Release of matrix proteins from mitochondria to cytosol during the prereplicative phase of liver regeneration

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Abstract 70% partial hepatectomy (PH) in the rat causes a release, into the cytosolic fraction, of mitochondrial matrix proteins, namely the mitochondrial isoform of aspartate aminotransferase (mAAT) and malate dehydrogenase (MDH), during the first 24 h after PH, when no growth of the residual liver is observed. After this time interval, the weight of the liver starts to increase and the normal weight is reached at 96 h after PH. This proliferative phase is characterized by a progressive recovery of the normal levels of intramitochondrial activities of mAAT and MDH. Mitochondria isolated at 24 h after PH show a membrane permeabilization to sucrose accompanied by a release of matrix enzymes; both are blocked by cyclosporin A. These results suggest an alteration of mitochondrial membrane integrity, during the prereplicative phase of liver regeneration, with the occurrence of an increased permeability that allows the passage into the cytosol of matrix enzymes.

Key words: Liver regeneration; Mitochondrion; Aspartate aminotransferase; Malate dehydrogenase; Cyclosporin A

1. Introduction

Liver regeneration is the capability of the organ to replace tissue mass after partial hepatectomy (PH) or injury [1]. Partial hepatectomy is followed by early changes in cellular energy metabolism which could be involved in the starting of liver regeneration [2]. Mitochondria are the main producers for cellular energy [3] and, in the prereplicative phase of liver regeneration (0–24 h after PH), they show: (i) decrease in oxidative phosphorylation capability [4], (ii) production of oxygen radicals [5–7], and (iii) decrease in mitochondrial glutathione levels [4]. In this phase of liver regeneration, the oxidative injury of mitochondria appears to be related to alterations of proteins [6,7] and lipids [5] of the inner mitochondrial membrane and to decreases of mitochondrial F_0F_1-ATP synthase activity and amount [8].

It has been reported that oxygen radicals, which can be implicated as potential mediators of the early regenerative process [9], increase the membrane permeability of isolated mitochondria [10], which, in turn, results in a release of matrix proteins [11].

In this study we show that, in the prereplicative phase of liver regeneration, a release, in the cytosolic fraction, of mitochondrial aspartate aminotransferase (E.C. 2.6.1.1) and malate dehydrogenase (E.C. 1.1.1.37) activities occurs. The proliferative phase of liver regeneration (which starts at 24 h after PH) is characterized by a progressive recovery in mitochondria of normal enzyme activities. Mitochondria, isolated 24 h after PH, show a CsA-sensitive permeabilization to sucrose accompanied by a CsA-sensitive release of matrix enzyme activities.

We infer that, during the prereplicative phase of liver regeneration, mitochondrial matrix proteins are released into the cytosol. The possible mechanism and physiological significance of this release are discussed.

2. Materials and methods

2.1. Partial hepatectomy

Three month old male Wistar rats were anaesthetized with ether/oxygen and the median and left lateral lobes of the liver (corresponding to 65–75% net weight of the whole liver) were excised [8]. After surgery, the rats, kept on a normal diet for the whole experimental period (0–120 h after PH) were killed by decapitation. The livers were removed, weighed and processed for isolation of cytosol and mitochondria. All operations were carried out under sterile conditions and at about 4°C. To prepare control liver cytosol and mitochondria, sham-operated rats were anaesthetized, without excision of the liver, then cytosol and mitochondria were isolated at the same time reported for rats subjected to liver regeneration. The study was approved by the state commission on animal experimentation.

2.2. Preparation of cytosol and mitochondria

Mitochondria were prepared as described in [12]. After precipitation of nuclei and mitochondria, the supernatant was used for preparation of cytosol by ultracentrifugation at $105\,000 \times g$ for 1 h. The final supernatant was used as cytosolic fraction.

Protein concentration was determined according to Bradford's method [13] using the Bio-Rad kit (Bio-Rad Laboratories. Inc) and albumin as standard.

2.3. Determination of mAAT and MDH activities in mitochondria and cytosol

Isolated mitochondria, suspended at about 10 mg protein/ml in a medium containing: 5 mM succinate-Tris, 10 mM Tris-MOPS, 0.2 M sucrose, 1 mM Pi-Tris and 0.1% Triton X-100, were used for determination of mAAT [14] and MDH [15] activities.

