REGULATORY PROPERTIES OF SUCCINATE DEHYDROGENASE: ACTIVATION BY SUCCINYL CoA, pH, AND ANIONS

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SUMMARY. Previous studies established that succinate dehydrogenase (SD) is reversibly activated by three types of compounds: substrates, reduced CoQ_{10}, and ATP. Each of these mechanisms have been shown to operate in intact mitochondria in regulation of SD activity. The present study demonstrates activation of the enzyme by succinyl CoA, which occurs at about the rate and to the extent observed with the same concentration of succinate and represents an example of feed-forward regulation. Further, in membranal and purified, soluble preparations lowering the pH in the range of 7.5 to 5.5 results in "spontaneous" activation: the lower the pH, the more extensive activation is observed. On raising the pH of SD thus activated, deactivation occurs to the level characteristic of the new pH. A number of anions (Cl^-, Br^-, $SO_4^=$, formate, ClO_4^-) increase the extent of activation observed on lowering the pH and appear to act by stabilizing the active conformation of SD.

INTRODUCTION

It has been known for many years that SD is reversibly activated by substrates and competitive inhibitors (1, 2) and the interconversion of the active and deactivated forms has been thought to represent a conformation change because of the high energy of activation (1) and large entropy change (3) involved. During the past year two additional mechanisms of activation have been discovered: (a) activation by $CoQ_{10}H_2$ (or by substrates which reduce CoQ_{10}) in mitochondria and membrane preparations (3, 4) and (b) activation by very low concentrations of ATP (or a compound in equilibrium with it), which is oligomycin-insensitive and has been observed only in mitochondria (5, 6). The same maximal activity is produced by each of the three types of activators and spontaneous deactivation occurs in each

case when the activator is removed. Collectively these activators could account for the known changes in succinate concentration and flux through succinate in metabolic transitions (6, 7). In addition, in membranal and soluble preparations deactivated SD is activated by IDP and ITP but not by IMP, AMP, ADP, GTP, GDP, cyclic AMP, or cyclic IMP (6, 8).

In further studies on the regulatory significance of the phenomenon it has been noted that succinyl CoA, the metabolic precursor of succinate, is a good activator. Further, it has been observed that on lowering the pH SD becomes "spontaneously" activated, the extent of activation being a function of pH, and of the type and concentration of anions present. These observations may provide a clue to the molecular mechanism of the regulation of SD.

MATERIALS AND METHODS

Except as noted below all methods and materials were as in previous work (3-5). Succinyl CoA was from Pabst Laboratories, methylmalonyl CoA the kind gift of Dr. H. G. Wood. HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) and MES (2-(N-morpholino)ethanesulfonic acid) were purchased from Calbiochem and were brought to the desired pH with KOH, thus avoiding the use of inorganic anions. For activation, the enzyme was incubated at 30° under the conditions stated; samples were removed at intervals and assayed at 15° in the PMS-DCIP assay but at fixed PMS concentration (3). Aliquots of the activation mixture were also incubated at the start and the end of the pre-incubation period with 20 mM succinate for 6 min at 38° to ascertain that no inactivation had occurred and the resulting activity, measured at 15°, was taken as 100% activation. The pH values stated are those of the complete incubation mixture at 30° at the end of the pre-incubation.

RESULTS AND DISCUSSION

Activation by succinyl CoA - Fig. 1 compares the rate and extent of activation of SD by succinate and succinyl CoA at 30°, using a soluble purified preparation (9). As in

Fig. 1, also with ETP and ETP_H preparations and at lower concentrations of the activator, the rate and extent of activation was comparable for succinate and succinyl CoA. Analysis of the succinyl CoA by TLC assured the absence of significant contamination by free succinate. Under the same conditions methylmalonyl CoA (10 to 20 mM) gave negligible activation, in line with the fact that methylmalonic acid is not an effective competitive inhibitor of SD.

