THE STEADY STATE ACTIVITY OF SUCCINATE DEHYDROGENASE IN THE PRESENCE OF OPPOSING EFFECTORS*†

I. THE EFFECT OF L MALATE AND CoQH₂ ON THE ENZYMIC ACTIVITY

Menachem GUTMAN and Nitza SILMAN

The Department of Biochemistry, The George S. Wise Center for Life Sciences, Tel-Aviv University, Tel-Aviv, Israel

(Received August 8, 1974)

Summary

Succinate dehydrogenase is subjected to positive and negative modulation. The negative modulators oxaloacetate and D- or L-malate transform the enzyme into a nonactive complex in which oxaloacetate is bound. The deactivation by malate involves its oxidation by the succinate dehydrogenase which then deactivates the enzyme.

In the present study we measured the activity of succinate dehydrogenase in the presence of two opposing effectors, L-malate as deactivator and CoQH₂ as an activator. With these opposing effectors present, the catalytic activity of succinate dehydrogenase assumes a steady state, the level of which is a function of the concentration of the two effectors. At low concentration of L-malate all of the succinate dehydrogenase activity is protected by CoQH₂, while at saturating malate concentrations only 60–70% of activity is protected.

Kinetic analysis of the approach to the steady state indicates that the protective effect of CoQH₂ is not due to its activator property but due to its ability of reduce the enzyme. This was verified by carrying out a redox titration of succinate dehydrogenase activity in the presence of L-

malate. A redox active component was characterized with E' = +25 mV and n = 1.8. When this component is reduced, L-malate cannot deactivate the succinate dehydrogenase, but when in the oxidized state the enzyme is susceptible to such deactivation. It is proposed that this group participates in the regulation of the activity of succinate dehydrogenase in the mitochondria.

Introduction

The activation of SDH by succinate and its analogues was first described by KEARNEY. In recent years the family of activators of this enzyme was expanded: physiological compounds like CoQH₂, ATP, ITP or certain anions at pH 6–6.5 all activate the enzyme. Variations in SDH activity were observed in intact mitochondria, in state 3 the activity of SDH declined while in state 4 the enzyme was activated. It was proposed, that the function of these variations was to lower in active mitochondria, the electron flux from succinate to oxygen, and by this to enhance the oxidation of NADH with the concomitant increase in the yield of ATP.

It was noted that the deactivation of SDH in mitochondria was very rapid, as if affected by some deactivator. The studies of KEARNEY and

† Abbreviations: SDH = succinate dehydrogenase. PMS – phenazine methosulfate. DCPIP – dichloro phenol-indophenol. CoQ (H₂) Ubiquinone 10 oxidized and reduced respectively. OAA oxaloacetate.
her colleagues demonstrated that OAA, D-malate and L-malate are deactivators of the enzyme. The deactivation of SDH by malate was due to its oxidation by SDH to OAA, a reaction previously observed. In rat-heart mitochondria in state 4, 50–80% of SDH is active. At these conditions the intramitochondrial concentrations of L-malate and OAA are 230 μM and 35 μM respectively, concentrations which are high enough to deactivate the SDH. Apparently the activators present in the mitochondria; succinate, CoQH₂ or ATP, are able to counteract the effects of the L-malate or the OAA.

In the experiments described in this publication we investigated the mechanism by which a combination of an activator and deactivator maintains the enzyme activity in a steady state.

At first we had to choose the most convenient combination of enzyme preparation, activator and deactivator. The simplest model, soluble purified enzyme, is not suitable because it is activated only by one type of physiological activator—the substrate. Furthermore, by detaching the enzyme from the respiratory system we isolate the phenomenon from all its metabolic interlocks operating in mitochondria. On the other hand, mitochondria are too complicated for our studies due to the presence of the matrix enzymes and the coupling mechanism. Consequently, submitochondrial particles (ETP or ETPₙ) are the materials of choice. The respiratory system of these particles is intact, they are free of matrix enzymes of permeability barriers and are activated by CoQH₂, succinate or anions.

In the present study we used CoQH₂ as an activator for the following reasons. Of the known physiological activators namely succinate, CoQH₂ and ATP, ATP cannot be employed as it activates only intact mitochondria. Succinate, is the natural choice, but both malate and OAA are competitive inhibitors of SDH as well as deactivators. Thus whilst studying the effect of malate or OAA versus succinate, we have to account both for activation-deactivation reactions as well as for the competitive relationships between the effectors. Therefore CoQH₂ was chosen as the most suitable activator. It can be generated by NADH and antimycin and, with the proper choice of antimycin concentration, the reduction level of CoQ can be kept constant for a considerable length of time (15–20 minutes).

The decision to use CoQH₂ as activator precludes the use of OAA as deactivator. As long as CoQH₂ is reduced by NADH, traces of malic dehydrogenase will reduce the OAA to malate. Consequently we used CoQH₂ and L-malate for our studies.

Materials and Methods

Beef heart mitochondria were prepared according to RINGLER et al. ETPₙ were prepared by the method of HANSEN and SMITH. Alcohol dehydrogenase was a Worthington preparation 345 μg/mg.

Succinate dehydrogenase activity was measured spectrophotometrically using the PMS-DCPIP method as modified by GUTMAN et al. In order to avoid activation in situ, the assay was carried out at 13 °C.

NADH generating system included NAD (330 μM), ethanol (100 mM), alcohol dehydrogenase (10 μg), and semicarbazide (3.3 mM), all added to 0.18 M sucrose, 50 mM HEPES, 5 mM Mg-acetate pH 7.4, 30 °C. This system could keep the NADH fully reduced in presence of 1 mg/ml of ETPₙ. The oxygen uptake measured with ETPₙ and the NADH generating system was identical to that measured with 330 μM NADH (1.2–1.5 μmoles NADH/min/mg).

Steady state reduction of CoQ was achieved by suspending ETPₙ at 1 mg/ml in the NADH generating system at 30 °C. Antimycin in various amounts was added in order to alter the steady state reduction of the quinone. Aerobiosis was achieved by vigorous stirring and verified by oxygen electrode. From the above mixture samples of 1 ml were withdrawn and injected into ice cold methanol-hexane 2:3 v/v. The method of KROGER and KLINGENBERG was used for the separation of the quinone and its spectrophotometric determination.

Determination of steady state activity of SDH. ETPₙ, washed by 0.25 M sucrose, 50 mM tris-acetate, 5 mM MgSO₄ pH 7.4, were suspended in 0.18 M sucrose, 50 mM tris-acetate, 5 mM MgSO₄ pH 7.4, to 20 mg/ml and activated by 1 mM malonate 30 min at 30 °C. The particles were then spun down and resuspended in the same buffer at 10–17 mg/ml. These particles were diluted in the