DIFFERENTIAL MOVEMENT OF MITOCHONDRIAL ASPARTATE AMINO TRANSFERASE AS A FUNCTION OF THE ENERGETICAL STATE OF THE MITOCHONDRIA

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Previous investigations (Penniston et al., 1968 ; Harris et al., 1968 ; Green et al., 1968a; Lehniger, 1959 ; Hackenbrock, 1968) have established that isolated rat liver mitochondria undergo reversible ultrastructural changes as a function of their energetical states. Major transformations regarding the folding of the inner membrane as well as the changes in their configurational state were indeed observed.

These and other data (Scottocasa et al., 1967 ; Levy et al., 1967 ; Green et al., 1968b; Schnaitman et al., 1967 ; Beattie, 1968) related to the localization of enzymatic proteins in the submitochondrial fractions suggested that the different energetical state of the mitochondria could be the reason for the apparently controversial results concerning the localization of diverse enzymes in the submitochondrial fractions. Altough there is general agreement that mitochondria have an outer and inner membrane system as well as a soluble matrix ; there is disagreement about the localization of some enzymes in the three submitochondrial compartments.

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Recent observations (Green et al., 1968a) describing the action of respiratory inhibitors and uncoupling agents (dinitrophenol, rotenone, antimycin A) or oxydizable substrates (pyruvate, malate, succinate) in the mitochondria, showed that some ultrastructural transformations could be associated with the variation of the energetical states of the latter.

In this report we shall present evidence, suggesting the existence of a differential movement of two enzymes between submitochondrial fractions as a function of the energetical state of the mitochondria.

Experimental methods

Rat liver mitochondria were prepared by the method of Harel et al. (1957) as described by Levy et al. (1967) special care being taken to remove as much of the light mitochondria as possible. All mitochondrial pellets were suspended in a medium that was 0.25 M in sucrose.

Aliquots of mitochondrial suspensions in 0.25 M sucrose were incubated at 37° for 10 min with 2-4 dinitrophenol (DNP) (0.184 µmoles/10 mg protein) or monopotassium phosphate (Pi) (0.1 mmoles/10 mg protein) and pyruvate (0.01 mmoles/10 mg protein) ; controls incubated only with sucrose were run simultaneously. In all cases pH was adjusted to a value of 7.4.

For submitochondrial fractionation the mitochondria were submitted to the action of digitonine according to the method of Schnaitman et al. (1967). Aspartate aminotransferase activity (AAT) (E.C. 2.6.1.1.) was determined by measuring the oxydation of NADH in presence of malate dehydrogenase at 340 mµ in a coupled reaction with the oxalacetate formed by the transamination reaction. Electrophoretical controls were run and showed that this AAT was only of the mitochondrial isozyme type. Mono-

amine-oxidase (MAO) (E.C. 1.4.3.4.1.) was assayed according Tabor et al. (1954). The production of benzaldehyde was followed spectrophotometrically at 250 mμ. The spectrophotometric measures were performed in a Cary 14 type spectrophotometer at 18°. The method of Lowry et al. (1951) was used for protein determination.

Results

Fractionation of mitochondria and localization of AAT and MAO activities after generation and discharge of an energized state.

When rat liver mitochondria are exposed to the action of [Pi + pyruvate] or DNP before the digitonine treatment, the distribution patterns of AAT and MAO are different. As shown in table I, the incubation with [Pi + pyruvate] or DNP influences the distribution of AAT. The enzyme in non-treated mitochondria (control) is localized mainly in the membraneous fractions whereas for the [Pi + pyruvate] or DNP mitochondria it is mainly found in the soluble matrix. In contrast the MAO distribution patterns remain unaffected, in our hands, by the preceding treatments and stays practically constant, and this whichever the energetical state of the mitochondria could be, suggesting the integrity of the structures involved in its retention.

