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THE ADENINE NUCLEOTIDE TRANSLOCATOR IN FOETAL, SUCKLING AND ADULT RAT LIVER MITOCHONDRIA

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SUMMARY

The adenine nucleotide content of rat liver mitochondria was shown to increase significantly after birth. On the other hand, it was found that the ligand-binding properties of the adenine nucleotide translocator were essentially the same in foetal, suckling and adult rat liver mitochondria. These results are compatible with the proposal that the accumulation of adenine nucleotides which occurs during mitochondrial biogenesis and maturation is effected by a pathway different from the adenine nucleotide translocator.

The entry of the adenine nucleotides, ATP and ADP, into the mitochondrial matrix through the barrier provided by the inner mitochondrial membrane is effected by an exchange-diffusion process catalysed by a specific translocator. The use of specific inhibitors of adenine nucleotide translocation, such as atractylate, carboxy-atractylate and bonkrekate, has clearly shown that mitochondria derived from a number of different species and tissues are completely dependent on the adenine nucleotide translocase for the influx and efflux of ADP and ATP.

Recent work has shown that the accumulation of [^{14}C]ATP by foetal rat liver mitochondria follows different kinetics than that of adult rat liver mitochondria (2). In foetal mitochondria [^{14}C]ATP uptake reached a maximal value within 10 - 30 seconds at 0 - 1^{o}C; this initial rapid uptake of ATP was followed by a small decrease in the [^{14}C]ATP retained over a period of 5 minutes (2). These and other results led to the proposal that foetal

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mitochondria possess an additional or alternative mechanism for adenine nucleotide permeation (2, 3). Another possibility that had to be considered was that the adenine nucleotide translocator is markedly different in these mitochondria. In the present investigation some ligand-binding properties of the adenine nucleotide translocator are compared. The results suggest that these properties of the adenine nucleotide translocator are essentially the same in foetal, suckling and adult rat liver mitochondria.

MATERIALS AND METHODS

Wistar rats were used as the source of liver mitochondria in all experiments. Liver mitochondria from foetal, suckling and adults rats were isolated as described by Pollak (3).

The binding of [^{3}H]CAT was measured by the sedimentation method in an Eppendorf centrifuge as described by Klingenberg et al. (4). Protein concentrations in these experiments were determined by a modified Biuret method (5).

The concentrations of ATP, ADP and AMP were determined by means of the luciferin-luciferase reaction, using coupled enzyme reactions (i.e. pyruvate kinase and phosphoenol pyruvate were added for the determination of ATP plus ADP; for the determination of total adenine nucleotides myokinase was added as well). The bioluminescence produced by the luciferase reaction was measured by means of a Packard 2211 scintillation spectrometer in the non-coincidence mode. A detailed description of this method has been submitted for publication to Anal. Biochem. (Sutton and Pollak, unpublished). For these experiments proteins were determined by the method of Lowry et al. (6), using bovine serum albumin as standard.

RESULTS AND DISCUSSION

A series of experiments were carried out to compare the affinities of the adenine nucleotide translocator of foetal, suckling and adult rat liver mitochondria to CAT in the absence and presence of ATP, in order to establish whether differences between foetal and adult rat liver mitochondria with respect to ATP uptake are due to differences in the adenine nucleotide translocator (Fig. 1).

Scatchard mass-action plots for the binding of CAT to the adenine nucleotide translocator indicate that the affinity of the translocator for this inhibitor of adenine nucleotide translocation is similar in foetal, suckling and adult rat liver mitochondria (Fig. 1). On the other hand the

Abbreviation: CAT = carboxy-atractylate

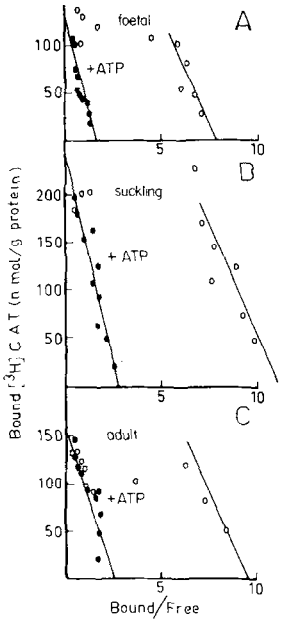

Figure 1.

Mass-Action plots of [^{3}H]CAT binding to foetal, suckling and adult rat liver mitochondria in the absence and presence of ATP. 300 µg protein in A: foetal, B: 2.5 day suckling and C: adult mitochondrial suspensions.