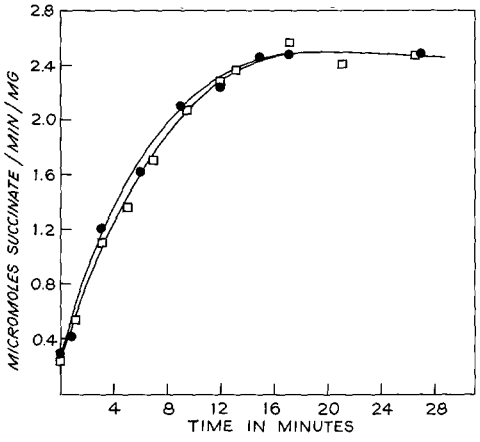

Fig. 1. Activation of SD by succinate and succinyl CoA. The enzyme used was a purified, soluble preparation (9), deactivated by passage though Sephadex G-25. Activation was run at 30° under N_2 at 2.1 mg protein/ml in Tris buffer, pH ~7.5. Symbols: squares, 10 mM succinate; circles, 10 mM succinyl CoA. For other conditions see text.

In view of the structural similarity to succinate, it appears likely that succinyl CoA, like malonate and fumarate, activates by combining at the substrate-binding site. The interest of the observation is that succinyl CoA is the immediate metabolic precursor of succinate both in the Krebs cycle and in its formation from odd numbered fatty acids and branched-chain amino acids. This may be considered a feed-forward regulation, since the accumulation of succinyl CoA in mitochondria would contribute to turning on SD, the enzyme whose function is to oxidize the deacylation product of the activator.

Effect of pH and of anions - Fig. 2 demonstrates the time course of the activation of SD in an ETP preparation on lowering the pH. Prior to the experiment the particles were in 0.2 M sucrose-50 mM MES buffer, pH 7.1 at 0°. At zero time the particles were five-

fold diluted with MES buffer to give a 0.1 M buffer concentration and pH 6.57 at 30°; samples were withdrawn, and assayed immediately at 15°, a temperature at which activation during spectrophotometric assays is negligible. Curve 2 of Fig. 2 is a plot of the % of maximal activity reached (based on the succinate-activated samples) against time of incubation at pH 6.57 and 30°. Curve 1 represents an identical experiment but with 100 mM NaCl included in the incubation mixture. It is seen that without added Cl^- about 41% activation is reached at this pH, while with Cl^- present the value is nearly 80%.

Fig. 3 demonstrates the effect of pH on the extent of activation obtained under these conditions with (Curve 1) and without (Curve 2) 100 mM NaCl present. Samples were taken for assay after 10, 15, and 20 min of incubation and gave the same activity at the latter two intervals, suggesting that an equilibrium has been attained; this is the value plotted on the ordinate. Again, the presence of 100 mM NaCl displaces the position of this apparent equilibrium.

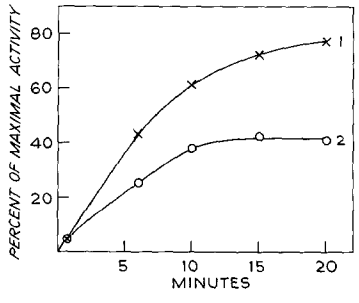

Fig. 2. Kinetics of activation of SD on lowering pH. ETP (10 mg/ml) in 0.2 M sucrose-50 mM MES (pH 7.1 at 0°) was diluted at zero time to 2 mg protein/ml with 0.1 M MES to yield pH 6.57 at 30° and incubated with (Curve 1) and without (Curve 2) 100 mM NaCl at 30°. Other conditions are in text.

If, indeed, the equilibrium between activated and deactivated forms is determined by the pH, it should be possible to reverse the activation by increasing the pH after a steady level of activation has been reached. That this is the case may be seen in Fig. 4. In this experiment an ETP_H sample was first activated at pH 6.11, 30°, in the presence of 100 mM NaCl (Curve 2, shaded circles). At point A an aliquot was removed and diluted

with an equal volume of the same activating medium (0.1 M MES buffer - 100 mM NaCl, pH 6.11) and the incubation at 30° was continued (open circles). It may be seen that dilution had no effect on the time course or final extent of activation. Another sample (crossed circles) was removed at point B and diluted with an equal volume of 0.1 M HEPES buffer - 100 mM NaCl, pH 8.0, yielding pH 7.11 at 30°. The time course of the ensuing deactivation is shown in Curve 3. Curve 1 is a control, showing activation of the enzyme, in the medium of Curve 3 (50 mM MES buffer, 50 mM HEPES buffer, and 100 mM NaCl at pH 7.11 (30°). Since the activation reached in Curve 1 and the deactivation attained in Curve 3 are nearly identical, it appears that pH indeed governs the equilibrium between activated and deactivated forms under these conditions.

