THE FORMATION OF ACETATE FROM PYRUVATE IN HOUSE FLY FLIGHT MUSCLE MITOCHONDRIA

A.TULP and K.VAN DAM

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

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1. Introduction

During our studies on the effect of malonate on the oxidation of pyruvate by isolated house fly flight muscle mitochondria we observed that under some conditions the oxygen uptake was much lower than expected on the basis of complete oxidation of the pyruvate that disappeared.

Incomplete oxidation of pyruvate in mitochondria from mammalian tissues has been reported by several groups of investigators [1–3]. In these mitochondria either acetate or acetoacetate is the product of an incomplete oxidation. Although the flight muscle mitochondria of carbohydrate utilizing insects are reportedly not very active with acetate as the substrate [4], the ratio of oxygen uptake to pyruvate disappearance suggested that acetate might be formed under our conditions. Furthermore the finding of Childress et al. [4] that in blowfly mitochondria malonate inhibits $^{14}CO_2$ production from pyruvate-3-^{14}C much more than from pyruvate-1-^{14}C indicated that in the presence of malonate the (oxidative) decarboxylation of pyruvate can still proceed to some extent.

The results presented in this paper show that under a number of different conditions a large proportion of pyruvate utilized by house fly mitochondria is converted to acetate.

2. Materials and methods

Mitochondria were isolated from the thoracic muscles of house fly according to van den Bergh [5]. Protein was determined according to Cleland and Slater [6]. Oxygen consumption was measured in differential manometers.

The standard reaction medium contained: 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$, 50 mM tris-Cl, 1 mM ADP, 35 mM phosphate, 5 mM pyruvate, 30 mM glucose, 3–5 I.U. hexokinase and substrates as indicated in the legends to the figure and table. The temperature was 25° and the pH 7.5.

Pyruvate was determined enzymically in neutralized perchloric acid extracts with lactate dehydrogenase and α-oxoglutarate with glutamate dehydrogenase. Acetate was determined at pH 8.0 according to Davis [2] with acetate-activating enzyme (isolated from an acetone powder of beef heart mitochondria [7]), citrate synthase plus malate dehydrogenase.

3. Results

Pyruvate oxidation by house fly mitochondria is inhibited by malonate and this inhibition is prevented by the dicarboxylic acids α-oxoglutarate, succinate and malate [8]. In the experiment of fig. 1 a balance study of the pyruvate oxidation in the presence of malonate *plus* α-oxoglutarate is presented.

At the end of the experiment the pyruvate had disappeared completely, whereas no significant change in the concentration of α-oxoglutarate was observed. The most striking feature was the $\Delta O/\Delta$pyruvate ratio (1.26 at the end of the experiment) which was much lower than the value of 5.0 expected for complete oxidation of pyruvate. Indeed, a large proportion of the pyruvate was con-

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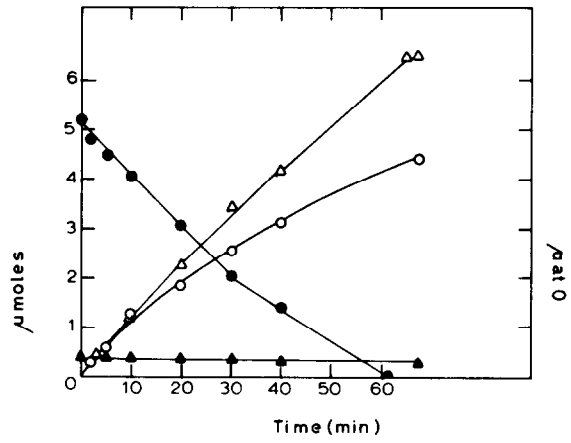

Fig. 1. Stoicheiometry of partially inhibited pyruvate oxidation by house fly mitochondria. To the standard reaction medium 20 mM malonate and 0.4 mM α-oxoglutarate were added. The protein concentration was 0.47 mg/ml.
●——● pyruvate ○——○ acetate
△——△ oxygen ▲——▲ α-oxoglutarate

verted to acetate by one-step oxidation. In the initial phase the ΔO/Δpyruvate was almost exactly one.

Formation of acetate also occurred under other conditions, such as in the presence of low concentrations of malonate (table 1). The progressive increase in acetate formation may be a result of the slow development of the inhibition by malonate due to its slow penetration into the mitochondria [8]. Even in the absence of any inhibitors, pyruvate can be converted to acetate; a mean Δacetate/−Δpyruvate of 0.20 was found (8 experiments). However the largest conversion to acetate is found under those conditions where the functioning of the Krebs cycle is inhibited.

4. Discussion

The finding that pyruvate is partially converted to acetate by house fly mitochondria immediately raises the question whether this also occurs in vivo and whether the acetate can subsequently be further oxidized by the mitochondria.

Indeed, in agreement with the observation of Childress et al. [4] who used blow fly mitochondria, we found that acetyl carnitine is oxidized by house fly mitochondria at a rate of 180 n atoms O/min/mg. Moreover, pyruvate oxidation by these mitochondria can be inhibited by fluoroacetate [9] indicating that at least some acetate-activating enzyme is present in the preparation. This activity is, however, relatively low since acetate *plus* carnitine is oxidized at a rate of only 24 natoms O/min/mg.

Although the flight muscle of the house fly is considered to be a carbohydrate-utilizing tissue, it apparently can also utilize acetate to some extent. It may be speculated that under conditions of very high muscular activity the bicarbonate concentration in the

Table 1
Formation of acetate from pyruvate under different conditions. Additions to the standard medium as indicated. The protein concentration was approximately 0.5 mg/ml in each case.

Expt.	Addition	−Δpyruvate (µmoles)	Δacetate (µmoles)	+Δacetate / −Δpyruvate
1	None	3.34	0.47	0.14
	20 mM malonate	0.69	0.74	1.07
	20 mM malonate, 20 mM α-oxoglutarate	4.24	1.92	0.45
2	20 mM malonate, 4 mM α-oxoglutarate	4.96	2.99	0.60
	20 mM malonate, 0.4 mM α-oxoglutarate	4.96	4.03	0.81
3*	0.2 mM malonate	0.54	0.12	0.22
		1.28	0.33	0.26
		2.42	1.21	0.50
		4.68	3.03	0.65

* These determinations were done after increasing times of incubation.

cell increases which, in turn, may inhibit the Krebs cycle at the level of succinate dehydrogenase [10]. The one-step oxidation of pyruvate could serve under those conditions as an emergency source of energy, whereas the resulting acetate could be later metabolized in the resting condition.

High concentrations of malonate completely inhibit the metabolism of pyruvate. Presumably, under those conditions the ratio acetyl coenzyme A/free coenzyme A is high due to a decreased oxaloacetate production. The suggestion of Garland and Randle [11] that such a high ratio inhibits pyruvate dehydrogenase would adequately explain this observation. Presumably, the deacylation of acetyl coenzyme A is the rate-limiting step in the acetate formation and this side path of pyruvate metabolism occurs when the Krebs cycle does not function optimally.

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