MULTIPLE CONTROL MECHANISMS FOR SUCCINATE DEHYDROGENASE IN MITOCHONDRIA

M. Gutman*, Edna B. Kearney, and T. P. Singer

Molecular Biology Division, Veterans Administration Hospital, San Francisco, California 94121 and Department of Biochemistry and Biophysics, University of California Medical Center, San Francisco, California 94122

Received May 27, 1971

SUMMARY. Succinate dehydrogenase (SD) in intact, respiring mitochondria undergoes activation and deactivation in response to the metabolic state of the mitochondria. Highest SD activity is observed in state 4 and lowest in state 2 or in the presence of uncouplers, while in state 3 the level of activation is intermediate and varies with the nature of the substrate. Transition from the active to inactive (unactivated) state of SD occurs in mitochondria with a lower energy of activation (10 Kcal/mole) than in soluble or membrane preparations (33 to 36 Kcal/mole). In addition to activation by succinate and $CoQ_{10}H_2$, the enzyme in mitochondria is uniquely activated by ATP (or a compound in equilibrium with it), a process which has not been previously observed in submitochondrial particles and is oligomycin-insensitive. In tightly coupled mitochondria reversible activation by all these agents may occur concurrently, but experimental conditions are described to study the action of each type of activator independently.

INTRODUCTION

The activity of succinate dehydrogenase (SD) in soluble or membranal preparations can be increased several-fold if the enzyme is preincubated with succinate or competitive inhibitors (1) and the enzyme returns to the deactivated (unactivated) state upon removal of the activator (2). The activation process is characterized by a high energy of activation (33 to 36 Kcal/mole) and is thought to be a conformation change in protein.

We have recently reported that in membrane preparations reduced CoQ_{10} and substrates capable of reducing endogenous CoQ also activate SD in the same manner (3, 4). Of the two types of activator mentioned succinate is not likely to play a major role in controlling SD activity in intact mitochondria, since its concentration does not vary greatly in different metabolic states, but the reduced/oxidized CoQ_{10} ratio may well exert a regulatory effect, since the % reduction of CoQ_{10} may change 10-fold in transitions between metabolic states (5) with consequent major changes in SD activity (3, 4).

---

*On leave of absence from Tel-Aviv University, Israel.

If the concentration of reduced CoQ_{10} regulated SD in mitochondria, the activity of the enzyme should be higher in state 4 than state 3. This is in accord with reports that the steady state concentration of succinate is lower and the labeling of malate by ^{14}C-succinate higher in state 4 than 3 (6, 7). Control of SD activity by $CoQ_{10}H_2$ may also explain the findings that uncouplers cause succinate accumulation (8) and in the presence of antimycin block the reduction of quinones by succinate (9), since uncouplers lead to nearly complete oxidation of $CoQ_{10}H_2$ with consequent deactivation of SD.

The experiments reported here demonstrate very rapid and extensive changes in SD activity in intact mitochondria compatible with metabolic regulation. The activation-deactivation in SD observed in mitochondria is in accord with changes in the reduced/oxidized CoQ_{10} ratio which are known to occur in metabolic transitions (5). The experiments further suggest a more direct role for ATP in activating SD in intact mitochondria which may, in part, explain the known effect of ATP in augmenting succinoxidase activity in such preparations (10).

MATERIALS AND METHODS

Rat liver mitochondria were prepared by the method of Schnaitman and Greenawalt (11); rat heart mitochondria were prepared and respiration measured with an O_2 electrode at 30° as per Pande and Blanchaer (12). Throughout this paper SD activity refers to the PMS-DCIP assay (13) conducted at 15° with 60 mM KP_i, 1 mM KCN, 52 μM DCIP, and 0.1 mM PMS present. $CaCl_2$ (0.75 mM) and phospholipase A (10 μg, 600 units/mg) were included to assure free penetration of PMS (13). Spurious activation by the substrate was avoided by the use of low temperature and by adding succinate as the last component in the assay. Aliquots removed from the O_2 electrode chamber for SD assay were immediately assayed.

RESULTS

Figs. 1 and 2 demonstrate the level of succinate dehydrogenase activity during transitions in metabolic states in rat liver mitochondria. The state 4 $\rightarrow$ 3 transition is shown in Fig. 1 with α-ketoglutarate as substrate. There is a rapid decline in SD activity accompanying the increased respiration elicited by ADP. Deactivation is more pronounced in the absence of exogenous substrate (Fig. 2, left side). Similarly, the release of respiratory control by DNP causes concurrent deactivation of SD (Fig. 2, right side). The decline in SD activity in these experiments is not an inactivation, since the addition of succinate (in the presence of cyanide) caused a rapid return of the original activity.

