THE CATALYTIC ACTIVITY OF SOLUBLE AND MEMBRANE-BOUND SUCCINATE DEHYDROGENASE

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1. Introduction

Most of the data available indicate that coenzyme Q is a natural electron carrier between succinate dehydrogenase and cytochromes ([1–4] and references therein). However the direct reaction between soluble reconstitutively active succinate dehydrogenase and ubiquinone or its analogs has not been shown yet. Two methods are employed for assay of soluble preparations of succinate-dehydrogenating enzymes: use of artificial electron acceptors and so called 'reconstitution test' [5,6]. The qualitative and quantitative interrelations of reconstitution activity and reactions with artificial electron acceptors has been extensively discussed by different groups [4, 6–8]. Most recently the reaction with artificial acceptor (low concentration of ferricyanide) has been shown to have some properties of reconstitution activity [9,10]. This finding seemed to have solved the long existing puzzle of the lack of correlation between the activity of the soluble enzymes measured with dyes and their reconstitutive capability. The problem is however complicated by the recent observation by Ackrell et al. [4] who have shown that the total activity of succinate dehydrogenase measured with PMS is increased when the soluble enzyme is incorporated into submitochondrial particle membrane. It has been speculated that the specific environment of the membrane influences the turnover number of the enzyme [4]. The similar hypothesis has been suggested on the basis of experimental data obtained by different approaches [11,12].

The extraction of phospholipids from succinate: cytochrome *c* reductase has been shown to decrease the activity of succinate dehydrogenase measured with PMS [11]. The same decrease of the enzyme turnover number occurs when ubiquinone is extracted from submitochondrial particles or when interaction between succinate dehydrogenase and coenzyme Q is prevented by TTA [12].

It is obvious that the hypothesis about 'allotopic' change of succinate dehydrogenase activity is valid only if the acceptor used for the turnover number measurement is able to reveal the full catalytic activity of the soluble enzyme.

Thus quantitative comparison of enzyme activity with different electron acceptors seems to be important.

The reactions of succinate dehydrogenase with ferricyanide and PMS has been characterized from this point of view [5,8–10]. Another artificial acceptor, WB, has been reported to serve as effective oxidant for the enzyme [7,13,14]. No quantitative data are available comparing WB and other electron acceptors.

This paper examines the reactivities of soluble and reconstituted succinate dehydrogenase towards WB and PMS. The data obtained show that only part of the soluble enzyme activity is revealed when PMS at infinite concentration is used as an oxidant; the activity of the enzyme measured with WB is about twice that with PMS. The turnover number of succinate dehydrogenase measured with PMS does, indeed, increase when the reconstitutively active enzyme is incorporated into membrane. No such an 'activation' occurs when WB is used as an oxidant.

Abbreviations: PMS phenazine methosulfate, TTA 2-thenoyltrifluoroacetone, DCIP 2,6-dichlorophenolindophenol, WB Wurster's Blue, a semiquindiimine radical of *N,N,N',N'*-tetramethyl-*p*-phenylendiamine

These observations suggest that the 'environment effect' on succinate dehydrogenase is due to the nature of the artificial acceptor used, rather than to real changes in catalytic capacity of the enzyme.

2. Materials and methods

Soluble succinate dehydrogenase and alkali-treated apo-particles were prepared from heart muscle essentially as described by King [14].

The enzyme was reconstitutively active and showed no activation phenomenon when preincubated with succinate. Succinate: PMS reductase activity was measured in a coupled system [15] as described by Singer [16]. Succinate: WB reductase activity was measured spectrophotometrically at 612 nm, using the millimolar extinction coefficient 12 [17].

Wurster's Blue was prepared from *N,N,N',N'*-tetramethyl-*p*-phenylendiamine according to the method of Michaelis [17]. Particle bound protein was determined by the biuret method [18] and soluble protein by the Lowry procedure [19].

