

# Regulation of Succinate Dehydrogenase in Higher Plants

## II. ACTIVATION BY SUBSTRATES, REDUCED COENZYME Q, NUCLEOTIDES, AND ANIONS<sup>1</sup>

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GUILLERMO OESTREICHER,<sup>2</sup> PATRICIA HOGUE, AND THOMAS P. SINGER

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

### ABSTRACT

The effect of various agents on the activation of succinate dehydrogenase in cauliflower (*Brassica oleracea*) and mung bean (*Phaseolus aureus*) mitochondria and in sonicated particles has been investigated. Reduced coenzyme Q<sub>10</sub>, inosine diphosphate, inosine triphosphate, acid pH, and anions activate the enzyme in mitochondria from higher plants in the same manner as in mammalian preparations. Significant differences have been detected in the behavior of plant and animal preparations in the effects of ATP, ADP, NADH, NAD-linked substrates, and of 2,4-dinitrophenol on the state of activation of the dehydrogenase. In mammalian mitochondria ATP activates, whereas ADP does not, and the ATP effect is shown only in intact mitochondria. In mung bean and cauliflower mitochondria, both ATP and ADP activate and the effect is also shown in sonicated and frozen-thawed preparations. In sonicated mung bean mitochondria NADH causes complete activation, as in mammalian submitochondrial particles, but in sonicated cauliflower mitochondria activation by NADH is incomplete, as is also true of intact, anaerobic cauliflower mitochondria. Moreover, neither NAD-linked substrates nor a combination of these with NADH can fully activate the enzyme in cauliflower mitochondria. In contrast to mammalian mitochondria, succinate dehydrogenase is not deactivated in cauliflower or mung bean mitochondria under the oxidized conditions brought about by uncoupling of oxidative phosphorylation by 2,4-dinitrophenol.

It was shown in the previous paper (9) that succinate dehydrogenase in cauliflower and mung bean mitochondria is activated by substrates with the same kinetic characteristics and same high activation energy as in animal tissues. Activation by succinate appears to be accompanied by the release of tightly bound oxaloacetate. After full activation, membrane-bound succinate dehydrogenase in higher plants has the same turnover number as in heart mitochondria.

The present paper extends these observations on the fine regulation of the enzyme from higher plants to a study of the characteristics of activation by CoQ<sub>10</sub>H<sub>2</sub><sup>3</sup>, adenine and inosine

nucleotides, anions, and acid pH. It is shown that in most respects activation of the plant enzyme by these agents parallels the behavior of the mammalian enzyme. Important differences have been noted, however, in the activation of the mammalian and plant enzymes by ATP and ADP and in the effect of uncouplers on the degree of activation.

### MATERIALS AND METHODS

ATP and ADP were products of P-L Biochemicals, Inc. All other biochemicals were from the Sigma Chemical Co. Cauliflower (*Brassica oleracea* L.) and mung bean (*Phaseolus aureus* L.) preparations and analytical procedures were as detailed in the previous paper (9).

### RESULTS

**Activation by CoQ<sub>10</sub>H<sub>2</sub>.** In submitochondrial particles from animal tissues, NADH activates succinate dehydrogenase. NADH apparently serves only to reduce endogenous CoQ<sub>10</sub> to CoQ<sub>10</sub>H<sub>2</sub>, the actual activator, because in CoQ-depleted membranes NADH does not activate, but succinate does (5). As would be expected, in intact mitochondrial NAD-linked substrates and all other metabolites capable of reducing CoQ<sub>10</sub> activate succinate dehydrogenase (6).

Activation of succinate dehydrogenase in sonicated mung bean mitochondria by CoQ<sub>10</sub>H<sub>2</sub> is shown in Figure 1. The experiment was performed by incubating the particles at 29 C with chemically reduced CoQ<sub>10</sub> under anaerobic conditions and in the presence of antimycin A to minimize oxidation of the reduced quinone. Aliquots were removed at varying intervals, chilled to 15 C and immediately assayed at that temperature for succinate dehydrogenase activity by the PMS-DCIP assay (9). At concentrations of the order of 150 to 250 μM CoQ<sub>10</sub>H<sub>2</sub> the same activation was reached as with succinate as activator (Fig. 1A). Figure 1B shows the kinetics of activation by CoQ<sub>10</sub>H<sub>2</sub> at 29 C.