The MDH activity in cytosol was measured as reported in [15].

Differential assay between mitochondrial and cytosolic isozymes of AAT in the cytosol was done using a method [16] that depends on the thermal instability of mAAT at 70°C under conditions where the cytosolic AAT is stable. One aliquot of the ultracentrifuged cytosol was incubated at 37°C for 15 min and another at 70°C for 15 min. After this incubation the AAT activity in both samples was determined at 25°C using the coupled assay of Karmen [14]. The AAT activity of the sample pre-incubated at 37°C was taken to be that of both isoenzymes, whereas that of the sample pre-incubated at 70°C was assumed to be solely due to the cytosolic isoenzyme. Activity of the mAAT was taken as the difference between the two values.

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Abbreviations: Tris, Tris (hydroxymethyl)-aminomethane; MOPS, 3-(*N*-morpholino)-propanesulfonic acid; mAAT, mitochondrial isoform of aspartate aminotransferase; MDH, malate dehydrogenase; PH, partial hepatectomy; CsA, cyclosporin A

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Activities were expressed as enzymatic units (μmol of product/min) per mg of mitochondrial or cytosolic proteins.

2.4. Determination of mitochondrial permeabilization to sucrose and 'in vitro' release of matrix enzyme activities by isolated mitochondria

For determination of mitochondrial permeabilization to sucrose (swelling), isolated mitochondria (1 mg protein/ml) were suspended in a medium containing: 0.2 M sucrose, 10 mM Tris-MOPS pH 7.4, 5 mM succinate-Tris, 1 mM Pi-Tris, 2 μM rotenone and 1 μg/ml oligomycin, 25°C.

The volume change of mitochondria was determined from the absorbance changes at 540 nm using a Beckman DU 7400 spectrophotometer equipped with magnetic stirring and thermostatic control [17].

For the assay of in vitro release of enzyme activities, isolated mitochondria (10 mg protein/ml) were suspended in the same medium of the swelling assay, above reported, and incubated at 25°C for 5 min. Then, the mitochondria were precipitated by centrifugation at $8000 \times g$ for 40 s. The supernatant was centrifuged, again, for 2 min at $10\,000 \times g$. The final supernatant was used for determination of mAAT and MDH activities as reported above. Where indicated, CsA (1.7 nmol/mg mitochondrial proteins) was added.

2.5. Statistics and graphs

Data are expressed as mean ± S.E.M. of five animals. Statistical comparisons between groups were made by Student's unpaired *t*-test.

Graphs (Figs. 1, 2 and 4) were done by using Grafit (Erithacus Software) program for IBM computer.

3. Results and discussion

Liver regeneration is characterized by a lag in the growth of the liver which lasts for about 24 h after PH (inset of Fig. 1). Cytosolic and mitochondrial fractions, isolated from rat livers during the first 24 h after PH, show progressive increase and decrease, respectively, of mAAT activity (Fig. 1). After this time, the regeneration of the liver mass is accompanied by a progressive restoration of the normal values of the enzyme activity in both fractions (Fig. 1). No change in the activity of the cytosolic isoenzyme is observed (not shown). Another matrix enzyme, the MDH, is released in the cytosol during the first 24 h after PH (Fig. 2). The normal value of the mitochondrial MDH activity is progressively restored during the replicative phase of liver regeneration (Fig. 2).

From these observations we infer that matrix enzymes can be released from mitochondria into the cytosol, during the prereplicative phase of liver regeneration, through a non-specific permeabilization of the mitochondrial membrane. It has been reported that reoxygenation of hypoxic liver [11] or of ischemic brain [18], which could generate oxygen radicals injuries of mitochondria, causes a release of mAAT activity into the cytosol. In addition, in isolated liver mitochondria, a release of mitochondrial matrix proteins through a CsA-sensitive pathway [19] or upon reoxygenation of anoxic mitochondria [20] has been observed.

Fig. 3 shows that a CsA-sensitive membrane permeabilization to sucrose occurs in mitochondria isolated 24 h after PH (trace b), when incubated in medium reported in Section 2. Incubation for 6–7 min either of control mitochondria (trace a) or of mitochondria isolated at 72 h after PH (trace c) did not cause any change in the absorbance at 540 nm. The permeabilization to sucrose observed for mitochondria prepared at 24 h after PH is accompanied by a CsA-sensitive release of matrix enzymes (mAAT, Fig. 4A and MDH, Fig. 4B) from mitochondria.

