THE SULFHYDRYL GROUPS AND STABILITY OF THE SOLUBLE SUCCINATE DEHYDROGENASE

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1. Introduction

It is well known that soluble succinate dehydrogenase (SDH) in contrast to the particle-bound enzyme is very unstable. The inactivation of soluble SDH has been studied [1–3] and two major factors, namely the presence of succinate (or malonate) and the absence of oxygen were found to be important for enzyme stability. The nature of the protective effect of substrate (or competitive inhibitor) on the enzyme is still unclear.

We have proposed [4] that oxaloacetate (OAA) binds with the enzyme through the formation of thiosemiacetate at the active site of SDH. Recently we have reported evidence that sulfhydryl groups of the enzyme are involved in apparently irreversible binding of OAA [5].

Since substrate or competitive inhibitors protect SDH against the aerobic inactivation, it seemed likely that the binding of SH-groups at the active site could prevent enzyme inactivation. It is shown in this report that the treatment of SDH with *p*-CMB prevents the loss of succinate ferricyanide reductase activity when the enzyme is incubated aerobically without substrate or competitive inhibitors.

2. Materials and methods

SDH was isolated essentially as described by King [6]. The enzyme-depleted particles were obtained from a Keilin–Hartree preparation by alkaline treatment [6]. The ferricyanide reductase activity was measured at 400 nm in 0.5 cm cuvettes containing: 0.1 M potassium phosphate; 10 mM succinate; 0.1 mM EDTA; 1 mg/ml bovine serum albumin and 3 mM ferricyanide (pH 7.8) at 20°C. The reconstitution was carried out in 2 ml samples containing: 0.1 M potassium phosphate (pH 7.8); 10 mM succinate; 0.1 mM EDTA; 1 mg/ml bovine serum albumin; 1.2 mg/ml alkali-treated particles; 0.26 mg/ml succinate dehydrogenase at 20°C and was measured with an oxygen electrode. The protein content was measured according to Lowry et al. (isolated SDH) [8] or Cornall et al. (alkali-treated particles) [9]. The experimental details are indicated in the legends to the figures.

3. Results

As seen from table 1, when SDH is preincubated with *p*-CMB (20 nmole/mg), almost complete loss of succinate-ferricyanide reductase activity is observed. Both mercaptoethanol and dithiothreitol reverse *p*-CMB-induced inhibition of the activity. It has to be pointed out that SH-containing compounds themselves do not affect the enzyme activity.

The result obtained can be compared with the data presented in fig. 1, where the effect of *p*-CMB on the reconstitution of succinate oxidase was studied.

The activation of oxygen consumption after a short lag was added to the alkali-treated particles (fig. 1,1). The following addition of antimycin A, and TMPD* show that reconstituted activity has the properties of the intact succinate oxidase. The addition of mercapto-ethanol (100 μM) does not influence the reconstitu

* Tetramethyl-*p*-phenylenediamine.

Table 1

The inhibition of ferricyanide reductase activity of SDH by *p*-CMB and reactivation of the enzyme in the presence of SH-containing compounds*.

Samples	Activity (μmoles $K_3Fe(CN)_6$ reduced per min per mg of protein	Activity %
Untreated SDH	2.34	100
Untreated SDH measured in the presence of 100 μM mercaptoethanol or 100 μM dithiothreitol	2.34**	100
SDH preincubated with *p*-CMB	0.37	15.5
SDH preincubated with *p*-CMB measured in the presence of mercaptoethanol (100 μM)	2.36**	100
SDH preincubated with *p*-CMB measured in the presence of dithiothreitol (100 μM)	2.4**	100

* SDH (3.2 mg/ml) was preincubated 20 min with *p*-CMB (20 nmoles/mg) at 0°C. After preincubation the control and inhibited activities were measured as described in Materials and methods. The SDH concentration in a cuvette was 96 μg/ml.

** A slow nonenzymatic reduction of ferricyanide by SH-containing compounds was observed. The figures in the table represent ferricyanide reductase activity sensitivive to malonate.

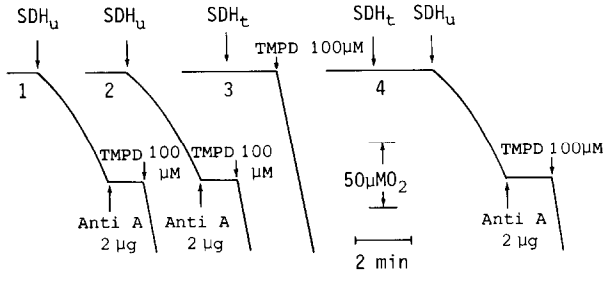

Fig. 1. The effect of *p*-CMB on the reconstitution of succinate oxidase from soluble SDH and alkali treated particles (experimental details in Materials and methods). SDH_u and SDH_t – the control and *p*-CMB-treated (20 nmoles/mg) enzymes respectively. Samples 2 and 3 contained 150 μM of mercaptoethanol.