These results on the activation of the enzyme in sonicated mung bean mitochondria by added CoQ<sub>10</sub>H<sub>2</sub> parallel the behavior of the mammalian enzyme (5). Similar experiments on sonicated cauliflower mitochondria, however, revealed a somewhat different response to CoQ<sub>10</sub>H<sub>2</sub> (Fig. 2). In this case, the maximal activation obtained on incubation with CoQ<sub>10</sub>H<sub>2</sub> varied in different preparations from 60 to 75% of that produced by succinate and neither longer incubation (Fig. 2A) nor a higher concentration of CoQ<sub>10</sub>H<sub>2</sub> (Fig. 2B) increased the degree of activation further. As discussed below, a similar difference was found between mung bean and cauliflower preparations when endogenous CoQ<sub>10</sub>H<sub>2</sub> (reduced by NADH or malate) was used to activate succinate dehydrogenase.

**Activation by NADH and NAD-linked Substrates.** Figure 3 shows the progress of activation of succinate dehydrogenase at

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<sup>2</sup> Predoctoral Fellow of the Organization of American States, on leave of absence from the University of Chile.

<sup>3</sup> Abbreviations: CoQ<sub>10</sub> and CoQ<sub>10</sub>H<sub>2</sub>: coenzyme Q<sub>10</sub> and its reduced form; DCIP: 2,6-dichlorophenolindophenol, DNP: 2,4-dinitrophenol; PMS: phenazine methosulfate.

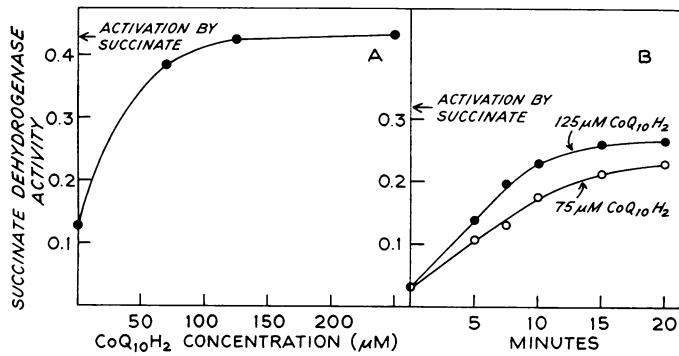


FIG. 1. Activation of succinate dehydrogenase in sonicated mung bean mitochondria by reduced  $\text{CoQ}_{10}$ . A 5 mM solution of  $\text{CoQ}_{10}$  in ethanol was titrated anaerobically with sodium borohydride (2 mg/ml), following the reduction spectrophotometrically at 275 nm. When the reduction was complete, the solution was acidified with 0.5 M acetic acid to destroy unreacted borohydride, a suitable aliquot of the  $\text{CoQ}_{10}\text{H}_2$  was placed in the side arm of a Thunberg tube, along with 0.3 M mannitol - 0.1% (w/v) bovine serum albumin - 0.1 mM EDTA - 0.02 M HEPES buffer, pH 7.5 to give 0.3 ml. The main compartment of the tube contained sonicated mitochondria (1.3 mg in A, 1.2 mg in B) and 3  $\mu\text{g}$  of antimycin A in 0.7 ml of the same mannitol - buffer solution. One such tube was prepared for each point shown in Fig. 1. The tubes were repeatedly evacuated, filled with  $\text{N}_2$ , then brought to 29.2 C, and the contents of the side arm tipped in. At the times stated, the tube was quickly chilled and a 50- $\mu\text{l}$  aliquot immediately assayed for succinate dehydrogenase activity at 15 C. The results are expressed on the ordinate as absorbance change per min per 0.36 mg of protein in A, and per 0.4 mg of protein in B. The horizontal arrow denotes the activity of the fully activated enzyme. The results are uncorrected for the small (about 7%) activation caused by the borate ion carried over but are corrected in B for the small inactivation caused by the ethanol in the  $\text{COQ}_{10}\text{H}_2$  solution. A: Final level of activation (at 15 min) obtained at various concentrations of added  $\text{CoQ}_{10}\text{H}_2$ . The specific activity of the preparation at 15 C was 0.09. B: Progress curve of activation. The specific activity at 15 C was 0.14.