Incubation medium: 50 mM KCl, 10 mM Hepes, 0.2 M sucrose (all adjusted to pH 7.2). [^{3}H]CAT added as indicated. Incubation carried out for 5 min. at 20°C in the absence of ATP: o; in the presence of 2 µM ATP:o. At the end of incubation, suspensions were sedimented, the pellet washed and solubilized with 2% lubrol; portions of both supernatants and pellet suspensions were counted for radioactivity.

decrease of CAT binding caused by the addition of ATP differs considerably in the mitochondria isolated from these three developmental stages. The tendency for the binding of CAT to the adenine nucleotide translocator to be lowered by the presence of ATP in foetal rat liver mitochondria and to a lesser extent in 2.5 day old suckling rat liver mitochondria may be ascribed to lower endogenous levels of adenine nucleotides of these mitochondria (7 and Table 1). The results also show that the maximal amount of CAT bound per mg of mitochondrial protein in foetal, suckling and adult rat liver mitochondria is of the same order and the saturation of the binding sites

TABLE 1

ADENOSINE NUCLEOTIDE CONTENT OF RAT LIVER MITOCHONDRIA DURING DEVELOPMENT.

Immediately on making up fresh mitochondrial suspensions, $HClO_4$ is added to a final concentration of 0.3 M at $2^{o}C$. The suspension is centrifuged in the cold and the supernatant neutralised with KOH. After centrifugation in the cold, portions of the supernatant are taken for adenine nucleotide determination as described in the methods section. Results presented ± S.E.M.

Developmental Age	Number of experiments	µmol/g mitochondrial protein ATP	ADP	AMP	Total adenine nucleotides
21.5 day foetal rat liver	6	1.14±0.16	0.89±0.08	0.96±0.20	2.97±0.31
2.5 day suckling rat liver	3	2.28±0.12	1.72±0.03	5.76±0.06	9.76±0.10
5 day suckling rat liver	2	3.07±0.96	3.63±0.50	6.16±0.48	12.87±0.97
Adult rat liver	5	3.19±0.31	3.89±0.36	5.63±0.58	12.76±0.98

for CAT occurs at similar CAT concentrations in the mitochondria of the three developmental stages that were investigated. The differences that do exist, in the total amount of CAT bound per mg of mitochondrial protein may be related to the number of translocator sites per mg of mitochondrial protein and do not necessarily represent essential differences in the nature of the translocator itself. The greater amount of CAT bound to the 2.5 day old suckling rat liver mitochondria is a typical overshoot phenomenon which has been demonstrated to exist also for other enzymes at that developmental stage (8).

CAT is known to be firmly bound to the translocator of adult rat liver mitochondria (9). In experiments designed to give an indication of the tightness of CAT binding to the translocator of foetal rat liver mitochondria,

TABLE 2.

THE STABILITY OF [^{3}H]CAT BINDING WITH FOETAL RAT LIVER MITOCHONDRIA.

Incubation medium contained 50 mM KCl, 10 mM Hepes, 1 mM EDTA, 0.2 M sucrose (all adjusted to pH 7.2) and foetal rat liver mitochondria (330 µg protein). Incubation volume 0.5 ml at 20°C. At 0 min. 5 µl 21.7 µM [^{3}H]CAT (16,000 d.p.m.) were added and incubated. The following ligands were added at the end of the initial incubation period as indicated: 5 µl 20 µM "cold" CAT, 5 µl 100 mM ATP or 5 µl 100 mM ADP.

Initial incubation with [^{3}H]CAT Time (min).	Additional incubation Time (min).	Ligand	[^{3}H]CAT bound µmol/g protein
Experiment 1.			
5	--	--	0.203
15	--	--	0.187
5	10	"cold" CAT	0.182
35	--	--	0.187
35	2	ATP	0.174
35	2	ADP	0.183
Experiment 2.			
10	--	--	0.147
5	5	ATP	0.135
20	--	--	0.139
5	15	ATP	0.140
50	--	--	0.146
5	45	ATP	0.135

these were incubated with [^{3}H]CAT and then subsequently incubated with either ATP, ADP or non-isotope-labelled ["cold"]CAT. In all instances CAT binding was only marginally affected, (Table 2). The results presented in Table 2 clearly indicate that in foetal rat liver mitochondria, CAT is as firmly bound to the adenine translocator as in adult rat liver mitochondria.

The results presented in Figure 1 and Table 2 therefore indicate that the adenine nucleotide translocators of foetal and adult rat liver mito-

chondria are essentially similar, bearing in mind the differences in adenine nucleotide concentration and membrane properties between foetal and adult rat liver mitochondria.

The question then still remains how mitochondria are able to increase their adenine nucleotide content during development as shown in Table 1. The results of this investigation are consistent with the proposal that during the biogenesis and maturation of mitochondria, adenine nucleotides are taken up by a pathway other than the adenine nucleotide translocator. The translocator effects the exchange of adenine nucleotides for oxidative phosphorylation and is not involved in the accumulation of adenine nucleotides within the mitochondria.

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