PYRUVATE UPTAKE IN RAT LIVER MITOCHONDRIA: TRANSPORT OR ADSORPTION?

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1. Introduction

Studies were performed in our laboratory to investigate certain aspects of pyruvate translocation into rat liver mitochondria, which is still a matter of controversy. Even though evidence has been collected in recent years showing that monocarboxylate uptake in mitochondria is not a controlled and carrier-limited translocation [1, 2], Papa et al. [3] reported recently that pyruvate is transported through a specific translocator into mitochondria. The method selected for our study was the technique of rapid centrifugation (with or without silicone layer), which was established as the method of choice for many carboxylic acid [1] and adenine nucleotide [1, 4] transport and exchange studies. Mitochondrial terminology such as "uptake" and "transport" (translocation of a substrate from one side of the membrane to the other) usually does not distinguish precisely between a translocation through a membrane system or a simple "surface limited" or "bulk phase limited" [5] adsorption of anions or cations on proteins and lipids. For these transport studies, we prepared different denatured, metabolically and structurally destroyed mitochondria as a control to normal mitochondria. We present evidence that it is not possible to justify the kinetic treatment [3] of the data obtained with the method of rapid centrifugation as representing active transport. Adsorption of pyruvate to mitochondrial proteins and/or lipids rather than specific transport to the matrix space appears to account for the binding of pyruvate by rat liver mitochondria.

2. Materials and methods

The experiments were performed in Fisher polystyrene centrifuge tubes of 1 ml capacity, centrifuged at 7000 *g* for 3 min (Fisher centrifuge, model 59). Two different methods for the rapid centrifugation of incubated mitochondria were used. Method 1 (with silicone): layers from bottom to top were 0.2 ml 15% $HClO_4$ (PCA), 0.1 ml silicone oil, and 0.7 ml buffer medium with the exact composition described by Papa et al. [3] (see legend in fig. 1). After incubation of mitochondria in the top layer at 37°, the mitochondria were spun down through the silicone layer into the PCA and aliquots were counted. In method 2 (no silicone), mitochondria were incubated in 0.7 ml medium as described for method 1 and were rapidly pelleted by centrifugation. The mitochondrial pellet was freed of supernatant fraction and dissolved in formic acid as described by Henderson and Lardy [4], and aliquots were counted. Variations between duplicates in both methods were less than 5%.

Sodium pyruvate (2-^{14}C) (NEN) was used for all studies with the same specific activity in various concentrations. Mitochondria were isolated from the 600 *g* × 10 min supernatant fraction of rat liver homogenate (prepared with 0.25 M sucrose, pH 7.4; 1:4, w/v) at 6500 *g* for 10 min and washed 3 times. Denatured mitochondria were prepared as follows: TCA-mitochondria were prepared by precipitating fresh mitochondria with 10% tricholoroacetic acid, washed several times and resuspended with 0.25 M sucrose. Heat-denatured mitochondria were prepared by boiling for 15 min in a water bath, precipitating by centrifugation, washing, and resuspension. PCA-mitochondria were prepared through denaturation with 30% $HClO_4$ (PCA), the pellet washed with sucrose, and

resuspended. All denatured mitochondrial suspensions were adjusted to the desired pH for the experiments. Calculation of the reciprocal plot (fig. 5) were performed with a UNIVAC least square fit program (UNIVAC model 1108 computer).

3. Results

Pyruvate uptake in normal mitochondria is higher at pH 6.9 than at pH 7.4; the difference becomes more apparent with increasing pyruvate concentrations (fig. 1A, B). Quantitatively similar results were obtained by using rapid centrifugation with or without a silicone layer. A biphasic pattern of the pyruvate uptake was observed at low substrate concentrations.