29 C in sonicated mung bean mitochondria by 1 mM NADH in the presence of antimycin A, included to minimize oxidation of NADH. In this, as in all other experiments of this type with mung bean preparations, NADH produced full activation. In contrast, in sonicated cauliflower mitochondria, under all conditions tested, including anaerobiosis and the inclusion of antimycin A, NADH produced only partial (40-68%) activation (Fig. 3). In untreated cauliflower mitochondria, where NADH is not expected to pass the inner membrane, activation by NADH was nevertheless considerably more extensive (Fig. 4 and Table I). Other NAD-linked substrates (L-malate alone,  $\alpha$ -ketoglutarate, L-glutamate in the presence of arsenite) gave similar degrees of activation to malate + pyruvate in fresh cauliflower mitochondria.

These findings may be related to the presumed presence of two respiratory chain-linked NADH dehydrogenases in plant mitochondria (1, 2), one located outside, the other inside the inner membrane. In intact mitochondria, NADH is expected to reduce the former, malate plus pyruvate the latter enzyme. It has also been demonstrated recently with the aid of the competitive inhibitor, rhein, and of NADH analogs that sonication inverts the inner membrane of cauliflower mitochondria, as in animal tissues, exposing the internal NADH dehydrogenase (Oestreicher and Singer, unpublished data). These facts should be considered in interpreting the different degrees of activation of succinate dehydrogenase produced by NAD-linked substrates and NADH, respectively, in untreated cauliflower mi-

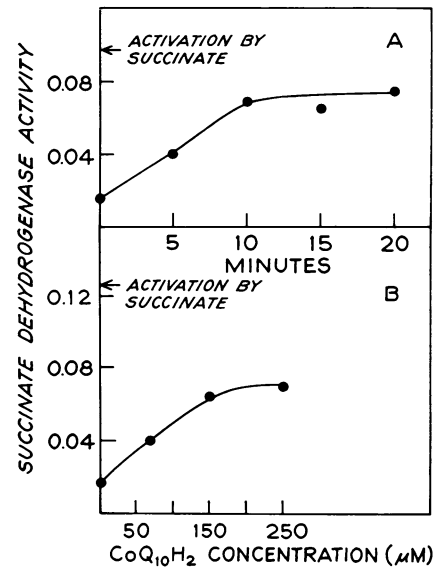


FIG. 2. Activation by  $\text{COQ}_{10}\text{H}_2$  in sonicated cauliflower mitochondria. A: Progress of activation in the presence of 250  $\mu\text{M}$   $\text{COQ}_{10}\text{H}_2$ ; B: effect of  $\text{COQ}_{10}\text{H}_2$  concentration on final extent of activation reached (at 20 min). Conditions were as in Fig. 1, except as follows. The suspending medium for the particles was 0.25 M sucrose - 0.02 M HEPES, pH 7.5. The amount of antimycin present was 5  $\mu\text{g}$ . Activity on the ordinate represents  $\Delta A_{275\text{ nm}}/\text{min} \cdot 0.14$  mg of protein in A and per 0.16 mg of protein in B. The amount of mitochondrial protein present during incubation was 1.4 mg in A, 1.6 mg in B, and their specific activities at 15 C were 0.098 and 0.116, respectively. The results are uncorrected for the slight (10-15%) activation by the borate carried over. The ethanol in the  $\text{CoQ}_{10}\text{H}_2$  solution caused no inactivation.