Deactivation by both ADP and the uncoupler are in accord with the lowering of the $CoQ_{10}H_2/CoQ_{10}$ ratio known to occur under these conditions (5). The more extensive de-

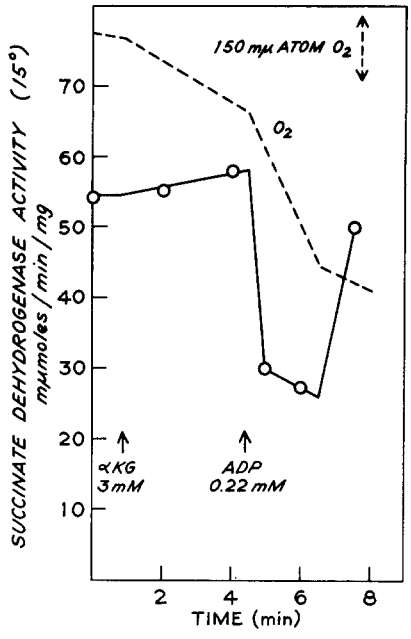

Fig. 1. Variation of SD activity in rat liver mitochondria in states 3 and 4. Conditions: 2.55 mg protein in 1.5 ml of 230 mM mannitol–70 mM sucrose–20 mM Tris–5 mM KP_i–5 μM EDTA, pH 7.4, at 30°. Dashed line indicates respiration on 3 mM α-ketoglutarate, solid line the SD activity (at 15°) of aliquots removed at intervals and assayed immediately.

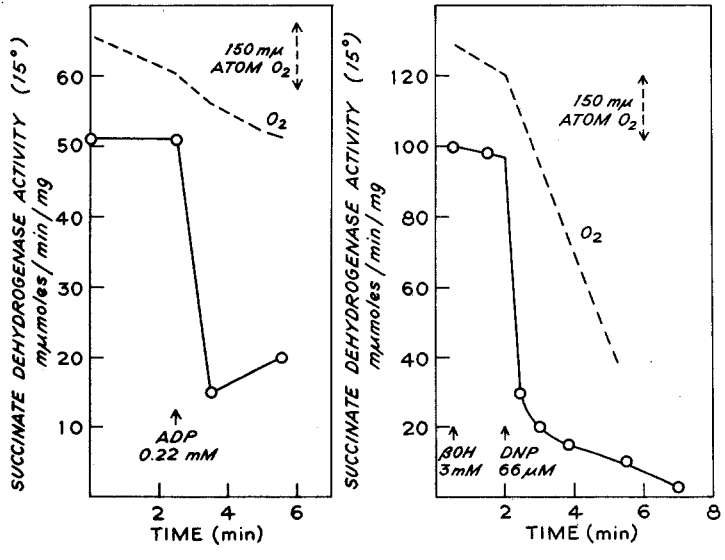

Fig. 2. Left side: variation of SD activity of rat liver mitochondria on endogenous substrate. Conditions were as in Fig. 1, except that the protein concentration was 3.5 mg/ml and no substrate was added. Right side: effect of 2,4 dinitrophenol on SD activity of rat liver mitochondria respiring on β-hydroxybutyrate. Conditions were as in Fig. 1 except that the protein concentration was 2.3 mg/ml.

activation elicited by DNP than by ADP (Fig. 2 vs. Fig. 1) suggested that, in addition to causing oxidation of $CoQ_{10}H_2$, the effect of DNP on the activation state of SD might be in part due to prevention of ATP synthesis. In other words, the presence of ATP might directly affect the state of activation of SD. In order to study separately activation involving $CoQ_{10}H_2$ and effects due to ATP itself, it was desirable to use a simpler system where prior deactivation was not necessary. Rat heart mitochondria were used, since in these SD is largely in the deactivated state, in contrast to rat liver mitochondria.

Fig. 3 shows the activation of SD initiated by pyruvate + malate in heart mitochondria in the presence of cyanide. It is seen that the same final activation was reached as by direct activation with succinate. In view of the high cyanide concentration present, extensive reduction of the various respiratory chain components unaccompanied by ATP production

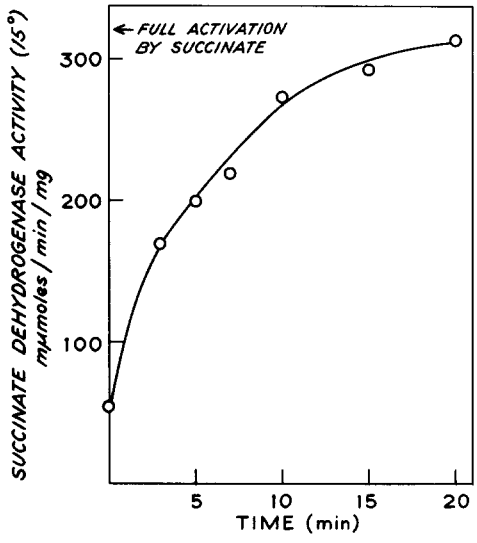

Fig. 3. Activation of SD in rat heart mitochondria initiated by pyruvate + malate in the presence of KCN. The mitochondria were suspended in the buffer given in Fig. 1 at 0.48 mg protein/ml; 1 mM KCN was added, and activation was initiated by 3 mM each of pyruvate and malate at 15°. At intervals aliquots were removed and immediately assayed for SD activity (shown on ordinate). The O_2 uptake of the preparation on pyruvate + malate at 30° was 500 mμatoms O_2/min/mg and the respiratory control ratio 6.

