IS THERE A 'TURNOVER CONTROL SITE' IN SUCCINATE DEHYDROGENASE?

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1. Introduction

It is well known that a number of compounds, including substrates, competitive inhibitors and anions, activate mammalian succinate dehydrogenase [1]. There is also growing evidence that the catalytic activity of the activated form of the enzyme is further increased in the environment of the inner membrane [2,3]. Recently, a third type of regulation of the activity of the enzyme was postulated by Gopher and Gutman [4]. Using beef heart ETP_H as experimental material, these authors noted that, following activation by malonate under conventional conditions, the catalytic activity measured at 3°C shows a pronounced lag, measuring the reduction of PMS, as well as inhibition of the reduction of cytochrome *b*. This lag was abolished by preincubating the activated enzyme at 3°C with succinate or fumarate. They interpreted these observations as indicating that the fully activated enzyme is only potentially active and reaches full activity only after succinate or fumarate occupy a postulated 'turnover control site', distinct from the catalytic site.

As shown below, the experiments described in [4] may be explained in a different and simpler way, without the need for postulating additional regulatory mechanisms or sites in succinate dehydrogenase.

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; ETP_H, phosphorylating inner membrane preparation; PMS, phenazine methosulfate

*The use of 10 mM PMS and 60 mM succinate as described [4] did not materially alter our results, except that the higher concentration of PMS caused gradual decay of enzymic activity

2. Materials and methods

ETP_H particles were made from beef heart mitochondria [5]. Activation by malonate and activity measurements with PMS–DCIP were carried out as described [4], except that 1 mM PMS and 20 mM succinate were used in the assay*. Activation by Br^- or NO_3^- was as described by Ackrell et al. [6].

3. Results and discussion

In this laboratory we have noted that in assays carried out at 15°C the malonate-activated enzyme shows a distinct lag, which is abolished if the enzyme is first washed with NaBr solutions to displace the malonate and assumed that the lag was an expression of the relatively slow dissociation of malonate from the catalytic site. It seemed likely that this is also the explanation of the observation [4]. In order to test this, the following experiments were performed.

Preparations activated with 2 mM malonate at 30°C, centrifuged and resuspended in buffer, showed a pronounced lag in assays at 3°C, if the reaction is started by adding succinate and dyes (fig.1, curve 1). In contrast, samples activated with Br^- (curve 2) or NO_3^- (curve 3) and similarly treated and assayed showed no lag. The activity eventually reached in malonate-activated samples was essentially the same as given in anion-activated samples from the start. Figure 2 illustrates that the lag was also abolished and maximal rate attained by 15 min incubation at 3°C with 2 mM fumarate (curve 1) or without fumarate (curve 2) or by washing the malonate-activated particles with Br^- solution prior to start of

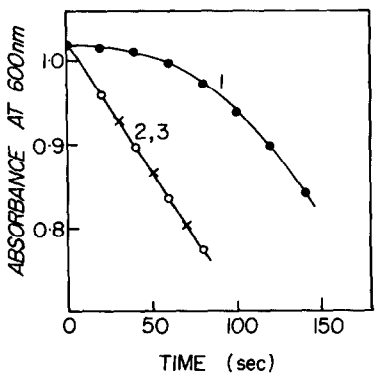

Fig.1. Kinetics of succinate dehydrogenase activity in ETP_H at 3°C. Curve 1: after activation with 2 mM malonate 30 min at 30°C, centrifugation and resuspension under the exact conditions of [4]. The reaction, in 3 ml total vol., was started by the simultaneous addition of ETP_H (20 μl) and of succinate (20 mM), DCIP (50 μM) and PMS (1 mM). Curves 2, 3: same as 1, except that activation was with 0.18 M sucrose, 45 mM Hepes, 90 mM semicarbazide, 450 mM Br^- (○) or NO_3^- (×) pH 7.0, for 20 min at 25°C.

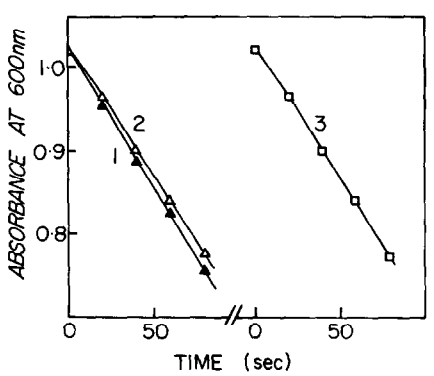

Fig.2. Kinetics of succinate dehydrogenase activity in ETP_H at 3°C. Curves 1, 2: identical to fig.1, curve 1, except that the enzyme was incubated in the assay mixture for 15 min at 3°C with (▲) or without (△) 2 mM fumarate prior to the addition of succinate, DCIP and PMS. Curve 3: as in fig.1, curve 1, except that the malonate-activated ETP_H was further treated with Br^- as in fig.1, curve 2, followed by centrifugation and resuspension as before.

the assay (curve 3). Hence, neither succinate nor fumarate are needed to abolish the lag and to measure linear rates from the start of the assay.

We conclude that the lag observed in the cold is the consequence of the slow rate of dissociation of inhibitory malonate from the substrate site under these conditions and that there is no need to postulate a 'turnover control site' in the enzyme. The authors of [4], in fact, considered inhibition by malonate as a possible explanation of their results but dismissed it on the grounds that

(a) The malonate carried over into the assay would be diluted out and displaced by the very high succinate concentration used in the assay.
(b) Preparations activated by NaBr instead of malonate also showed the lag and fumarate effect, although no documentation of this was presented.

Point (a) assumes that the dissociation of malonate is far more rapid than catalytic activity, which is not the case (fig.1, curve 1). Point (b) is negated by the experiments of fig.1, curve 2.

Acknowledgements

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