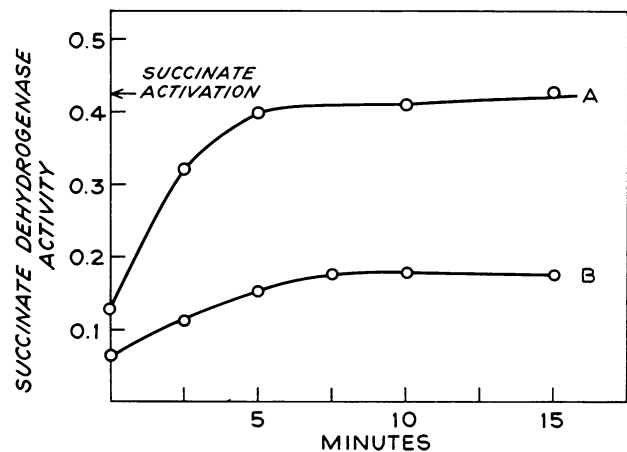


FIG. 3. Activation by NADH in sonicated mung bean and cauliflower mitochondria. A: Sonicated mung bean mitochondria; B: frozen-thawed and sonicated cauliflower mitochondria. Experimental conditions for the mung bean preparation were as in Fig. 1A and for cauliflower as in 2A, except that 0.99 mg of protein was present in the incubation in curve B and that in both experiments 1 mM NADH replaced  $\text{CoQ}_{10}\text{H}_2$ . Although the specific activities of the two preparations were slightly different (0.09 for mung bean, 0.077 for cauliflower), the activities are normalized to permit comparison.

tochondria and in comparing activation by NADH before and after sonication.

The ratio of activation produced by NADH to that produced by malate plus pyruvate in fresh mitochondria is remarkably constant (Table I). Moreover, the same ratio is ob-

tained on dividing the maximal activation produced by NADH in fresh mitochondria by the activation reached in sonicated preparations, both in the presence of antimycin A. Either calculation measures the relative degrees of activation caused by the action of the external and internal NADH dehydrogenases, respectively, at apparent equilibrium. These findings are further discussed in the last part of this paper.

**Activation by ATP and ADP.** In mitochondria from animal tissues (6) and bakers' yeast (4), ATP activates succinate dehydrogenase in an oligomycin-insensitive manner. ADP does not activate but induces deactivation indirectly by initiating the state 4 → state 3 transition and concomitant oxidation of  $\text{CoQ}_{10}\text{H}_2$ . Activation by ATP is not seen in damaged mitochondria or in submitochondrial preparations (6).

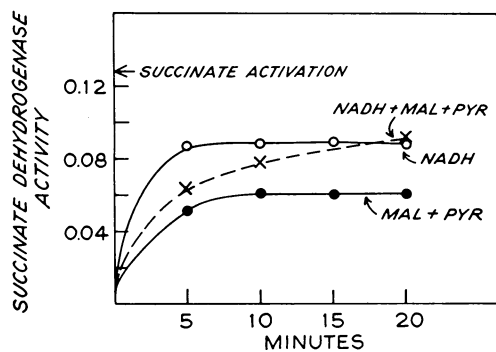


FIG. 4. Activation by 1 mM NADH and by 20 mM pyruvate plus 20 mM malate in cauliflower mitochondria. A series of Thunberg tubes contained 0.34 mg of fresh cauliflower mitochondria and 6  $\mu\text{g}$  of antimycin A in a final volume of 0.5 ml of 0.25 M sucrose - 0.02 M HEPES, pH 7.5. The side arms of the tubes contained 0.5  $\mu\text{mole}$  of NADH, 10  $\mu\text{moles}$  each of pyruvate and malate, or both, as indicated in the curves. After repeated evacuation and filling with  $\text{N}_2$ , the tubes were brought to 29.2 C, tipped, and incubated. At the times shown one tube from each set was removed and a 40- $\mu\text{l}$  aliquot immediately assayed for succinate dehydrogenase activity as 15 C. The results on the ordinate are expressed as  $\Delta A_{300\text{ nm}}/\text{min} \cdot 0.17\text{ mg}$  mitochondrial protein.

Table I. *Activation of Succinate Dehydrogenase in Cauliflower Mitochondria by NADH and by Pyruvate Plus Malate*

Mitochondria (3.4-5 mg protein in the different experiments) were suspended in 0.5 ml of final volume of 0.25 M sucrose-0.02 M HEPES buffer, pH 7.5, in the presence of 6  $\mu\text{g}$  of antimycin A in the main compartment of Thunberg tubes. The side arms contained 0.05  $\mu\text{mole}$  of NADH or 10  $\mu\text{moles}$  each of pyruvate and L-malate. After three cycles of evacuation and flushing with  $\text{N}_2$ , where indicated, the tubes were warmed to 29.2 C. At 2.5-min intervals, tubes were removed into ice, a 40- $\mu\text{l}$  aliquot rapidly withdrawn and immediately assayed at 15 C for succinate dehydrogenase activity, until the degree of activation became constant. Percentage of activation was calculated taking activation by succinate (9) as 100.