The solubilization of an important part of the membrane bound AAT in two basically different energetical states and the fact that under the same experimental conditions, localization of MAO activity remains unchanged, brings up the question of a possible differential movement of AAT, and perhaps other enzymes, as a function of the energetical state of the mitochondria. In order to study the eventuality of an oriented AAT flux whole mitochondria were exposed to [Pi + pyruvate] or DNP for 10 min at 37° and the enzymatic activity measured in the resi-

TABLE I

Localization of AAT and MAO in digitonin-treated mitochondria as a function of different energetical states

		Control*		[Pi + pyruvate]*		DNP*	
	Sample	Sp.Act.	%++	Sp.Act.	%++	Sp.Act.	%++
PROTEIN	Outer membrane		2.6		10.9		2.9
	Inner membrane		52.7		25.1		36.9
	Soluble matrix		44.8		63.8		60.0
ASPARTATE AMINO TRANSFERASE	Outer membrane	146.14	7.0	8.58	1.6	11.72	0.55
	Inner membrane	77.17	82.8	5.83	2.5	8.72	5.33
	Soluble matrix	8.60	10.2	88.84	95.7	99.87	94.04
MONOAMINO OXIDASE	Outer membrane	251	8.7	111.3	9.5	191.3	6.9
	Inner membrane	115.3	81.0	451.0**	83.4	170.6	80.7
	Soluble matrix	14.6	10.2	15.0	7.5	12.0	12.3

Aspartate amino transferase activity is expressed in mμmoles x 10^{-2} NADH oxidized/min/mg protein. Monoamino oxidase is expressed in mμmoles benzaldehyde produced/min/mg protein. ++ as % of total recuperation. * for experimental conditions see text. ** the MAO seems to be activated in the inner membrane in our experimental conditions.

dual mitochondria and in the supernatant fluid. Under these conditions, as shown in table II, 40 % of AAT activity in the [Pi + pyruvate] treated mitochondria leaves the residual mitochondria, as for the DPN treated mitochondria all the AAT activity remains trapped inside the organelle and MAO distribution remains unchanged.

Nevertheless the MAO has been described as a mitochondrial outer membrane marker by Schnaitman et al.(1967) and has been shown to be bound to the inner membrane by Allmann et al.(1968). As a matter of fact this apparent contradiction may be under - stood if one considers that it has effectively the highest specific activity in the outer membrane and that the greatest concentrations of enzymatic activity is located in the inner membrane.

Thus in the [Pi + pyruvate] state an important portion of the AAT moves toward the extramitochondrial space and in the DNP state all the enzyme moves toward the matrix space. Nevertheless we think that this movement is not an exclusive privilege of AAT and could also involve enzymes of the major synthetic and degradation pathways of the mitochondria and of the respiratory chain for instance. As for Estrada's (1964) finding concerning the DNP external flux of mitochondrial AAT, it has been shown by us that this effect was due uniquely to the action of the added 0.125 M KCl in the incubation medium.

On the other hand, the MAO seems to constitute an excellent marker of the mitochondrial membrane, under our experimental conditions.

Localization of other mitochondrial enzymes as a function of the energetical state of the mitochondria is presently under investigations in this laboratory and will be presented in a subsequent paper.

TABLE II

Localization of AAT and MAO in mitochondria as a function of energetical states

		Control*		[Pi + pyruvate]*		DNP*	
	Sample	Sp.Act.	%**	Sp.Act.	%**	Sp.Act.	%**
PROTEIN	Residual mitochondria		82.31		73.87		81.74
	Supernatant fluid		17.69		26.13		18.26
ASPARTATE AMINO TRANSFERASE	Residual mitochondria	62.41	91.13	56.75	59.36	63.15	93.19
	Supernatant fluid	28.24	8.87	109.86	40.65	20.64	6.81
MONOAMINO OXIDASE	Residual mitochondria	76.0	97.30	138.6	95.97	79.3	96.01
	Supernatant fluid	9.6	2.70	16.3	4.03	14.6	3.99

Aspartate amino transferase activity is expressed in mμmoles x 10^{-2} NADH oxidized/min/mg protein. Monoamino oxidase is expressed in mμmoles benzaldehyde produced/min/mg protein. * for experimental conditions see text. ** as % of total recuperation.

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