BBA 46125

# PERMEABILITY OF HOUSEFLY MITOCHONDRIA TOWARDS DICARBOXYLATE IONS

A. TULP, H. STAM AND K. VAN DAM

*Laboratory of Biochemistry, B.C.P. Jansen Institute*, University of Amsterdam, Amsterdam (The Netherlands)*

(Received January 13th, 1971)

SUMMARY

Studies with labelled substrates show that housefly mitochondria are permeable towards dicarboxylate anions, provided the phosphate concentration is low. Dicarboxylate anions compete with each other for entry. Phosphate also competes with the dicarboxylate anions for translocation and this effect is prevented by the sulphydryl reagent mersalyl.

## INTRODUCTION

Studies in several laboratories seemed to indicate that mitochondria from flight muscle of carbohydrate-utilizing insects are impermeable towards added Krebs-cycle intermediates[1-3]. As a consequence, it has been suggested that these mitochondria genetically lack the di- and tricarboxylate ion translocators that have been thoroughly studied in mammalian mitochondria.

The methods applied to reach this conclusion are all of rather indirect nature. VAN DEN BERGH AND SLATER[1] and SACKTOR AND CHILDRESS[2] showed that ultrasonic disruption of the insect mitochondria gives rise to a considerable stimulation of the oxidation of Krebs-cycle intermediates, indicating that the rate-limiting step was between the substrates and the enzyme system oxidizing these. According to these authors, the slow oxidation that was observed was brought about by a relatively small number of partly damaged mitochondria. Furthermore, CHAPPELL AND HAARHOFF[3] demonstrated that housefly mitochondria do not swell in isotonic solutions of the ammonium salts of Krebs-cycle intermediates in the presence of low concentrations of phosphate.

Additional evidence for the impermeability is derived from the properties of pyruvate oxidation. SACKTOR AND CHILDRESS[2,4] have shown that preincubation of blowfly mitochondria in the absence of pyruvate greatly diminishes the rate of pyruvate oxidation. Only L-proline and none of the Krebs-cycle intermediates is able to restore the oxidation. Apparently, under these conditions only L-proline is able to enter the compartment where pyruvate oxidation takes place. VAN DEN BERGH[5] showed that in housefly mitochondria glutamate can restore exhausted Krebs-cycle

* Postal address: Plantage Muidergracht 12, Amsterdam, The Netherlands.

activity as well. He suggested that the glutamate:pyruvate transaminase facilitates the entry of α-oxoglutarate (formed in the reaction) into the matrix space.

We thought that more direct proof for the (im)permeability of the membrane could be obtained by measuring the uptake of labelled Krebs-cycle intermediates into the matrix space of housefly mitochondria. This method was also used by KLINGENBERG[6] to show that α-glycerol phosphate is a non-penetrant anion in insect and hyperthyroid rat-liver mitochondria.

Preliminary experiments indicated that malate, succinate and citrate can penetrate rapidly into the matrix space of housefly mitochondria, provided that the phosphate concentration is low. Under the same conditions, it was shown that succinate can be rapidly oxidized[7].

## RESULTS AND DISCUSSION

In a previous communication it was shown that housefly mitochondria are capable of rapid oxidation of succinate[7]. The relationship between oxidation velocity and succinate concentration in the presence of different concentrations of phosphate is given in Fig. 1. Under these conditions the succinate oxidation is completely uncoupled for reasons that are still under investigation.

The $K_m$ for succinate is relatively high (approx. 5 mM) in contrast to the $K_m$ for succinate oxidation in sonicated housefly mitochondria (0.4 mM; not shown). Addition of phosphate markedly increases the $K_m$ and leads to a sigmoidal relationship between substrate concentration and velocity of oxidation. The chemically related arsenate gives similar results. Succinate dehydrogenase and fumarate hydratase were excluded as the site of the phosphate effect, because the housefly mitochondrial succinate dehydrogenase is inhibited only weakly in a competitive manner by phosphate ($K_i$ = 42 mM) and fumarate hydratase from this source is even slightly stimulated by added phosphate. Therefore, we measured the effect of phosphate on the rate of

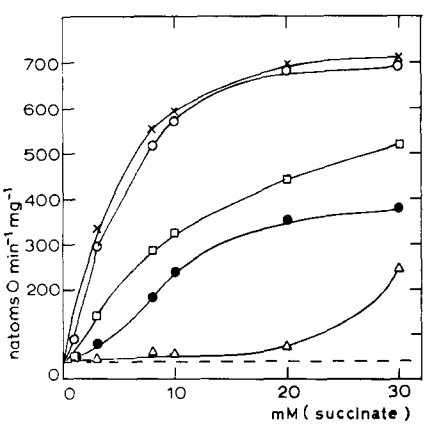


Fig. 1. The inhibition of succinate oxidation by phosphate. The reaction mixture contained 5 mM Tris chloride, 1 mM ADP, 2 mM EDTA, 5 mM $MgCl_2$, 50 mM KCl, 2 μg rotenone and succinate as indicated. The volume was 2.0 ml; pH 7.5; 25°. Further additions were: ○, no additions; □, 0.5 mM phosphate; ●, 1 mM phosphate; △, 5 mM phosphate; ×, 5 mM phosphate (mitochondria were preincubated for 2 min at 0° with 20 nmoles mersalyl per mg protein).

accumulation of dicarboxylic acids, exemplified for the case of malonate in Fig. 2. Again, it is obvious that in the absence of phosphate a rapid entry of malonate into the mitochondria takes place. Addition of phosphate markedly reduces the rate and this effect of phosphate is completely prevented by mersalyl, just as in the case of succinate oxidation.

