Correlation of the Effects of Citric Acid Cycle Metabolites on Succinate Oxidation by Rat Liver Mitochondria and Submitochondrial Particles

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Abstract

1. Succinate dehydrogenase is inhibited by citrate and β-hydroxybutyrate in a complex manner, both in mitochondria and submitochondrial particles. Kinetics of inhibition in the particles points to a competitive component in the mechanism involved.

2. Pyruvate, α-ketoglutarate, malate, and glutamate stimulate oxidation of succinate by mitochondria.

3. Stimulation by α-ketoglutarate and glutamate is not influenced by the presence of rotenone.

4. Stimulation by pyruvate is higher in the absence of rotenone and increases significantly in the presence of K+ and valinomycin. Pyruvate supplies in mitochondria reducing equivalents for malate dehydrogenase operating in the reverse direction—reduction of oxaloacetate to malate.

5. Stimulation by malate is higher in the presence of rotenone.

Introduction

It is known that substrates of various respiratory chain-linked flavoproteins inhibit the oxidation of each other by mitochondria or particulate preparations. Succinate inhibits DPNH, choline, α-glycero-phosphate oxidase activity and vice versa [1, 2, 3, 4, 5, 6]. As turnover of succinate dehydrogenase (EC 1.3.99.1), 18,000/min., is much higher than the activity of the respiratory chain it feeds [1, 7] the uncontrolled succinate oxidation would restrict the oxidation of DPNH.
The regulatory mechanism for SDH in heart muscle was postulated by Singer et al. [1, 8, 9, 10, 11, 12, 13, 14] in which CoQH₂, succinyl-CoA and ATP are the regulatory factors. SDH activity depends on the metabolic state of the mitochondria and declines in the state 4→3 transition and on adding uncouplers [9, 15, 16]. Deactivation of SDH in state 3 is associated with the change in CoQ/CoQH₂ ratio which can be as high as tenfold in transition from the controlled to the active state and almost complete oxidation of CoQH₂ occurs in the presence of uncouplers [17]. In addition the ATP generated, due to its activating action on SDH in mitochondria [1, 9, 18], may also play a role in determining what fraction of the enzyme is in the activated state at any given moment. Thus competition between substrates involves electron transport from the dehydrogenase to the respiratory chain, presumably the CoQ pool. Ideal conditions for fully active SDH in mitochondria are also those for reversed electron transport.

This mechanism, however, takes into account only one aspect of the multiple types of control operating in mitochondria for SDH. The enzyme seems to be controlled by a variety of substrates and inorganic phosphate. Fumarate and malate are competitive inhibitors with less affinity for the enzyme than succinate (Kₐ respectively 2.6, 4.9 and 0.1 mM) [19, 20, 21]. Inorganic phosphate is weakly inhibitory (Kₐ 20 mM) and binds at the active site [22]. Oxaloacetate is highly competitive to succinate (Kₐ 4.5 μM) and also binds to the enzyme in a pseudo-irreversible manner at a 1:1 molar ratio [23, 24, 25]. The activity of SDH in soluble or membrane preparations can also be increased several-fold if the enzyme is preincubated with succinate, inorganic phosphate, fumarate or malonate [8, 26, 27, 28, 29]; the enzyme returns to the deactivated (unactivated) state upon removal of the activator [30].

Overall regulatory mechanisms for succinate oxidation in intact mitochondria by SDH are, thus far, not clearly understood [24, 25, 31]. The enzyme still seems to be affected mostly by oxaloacetate [25]. Investigations correlating effects of several metabolic substrates on succinate oxidation in mitochondria and submitochondrial particles are presented in this report. It was found that SDH is inhibited only by citrate and β-hydroxybutyrate in a complex manner, both in mitochondria and particles. Pyruvate, α-ketoglutarate, malate and glutamate stimulate oxidation of succinate by mitochondria. Stimulation by α-ketoglutarate and L-glutamate is not influenced by the presence of rotenone. Stimulation by pyruvate is higher in the absence of rotenone and that of malate is higher in the presence of rotenone. Results indicate that citrate and β-hydroxybutyrate inhibit oxidation of succinate affecting enzyme. Mechanism of action of other substrates is discussed in the light of data available in the literature: α-ketoglutarate and malate remove oxaloacetate by exchange translocation on dicarboxylate carrier;