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THE EFFECT OF MERSALYL ON THE OXIDATION OF SUCCINATE BY HOUSEFLY MITOCHONDRIA

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SUMMARY

1. Succinate is oxidized very rapidly by housefly flight-muscle mitochondria in the presence of rotenone.

2. Phosphate (or arsenate) is a strong inhibitor of this succinate oxidation.

3. The inhibitory effect of phosphate (or arsenate) can be overcome by mersalyl and other sulphydryl reagents.

4. Under conditions of rapid succinate oxidation the intramitochondrial phosphate concentration is low.

5. It is suggested that phosphate competes with succinate for entry into the mitochondria.

INTRODUCTION

The impermeability of housefly flight-muscle mitochondria to added intermediates of the Krebs cycle has been shown under several conditions[1,2]. VAN DEN BERGH AND SLATER[1] postulated that this impermeability has physiological significance as a mechanism to prevent these intermediates from leaking out of the mitochondria during active oxidation of pyruvate.

However, TULP AND VAN DEN BERGH[3] demonstrated that under certain conditions malonate does penetrate slowly into housefly mitochondria. The rate of this penetration was comparable to the rate of succinate oxidation under similar conditions, *i.e.* in the presence of phosphate. Also, VAN DEN BERGH[4] has shown that succinate can be oxidized much more rapidly in the absence of phosphate and presence of Amytal (to prevent formation of oxaloacetate).

In this paper experiments are reported confirming that succinate can be oxidized rapidly by housefly mitochondria, provided the phosphate concentration is low. Furthermore, the effect of the sulphydryl-binding reagent mersalyl, known to inhibit phosphate translocation[5,6], was investigated.

The fact that succinate can be oxidized rapidly under certain circumstances indicates that an active system exists for its translocation. Under more physiological conditions, however, the conclusion of VAN DEN BERGH AND SLATER[1] that housefly

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mitochondria are relatively impermeable for Krebs-cycle intermediates such as succinate remains valid.

RESULTS AND DISCUSSION

The rate of oxidation of succinate by housefly mitochondria, in the presence of rotenone, is plotted in Fig. 1 as a function of the concentration of added phosphate.

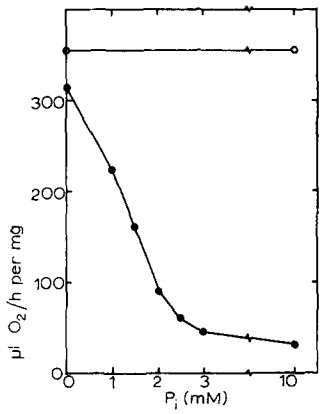

Fig. 1. Inhibition of succinate oxidation by phosphate and its release by mersalyl. Housefly mitochondria were incubated in differential manometers in a medium containing 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.5), 60 mM sodium succinate, 1 mM ADP and 2 µg/ml rotenone. The phosphate concentration is indicated in the figure. The protein content[7] was 1.0 mg/ml. The reaction volume was 1 ml; final pH, 7.5. Temp., 25°. The reaction was started by addition of the mitochondrial suspension. ●—●, no further additions; ○—○, 20 µM mersalyl.

Low concentrations of phosphate inhibit quite strongly. In another experiment (not shown) it was found that arsenate behaves like phosphate. The inhibition by phosphate (or arsenate) is completely prevented by the simultaneous addition of mersalyl, which is known to inhibit phosphate translocation[5,6]. The slight stimulation of the oxidation by mersalyl in the absence of added phosphate is probably due to a release of the inhibition caused by the endogenous phosphate of the mitochondria. Two other sulphydryl-binding reagents, *N*-ethylmaleimide or thiaminpropyldisulphide, behaved in the same way as mersalyl.

The maximal rate of succinate oxidation found was approx. 450 nmoles/mg per min, which may be compared with a maximal rate of approx. 200 nmoles succinate per mg per min for rat-liver mitochondria. In the presence of 30 mM phosphate this rate is lowered by more than 90 %.

It should be noted that the rapid oxidation of succinate in the absence of phosphate can not be stimulated by addition of compounds like 2,4-dinitrophenol. Thus, it appears to be uncoupled or loosely coupled. The factors responsible are currently under investigation.

