DOES THE ACTIVATION OF SUCCINATE DEHYDROGENASE AFFECT THE PHOSPHORYLATING SYSTEM IN SUB MITOCHONDRIAL PARTICLES?

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1. Introduction

Succinate dehydrogenase (SDH) is known to undergo activation and deactivation reactions [1]. These reactions were observed in intact mitochondria, submitochondrial particles or purified enzyme. It had been shown that the activation of SDH accelerates all of the reactions in which SDH participates: succinoxidase [2], the rate of the reduction of quinones and the *b* cytochromes, and the energy linked NAD reduction by succinate [3]. The modulation of SDH activity was observed to take place in intact mitochondria during transition from one metabolic state to another [4]. Based on these observations it was suggested that the role of activation is to help in the selection of the substrate which is oxidized by the mitochondria [5]. This hypothesis was recently supported by the observation that active SDH can act as an operational inhibitor of NADH oxidase activity, in mixed substrate respiration [6].

Recently Kupriyanov and Saks [7] observed that under conditions where SDH is known to be activated, the energy linked reverse electron transport from ascorbate + TMPD to NAD was accelerated. In this reaction SDH does not participate as an electron carrier, thus they generalized the stimulating effect of activation on the rate of energy linked reverse electron transport; assuming that the conformation change associated with the activation of SDH changes the properties of adjacent enzymes in the membrane i.e. the coupling factors, or even the properties of the whole membrane. As a result of such changes energy-linked reactions are more efficient, and the sensitivity of the coupling system against tryptic digestion decreases. It had been shown by Luzikov et al. [8] that energising the sub-mitochondrial membrane by ATP or by electron flux in presence of phosphate acceptor increases the resistance of the phosphorylating system to tryptic digestion. Such increased resistance was observed after subjecting the particles to conditions that are known to activate the SDH [7].

The suggestion that a conformation change of one enzyme in the membrane can propagate changes in other components of the membrane merits further investigation. Until now all of the reactions, the rates of which were dependent on the activation level of SDH, were measured at various activation levels and a correlation between the two parameters was observed. Succinoxidase and the rate of reduction of the *b* cytochromes depend hyperbolically on the activation of SDH, and near maximal rates are attained when SDH is about 60% active. On the other hand, the energy-linked NAD reduction by succinate responded linearly to the activation level of SDH [3]. Consequently we investigated the rate of reduction of NAD by ascorbate + TMPD at various levels of SDH activity, especially because in the previous report [7] the activation of SDH was not controlled but the enzyme was assumed to be active.

2. Materials and methods

ETPH were made according to Hansen and Smith [9].

Activation of SDH was obtained by incubation of ETPH (2 mg/ml) with 1 mM malonate (30°C) and samples were drawn, cooled and washed by centrifugation [3]. The activity of SDH was measured at 13°C

as described previously [3], reverse electron transport was measured as described by Ernster and Lee [10] with succinate or with TMPD and ascorbate (0.3 mM each). Trypsin digestion was carried with 15 μg trypsin/ml in a 15 mg ETPH protein/ml. The digestion was stopped by removing samples from the digestion mixture and quenching it by lima bean trypsin inhibitor 5 μg/μg trypsin.

3. Results and discussion

When studying the effect of activation of SDH on the reduction of NAD by TMPD + ascorbate, an internal standard for the other effects of the activation of SDH is a helpful tool. Since the energy-linked reduction of NAD by succinate is sensitive to the activation of SDH, and the ATP is the driving force for the two reactions, we compared the effect of activation on the two energy linked reactions. Fig. 1 shows the variation

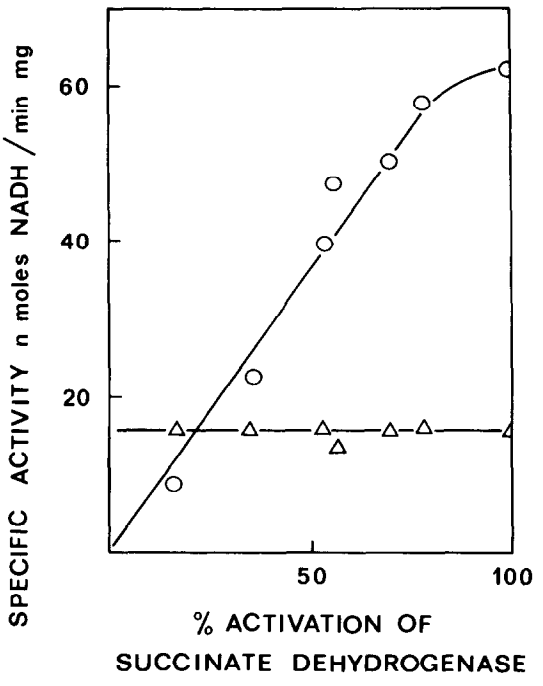

Fig. 1. The dependence of energy-linked reduction of NAD on the activation level of SDH. ETPH were activated to various levels of activity and the reduction of NAD in the presence of ATP (2 mM) was measured with succinate (○—○) or TMPD + ascorbate (△—△) as electron donors.

of the rates of reduction of NAD by succinate or TMPD + ascorbate at various levels of SDH activity. These results indicate that the stimulation by activation of SDH is limited only to the reaction in which SDH participates as a carrier.