The pH-dependent activation of the enzyme occurs qualitatively in the same manner in highly purified, soluble preparations of SD but the extent of activation reached at acid pH values is less. The cation component of salts is apparently without effect, since at equimolar concentrations NaCl, KCl, and Tris Cl displaced the equilibrium to the same extent. On the other hand, different anions yield quantitatively different results. Preliminary results with ETP and soluble preparations at two pH values (6.6 and 7.5) in the 50 to 200 mM range suggest that the effectiveness may range ClO_4^-, formate> Br^- > Cl^-. In addition $SO_4^=$, but not acetate, displaces the equilibrium in favor of the activated enzyme. Activation by formate may be a mixed effect, since it is both an anion and a weak competitive inhibitor (10). As to ClO_4^-, it may be recalled that the preparation of Davis and Hatefi (11), which is extracted with 0.8 M ClO_4^- from Complex II, is fully activated. Concurrent studies in this laboratory have shown that complete activation of SD occurs even at 0° on treatment of the particles with the concentrated ClO_4^- solution and that the resulting preparation is very difficult to deactivate (8, 12).

The observations described may provide an important clue to the nature of the conformation changes involved in the reversible activation of the enzyme. If we assume that activation of SD by pH and Cl^- reaches the same maximal activity as activation by succi-

nate, $CoQ_{10}H_2$, etc. (which has not been established), then the midpoint in the titration curve of Fig. 3 (pH 6.3 to 6.4) suggests the possible involvement of a histidine group, although the curves in Fig. 3 appear to cover over 2 pH units. Actually, however, Curve 1 seems to level off at 80% activation and in no case was greater than 80% activation attained by pH and anions (although the enzyme could be 100% activated by subsequent in-

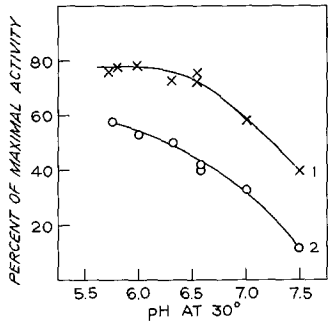

Fig. 3. Effect of pH on extent of activation reached at various pH values. Conditions were as in Fig. 2, except that the pH of the incubation mixtures for activation was as given on the abscissa. Curve 1 with, Curve 2 without 100 mM NaCl present.

cubation with succinate), so that it is conceivable that the span between zero and maximem possible activation falls within the 2 pH units required for a single ionizing group and that its pK is somewhat higher (6.6 at 30°). In either event the ionization of one or more histidine groups seems indicated. It is not likely, however, to be the histidine residue to which the flavin is attached (13), since this has a pK = 4.6.

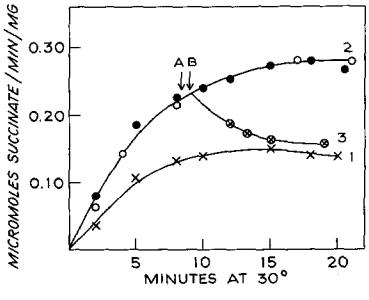

Fig. 4. Reversibility of pH activation. For experimental conditions and details see text.

These findings are reminiscent of the behavior of L-amino acid oxidase which is spontaneously activated on lowering the pH, inactivated on raising it, with a $pK' = 6.55$ at 38°, the active form of which is stabilized by monovalent anions, the process being characterized by a very high energy of activation (64). In this case also, the activation-deactivation was thought to reflect conformation changes governed by the ionization of a histidine group.

ACKNOWLEDGMENTS

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