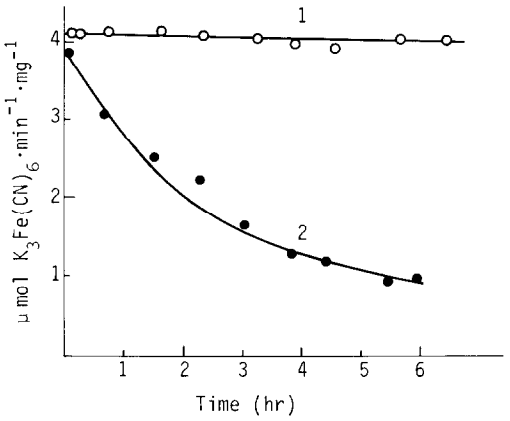

Fig. 2. The effects of *p*-CMB on the stability of SDH aged without substrate. SDH (2.8 mg/ml) was incubated in air at 0°C in 0.1 M phosphate buffer (pH 7.8) containing 0.1 mM EDTA. The activity was measured as described in Materials and methods in the presence of dithiothreitol (200 μM). Curve 1 – *p*-CMB-treated enzyme (20 nmoles/mg), curve 2 – control.

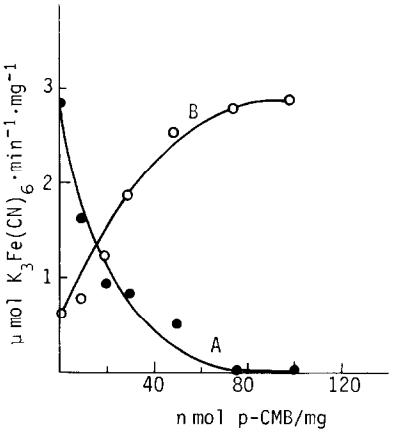

Fig. 3 The titration curves for the inhibitory (A) and stabilization (B) effects of *p*-CMB on SDH. SDH 2.4 mg/ml was treated 20 min at 0°C with different concentration of *p*-CMB and the activity was measured (A). After the treatment the samples were incubated aerobically 25 min at 25°C, and succinate ferricyanide reductase activity was measured (B) as described in Materials and methods in the presence of mercaptoethanol (100 μM). The final SDH concentration at a cuvette is 0.12 mg/ml.

tion process (fig. 1, 2). No reconstitution was found when *p*-CMB-treated SDH was added to the reaction mixture, however the subsequent addition of the fresh enzyme to the same system caused the formation of intact succinate oxidase (fig. 1, 4). The presence of mercaptoethanol cannot reverse the *p*-CMB-induced inhibition of the reconstitution but does activate the oxygen consumption in the presence of artificial electron acceptor (TMPD) (fig. 1, 3).

Therefore the data presented show that *p*-CMB causes the inhibition of both reconstitution process and activity of the enzyme towards the artificial electron acceptor. The latter in contrast to the former can be completely recovered in the presence of SH-compounds.

Since, as noted above, an inactivation of SDH can be prevented by substrate or competitive inhibitors (2, 3], and on the other hand there is evidence that the sulfhydryl group is responsible for the binding of OAA [5], experiments were done, where the effect of *p*-CMB on the enzyme stability was studied. As seen in fig. 2 when SDH was treated with *p*-CMB the complete prevention of inactivation was observed under the condition where the control enzyme was found to be almost fully inactivated.

The titration experiments (fig. 3) showed that the same quantity of *p*-CMB is required for both inhibition of succinate ferricyanide reductase activity and stabilization of the enzyme (~ 50 nmoles/mg).

4. Discussion

The nature of the protective effect of substrate, competitive inhibitor [2, 3] and *p*-CMB (this paper) on the enzyme is still unclear.

There are at least two possibilities for the stabilizing effect of succinate or competitive inhibitors on SDH. These compounds may be directly involved in binding to the oxygen-sensitive site of SDH. Alternatively, substrate or competitive inhibitors may induce the conformational change of SDH in such a manner that the oxygen-sensitive site becomes 'hidden'. The protective effect of *p*-CMB found, together with the data on OAA binding [4, 5] suggests that the first alternative is correct. Since OAA and *p*-CMB behave kinetically as competitive inhibitors [4] it seems likely that the same sulfhydryl group of the enzyme takes part in: i) the first step of succinate oxidation; ii) the inactivation of SDH in presence of oxygen and iii) the irreversible binding of OAA [4, 5].

The high reactivity of that SH-group to oxygen (and probably to the substrate) is possibly due to the coordination of sulfide-ion by an electropositive charge. The p*K*-value for the group responsible for succinate binding (~ 7.8) [10] is in a good treatment with this hypothesis.

The inability of SH-compounds to reactivate *p*-CMB-inhibited reconstitution is probably due to the reaction with other than substrate binding sulfhydryl. The evidences for the existence of different kinds of SH-groups in SDH have been reported [11].

Whatever, the mechanism of the *p*-CMB-induced enzyme stabilization and the chemical properties of SH-group are, the data reported in this paper provide a useful tool for preventing inactivation of soluble SDH.

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