One possible explanation for these observations is that the oxidative damage of mitochondrial membrane proteins, ob-

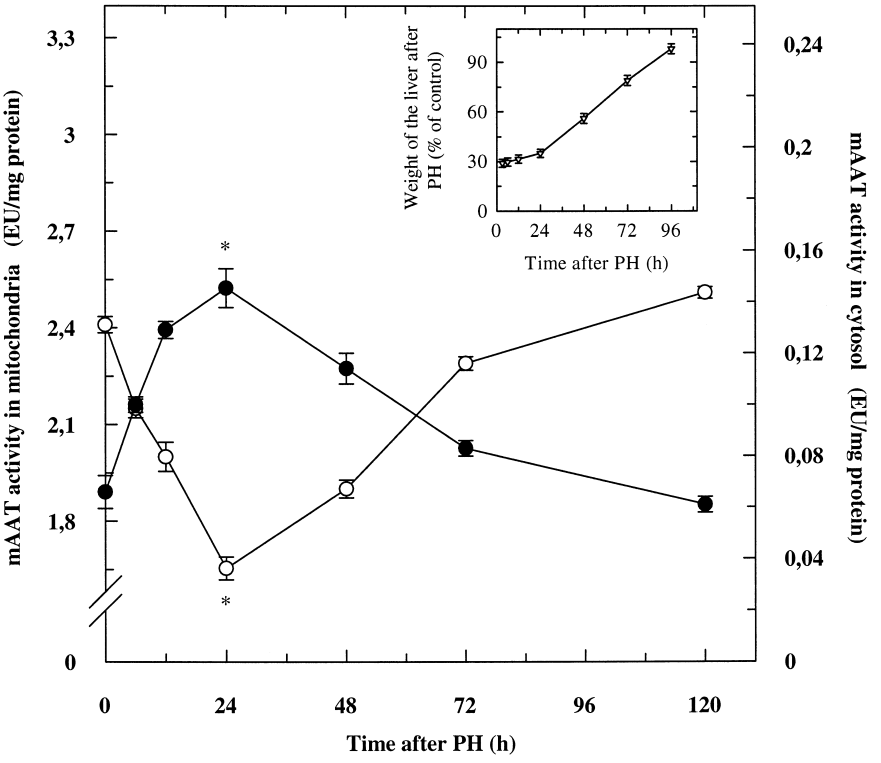

Fig. 1. Time course of changes in mAAT activity in mitochondria (○) and in cytosol (●) during liver regeneration after PH. For experimental details see Section 2. In the inset is reported the time course of changes of liver mass expressed as percentage of the weight of the liver of sham-operated rats which was 12.3 ± 1 g. $^*P < 0.001$ versus control.

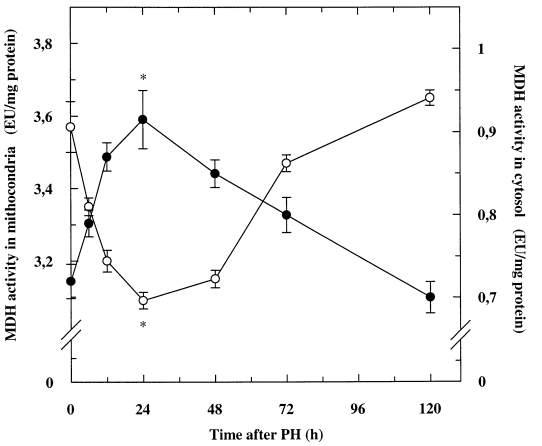

Fig. 2. Time course of changes in MDH activity in mitochondria (○) and in cytosol (●) during liver regeneration after PH. For experimental details see Section 2. $*P < 0.001$ versus control.