Conditions	Activation by		% Activation by NADH by Malate + pyruvate	% Activation by NADH before Sonication and after Sonication
	Malate + pyruvate	NADH		
Mitochondria, $\text{N}_2$ , antimycin	58	91	1.6	ratio
	52	78	1.5	
	47	77	1.6	
Mitochondria, antimycin After sonication		62		1.6
		39		

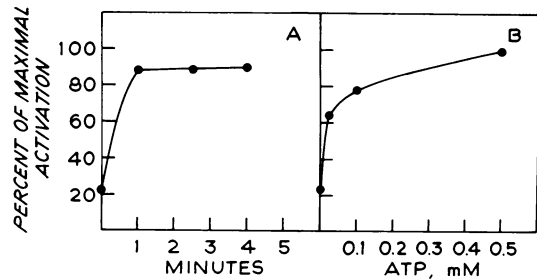


FIG. 5. Activation of succinate dehydrogenase by ADP and ATP in mung bean mitochondria. A: Activation by 0.8 mM ADP; B: final extent of activation by various concentrations of ATP. Fresh mitochondria (1.8 mg of protein) suspended in 0.25 ml final volume of the medium specified in Fig. 1A and containing 5  $\mu\text{g}$  of oligomycin were incubated in test tubes at 30 C. In Fig. 5A activation was started by adding 5  $\mu\text{l}$  of 0.04 M ADP, in Fig. 5B by adding suitable aliquots of 5 mM ATP. Samples of 50  $\mu\text{l}$  were removed at various times and immediately assayed for succinate dehydrogenase activity at 15 C. In Fig. 5A only the final extent of activation (measured after 5 min at 30 C) is shown. Activities are expressed on the ordinate as per cent of the activity of a succinate activated sample.

Figure 5B shows activation of succinate dehydrogenase by ATP in untreated mung bean mitochondria in the presence of oligomycin. Full activation was reached at relatively low concentrations of ATP. Activation was quite rapid at 30 C; with 0.1 mM ATP the reaction was complete in 1 min. At comparable concentrations ADP, in the presence of oligomycin, also produced 90 to 100% activation (Fig. 5A), in contrast to its effects in heart mitochondria (6).

In cauliflower mitochondria the effects of ATP and ADP were again more complicated (Fig. 6). Activation was incomplete at all concentrations of ATP and ADP tested, with or without oligomycin. Activation by ATP is faster in the presence of oligomycin than in its absence (Fig. 6A), showing the presence of an oligomycin-sensitive ATPase. The action of ADP is not due to ATP synthesis by oxidative phosphorylation, since oligomycin does not prevent the effect (Fig. 6C), although an ADP to ATP conversion by other mechanisms (e.g. adenylate kinase) cannot be excluded. Activation by ADP declined after reaching a maximum (Fig. 6B). Although not shown, the inclusion of 1 mM arsenite under the conditions of Figure 6B decreased the activation by 0.8 mM ADP in untreated mitochondria and greatly accelerated the deactivation after 5 min, so that the ADP effect may be, in part, related to GTP synthesis.

As shown in Figure 6, A and B, activation by both ATP and ADP is invariably seen also in sonicated and frozen-thawed cauliflower preparations, although to a lesser extent than in untreated ones. This is in direct contrast to the lack of activation by either nucleotide in damaged mammalian mitochondria (6).

**Activation by ITP and IDP.** In submitochondrial and soluble preparations from animal tissues, IDP and ITP, at moderate concentrations, activate the dehydrogenase (6). IMP, cyclic IMP, and various other mono- and dinucleotides, including cyclic AMP, at comparable concentrations do not seem to activate. Activation by IDP and ITP has not been tested in intact mitochondria because these nucleotides do not penetrate mammalian mitochondria.

Figure 7 shows the activation of succinate dehydrogenase in sonicated cauliflower mitochondria by 5 mM IDP at 30 C. Under these conditions ITP also activated the enzyme, although quantitatively it was perhaps somewhat less effective. These results are quantitatively similar to those reported for the beef heart enzyme (6, 8).

