Vol. 85, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 14, 1978 Pages 1173-1179

ACTIVATION OF SUCCINATE DEHYDROGENASE BY BICARBONATE

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Received October 16, 1978

SUMMARY

Succinate dehydrogenase (SD) of mitochondria from rat liver or kidney is to a large extent in the active form as isolated, whereas SD activity of heart and skeletal muscle mitochondria can be activated as much as ten-fold over the basal activity when isolated. Incubation of the latter at 37° with bicarbonate resulted in more extensive activation of SD than when succinate was the activator. Activation by bicarbonate was not readily reversed by washing unless succinate was also present. The data indicate that bicarbonate and succinate share the same site for activation of SD. A physiological role for bicarbonate in regulation of SD activity in muscle is suggested.

INTRODUCTION

Kearney et al. (1,2) first reported that succinate dehydrogenase (SD) is activated by its combination with succinate or any substance capable of binding at the active center. This original observation has been the subject of numerous subsequent studies (e.g. refs. 3-6). This activation has now been explained, at least in some instances, by the effector-mediated release of tightly-bound oxalacetate (7).

In the course of recent studies with liver mitochondria we found that high concentrations of bicarbonate** strongly suppressed glutamate oxidation, and that this effect resulted specifically from inhibition of SD (J. Swierczynski and E. J. Davis, unpublished observations). This observation confirmed an earlier report (8) that bicarbonate can act as a competitive inhibitor of purified soluble SD. On the other hand, in a separate study (J. Bremer and E. J. Davis, in preparation) it was observed that bicarbonate has a strong stimulatory effect on pyruvate-supported respiration by mitochondria isolated from skeletal

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** The equilibrium mixture of CO_2, H_2CO_3 and HCO_3^- will be referred to as bicarbonate.

0006-291X/78/0853-1173$01.00/0

muscles if only endogenous substrates other than pyruvate were present. One possible explanation for this effect could be that bicarbonate may also act as an activator of SD, as has been shown for other competitive inhibitors (2-4). In this view, SD would be in a relatively inactive form in the absence of activating concentrations of succinate, but could be converted to its catalytically active form by bicarbonate. In the present study we report that bicarbonate can indeed activate membrane-bound SD in mitochondria taken from several tissues. The extent of activation over that of the mitochondria as isolated is much greater for heart and skeletal muscle than for mitochondria from liver and kidney cortex tissues.

MATERIALS AND METHODS

Mitochondria from rat tissues were prepared as follows: heart, as previously reported (9) except that the isolation medium contained 1 mM EDTA, and the pH was 7.6; skeletal muscle, essentially as described in (10); and liver and kidney cortex following Johnson and Lardy (11). Mitochondria from all sources were finally suspended in .25 M sucrose, 10 mM Tris and 1 mM EDTA to pH 7.6. Protein was determined by the biuret method. SD activity was determined spectrophotometrically with phenazine methsulfate (PMS) and 2,6-dichlorophenolindophenol (DCIP) as the electron acceptor system as described by Kimura et al. (4). The reaction mixtures contained 50 mM potassium phosphate buffer (pH 7.4), 1 mM NaCN, 0.55 mM $CaCl_2$, 20 mM succinate, 50 µM DCIP, 1 mM PMS and 40-80 µg mitochondrial protein to a final volume of 2.5 ml. Enzyme activity was determined at 15° by following the decrease of absorbance at 600 nm using a Unicam SP1800 spectrophotometer, and expressed as nmol DCIP reduced/min/mg protein. Bicarbonate solutions were prepared as follows: $KHCO_3$ was dissolved in 0.1 M HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer and the pH adjusted to 7.6 with HCl. The freshly-prepared solutions were kept in closed containers during experiments.

Preincubations for activation studies were routinely carried out at the specified temperature (usually 37°) in 0.1 M HEPES buffer (pH 7.6) containing 2 µg/ml of rotenone, with or without bicarbonate or succinate. In control experiments, preincubations were carried out in a more 'physiological' medium containing 100 mM KCl, 20 mM Tris-HCl, 10 mM potassium phosphate, 2 mM $MgCl_2$ and varying concentrations of $KHCO_3$ to a pH of 7.6. The course and extent of activation was not distinguishable from that when HEPES was the suspending medium.

