ON THE REGULATION OF SUCCINATE DEHYDROGENASE IN BRAIN MITOCHONDRIA

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1. Introduction

The first findings that suggested that succinate dehydrogenase could be a point of metabolic control were those in which oxaloacetate was found to be a potent inhibitor of succinate dehydrogenase and that this inhibition was reversed by ATP [1, 2]. Since that time, it has been made clear that the aerobic oxidation of succinate in mitochondria incubated with uncouplers is inhibited by oxaloacetate, and that this process is prevented or reversed by ATP most likely through the removal of oxaloacetate [3–7]. Indeed in kidney mitochondria, Papa et al. [7] found that the prevention of the inhibition of succinate oxidation by ATP correlated with a decrease and rise in the levels of oxaloacetate and phosphoenolpyruvate, respectively. However, these studies have been carried out in mitochondria in which oxidative phosphorylation was uncoupled and therefore it has not been shown conclusively whether succinate dehydrogenase is subject to the same metabolic control when active synthesis of ATP is taking place.

Very recently, Singer et al. [8] suggested that the activity of succinate dehydrogenase could be regulated by $CoQ_{10}H_2$ and ATP. Since $CoQ_{10}H_2$ and ATP activate succinate dehydrogenase [9–11], most probably Singer's hypothesis is correct. Nevertheless, in this communication it will be shown that the activity of succinate dehydrogenase of brain mitochondria incubated in conditions of active oxidative phosphorylation is regulated mainly by the levels of oxaloacetate and succinate and that, in our experimental conditions, endogenously generated ATP is of less importance in the regulation of the enzyme.

2. Materials and methods

Mitochondria from the brain of rats of our local colony were prepared in 0.25 M sucrose and 1 mM EDTA pH 7.3 as described previously [10]. Oxygen uptake was measured with a Clark type electrode (Yellow Springs Instrumental Co.) in the conditions indicated in the respective figures.

3. Results

Fig. 1A shows that brain mitochondria have a state 3/state 4 respiratory ratio of 5.1 with malate–glutamate as oxidizable substrates; on the other hand, with succinate, ADP induces an initial state 3 rate that is gradually inhibited to state 4 respiratory rate or lower. This latter rate of oxygen uptake is not increased by ATP, 2,4-dinitrophenol or both (fig. 1B). Traces C and D of fig. 1 show that oxaloacetate is responsible for the inhibition of coupled oxidation of succinate since glutamate plus arsenite and pyruvate, which remove oxaloacetate through the transamination and condensing reactions respectively, release the inhibition. This release of succinate oxidation is coupled to ATP formation since oligomycin decreases the rate of oxygen uptake to the original state 4 rate (trace C).

Since oxaloacetate is a competitive inhibitor of succinate dehydrogenase [12], it was investigated whether in coupled mitochondria, succinate could overcome the inhibiting effect of oxaloacatate and also whether the concentration of succinate affected the enzymatic activity independently of oxaloacetate formation. The

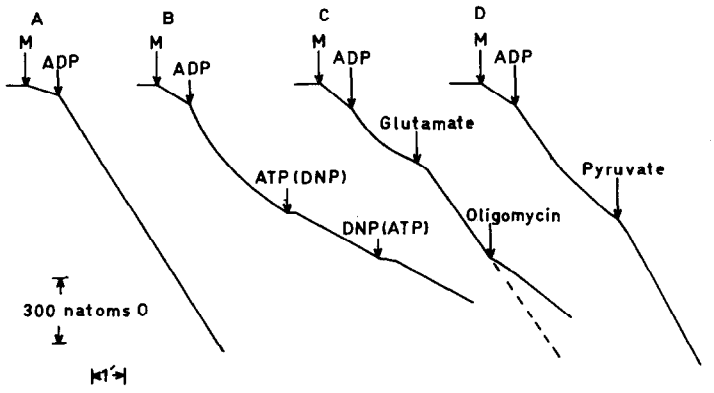

Fig. 1. The aerobic oxiation of succinate by brain mitochondria. Mitochondria (7 mg of protein) were incubated in 10 mM phosphate, 8 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.3) and 5 mM glutamate and 5 mM malate in trace A, and 5 mM succinate in traces B, C and D. The reaction was started by the addition of the mitochondria (M), followed by the addition of 2.2 mM ADP where shown. In B, 3.6 mM ATP and 10^{-4} M DNP were added as shown; the inverse order of addition (in parenthesis) resulted in the same trace. The mixture in trace C contained 10 mM arsenite from the beginning of the experiment; 10 mM glutamate, and 5 μg of oligomycin were added as shown; the dashed line indicates the polarographic trace without added oligomycin. In trace D, 10 mM pyruvate was added where indicated. The respiratory rates with pyruvate and glutamate + arsenite were 0.010 and 0.011 natoms min^{-1} mg^{-1}, respectively.

polarographic traces of fig. 2, A and B, were obtained by incubating mitochondria in the presence and absence of rotenone at various concentrations of added succinate. Since rotenone inhibits the oxidation of NADH [13] and thus the formation of oxaloacetate from malate, the traces obtained with rotenone indicate the effect of succinate concentration on the aerobic oxidation of succinate without the influence of oxaloacetate, while the traces obtained in the absence of rotenone are a reflection of succinate concentration as influenced by oxaloacetate formation. In both conditions the results show that, as the concentration of succinate is increased, the longer the time required for the appearance of the inhibited state, and that at equal concentrations of succinate, the time required for the appearance of the inhibited state is shorter when oxaloacetate formation is taking place than when the generation of oxaloacetate is precluded.