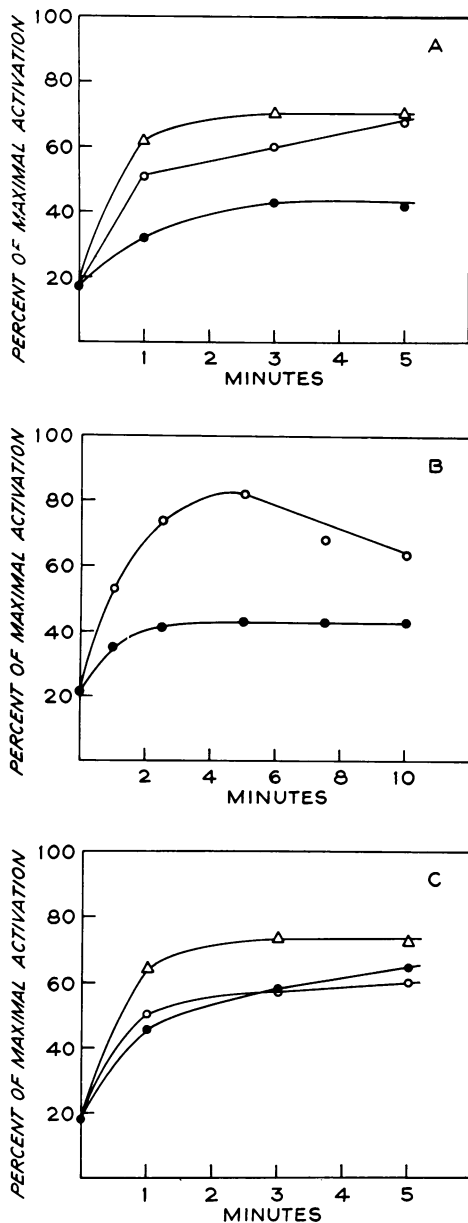


FIG. 6. Effect of ADP and ATP on cauliflower succinate dehydrogenase. A: Activation by 0.5 mM ATP with ( $\Delta$ ) and without ( $\circ$ ) oligomycin in untreated mitochondria; same, without oligomycin, in frozen-thawed mitochondria ( $\bullet$ ). A final volume of 0.25 ml contained 0.1 ml of fresh or frozen-thawed mitochondria (3 mg of protein) with or without 5  $\mu$ g of oligomycin, 0.125 ml of 0.25 M sucrose - 0.02 M HEPES - 0.1% (w/v) bovine serum albumin - 0.1 mM EDTA, pH 7.5, and 25  $\mu$ l of 5 mM ATP to start the activation. The suspension was incubated at 29.2 C and 25- $\mu$ l aliquots were removed and immediately assayed at 15 C. The degree of activation is compared with the maximal activation produced by succinate. B: Activation by 0.8 mM ADP in the presence of oligomycin in fresh ( $\circ$ ) and in frozen-thawed and sonicated ( $\bullet$ ) mitochondria. Conditions were as in A, except that the suspending medium during activation was 0.3 M mannitol - 0.1 mM cysteine, pH 7.5. C: Comparison of activation by ADP and ATP in untreated cauliflower mitochondria.  $\Delta$ , 0.5 mM ATP plus 5  $\mu$ g oligomycin;  $\bullet$ , 0.5 mM ADP plus 5  $\mu$ g oligomycin;  $\circ$ , 0.5 mM ADP without oligomycin. Conditions were as in A.

**Activation by Anions and Acid pH.** It was shown in the previous paper (9) that succinate dehydrogenase in sonicated cauliflower mitochondria is nearly completely activated by 0.2 M

$\text{Br}^-$  at pH 6 and 30 C in a few minutes. Figure 8 demonstrates that, as in animal tissues, the cauliflower enzyme is activated on merely lowering the pH from 7.2 to pH 6.1 to 6.5 (at 30 C) and incubating the particles at 30 C without added anions. The lower the pH of incubation, the greater is the degree of activation reached. If 0.1 M  $\text{Br}^-$  is included in the incubation medium the extent of activation reached is greatly increased. Although the specificity for different anions has not yet been tested with the plant enzyme, in other respects these observations parallel published results for the behavior of the beef heart enzyme (7, 8).