---

may be expected under these conditions. Since among respiratory chain components only $CoQ_{10}H_2$ has been so far demonstrated to activate SD and since ATP production is absent, the data are consistent with activation of SD by way of reduced quinone.

The experiment of Fig. 4, on the other hand, was designed to explore activation by ATP which is not mediated by $CoQ_{10}H_2$ or succinate. In this experiment no substrate was

added, ATP hydrolysis was blocked by oligomycin and the reduction of CoQ_{10} by endogenous substrate inhibited with piericidin A. It is seen that ATP, in the presence or absence of

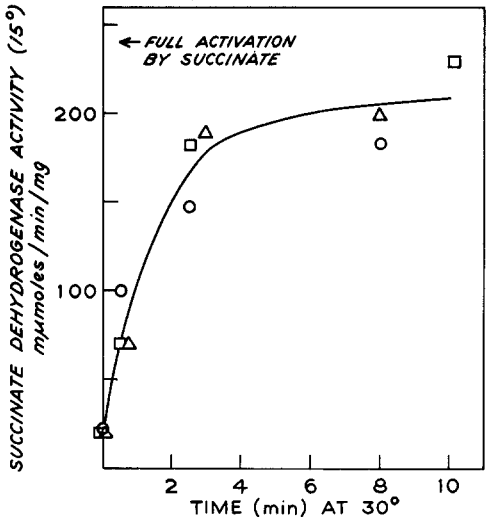

Fig. 4. Activation of SD in rat heart mitochondria initiated by ATP. The mitochondria were suspended in the buffer of Fig. 1 at 1 mg protein/ml at 30° and 1 mM ATP was added at 0 time. Aliquots were assayed for SD activity as in Fig. 1. Without ATP no change in SD activity occurred. Symbols: △ = ATP alone; □ = ATP + oligomycin (2 μg/mg of protein); 0 = ATP + piericidin A (1.25 mμmole/mg of protein).

oligomycin or piericidin caused substantially the same activation. The activation triggered by ATP cannot be mediated by the phosphorylation system, since oligomycin was present. Thus the existence of a third type of activation by ATP (or a compound in equilibrium with it) is suggested by this experiment. In order to preclude the interpretation of this ATP effect as being due to the removal of endogenous oxalacetate and consequent reversal of an oxalacetate inhibition, the experiments of Fig. 4 were repeated with DNP, arsenite, oligomycin, and glutamate present, the latter serving to transaminate oxalacetate to aspartate. Activation of SD by ATP occurred to an extent comparable to that seen in Fig. 4.

An important feature of these experiments is that the temperature coefficient for the activation of SD by electrons from DPN-linked substrates is very much lower (10 Kcal/mole) than that observed by activation by $CoQ_{10}H_2$ or succinate in membrane and soluble preparations (33 to 36 Kcal/mole). Although at 37° activation in mitochondria or in ETP_H occurs at comparable rates and rapidly enough to be of regulatory significance, at 15° activation is still fast in mitochondria ($k = 0.35\ min^{-1}$) but extremely slow in ETP_H ($k = 0.025\ min^{-1}$).

DISCUSSION

The data presented clearly demonstrate that SD activity is controlled by the metabolic

state of the mitochondria. The three known mechanisms for modulation of SD include activation by the substrate, which may not play a major role in intact mitochondria, by the prevailing level of reduced CoQ_{10}, and by the ATP concentration. Thus substrate flux, DPN/DPNH, $CoQ_{10}H_2/CoQ_{10}$, and ATP/ADP ratios, which are interrelated, enter into determining the level of SD activity. Of these the ATP effect on SD activity has not been previously noted in mammalian preparations, although Gregolin reported similar effects for yeast mitochondria some years ago (14). His observations and findings of increased succinoxidase activity in the literature, however, have been usually interpreted as a removal of oxalacetate inhibition by ATP (10, 15), although it has been pointed out (16) that oxalacetate may not be accessible to SD in mitochondria and, therefore, alternate explanations of the activation of ATP remain open.