The experimental results up to this point indicate that both succinate and oxaloacetate levels are important regulatory factors of the coupled oxidation of succinate. Nevertheless, in an uncoupled system, ATP has been reported to reverse and to prevent the oxaloacetate induced inhibition of succinate oxidation [3–7, 10] and furthermore ATP directly activates succinate dehydrogenase [10, 11]. Thus it was necessary to investigate to what extent ATP generated in oxidative phosphorylation affected the inhibition of the coupled aerobic oxidation of succinate. This has been judged by a comparison of the polarographic traces of the aerobic oxidation of succinate by mitochondria incubated in the presence and absence of an ATP trap (fig. 3). Apparently, the role of ATP in supporting succinate oxidation is only of limited value (fig. 3) since the time required for the appearance of the inhibited state is only a little longer when ATP is not transformed into glucose-6-phosphate.

4. Discussion

The inhibition of the aerobic oxidation of succinate has been repeatedly observed in mitochondria incubated in uncoupled conditions [3–7, 10, 14], most probably this is due to the fact that in the presence of uncoupler, the level of ATP is extremely low, thereby facilitating the inhibiting action of oxaloacetate. Therefore, it is of interest that the aerobic oxidation of succinate in brain mitochondria is inhibited by oxaloacetate in conditions of active phosphoryla-

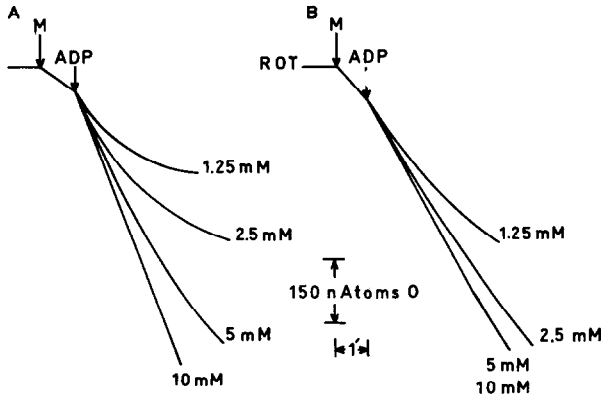

Fig. 2. Effect of succinate concentration on the aerobic oxidation of succinate of brain mitochondria. The oxygen uptake of mitochondria incubated separately with the indicated concentrations of succinate are shown. The rest of the incubating conditions were as in trace B of fig. 1. In B the experimental mixtures contained 7 μM rotenone (Rot) from the beginning of the experiment; the oxygen uptake pattern with 5 and 10 mM succinate was identical.

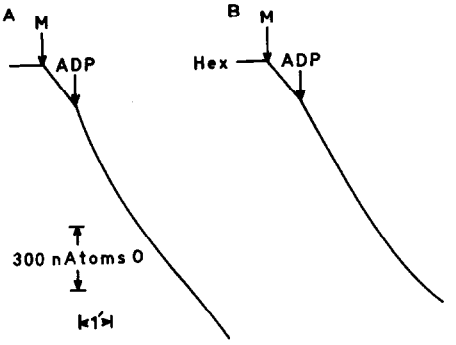

Fig. 3. Effect of ATP on the aerobic oxidation of succinate of brain mitochondria. The incubating conditions were as in fig. 1, trace B, except that in B the mixture contained 5.5 mM glucose and 10 units of hexokinase.

tion. Moreover, this inhibition is not affected by added ATP which, in the uncoupled state, reverses oxaloacetate inhibition [3–7, 10] and directly activates succinate dehydrogenase [10, 11]. In order to explain this apparent discrepancy, the following factors may be evoked:

First, brain mitochondria lack the reaction for removal of oxaloacetate in an energy dependent form; indeed, brain mitochondria are largely devoid of phosphoenolpyruvate carboxykinase [15], an enzyme that has been demonstrated to be an important regulatory factor of oxaloacetate levels [7].

Second, in our experimental conditions we have employed relatively large concentrations of ADP which would favour strongly the uptake of ADP and the release of ATP by the mitochondria through the adenine nucleotide carrier [16, 17]. In this respect, if Singer's hypothesis is correct on ATP being a regulatory factor of succinate dehydrogenase [8], the affinity for ATP of the enzyme incubated in coupling conditions would have to be higher than that of the carrier.

In our experimental conditions, we have observed that the coupled oxidation of succinate can be induced to an optimum rate either by removal of oxaloacetate or by increasing substrate concentration. Therefore, in the light of the present findings, and in agreement with Singer et al. [8], we think that succinate dehydrogenase is subject to metabolic control by ATP and $CoQ_{10}H_2$ but that the model of regulation should also include oxaloacetate to succinate ratios, as well as ADP and ATP levels as controlled by the adenine nucleotide carrier.

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