A maximal oxidation rate was found whether mersalyl was added immediately or after a period of inhibited respiration, indicating that the inhibition is reversible. However, it was noted that after incubation with phosphate, there was a lag period before mersalyl exerted its maximal effect.

TABLE I

MITOCHONDRIAL PHOSPHATE CONTENT UNDER DIFFERENT CONDITIONS

Housefly mitochondria were incubated at a concentration of 1.3 mg/ml under the conditions described in Fig. 1. 0.5-ml samples were withdrawn after 10 min and centrifuged through silicone into 0.1 ml $HClO_4$ (15%), as described by VAN DAM AND TSOU[9]. In the supernatant and acid layers P_i was determined according to the method of WAHLER AND WOLLENBERGER[10]. The values found were corrected for the amount of P_i expected to be carried in the sucrose-permeable space that was determined in a parallel experiment using [^{14}C]sucrose[9].

Additions	P_i (*nmoles/mg*)	
	Expt. 1	*Expt. 2*
None	13.5	19.1
P_i (4 mM)	45.0	42.0
P_i + mersalyl (20 μM)	18.0	21.2
Mersalyl	5.6	—

To test the possibility that the rate of succinate oxidation by housefly mitochondria is governed by the intramitochondrial concentration of phosphate, the intramitochondrial concentration of phosphate under the different conditions was measured, using the technique of centrifugation through silicone[8,9] to determine the mitochondrial contents. Addition of 4 mM phosphate, which strongly inhibits the oxidation of succinate, leads to an increased phosphate content in the mitochondrial matrix (Table I). Addition of mersalyl together with the phosphate reduced the phosphate content approximately to its original level. Addition of mersalyl alone, which slightly stimulates oxygen uptake, slightly decreased the amount of phosphate present in the mitochondria.

To distinguish between the possibilities that the inhibitory effect of phosphate is connected with succinate oxidation or its penetration into the mitochondria, the experiment described in Fig. 2 was carried out. In mitochondria broken by sonic treatment, phosphate no longer has an inhibitory action. At the same time, concentrations of mersalyl that stimulate oxidation of succinate in the intact mitochondria

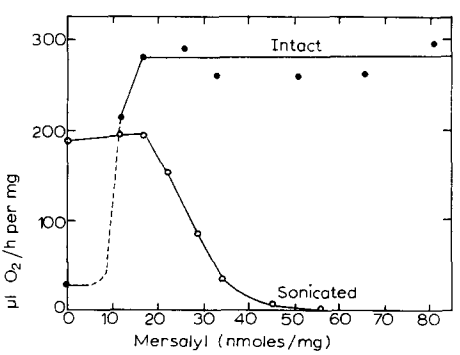

Fig. 2. Effect of mersalyl on succinate oxidation in intact and broken mitochondria in the presence of phosphate. The reaction medium was as described in Fig. 1. Phosphate concentration, 10 mM. Mitochondria were sonicated in an MSE sonic disintegrator at 60 kHz for 4 periods of 30 sec at 0°. The protein concentration was 1.24 mg/ml for the intact and 1.78 mg/ml for the sonicated mitochondria. In the latter case 10 μM cytochrome *c* was added to the reaction mixture. The initial velocity of the reaction is plotted.

have no effect on the broken particles. Higher concentrations of mersalyl inhibit but this effect is probably due to the long-known inhibition of succinate dehydrogenase by sulphydryl reagents[11]. The effects of mersalyl were completely reversible. Addition of dithioerythritol resulted in a restoration of the respiration found in the absence of mersalyl, both in intact and broken mitochondria.