RESULTS AND DISCUSSION

The time course of activation of SD by bicarbonate in heart mitochondria is presented in Fig. 1. At 37° maximum activation was obtained after 5 to 6 min, and the basal activity was also spontaneously stimulated somewhat at this temperature. If activation was carried out at 25°, only moderate activation by

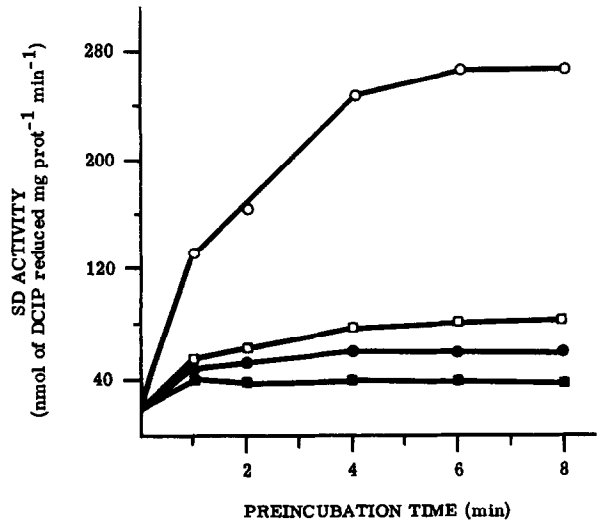

Fig. 1. Time course of the activation of succinate dehydrogenase by $KHCO_3$. Rat heart mitochondria (4 mg) were preincubated at 37° C in 10 ml of 0.1 M HEPES (pH 7.6) + 2 µg/ml rotenone with (O-O) or without 20 mM $KHCO_3$ (●-●); or at 25° C with (□-□) or without 20 mM $KHCO_3$ (■-■) for various time periods. At the end of the incubation period, the tubes were cooled to 0° C to prevent further activation. SD activity was then determined as described under Materials and Methods.

bicarbonate was observed within this time period. However, if the preincubation period was extended to 20 min, subsequently measured SD activity reached about 240 nmols/min/mg protein (data not shown).

SD activity of mitochondria from all four tissues was slightly stimulated during a preincubation period at 37° (Table I). This finding is in accord with results previously reported by Thorn (12) and by Kimura et al. (4). The basal activity of kidney or liver SD without prior incubation at 37° was about 30 or 50%, respectively, of the maximum obtainable after activation with either succinate or bicarbonate. Additional activation due to either bicarbonate or succinate, over the spontaneous activation at 37° was very slight. In contrast, the basal activity of SD of heart and skeletal muscle mitochondria was stimulated many-fold on prior incubation with 20 mM bicarbonate or succinate. The maximum activation attained with bicarbonate was, in fact, substantially greater than that with succinate for mitochondria from both muscle tissues.

TABLE I

ACTIVATION OF SD BY $KHCO_3$ AND SUCCINATE

Pre-Treatment	SD Activity (nmol DCIP reduced mg prot^{-1} min^{-1})			
	heart	skeletal muscle	liver	kidney
None	28	24	72	53
Preincubated 7 min at 37°	50	40	93	110
Preincubated 7 min at 37° with 20 mM $KHCO_3$	280	280	125	154
Preincubated 7 min at 37° with 20 mM succinate	220	200	151	179

Mitochondria (0.4-0.8 mg protein/ml) were suspended in 0.1 M HEPES, pH 7.6, containing 2 µg rotenone/ml. Pre-treatment was then carried out in this medium plus additions as indicated. Values are means of three determinations.

Fig. 2 shows a comparison of the extent of activation of SD in mitochondria from different tissues as a function of bicarbonate concentration. As seen from this figure, bicarbonate caused very marked activation of SD in heart and skeletal muscle mitochondria even at quite low concentrations (about 3-fold at 5 mM), reaching 6-7 fold activation at higher concentrations. On the other hand, bicarbonate maximally elevated SD activity of liver and kidney cortex mitochondria on incubation at 37° less than 40%.

Kimura et al. (4) have shown that the activation of SD with succinate could be reversed by removal of succinate by washing, whereas competitive inhibitors are more firmly bound to the active site and are therefore less easily removed. Since bicarbonate can also function as an inhibitor of SD (8), we compared the reversibility of the activation by succinate and bicarbonate, and combinations of the two. As shown in Table II, Experiment 1, activation by succinate was completely reversed on repeated washing, as expected from published reports (4). Prior activation by bicarbonate was reduced from about 6-fold to 4-fold of the basal SD activity by a single wash, a second wash having no further