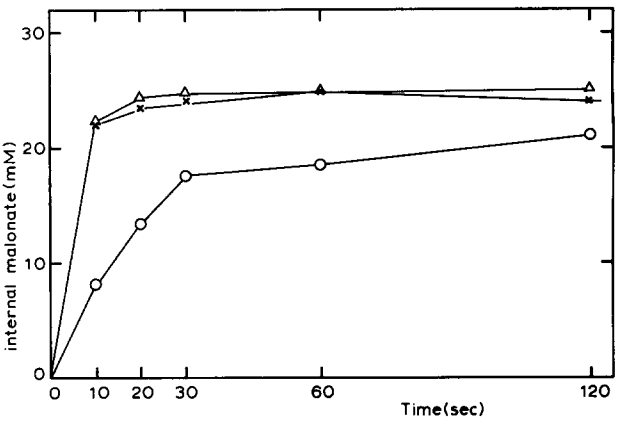


Fig. 2. The inhibition of the rate of uptake of malonate by phosphate. The reaction mixture contained 5 mM Tris chloride, 2 mM EDTA, 5 mM $MgCl_2$, 50 mM KCl, 5 mM malonate and 2 $\mu$g rotenone. The volume was 2.0 ml, pH 7.5, 0°. At the times indicated 0.20-ml samples were withdrawn for analysis. Further additions were: △, no additions; ○, 25 mM phosphate; ×, 25 mM phosphate (mitochondria were preincubated for 2 min at 0° with 20 nmoles mersalyl per mg protein).

An even greater inhibition of malonate entry is observed in the presence of phosphate and pyruvate, *i.e.* under normal metabolizing conditions. In this case it takes several minutes before the malonate concentration in the matrix has reached its maximal level (Fig. 3). This observation correlates nicely with the findings that it takes some time before malonate inhibits pyruvate oxidation maximally[8] (Fig. 4).

Competition between dicarboxylate anions for entry under metabolizing conditions is also illustrated in Fig. 3. Both butylmalonate and α-oxoglutarate strongly inhibit the malonate uptake into the mitochondria. If α-oxoglutarate is added after the intramitochondrial malonate concentration has reached its maximal level it causes a slow expulsion of the malonate. Addition of butylmalonate, on the other hand, does not lead to rapid release of the accumulated malonate.

These observations are again in line with the results of the experiments with pyruvate oxidation which show that butylmalonate cannot release the malonate inhibition and is only able to retard the onset of the inhibition if added before the malonate (Fig. 3). In other experiments it was found that malonate in the presence of butylmalonate inhibited pyruvate oxidation completely only after 20 min. Such a behaviour should be expected on the basis of the suggestion of CHAPPELL *et al.*[9] that butylmalonate is not able to cross the mitochondrial membrane but can bind to the dicarboxylate translocator. In such a model the movement of the dicarboxylate ions should be retarded but the equilibrium concentrations, which are determined by the thermodynamic properties of the system, should not be affected.

Evidently, housefly mitochondria possess a dicarboxylate ion translocator very similar to those of other mitochondria. The uniqueness of the housefly mitochondria lies in the strong inhibition of this translocator by phosphate. Thus, under physiological conditions the movement of dicarboxylate ions will be very slow, resulting in a strong retention of Krebs-cycle intermediates by the housefly mitochondria.