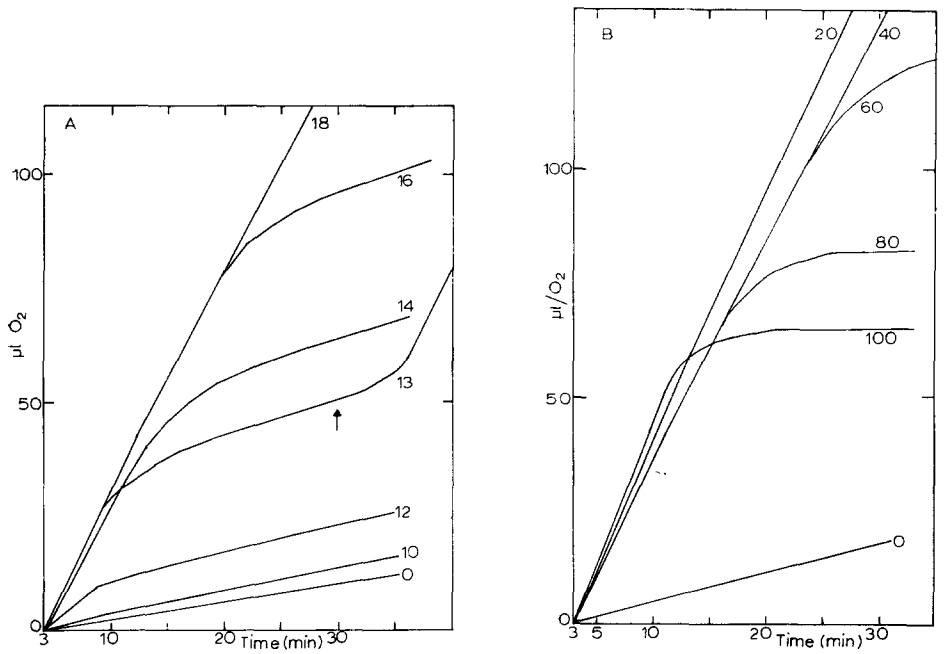

Fig. 3. Effect of different concentrations of mersalyl on the kinetics of succinate oxidation in th presence of phosphate. The reaction conditions were as described in Fig. 1. Phosphate concentra- tion, 12.5 mM. Mersalyl was added to the concentration (μM) as indicated in the figure. In A the protein content was 0.94 mg/ml. At the arrow another 10 μM mersalyl was added. In B, the protein content was 1.24 mg/ml.

The kinetics of succinate oxidation at different concentrations of mersalyl are illustrated in Fig. 3. At low concentrations (Fig. 3A) the inhibition by phosphate is temporarily released and the oxidation returns to the level found without mersalyl. The release of inhibition is maintained longer with higher concentrations of mersalyl. It appears that mersalyl acts initially on the phosphate-translocating system[5,6], and is later transferred to some other sulphydryl group, possibly located deeper inside the mitochondria. Support for this view comes from the fact that addition of a second small amount of mersalyl again releases the phosphate inhibition (Fig. 3A, at arrow). Much higher concentrations of mersalyl also stimulate respiration only temporarily (Fig. 3B), but in this case inhibition sets in more rapidly the higher the concentration of mersalyl and the final level of respiration is lower than that in the absence of mersalyl. This inhibition is not released by a second addition of mersalyl (not shown). This inhibition in this case is probably due to binding of mersalyl to sulphydryl groups of succinate dehydrogenase[11], as in the case of sonicated mitochondria (*cf.* Fig. 2).

Thus, housefly mitochondria appear to contain a phosphate-translocating system sensitive to low concentrations of sulphydryl reagents as already described for other mitochondria by TYLER[5] and FONYÓ[6]. Higher concentrations of mersalyl also react with other sulphydryl-containing proteins in the mitochondria, for instance succinate dehydrogenase.

We interpret the effects of phosphate and mersalyl on succinate oxidation in housefly mitochondria as follows. During succinate oxidation succinate exchanges for its oxidation product malate[2]. In housefly mitochondria the exchange system can be saturated by low concentrations of phosphate and much less succinate can enter. It may well be that the actual physiological function of this carrier system is to bring phosphate into the mitochondria: during active pyruvate oxidation up to 2.5 μmoles P_i per mg per min have to enter for synthesis of ATP.

It has been shown recently that in rat-liver mitochondria the exchange of dicarboxylic acid anions is not inhibited by mersalyl while the exchange of a dicarboxylic acid anion against phosphate is[12]. It seems, then, that it is the access of phosphate to the carrier that is inhibited by mersalyl. These data fit very well with the observations presented here: addition of mersalyl to housefly mitochondria prevents the inhibitory effect of phosphate and allows the succinate–malate exchange to proceed at maximal speed.

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