Respiratory inhibitors did not affect pyruvate accumulation in normal or TCA-denatured mitochondria (fig. 2A). At pH 6.9, the TCA-denatured mitochondria show a higher binding of pyruvate than normal mitochondria at all protein concentrations tested. TCA-mitochondria revealed little pH dependency for pyruvate binding, whereas normal mitochondria show increasing pyruvate uptake at decreasing pH (fig. 2B). At pH 5.0, normal mitochondria bind as much pyruvate as TCA-denatured mitochondria.

Other examples of the binding capacity of different

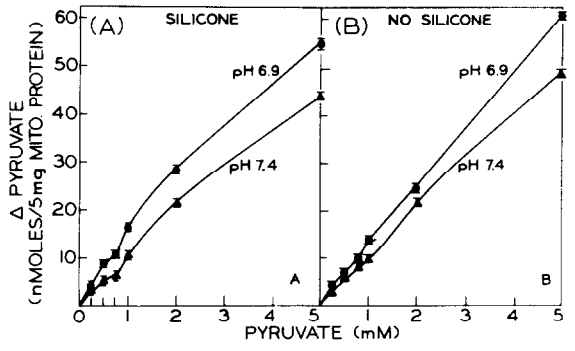

Fig. 1. Pyruvate uptake in the normal mitochondrial pellet after rapid centrifugation with (A) and without (B) silicone layer. Five mg of mitochondrial protein were incubated for 1 min at 37° in 0.7 ml medium (pH 6.9 and 7.4) containing: 200 mM sucrose; 20 mM Tris-HCl; 1 mM $MgCl_2$; 0.5 mM EDTA; 1 μg/ml rotenone; 0.5 μg/ml antimycin; 10 μg/ml oligomycin; and 1 mM arsenite. Pyruvate was then added at indicated concentrations. Two min after the addition of ^{14}C-pyruvate, mitochondria were rapidly centrifuged from the medium. The variation between duplicate measurements is depicted by horizontal bars.

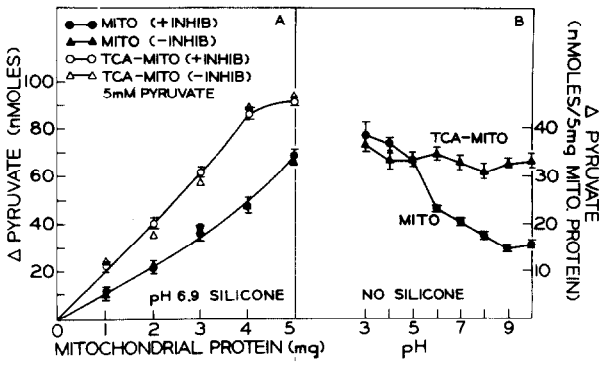

Fig. 2. (A) Effect of various concentrations of mitochondrial proteins and of respiratory inhibitors on the binding of pyruvate. Normal and TCA-denatured mitochondria are compared in the presence and absence of inhibitors (see legend to fig. 1) at a fixed pyruvate concentration. (B) Effects of pH on binding of pyruvate in normal and TCA-denatured mitochondria. Medium and inhibitors were as described under fig. 1; pyruvate was 2 mM. Mitochondria were prepared as in Materials and methods section.

denatured mitochondria are shown in fig. 3, where boiled as well as PCA-denatured mitochondria reveal concentration-dependent binding of pyruvate. Comparison with corresponding undenatured mitochondria (fig. 1B) shows that both boiling and treatment with PCA enhance pyruvate binding.

Uptake of pyruvate over a wide range of concentrations in normal mitochondria was compared with TCA-denatured mitochondria at pH 7.4 (fig. 4). Further analysis of the data of fig. 4 is presented in fig. 5. The apparent cooperativity of pyruvate "uptake" by mitochondria as shown in the double reciprocal plot is mimicked by the denatured mitochondria. Almost

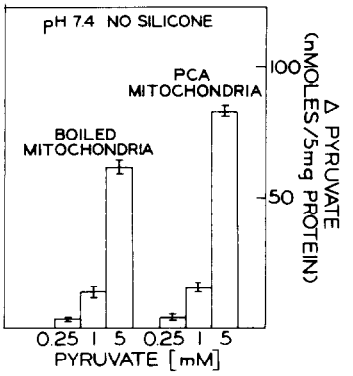

Fig. 3. Pyruvate uptake in boiled and PCA-denatured mitochondria. Medium and incubation procedures as described under fig. 1.