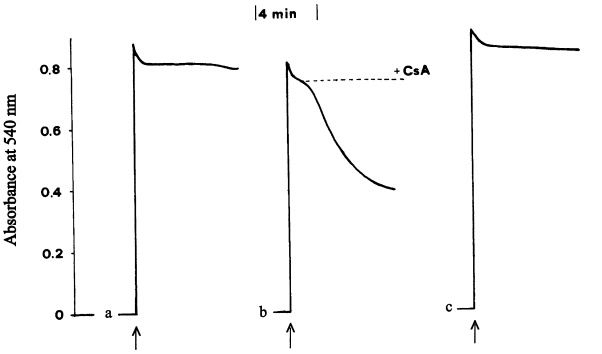

Fig. 3. Analysis of mitochondrial permeabilization to sucrose following the absorbance variations at 540 nm. For experimental details see Section 2. Trace a: control mitochondria; trace b: mitochondria isolated 24 h after PH; trace c: mitochondria isolated 72 h after PH. The dotted line in trace b refers to changes of absorbance of liver mitochondria isolated 24 h after PH and incubated in the medium for swelling assay in the presence of CsA (1.7 nmol/mg mitochondrial proteins). Where indicated (arrows), mitochondria (1 mg/ml) are added.

served during the early phase of liver regeneration [5–7], can favor a non-selective permeabilization of the inner membrane which has been suggested to be a part of the machinery required for turnover of matrix proteins [19]. It remains to clarify if the well known permeability transition pore (which allows the passage of ions and molecules with a molecular mass of < 1.500 Da [21,22]) contributes to the CsA-sensitive permeabilization of the mitochondrial membrane to the matrix enzymes (i.e. causing disruption of the inner membrane following mitochondrial swelling) or if the CsA-sensitive non-selective permeabilization to the matrix enzymes is a different system activated by oxidative stress. Whatever the mechanism for the release of matrix proteins is, the fact that this phenomenon has been observed in three different pathophysiological conditions (i.e. liver regeneration in the present paper, reoxygenation of hypoxic liver in [11] and reperfusion of ischemic brain in [18]) suggests that it could be of pathophysiological importance, not only because it may lead to loss of essential proteins from the mitochondria, but also because some of these proteins may convey important message to other intracellular organelles. Zamzani et al. [23] suggested that a protein released by mitochondrial depolarization and by a permeability transition can induce morphological changes in the nucleus of an apoptotic type.

It has been postulated that permanent opening of the transition pore can be involved in the selection of mitochondria by the cell, which will eliminate the oxygen radicals accumulating mitochondria [22].

The data in this paper show that release of enzyme activities from mitochondria (which are synchronous with oxygen radicals accumulation and protein damage of the inner mitochondrial membrane [4,6–8]) is transient and 72 h after PH the normal conditions are recovered. This could imply either that the mitochondrial membrane permeability to the matrix proteins, during the prereplicative phase of liver regeneration, could represent a mechanism for hepatocytes to select well functioning mitochondria (not damaged by oxygen radicals) or that transient protein release from mitochondria can act in the signal mechanism for cell proliferation.

In conclusion, the findings here reported allow for the possibility that, in the prereplicative phase of liver regeneration, a transport mechanism is present in mitochondria as part of the machinery required for turnover of matrix proteins; how this is involved in the proliferative signals remains to be clarified.

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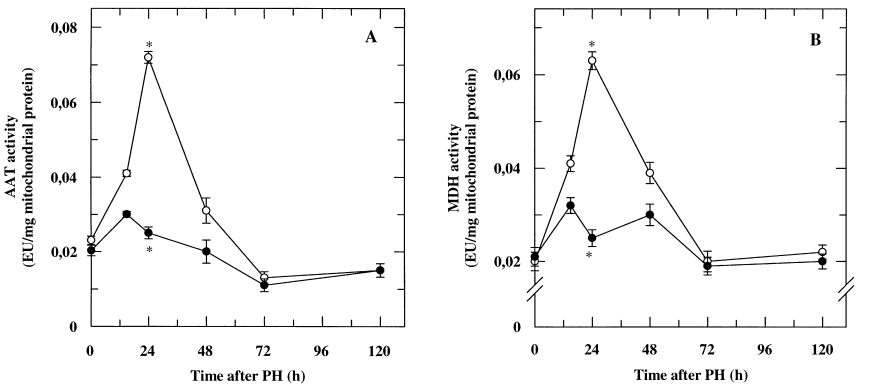

Fig. 4. Release of AAT (A) and MDH (B) from isolated mitochondria during rat liver regeneration. Isolated mitochondria are incubated for 5 min in the same medium reported for swelling assay without (○) or in presence of CsA (●). The amounts of enzyme activities released from mitochondria are shown as enzymatic units per mg of incubated mitochondrial protein. $*P < 0.01$ versus control.

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