**Effect of DNP.** In heart and liver mitochondria uncouplers, like DNP, cause rapid and extensive deactivation of succinate dehydrogenase, presumably because they elicit almost complete oxidation of the  $\text{CoQ}_{10}$  pool (5). Early in this study it was noted that 66  $\mu$ M DNP not only failed to deactivate the enzyme

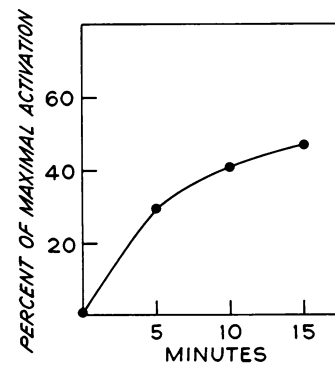


FIG. 7. Activation by IDP. Sonicated cauliflower mitochondria (3.6 mg of protein) were incubated at 30 C in 0.8 ml (final volume) of the suspending medium specified in Fig. 4 with 5 mM IDP. Samples of 0.2 ml were withdrawn and immediately assayed at 15 C. Activity on the ordinate is compared with that of a sample maximally activated by succinate.

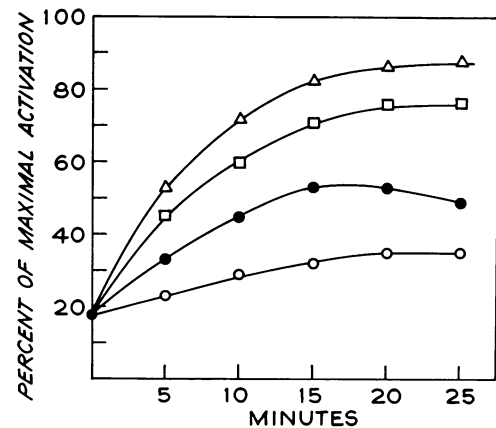


FIG. 8. Activation of succinate dehydrogenase in sonicated cauliflower mitochondria at acid pH with and without 0.1 M  $\text{Br}^-$ .  $\circ$ : pH 6.5;  $\bullet$ : pH 6.1;  $\square$ : pH 6.5 plus 0.1 M  $\text{Br}^-$ ;  $\Delta$ : pH 6.1 plus 0.1 M  $\text{Br}^-$ . Sonicated mitochondria (5.7 mg of protein), suspended in 0.7 ml of 0.3 M mannitol - 0.1% (w/v) bovine serum albumin - 0.1 mM EDTA, pH 7.5, were brought to 30 C. The pH of the suspension at 30 C was 7.20. At 0 time 0.1 ml of 0.2 M 2-(N-morpholino)ethanesulfonate buffer, pH 6.05 or 6.5, as appropriate, with or without 1 M  $\text{Br}^-$  present, was added to start the activation. Aliquots of 40  $\mu$ l were removed at the times shown and immediately assayed at 15 C. Activation on the ordinate is compared with a succinate activated sample. When maintained at the original pH at 30 C a control sample showed no change in activity in the course of the experiment.

which is present in partly activated states in cauliflower mitochondria (9), but did not seem to interfere with activation by NAD-linked substrates, such as pyruvate plus malate, in the aerobic steady state, although this type of activation is almost certainly mediated by  $\text{CoQ}_{10}$ . This concentration of DNP was sufficient to uncouple respiration from phosphorylation completely.

The action of DNP on the state of activation of the dehydrogenase was more extensively studied in mung bean mitochondria. Mung bean mitochondria with a respiratory control ratio of 4 to 5 on either malate or succinate were titrated with DNP to determine the concentration required for complete uncoupling. DNP at a concentration (1.3 mM) significantly in excess of that required for uncoupling respiration from phosphorylation was then added to mung bean mitochondria, and the suspension was incubated at 30 C, aliquots being periodically removed and assayed for succinate dehydrogenase activity. Although the enzyme was 21 to 40% activated prior to the addition of the uncoupler, so that any deactivation would have been readily detected, no such effect was noted.