3. Results

Figure 1 demonstrates the phenomenon of increase in catalytic activity of succinate dehydrogenase after enzyme binding to particles containing the rest of the respiratory chain. As seen from fig.1, at fixed succinate dehydrogenase: apo-particles ratio, both activity measured at any single concentration of PMS and activity extrapolated to infinite dye concentration, are increased about two times when soluble enzyme is incorporated into membrane. The degree of this increase depends on the amount of particles used in the reconstitution (fig.2) and reaches a plateau when the ratio succinate dehydrogenase: apo-particles is about 20 times less than that in an intact Keilin-Hartree preparation [20] (soluble enzyme with an average flavin content of about 3 mol/mg protein was used). At this ratio the total increase in dehydrogenase activity is about 2.5 times, which seems to be the upper limit for the experimental conditions used. It is worth noting that quantitative evaluation of the data presented in fig.2 suggests that the apparent activation of the enzyme depends on the content of some component

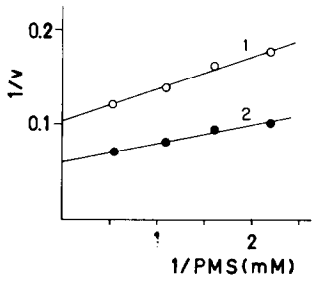

Fig.1. The succinate: PMS reductase activities of soluble (1) and membrane-bound (2) succinate dehydrogenases. Reconstitution was done as follows: alkali-treated particles were mixed with soluble enzyme in 50 mM phosphate buffer (pH 7.4) containing 10 mM succinate and incubated at 25°C for 3 min; antimycin A, final concentration 10^{-5} M, was then added and samples were placed in ice. Protein content in reconstitution system was: succinate dehydrogenase 0.4 mg/ml, alkali-treated particles 10 mg/ml. The samples were assayed at 25°C in a mixture containing 10 mM Tris–sulfate buffer (pH 7.8), 10 mM potassium succinate 0.1 mM EDTA, 0.05 mM DCIP, 1.5 mM KCN and different concentrations of PMS. The reaction was started by addition of the enzyme. No succinate: PMS activity of the alkali-treated particles was observed. All the activities are expressed as μmoles succinate oxidized/min/mg soluble enzyme.

in the reconstituted system belonging to the apo-particles, rather than to the physical incorporation of soluble enzyme into the membrane. Indeed, the enzyme preparation used is about 90% reconstitutively active and increase of its activity still occurs even when the amount of apo-particles added is much greater than necessary to bind all the soluble enzyme.

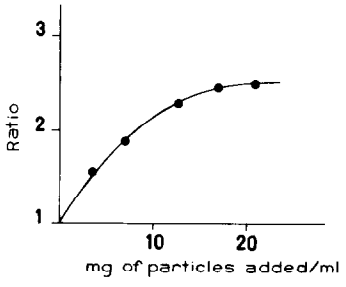

Fig.2. The succinate: PMS reductase activity of succinate dehydrogenase as a function of concentration of the alkali-treated particles. The ordinate is the ratio of activity of the reconstituted system to that of the soluble enzyme. The conditions for reconstitution and assay were the same as in fig.1. The concentration of soluble enzyme in the reconstitution mixture was 0.4 mg/ml.

The data presented in fig.1 and 2 appear to be consistent with the hypothesis of a specific environment effect on catalytic capacity of succinate dehydrogenase [4,11,12]. However no direct evidence exists that the artificial electron acceptor (PMS) is capable of revealing full catalytic activity of dehydrogenase. The simplest way to show that the measured activity of soluble dehydrogenase is limited by the electron acceptor applied, is to find another acceptor which is able to give a higher measured turnover number of the enzyme under identical conditions. The data in fig.3 show that the free radical WB is such an acceptor. The activity of the soluble enzyme measured with WB is about twice that with PMS when extrapolated to infinite dye concentrations. Since this finding suggests that PMS does not measure the full dehydrogenase capacity, it was of interest to find out whether the 'activation' phenomenon described is due to inefficiency of PMS as an electron acceptor for the soluble enzyme.