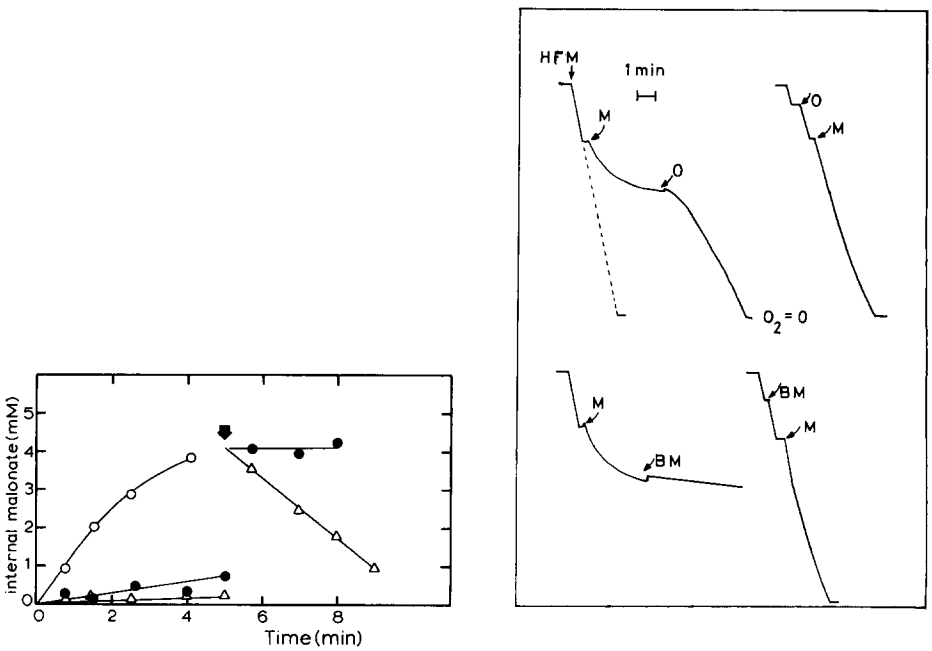


Fig. 3. The slow uptake of malonate during pyruvate oxidation. The reaction mixture contained 25 mM Tris chloride, 1 mM ADP, 25 mM phosphate, 1 mM EDTA, 2.5 mM $MgCl_2$, 10 mM pyruvate, 1 mM malonate and 1–2 units hexokinase. The pH was 7.5, the temperature was 25°. 1 mg mitochondrial protein was present. Other additions were: ○, no additions; △, 10 mM α-oxoglutarate; ●, 20 mM butylmalonate. At the arrow 10 mM α-oxoglutarate or butylmalonate was added.

Fig. 4. Effects of α-oxoglutarate and butylmalonate on malonate inhibited pyruvate oxidation. The reaction mixture was as described in Fig. 3. Where indicated 20 mM malonate, 10 mM α-oxoglutarate or 20 mM butylmalonate were added. 0.5 mg mitochondrial protein was present. Abbreviations: HFM, housefly mitochondria; M, malonate; O, oxoglutarate; BM, butylmalonate.

## MATERIALS AND METHODS

Mitochondria from flight muscle of the housefly were prepared according to VAN DEN BERGH[5]. Protein was determined by the biuret method as described by CLELAND AND SLATER[10]. Uptake of [$^{14}$C]malonate (Radiochemical Centre, Amersham) was measured by the procedure of VAN DAM AND TSOU[11]. $O_2$ consumption was measured with the vibrating platinum electrode of an Oxygraph (Gilson Medical Electronics).

For the assay of fumarate hydratase and succinate dehydrogenase the mitochondrial suspension was fragmented during four 30-sec bursts in a MSE sonic desintegrator (60 W, 60 kHz) at 0°. Fumarate hydratase was assayed spectrophotometrically according to RACKER[12] in the 100000 × *g* supernatant of the sonicated

mitochondria. Succinate dehydrogenase was assayed according to VEEGER *et al.*[13] in a suspension of the sonicated mitochondria.

ACKNOWLEDGEMENTS

Mr. F. J. Van Hemert is thanked for his expert technical assistance.

REFERENCES

1 S. G. VAN DEN BERGH AND E. C. SLATER, *Biochem. J.*, 82 (1962) 362.
2 B. SACKTOR AND CH. C. CHILDRESS, *Arch. Biochem. Biophys.*, 120 (1967) 583.
3 J. B. CHAPPELL AND K. N. HAARHOFF, in E. C. SLATER, Z. KANIUGA AND L. WOJTCZAK, *Biochemistry of Mitochondria*, Academic Press and Polish Scientific Publishers, London and Warsaw, 1967, p. 75.
4 CH. C. CHILDRESS AND B. SACKTOR, *Science*, 154 (1966) 268.
5 S. G. VAN DEN BERGH, *Respiration and Energy Production in the Flight Muscle of the Housefly Musca domestica L*, Ph. D. Thesis, Amsterdam, 1962, Rototype, Amsterdam.
6 M. KLINGENBERG, *European J. Biochem.*, 13 (1970) 247.
7 A. TULP AND K. VAN DAM, *Biochim. Biophys. Acta*, 189 (1969) 337.
8 A. TULP AND S. G. VAN DEN BERGH, *Abstr. 5th Meeting Federation European Biochem. Socs., Prague, 1968*, p. 50.
9 J. B. CHAPPELL, P. J. F. HENDERSON, J. D. MCGIVAN AND B. H. ROBINSON, in P. N. CAMPBELL AND P. N. CHURCHILL, *The Interaction of Drugs and Subcellular Components on Animal Cells*, London, 1968, p. 71.
10 K. W. CLELAND AND E. C. SLATER, *Biochem. J.*, 53 (1953) 547.
11 K. VAN DAM AND C. S. TSOU, *Biochim. Biophys. Acta*, 162 (1968) 301.
12 E. RACKER, *Biochim. Biophys. Acta*, 4 (1950) 211.
13 C. VEEGER, D. V. DERVARTANIAN AND W. P. ZEYLEMAKER, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 13, Academic Press, New York, 1969, p. 81.