Localization of the α-oxoacid dehydrogenase multienzyme complexes within the mitochondrion

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Received 10 October 1990

Bovine kidney mitochondria were separated into matrix and membrane fractions by treatment with digitonin and Lubrol PX. While malate dehydrogenase was found essentially in the matrix fraction, both the pyruvate and the α-oxoglutarate dehydrogenase multienzyme complexes remained bound to the inner membrane fraction and became solubilized only after repeated treatments with detergents. Thus both multienzyme complexes must be associated with the inner membrane rather than located within the matrix space.

Mitochondrion; Inner membrane; Association; Pyruvate dehydrogenase complex; α-Oxoglutarate dehydrogenase complex

1. INTRODUCTION

The pyruvate and the α-oxoglutarate dehydrogenase complexes are found in bacteria in the soluble fraction of the cytoplasm, while in eukaryotic cells these enzyme complexes are located exclusively in the mitochondria [1,2]. Their exact localization within the mitochondrion, however, remains unclear. Various reports claim for a localization within the matrix space [3,4], though detergents, like Triton X-100, are necessary for the isolation of both of these enzyme complexes. Nestroescu et al. reported the localization of the pyruvate dehydrogenase complex in the inner mitochondrial membrane applying histochemical methods [5]. Similar results were obtained by Sumegi et al. [6], using antibodies against the pyruvate dehydrogenase complex. In this study stepwise fractionation with digitonin and Lubrol PX was undertaken in order to localize the pyruvate and the α-oxoglutarate dehydrogenase complex within the bovine kidney mitochondrion.

2. MATERIALS AND METHODS

2.1. *Chemicals*

Digitonin and Lubrol PX were obtained from Sigma Chemical Co. (St. Louis, MO); dithioerythritol (DTE) and substrates and the cofactors for the enzyme assays were from Boehringer Mannheim GmbH (Mannheim, Germany); all other chemicals were from Merck AG (Darmstadt, Germany).

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Abbreviations: DTE, dithioerythritol; EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

2.2. *Fractionation of mitochondria*

Mitochondria from beef kidney were prepared and fractionated according to the method of Schnaitman and Greenawalt [7]. From 500 g of beef kidney 50 ml of mitochondria suspension (40 mg protein/ml) were obtained. All fractionation steps were carried out at 4°C.

2.3. *Enzyme assays and protein determination*

For determination of the activities of the pyruvate and the α-oxoglutarate dehydrogenase complexes, aliquots of 10 μl were given to 1 ml of a solution containing 0.6 M $MgCl_2$, 0.15 mM EDTA, 0.6 mM KCN, 1.4 mM thiamine diphosphate in 66 mM Hepes/KOH, pH 7.4. Under stirring, 3 mg of Lubrol PX per mg protein were added and the mixture was allowed to stand at room temperature for 15 min. Thereafter 1 ml of a solution of 0.86 mM coenzyme A, 5.0 mM $MgCl_2$, 5.0 mM NAD, 6.0 mM DTE in 133 mM Hepes/KOH, pH 7.4, was added. The reaction was started by addition of 50 μl 0.1 M pyruvate or α-oxoglutarate, respectively, to 1 ml of this solution. For the test of the malate dehydrogenase, an aliquot of 10 μl was given to 1 ml of a mixture of 0.22 mM NADH in 66 mM Hepes/KOH, pH 7.4, and 0.3 mg Lubrol PX per mg protein and preincubated for 15 min at room temperature. The enzyme reaction was started by addition of 10 μl 50 mM oxalacetate.

The protein concentration was determined according to the Lowry method [8] modified by Hartree [9].

3. RESULTS

The stepwise fractionation of mitochondria was initiated by treatment of a suspension of bovine kidney mitochondria with digitonin. A 2% aqueous digitonin solution was added dropwise into 20 ml (40 mg/ml protein) of a mitochondria suspension until a concentration of 0.12 mg digitonin per mg protein was reached. After stirring for 15 min, 60 ml of the 'isolation medium' (220 mM mannitol, 70 mM sucrose, 0.5 mg/ml bovine serum albumin in 2 mM Hepes/KOH, pH 7.4) were added and the suspension was centrifuged at 8000 × *g* for 10 min. The pellet was washed twice with 20 ml of isolation medium and centrifuged after each washing as described above. The combined super-

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00145793/90/$3.50

Table I

Distribution of the activities of the malate dehydrogenase (MDH), the pyruvate dehydrogenase complex (PDHC) and the α-oxoglutarate dehydrogenase complex (OGDHC) between different fractions after treatment of bovine kidney mitochondria with detergents

Fraction	Volume (ml)	Protein (mg/ml)	MDH	PDHC	OGDHC
			(mU/ml)		
LSP pellet	20	40	105	28.9	31.1
LSP supernatant	120	4.5	568	0.0	0.0
Inner membrane fraction I	20	7.5	58.9	13.1	11.2
UZ supernatant I	20	2.5	35.4	6.2	8.1
Inner membrane fraction II	20	7.1	9.2	9.7	8.2
UZ supernatant II	20	0.34	50.6	2.0	2.6
Inner membrane fraction III	20	6.8	0.96	5.1	6.8
UZ supernatant III	20	0.25	6.14	0.72	0.8

natants (LSP supernatant) of the low-speed pellet were tested for their respective activities of the pyruvate and the α-oxoglutarate dehydrogenase complexes in comparison to the activity of the malate dehydrogenase as a marker enzyme for the soluble fraction of the mitochondrion. While no activity of either multienzyme complex could be detected in the LSP supernatant, high amounts of the malate dehydrogenase were found (Table I).

Further fractionation of the mitochondria was achieved by treatment with Lubrol PX. The low-spin pellet was suspended in 20 ml of isolation medium containing 0.1 mg of Lubrol PX per mg protein. After stirring for 15 min the suspension was centrifuged for 30 min at 144 000 × g. Under these conditions no detectable amounts of already solubilized multi-enzyme complexes sedimented out into the pellet. The supernatant (UZ supernatant I) and the pellet (inner membrane fraction I) were tested for their enzyme activities. From Table I it can be seen that only about one-third of the total pyruvate dehydrogenase activity and 42% of the α-oxoglutarate dehydrogenase activity became solubilized under these conditions. The treatment of the pellet with Lubrol PX and the centrifugation was repeated two times (fraction II and III, Table I). While the malate dehydrogenase activity was completely depleted from the inner membrane fraction II, only relatively small fractions of both multi-enzyme complexes were further released by the two procedures and about 20% of the total activities remained bound even after the last extraction step.

4. DISCUSSION

Solubilization of both the pyruvate and the α-oxoglutarate dehydrogenase complexes requires more intense treatment with detergents than necessary for the solubilization of the malate dehydrogenase, which is localized in the matrix space. Though these multi-enzyme complexes cannot be considered as integral membrane proteins, they must be associated to the inner mitochondrial membrane in a non-dissociable form and a localization in the matrix space as a 'soluble' enzyme as was reported by different groups [1,2] can be excluded. Srere [10] postulated a higher organization of citric acid enzymes within an inner membrane-matrix compartment. Whether the association of the α-oxoacid dehydrogenase complexes with the inner mitochondrial membrane may be regarded as part of such a higher organization must be left open.

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