Restricting ourselves to the present experiments, it appears to us that under the conditions of Fig. 4 at least oxalacetate removal does not provide a satisfactory explanation of the ATP effect. While activation by ATP may not be a direct one in the sense that another nucleotide may be the actual activator, it certainly does not involve the phosphorylation system since oligomycin does not inhibit it.

The studies presented help explain several observations in the literature, such as the accumulation of succinate in state 3 (6), increased flux of succinate to malate in state 4 (7) and provide another factor to be considered in explaining the depression of succinoxidase activity by uncouplers (8, 9).

The reversible activation of SD by substrates (1) and $CoQ_{10}H_2$ (3, 4) has been thought to involve a conformation change in the enzyme. Although activation of the enzyme in mitochondria is characterized by a much lower energy of activation than in soluble or membranal preparations, this should not be taken to suggest that a different mechanism of activation occurs in mitochondria. Possibly, the conformation of the enzyme in intact mitochondria is sufficiently different to facilitate the reversible conformation change proposed, resulting in a lower energy of activation.

One may inquire into the physiological purpose behind the multiple types of regulation of SD. Studies in this laboratory have shown that even under conditions of extensive deactivation (Figs. 1 and 2) SD activity does not become rate-limiting in mitochondrial respiration. The modulation is such as to depress SD activity but not below the level needed for operation of the Krebs cycle. One possible explanation, presently speculative in nature but worth considering, of the purpose of this regulation is as follows. It is known (17-19) that substrates of the respiratory chain-linked flavoproteins (succinate, DPNH, α-glycerophosphate, choline) competitively interfere with the oxidation of each other, lowering observed oxidase activities sometimes below that seen with one substrate alone. Thus unconstrained SD acti-

vity would result in lowered rate of DPNH oxidation, hence in a lowered rate of ATP synthesis. Perhaps when increased ATP synthesis is called for (high ADP/ATP) the observed constraint on SD activity serves the purpose of accelerated ATP synthesis, provided that the constraint on SD is not sufficient to become rate-limiting in the Krebs cycle, as is the case. Succinate which accumulates during the phase of constrained SD activity is then rapidly oxidized when the ADP/ATP ratio reaches a low level (by the "direct" effect of ATP and the increased $CoQ_{10}H_2/CoQ_{10}$ ratio). Such a mechanism might provide a rapid regulation of this key step in the Krebs cycle.

ACKNOWLEDGMENTS

This research was supported by grants from the American Cancer Society (P-531) and the U. S. Public Health Service (HE 10027).

REFERENCES

1. Kearney, E. B., J. Biol. Chem., 229, 363 (1957).
2. Kimura, T., Hauber, J., and Singer, T. P., J. Biol. Chem., 242, 4987 (1967).
3. Gutman, M., Kearney, E. B., and Singer, T. P., Biochem. Biophys. Res. Communs. 42, 1016 (1971).
4. Gutman, M., Kearney, E. B., and Singer, T. P., Biochemistry, in press.
5. Klingenberg, M., in Biological Oxidations (Singer, T. P., Ed.), Wiley, N.Y., 1968, p. 3.
6. LaNoue, K., Nicklas, W. J., and Williamson, J. R., J. Biol. Chem., 245, 102 (1970).
7. McElory, A. F., and Williams, G. R., Arch. Biochem. Biophys., 126, 492 (1968).
8. Tsuiki, S., Sukeno, T., and Takeda, H., Arch. Biochem. Biophys., 126, 436 (1968).
9. Kröger, A., and Klingenberg, M., Biochem. Z., 344, 317 (1966).
10. Greville, G. D., in Regulation of Metabolic Processes in Mitochondria (Tager, J. M., et al., Eds.), Elsevier, Amsterdam, 1966, p. 86.
11. Schnaitman, C., and Greenawalt, J. W., J. Cell Biol., 38, 158 (1968).
12. Pandee, S. V., and Blanchaer, M. C., J. Biol. Chem., 246, 402 (1971).
13. Arrigoni, O., and Singer, T. P., Nature, 193, 1256 (1962).
14. Gregolin, C., and Scalella, P., Biochim. Biophys. Acta, 99, 187 (1965).
15. Papa, S., Tager, J. M., and Quagliariello, E., in Regulatory Functions of Biological Membranes (Järnefelt, J., Ed.), Elsevier, Amsterdam, 1968, p. 264.
16. Jones, A. E., and Gutfreund, H., Biochem. J., 87, 639 (1963).
17. Wu, C. Y., and Tsou, C. L., Scientia Sinica, 4, 137 (1955).
18. Kimura, T., Singer, T. P., and Lusty, C. J., Biochim. Biophys. Acta, 44, 284 (1960).
19. Ringler, R. L., and Singer, T. P., J. Biol. Chem., 234, 2211 (1959).