AN ATP REQUIREMENT FOR SUCCINATE-LINKED DPN REDUCTION[1]

Britton Chance and Bunji Hagihara[2]

Johnson Research Foundation
University of Pennsylvania
Philadelphia, Pennsylvania

Received June 24, 1960

In previous studies (Chance and Hollunger, 1960), it was pointed out that succinate induces an increased reduction of pyridine nucleotide, particularly in heart and kidney mitochondria. An energy requirement for this reaction was deduced from the fact that the reduction is found to be sensitive to uncoupling agents and from observations of recovery metabolism that follows the reduction of pyridine nucleotide in mitochondria by succinate addition. Previous attempts to inhibit partially the reduction of pyridine nucleotide by uncoupling agents and then to drive the reaction to completion by the addition of ATP without removal of the uncoupler were unsuccessful. It is now found that one or two days aging of pigeon heart mitochondria leads to a readily demonstrable requirement for ATP in pyridine nucleotide reduction. This paper describes the acceleration of this reduction in aged pigeon heart mitochondria by directly added ATP[3] or by ATP formed from phosphorylation of added ADP. These observations provide important and direct support for an energy requirement for succinate-linked pyridine nucleotide reduction. Such a reaction has the over-all properties predicted by Krebs and Kornberg (1957).

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1 This research has been supported in part by a grant from the National Science Foundation.

2 Permanent address: Department of Biology, University of Osaka, Japan.

3 The ATP activation of succinate utilization in DNP-pretreated rat liver mitochondria has been observed by Dr. G. R. Williams (1960) and also by Dr. M. Klingenberg (personal communication) who obtained similar results in recent experiments with Dr. Lars Ernster.

Materials and Methods. These are described in the preceding communication (Chance and Hagihara, 1960) and in the figure legend.

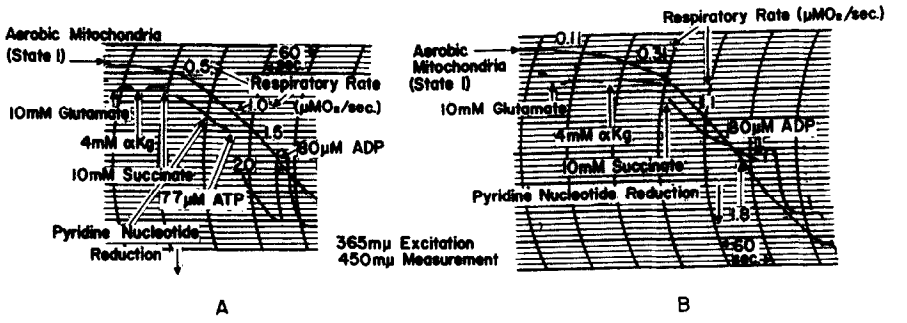

Fig. 1. Combined polarographic and fluorometric recordings illustrating the effects of ATP addition and ADP phosphorylation on respiration and pyridine nucleotide reduction in pigeon heart mitochondria. The data were recorded on separate charts which were superimposed and the fluorometric recording traced onto the chart for the polarographic recording. The respiratory rates in μM O_2/sec. are printed adjacent to the relevant portions of the platinum microelectrode traces. The times of addition of reactants and their concentrations are indicated on the diagram. Breaks in the fluorometric trace represent an interruption of the recording by the addition of the reagents. Mitochondria suspended in aerobic, Mg^{++}-free medium containing 0.23 M mannitol, 0.07 M sucrose, and 0.02 M phosphate, pH 7.2. Optical path, 10 mm. 26° C. (Expt. 97e).

Experimental Results. Fig. 1A shows the respiratory rates and pyridine nucleotide reduction occurring in a suspension of pigeon heart mitochondria. No measurable respiration is induced by glutamate addition, although a small downward deflection of the fluorescence trace indicates a small reduction of pyridine nucleotide. The addition of α ketoglutarate causes a slight acceleration of respiration to 0.25 μM O_2/sec., but no measurable pyridine nucleotide reduction. Succinate addition causes, in the first 30 sec., a doubling of the respiratory rate and two minutes later the respiratory rate has

again doubled (1 μM O_2/sec.). The downward deflection of the fluorescence trace proceeds slowly over the course of a minute and a half, when 77 μM ATP is added. As soon as the recording is resumed after the stirring artifact has subsided, it is seen that the fluorescence intensity is nearly doubled. In the next 30 sec., the fluorescence reaches a value which is roughly 3 times that obtained in the absence of ATP. The respiratory rate is slightly accelerated in this interval and reaches a value of 1.5 μM O_2/sec. At this point, 80 μM ADP is added to cause the state 4-to-3 transition. The respiratory rate increases to 2 μM O_2/sec. and the reduction of pyridine nucleotide is diminished by approximately 50 percent. When the added ADP has been phosphorylated, the reduction of pyridine nucleotide increases to the value prior to adding ADP. This experiment illustrates the slow initiation of respiratory activity and of pyridine nucleotide reduction by succinate and the acceleration of both these processes by the addition of ATP.[3]

In a similar recording with another aliquot of pigeon heart preparation (Fig. 1B), the events are similar up to and including the addition of succinate and the slow increase of pyridine nucleotide reduction. In this case, however, the reaction is allowed to run for a little more than two minutes and it is seen that the extent of reduction achieved does not equal that obtained 30 sec. after the addition of ATP in Fig. 1A. ADP is added and the slight oxidation of pyridine nucleotide is observed. There follows a large and rapid reduction of pyridine nucleotide after the ADP is exhausted. Thus the phosphorylation of ADP to ATP has likewise caused a rapid reduction of pyridine nucleotide. If the kinetics of the reduction following the addition of ATP in Fig. 1A are compared with the reduction following the phosphorylation of 80 μM ADP, it can be seen that the latter is more effective. In control experiments, ATP is added before succinate and no reduction of pyridine nucleotide is observed until succinate is added.

Discussion. These experiments give further evidence for the role of high-energy intermediates in the reduction of pyridine nucleotide in the

presence of succinate. An ATP acceleration for the reaction can be demonstrated in mitochondria that are sufficiently aged that neither glutamate nor α ketoglutarate produces sufficient internally generated high-energy intermediates to cause a rapid reduction of pyridine nucleotide on addition of succinate. The data suggest that the reduction is accelerated more effectively by the phosphorylation of ADP than by external ATP.

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