In order to check whether the lack of stimulation of the reductions by TMDP + ascorbate was due to non optimal conditions for the assay we repeated the experiment under the specified conditions [7], at varying ascorbate and TMPD concentrations, and after preincubation of the ETPH with ATP [11]. It can be seen from fig. 2 that the reduction of the NAD by TMPD + ascorbate commence immediately after the initiation of the reaction by ATP and the rate is linear with time so that the initial rate can be calculated without difficulties. The lag period observed in the reactions when succinate is the electron donor is due to activation in situ of the SDH, and is observed only in preparations in which SDH is only partially activated. When the SDH is fully activated the lag period disappears.

The fact that activation of SDH did not stimulate the reduction by TMPD + ascorborate, led us to re-examine the effect of SDH activation on the sensitivity of the phosphorylation system to trypsin. According to Kupriyanov and Saks [7] activation of SDH protects energy-linked reduction of NAD by succinate against tryptic digestion. This protection is visualized by a lag period during which the activity is not affected by trypsin. In non activated particles, the activity falls immediately after the addition of the trypsin.

In our experiment we lowered the trypsin concentration from 18 μg/ml to 15 μg/ml so as to prolong the lag period. At certain times during the digestion samples were withdrawn and quenched with lima bean trypsin inhibitor. At this step we deviated from the conditions of Kupriyanov and Saks [7] by allowing the SDH to be activated before we assayed for the energy-linked NAD reduction by succinate. By that, we digested either active or non active ETPH, but assayed them when they were fully activated. This procedure eliminates variations by the in situ activation as depicted in fig. 2.

The results given in fig. 3 are compatible with the conclusions of Luzikov et al. [9] where it calls for ATP or electron flux plus phosphate acceptor to protect the phosphorylating system against trypsin.

Our results clearly demonstrate that an energy-linked

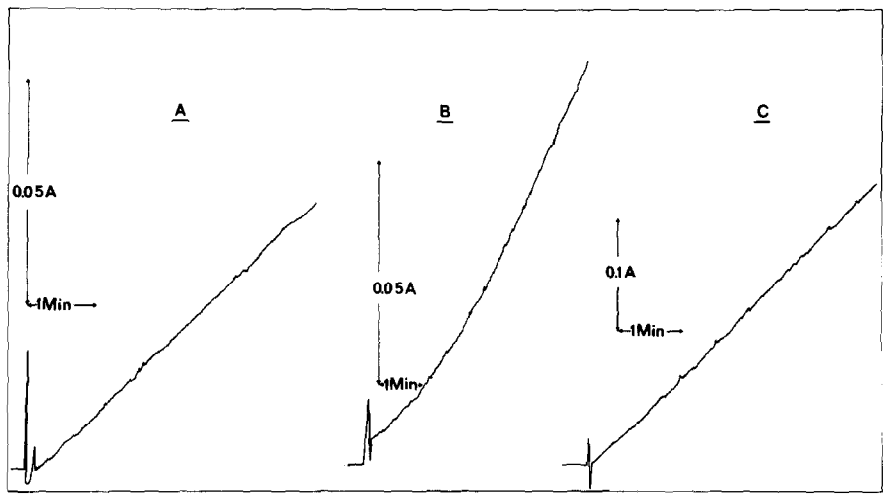

Fig. 2. Recorder tracing of the energy linked reduction of NAD. A) Reduction by TMPD (0.3 mM) + ascorbate (0.3 mM). The reaction was started by ATP (2 mM), ETPH (0.15 mg/ml). B) Reduction by succinate with non activated ETPH (0.15 mg/ml). C) Reduction by succinate carried with fully activated ETPH (0.15 mg/ml). Note the difference in scale in the three runs.

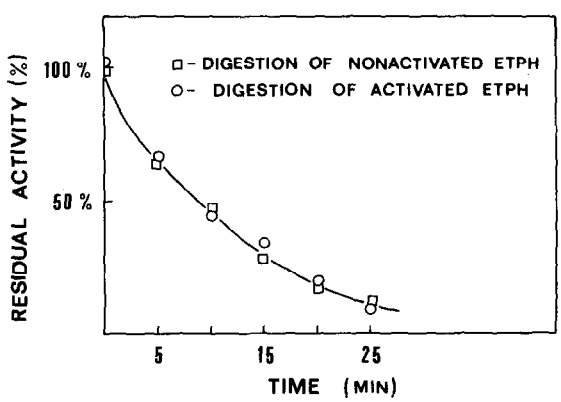

Fig. 3. The decay of energy-linked NAD reduction by succinate during tryptic digestion. ETPH activated (○—○—○) or non activated (□—□—□) (15 mg/ml in 0.18 M sucrose, 50 mM Tris–acetate, 5 mM $MgSO_4$ pH 7.4) were digested by crystalline trypsin (C.F. Boehringer) 15 μg/ml. At indicated times samples of 0.5 ml were withdrawn, quenched by lima bean trypsin inhibitor and cooled to 0°C. From these, samples for the reduction of NAD were taken. Before assay the ETPH were allowed to stay in presence of KCN, succinate and NAD for 10 min at 30°C and the reaction was started by ATP. It was verified that after such treatments the SDH was fully activated. (100% = 95 nmol NADH per mg protein per min).

redox reaction that does not include SDH in its pathway, is not affected by the activation of SDH. Furthermore, by controlling possible variables, it was shown that activation of SDH does not confer to the coupling membrane changes similar to those obtained by energising it [8]. We wish to stress that although activation of SDH might cause changes in the membrane where it is embedded, as proposed by Kupriyanov and Saks [7], their experiments do not provide sufficient proof for this assumption.

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