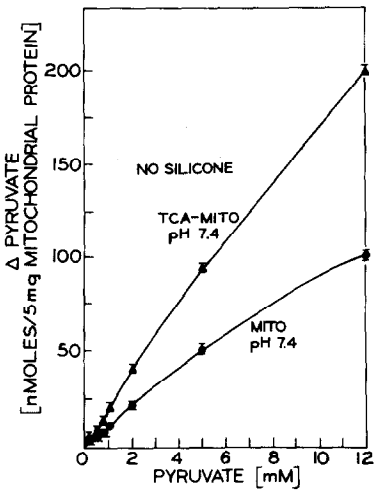

Fig. 4. Pyruvate uptake in normal mitochondria compared with TCA-denatured mitochondria. Medium and procedures as described under fig. 1.

parallel curves are evident for normal and denatured mitochondria in the log–log plot (fig. 5). In the Hill plot, points for the TCA- and normal mitochondria fall on a common line (fig. 5).

4. Discussion

This report is mainly concerned with the validity of using substrate accumulation methods to determine rates and regulation of transport. It has been shown that differently denatured mitochondria, which are structurally and metabolically destroyed, can take up pyruvate in a manner comparable to normal mitochondria. Normal and denatured mitochondria reveal very similar concentration-dependent uptake curves with a special biphasic pattern at low concentrations of

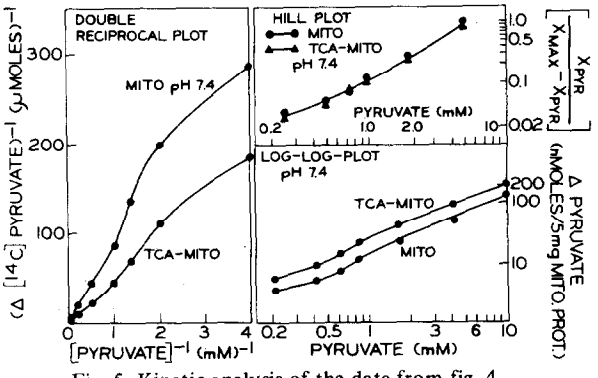

Fig. 5. Kinetic analysis of the data from fig. 4.

pyruvate. Therefore, it is not justifiable to apply kinetic treatment to the experimental data, assuming the process to be active transport. Further analysis of the biphasic pyruvate uptake curves (fig. 4) of normal and denatured mitochondria revealed in the reciprocal plot (fig. 5) a similar complex cooperativity pattern in respect to pyruvate binding. The parallel log–log plot curves and the identical Hill plots emphasize that normal and denatured mitochondria have relatively the same binding properties.

The pyruvate exchange and pyruvate uptake described by Papa et al. [3] as obeying so-called saturation kinetics, do not establish that specific transport occurs under the employed conditions. Saturability and ion competition are evidence only for the presence of a limited number of adsorption sites throughout the entire protein–lipid–water system [5]. Our data suggest that the accumulation of pyruvate under the described conditions is adsorption. The remarkably higher binding of pyruvate in normal mitochondria by change of the pH from 6 to 5 (fig. 2b) reflects increased exposure of ion adsorption sites of mitochondrial proteins and lipids through extreme swelling and rupture of the mitochondria as observed by electron microscopy [6]. Denatured mitochondria show throughout the tested pH range (fig. 2B) a relatively constant pyruvate adsorption, which is comparable to the binding to pyruvate by "normal" mitochondria below pH 5.

Acknowledgements

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