Hypothesis

ON THE NEED FOR REGULATION OF SUCCINATE DEHYDROGENASE

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It has been known since 1955 that substrates and competitive inhibitors of succinate dehydrogenase activate the enzyme by a process which is believed to involve a conformation change in the enzyme [1, 2]. The activation is completely reversible, since on removal of the substrate the activated enzyme rapidly reverts to the unactivated (inactive) form [3]. This type of activation has been observed in soluble and particulate preparations, in inner membrane preparations and in mitochondria [2–4]. More recently a second type of reversible activation of the enzyme was discovered [5, 6]. It was noted that in inner membrane preparations (ETP or ETP_H) NADH also activates the enzyme reversibly. Studies with suitable inhibitors indicated that NADH itself is not the direct activator, but merely serves to reduce endogenous ubiquinone, so that the reduced quinone appears to be the immediate activating agent. This conclusion was verified by two experimental approaches: (1) reduced ubiquinone ($CoQ_{10}H_2$) is at least as good an activator as NADH in membrane preparations and (2) in pentane-extracted preparations, which are devoid of CoQ_{10}, NADH no longer activates, but succinate or malonate still do; on reincorporation of the CoQ_{10} activation by NADH is restored [5, 6]. Activation via $CoQ_{10}H_2$ is characterized by the same kinetic and thermodynamic parameters as by substrates, and thus might involve the same type of conformational alteration.

Since the reduced/oxidized CoQ_{10} ratio undergoes extensive changes in response to the metabolic state of mitochondria [7] and since at 37° both activation and deactivation are quite rapid, and proceed at about the same rate, it has been suggested that this type of reversible activation may have regulatory significance [5, 6].

The role of the activation of succinate dehydrogenase in tightly coupled mitochondria has been examined in rat heart and liver with surprising results [8]. It was observed that, besides activation by succinate and by substrates, which reduce CoQ_{10}, ATP, or a compound in equilibrium with it, also activates the enzyme, under conditions which preclude mediation by $CoQ_{10}H_2$ or substrate. Being oligomycin-insensitive, activation initiated by ATP also cannot involve components of the energy conservation apparatus.

On probing the question whether succinate dehydrogenase activity ever becomes rate-limiting in the operation of the Krebs cycle in mitochondria, it was observed that while the enzyme underwent rapid and major (up to 6-fold) changes in activity in response to the metabolic state, its activity never declined to a point where it became rate-limiting in the oxidation of NAD-linked substrates (e.g., pyruvate plus malate) [8]. Thus on going from the controlled to the active state, the addition of ADP causes deactivation of succinate dehydrogenase, accompanying the increased rate of respiration, but succinate dehydrogenase activity remains sufficient to keep up with the rate of oxidation of pyruvate + malate.

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The rapid deactivation of the dehydrogenase in the state 4 → 3 transition and by uncouplers [8] helps explain data in the literature that the steady state concentration of succinate is lower and the labeling of malate by ^{14}C-succinate higher in state 4 than in 3 [9, 10] and that uncouplers cause succinate accumulation [11].

The existence of three different mechanisms for the reversible activation of succinate dehydrogenase in mitochondria raises the question of the physiological purpose of this intricate control. It is the purpose of this communication to present a possible hypothesis concerning the need for regulation of succinate dehydrogenase.

Several years ago Wu and Tsou [12] and our laboratory [13–15] noted that substrates of the various respiratory chain-linked flavoproteins (NADH, succinate, choline, α-glycerophosphate) mutually interfere with the oxidation of each other. This mutual inhibition is not merely a question of exceeding the maximum capacity of the respiratory chain for electron transport, for in liver mitochondria, for instance, the simultaneous presence of a rapidly oxidized substrate (succinate) and a slowly oxidized one (choline) results in a *lower* O_2 uptake than with succinate alone. On inhibiting choline oxidase with Amytal, the high rate observed with succinate alone is then restored [14, 15]. This curious behavior suggested at the time [16] that the electron transport system can orient itself toward whichever flavoprotein is in the reduced form at a given moment, being thereby unavailable to accept electrons from other flavoproteins, a suggestion which appears to be consistent with recent work on conformation changes in membrane systems.

Extending this hypothesis and the experimental facts on which it was based to the present situation, it is quite clear that uncontrolled succinate dehydrogenase activity would restrict the reoxidation of NADH, provided that a sufficient flux of succinate exists to keep the dehydrogenase largely in the reduced state. Since succinate oxidation yields only 2 moles of ATP, whereas the three sites of NADH oxidation in the cycle yield collectively 9 moles, maximal rate of ATP synthesis would require some contraint on succinate dehydrogenase activity, so as to permit maximal rate of NADH oxidation; but ideally, the constraint should not be great enough for succinate dehydrogenase to become rate-limiting in the operation of this cycle. This condition appears to be met, since even on extensive deactivation, as in the state 4 → 3 transition, succinate dehydrogenase does not become rate-limiting in the oxidation of pyruvate + malate [8]. This is not surprising, in view of the high overall succinate dehydrogenase activity in mammalian mitochondria [17].

The proposed regulation would operate as follows. When the ATP/ADP ratio is low, and ATP synthesis is called for, the dehydrogenase would be deactivated, partly because of the low concentration of ATP (an activator), partly because reduced CoQ_{10} concentration is low in state 3. This permits increased rate of NADH oxidation, and, hence, increased ATP synthesis, triggered in part by the ADP (transition to state 3), in part by permitting the respiratory chain to deal with electrons originating from NADH, rather than succinate. During this phase (state 3) succinate should accumulate, as has been observed experimentally [9]. When the ATP/ADP ratio reaches a high level (state 4), succinate dehydrogenase would be turned on partly by accumulated succinate, partly by the high $CoQ_{10}H_2/CoQ_{10}$ ratio, partly by ATP, resulting in removal of the accumulated succinate.

In order for this hypothesis to be plausible, a requisite is that sources of succinate other than from α-ketoglutarate oxidation should exist; otherwise, the NADH-linked α-ketoglutarate oxidation step, the source of succinyl CoA, would itself limit succinate flux. It is known, however, that considerable amounts of succinate are formed from the oxidation of odd numbered fatty acids [18] and of branched chain amino acids and methionine [19] via methylmalonyl CoA.

This hypothesis of the physiological purpose of the multiple mechanisms for the regulation of succinate dehydrogenase may not be the only plausible one, and may not be cogent in all metabolic situations. Thus, activation of succinate dehydrogenase is reflected in an increase in reversed electron flow [6], and this process has been implicated in fatty acid chain elongation, and consequent storage of reducing equivalents in heart mitochondria [20]. This hypothesis does seem to fit known facts, however, and is presented here in the hope of stimulating discussion and further experimental work.

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