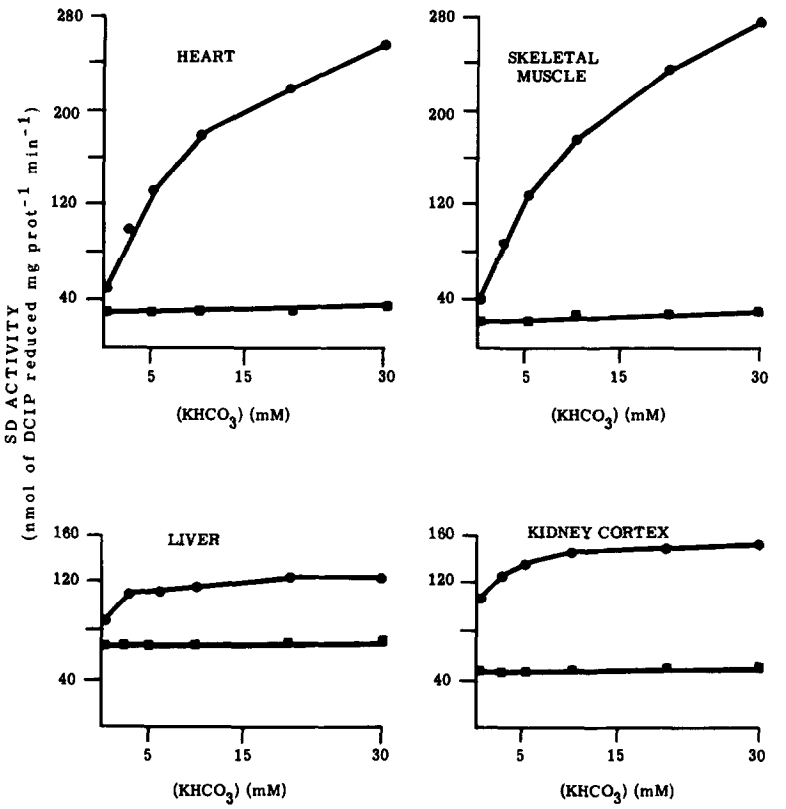

Fig. 2. Comparison of activation of succinate dehydrogenase of different rat tissues by $KHCO_3$. 4 mg (heart and skeletal muscle mitochondria) or 8 mg (liver and kidney cortex mitochondria) were preincubated at 37° C (●-●) or at 0° C (■-■) for 7 min in 10 ml of 0.1 M HEPES (pH 7.6) + 2 µg/ml rotenone with indicated concentrations of $KHCO_3$. At the end of incubation period the samples were cooled to 0° C, followed by measurement of SD activity at 15° as described under Materials and Methods.

effect. Now if SD was activated by a prior incubation of mitochondria with both succinate and bicarbonate, SD was activated only to the level obtained with succinate alone. Repeated washing completely reversed the activation, as was the case when only succinate was the activating agent. Thus, it is apparent that bicarbonate causes activation by binding the enzyme at the succinate-binding site, but that it is more tightly bound than is succinate. When both are present during the activation, however, succinate can displace or prevent the binding of bicarbonate. This conclusion is further substantiated by Experiment 2 in Table II. First, SD was activated by incubation with bicarbonate.

TABLE II

EFFECT OF WASHING OF HEART MITOCHONDRIA ON SD ACTIVITY AFTER PREVIOUS INCUBATION WITH $KHCO_3$ OR SUCCINATE

Experiment 1	SD Activity (nmol DCIP reduced $min^{-1}mg^{-1}$)		
Additions to Preincubation	Initial	Once-washed	Twice-washed
None	50	45	45
20 mM Succinate	216	60	45
20 mM $KHCO_3$	285	180	180
20 mM succinate plus 20 mM $KHCO_3$	210	60	45

Experiment 2	SD Activity
Treatment (sequential)	
Pretreated with 20 mM $KHCO_3$	280
Centrifuged, resuspended and incubated with 20 mM succinate for 7 min at 37°	210
Once washed	50

Experimental conditions as described in Materials and Methods and in Table I. Washing medium was .25 M sucrose containing 10 mM Tris and 1 mM EDTA, pH 7.6. Results are from a single experiment, which are essentially identical to several others which were carried out.

When these mitochondria were centrifuged and resuspended, followed by an incubation period in the presence of succinate, SD activity was reduced to the level of activation due to succinate alone. Subsequent washing then completely reversed the activation originally effected by bicarbonate.

It has been suggested (discussed e.g. in ref. 13) that SD activity is under multiple regulation by several effectors. In this context, since fully activated SD activity is well in excess of the capacity of the electron transport chain, uncontrolled oxidation of succinate derived from several possible metabolic sources would inhibit NADH oxidation, lower the yield of ATP, and upset the balance of fluxes and steady-state distribution of metabolites in the citrate cycle.

It is striking that the activating effect by bicarbonate which we observed on SD activity of mitochondria as isolated is most pronounced for muscle mitochondria. Since the rate of CO_2 production through citrate cycle oxidations by both heart and skeletal muscle can fluctuate many-fold, it is suggested that bicarbonate may exert an important regulatory role on the citrate cycle through its effects on SD.

ACKNOWLEDGEMENTS

Supported by USPHS Grants AM13939, AA00289 and the Grace M. Showalter Trust. J. S. is recipient of a Fogarty International Fellowship.

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