reduction of the endogenous pyridine nucleotide of the intact heart mitochondrial system. In the present study it was consistently observed that there is a pronounced time lag phase in DPN reduction when ATP was added to initiate the reaction. This is illustrated by the tracing of the kinetics of DPNH fluorescence presented in Fig. 1, curve A. The lag phase observed was solely dependent on ATP, as reversal of the order of addition of the components of the system did not show a lag phase, i.e. when ATP was added about 1 minute prior to the addition of succinate or DPN, no lag phase was apparent (Fig. 1, curve B).

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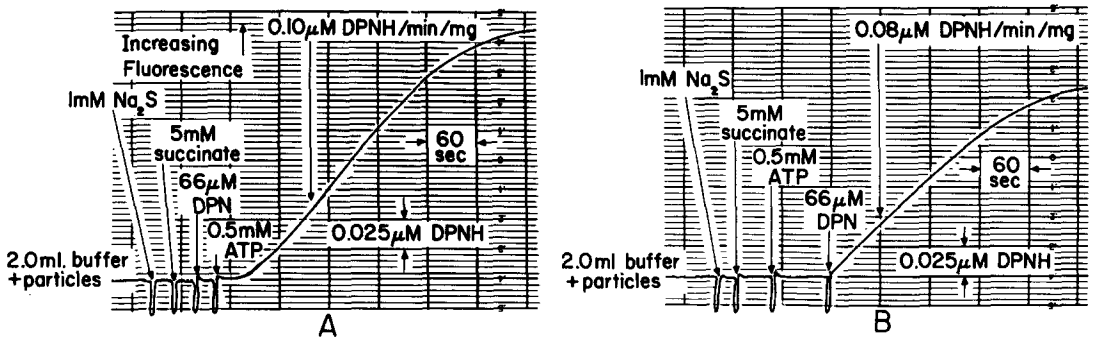

Fig. 1: Kinetics of DPN reduction by succinate and ATP utilizing sonic particles derived from beef heart mitochondria, prepared according to Linnane and Ziegler (5). DPNH formation was followed fluorometrically as described by Estabrook and Maitra (6). The submitochondrial particles were suspended in 2 ml of buffer (80 mM KCl, 3 mM Mg Cl2, 10 mM triethanolamine hydrochloride pH 7.5) to a final protein concentration of 0.5 mg/ml. Subsequently 10 μL 0.2 M sodium sulfide, 10 μL 1 M sodium succinate, 5 μL DPN solution (20 mg/ml) and 10 μL 0.1 M ATP were added in the order indicated (curve A). In curve B, DPN was added as the last component of the system, to initiate the reaction.

With these beef heart mitochondrial particles, no lag phase was observed in succinate oxidation. Since these submitochondrial particles are partially uncoupled, no high energy intermediates are necessary for the initiation of succinate oxidation, as is the case in aged pigeon heart mitochondria (c.f. Chance and Hagihara (4)). The reduction of DPN, however, still requires intermediates of oxidative phosphorylation, generated from ATP. One may therefore conclude that the lag phase observed in DPN reduction is not associated with succinic dehydrogenase or those components reacting between succinate and the cytochrome system.

The presence of magnesium ions in the reaction medium is obligatory for DPN reduction. Fig. 2 shows the relation between the magnesium ion concentration and the rate of DPN reduction as well as the duration of the lag phase. The initial ATP concentration was 0.5 mM in these experiments. A maximum rate of reduction and a minimum lag phase in DPN reduction is obtained with a magnesium ion to ATP ratio of about 10:1. This high ratio suggests that the magnesium-ATP complex is the true reactant, rather than free ATP. However, if the only function

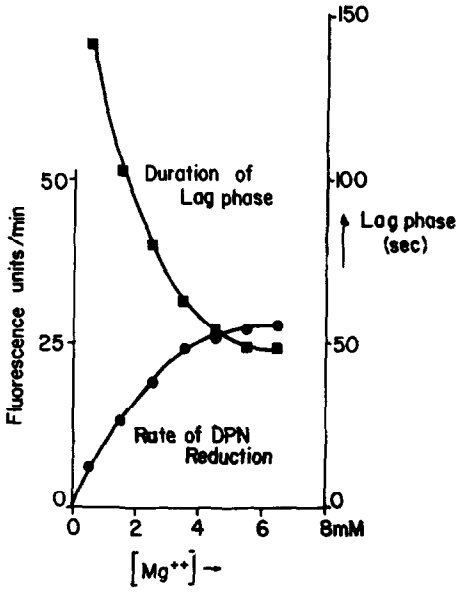

Fig. 2: Relation between the magnesium ion concentration and the rate of DPN reduction, measured in arbitrary fluorescence units (●) and duration of the lag phase in seconds (■). Experimental conditions are as described in Fig. 1, except for the magnesium ion concentration.

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of the magnesium ion was to participate in the magnesium-ATP complex, one would expect to find maximum rates at much lower magnesium ion concentrations. The fact that this is not observed would suggest that magnesium has an additional function.

In this respect these particles differ significantly from intact mitochondria, where magnesium was found to be inhibitory (1) to the succinate linked reduction of endogenous pyridine nucleotide.

The role of cytochrome b in the succinate linked reduction of DPN is controversial. In the present study the steady state reduction of cytochrome b was measured at 430 mμ, using 410 mμ as a reference wavelength, employing the dual wavelength technique described by Chance (7). It was found that after addition of sulfide, succinate and DPN to the particles suspended in the reaction medium, about 75% of the

cytochrome b was reduced. Addition of ATP caused a further reduction of cytochrome b, without an apparent lag phase. This reduction was independent of the magnesium ion concentration. A comparison of the influence of inhibitors on this additional cytochrome b reduction and the succinate linked DPN reduction, showed that both reactions are inhibited by oligomycin and by the uncoupling agent dibromophenol. Amytal inhibited this additional cytochrome b reduction, but 3 times higher concentrations of Amytal were required than that observed for the inhibition of DPN reduction. The additional cytochrome b reduction was not inhibited by octylguanidine up to concentrations which completely inhibited DPN reduction. Both reactions (cytochrome b and DPN reduction) were found to be inhibited by phenethylbiguanide (DBI), to the same extent when determined as a function of DBI concentration.

This ATP dependent cytochrome b reduction was also observed in the absence of added DPN, although to a lesser extent. (See also Chance (8)) To ensure that endogenous DPN was not contributing to this reaction, sensitive fluorometric measurements for DPNH indicated no associated reduction of bound DPN. This indicates that the ATP dependent reduction of cytochrome b is either an independent process or precedes DPN reduction. In addition the ATP dependent cytochrome b reduction is also observed, both in the presence and absence of added DPN, when paraphenylenediamine is used as electron donor. Here again it was found that this additional cytochrome b reduction was inhibited by oligomycin and DBI.

Pressman (9) has given evidence that DBI is a specific inhibitor for the phosphorylation associated with cytochromes b and c_1. These results rule out the hypothesis that the ATP dependent cytochrome b reduction is an independent process and therefore it is concluded that it precedes DPN reduction. This is further supported by the Antimycin A sensitivity of the overall reaction. The suggestion by Low *et al* (3) that Antimycin A elicits an ATP-ase activity could not be confirmed in the present study. The fact that higher concentrations of Antimycin A

are needed for the succinate linked reduction of DPN than for inhibition of succinate or DPNH oxidation may merely be a reflection of the difference in the location of the rate limiting step in the sequence of carriers participating in the succinate linked reduction of DPN.

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