As seen from table 1 no activation occurs when WB is used instead of PMS to compare the total activities of soluble and particle bound dehydrogenases. This table also shows that apparent activation of the enzyme as revealed by PMS-mediated DCIP reduction can be prevented when electron flow between dehydrogenase and coenzyme Q in the reconstituted system, is inhibited by TTA. Although no increase of total activity is observed when WB was used as acceptor, the reduction of this dye is also partially inhibited by TTA in the reconstituted system. This indicates that part of the electron flow from succinate dehydrogenase to WB in the reconstituted system passes through the component located after the TTA sensitive site.

Table 1
The reduction of different electron acceptors by soluble and membrane-bound succinate dehydrogenase preparations[a]

Acceptor	PMS		WB	
	μmoles succinate/min/mg soluble enzyme			
Enzyme preparation	−TTA	+TTA	−TTA	+TTA
Soluble enzyme	10.0	10.0	25.0	25.0
Reconstituted system	16.8	11.6	25.0	11.8

[a]All the activities were obtained by extrapolation to infinite acceptor concentration. 0.3 mM TTA was present in assay mixture where indicated. The reconstitution and assays were performed as in fig.1.

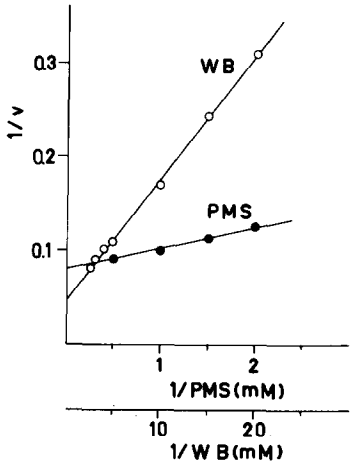

Fig.3. Comparision of succinate: PMS and succinate: WB reductase activities of soluble succinate dehydrogenase. For assay conditions see fig.1.

4. Discussion

Problems about the turnover numbers of different preparations of succinate dehydrogenating enzymes or enzyme systems has occupied a lot of the biochemical literature and has been extensively reviewed [4,7,21]. It has been known for a long time that different soluble (reconstitutively active or inactive) and particulate preparations of succinate dehydrogenase, such as mitochondria, submitochondrial particles, and complex II, have different turnover numbers, based on the acid nonextractable flavin content and the maximal activities obtained, as a rule, with PMS as electron acceptor [21 and references therein].

The turnover number of succinate dehydrogenase is an important problem from two points of view. First, the components of the respiratory chain are not present in a simple one to one ratio [7,20]. Thus the question of how one molecule of dehydrogenase can provide electrons to several cytochrome molecules remains to be answered both structurally and kinetically. Second, the effect of the natural lipid milieu on succinate dehy-

drogenase is not clear yet, although a definite answer to this question may give a clue to the solution of the basic problem, to what extent can this component of the respiratory chain be purified without loss of natural properties.

The results presented in this paper demonstrate that no change in succinate dehydrogenase activity occurs when soluble enzyme becomes bound to the membrane. An apparent increase of catalytic activity (fig.1 and 2) is evidently due to inability of PMS to reveal the full turnover number of the soluble enzyme. This conclusion is based on two experimental facts: (1) The V_{max} for the soluble enzyme with WB is about twice that with PMS (fig.3) and (2) no increase of activity was observed when WB was used as electron acceptor for the reconstituted system (table 1). The question then arises, which are the electron pathways involved in reduction of the two artificial acceptors. As seen from table 1, some component located after the TTA sensitive site is involved in reduction of WB in the reconstituted system. It seems logical to propose that this component is coenzyme Q.

This proposal is consistent with the reports that any kind of treatment which prevents interaction between dehydrogenase and ubiquinone (solubilization [4], cyanide treatment [22], extraction of coenzyme Q [12], TTA inhibition [12]) will decrease an apparent catalytic activity measured with PMS. Our experiments on the effect of extraction and reincorporation of coenzyme Q on catalytic activity of dehydrogenase also support this hypothesis [23].

Perhaps the most important observation reported in this paper is that the widely used artificial electron acceptor PMS is not capable of measuring the full catalytic activity of soluble succinate dehydrogenase. Free radical WB, first introduced for succinate dehydrogenase assay by King's group [7,13,14] seems to be more suitable for this purpose.

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