### DISCUSSION

The experiments presented in this and the preceding paper (9) indicate that the regulatory properties of mung bean succinate dehydrogenase are rather similar to those of the mammalian enzyme. The differences are that in mung bean mitochondria the uncoupler DNP does not seem to cause deactivation of the dehydrogenase and that ADP, as well as ATP, can activate. In cauliflower preparations, two additional differences from the mammalian enzyme have been noted. First, activation by both ATP and ADP may be observed in sonicated and frozen-thawed and sonicated preparations, while in animal tissues only ATP activates and the effect is observed only in intact mitochondria. Second, in cauliflower mitochondria neither NAD-linked substrates, nor NADH, nor a combination of the two activate succinate dehydrogenase completely in the presence of antimycin A under anaerobic conditions.

While activation by added NADH in plant mitochondria may well be due to the presence of an "external" NADH dehydrogenase capable of reducing the  $\text{CoQ}_{10}$  pool, the existence of dual NADH dehydrogenases would not explain the incomplete activations by NADH and by NAD-linked substrates in cauliflower preparations. The data in Figures 3 and 4 and Table I suggest that some type of equilibrium is established between activated and deactivated enzyme when reducing equivalents are donated by malate plus pyruvate or by NADH, even under anaerobic conditions or in the presence of antimycin. When malate plus pyruvate are added to cauliflower mitochondria two juxtaposing effects may occur: on one hand, malate oxidation would reduce  $\text{CoQ}_{10}$  and thus activate the dehydrogenase; on the other hand, it would generate oxaloacetate, leading to deactivation of the enzyme. The fact that the conditions were anaerobic would severely limit the amount of oxaloacetate produced, but since a molar equivalent of oxaloacetate suffices for extensive loss of succinate dehydrogenase activity (8), there may be sufficient oxidizing equivalents present in the mitochondria to permit the accumulation of this amount of oxaloacetate. Thus the apparent equilibrium in the case of malate plus pyruvate activation may represent the balance of oxaloacetate formation and its reduction to malate by  $\text{CoQ}_{10}\text{H}_2$ . The difference from the behavior of rat liver mitochondria (6), where NAD-linked substrates activate nearly completely, may reflect different rates of oxaloacetate removal by metabolic reactions, such as transaminase action.

The possible explanation advanced for the incomplete activation by pyruvate plus malate in cauliflower mitochondria cannot hold, of course, for the incomplete effect of NADH. The possibility has been considered that the apparent equilibria reached with either NADH or malate plus pyruvate might reflect different degrees of reduction of the  $\text{CoQ}_{10}$  pool by the "internal" and "external" NADH dehydrogenases, with neither capable of reducing the pool to a sufficient degree to permit full activation. This notion and the functional compartmentation of the  $\text{CoQ}_{10}$  pool which it implies is not supported, however, by the observation (Fig. 4) that with both NADH and malate plus pyruvate present the degree of activation was in between the values obtained with either type of activator alone. The fact that the inclusion of malate plus pyruvate in a system undergoing activation by externally added NADH slows down the process, supports the idea that oxaloacetate formation, even in anaerobiosis, may be at least partly responsible for the incomplete activation.

The significance of the observations described in this paper is not restricted to their comparative biochemical interest, for plant mitochondria offer a singularly useful experimental material for exploring the extent to which movements of oxaloacetate in and out of a tight binding site in succinate dehydrogenase participate in the rapid activation-deactivation of the enzyme under physiological conditions (8). This is so because oxaloacetate can freely penetrate the inner membrane of higher plant mitochondria (3), in contrast to animal tissues.

In mammalian mitochondria, the activation of succinate dehydrogenase in state 4 and its deactivation in state 3 fit known facts about the accumulation of succinate and of succinyl CoA in state 3, the higher rate labeling of malate by  $^{14}\text{C}$ -succinate in state 4 than in state 3, and the oxidation reduction ratio  $\text{CoQ}_{10}/\text{CoQ}_{10}\text{H}_2$  in different metabolic states (6). The facts that ADP activates, rather than deactivates, succinate dehydrogenase in plant mitochondria and that DNP, while acting as an uncoupler, fails to deactivate the dehydrogenase in plants indicate that, despite similarities in the action of activating agents on the plant and animal enzymes, the metabolic significance of the activation cannot be the same in plants as has been proposed for animal tissues (8). It is also evident that considerable additional work on intact plant mitochondria will be required before a rational hypothesis for the physiological purpose of the activation in higher plants can be developed.

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