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► **To cite this version:**

Robert Wattiaux, Simone Wattiaux-de Coninck, Jacqueline Thirion, Marie Christine Gasingirwa, Michel Jadot. Lysosomes and Fas-mediated liver cell death. *Biochemical Journal*, 2006, 403 (1), pp.89-95. 10.1042/BJ20061738 . hal-00478700

HAL Id: hal-00478700

<https://hal.science/hal-00478700>

Submitted on 30 Apr 2010

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Lysosomes and Fas mediated liver cell death .

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KEYWORDS

Apoptosis, lysosomes, Fas, liver, Jurkat cells.

ABBREVIATIONS

Ac-DEVD-AMC : N-Acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin

FADD : Fas Associated protein with death domain

SHORT TITLE:

Lysosomes and apoptosis

SYNOPSIS

A number of studies, mostly performed « ex vivo », suggest that lysosomes are involved in apoptosis as a result of a release of their cathepsins into the cytosol owed to a fragilisation of the membrane of these organelles. These enzymes could then be contributing to the permeabilization of the outer mitochondrial membrane, they could also activate effector caspases. The work presented here aims at testing if the membrane of liver lysosomes is disrupted during Fas-mediated cell-death of hepatocytes « in vivo », a process implicated in several liver pathologies. Apoptosis was induced by injecting mice with antiFas antibody (aFas). The state of the lysosomes was assessed by determining the proportion of lysosomal enzymes (β -galactosidase, β -glucuronidase, cathepsin C and cathepsin B) present in homogenate supernatants, devoid of intact lysosomes, obtained by high speed centrifugation and in some cases, by analyzing the behaviour in differential and isopycnic centrifugation of β -galactosidase taken as reference enzyme of these organelles. Apoptosis was monitored by measuring caspase-3 activity with a specific tetrapeptide substrate (DEVDase) and the release of sulfite cytochrome c reductase, an enzyme located in the intermembrane space of mitochondria. Results show that an injection of 10 μ g aFas causes a rapid and large increase of DEVDase activity and of unsedimentable sulfite cytochrome c reductase. This neither modifies the proportion of unsedimentable lysosomal enzyme in the homogenates nor the behavior of lysosomes in centrifugation. Experiments performed with a lower dose of aFas (5 μ g) which ensures a longer lifespan of the animals indicate that lysosomal hydrolase unsedimentable activity increases in the

homogenate after injection but with a marked delay with respect to the increase of DEVDase activity and of unsedimentable sulfite cytochrome c reductase. Comparative experiments «ex vivo» performed with Jurkat cells show that after treatment with aFas, an increase of unsedimentable lysosomal hydrolases can be observed but much later than caspase-3 activation and together with a release of cytosolic enzymes, dipeptidylpeptidase III and DEVDase into the culture medium. It is proposed that the fragilisation of lysosomes that is observed after aFas treatment «in vivo» and «ex vivo» results from a necrotic process that takes place late after initiation of apoptosis.

INTRODUCTION

The involvement of lysosomes in apoptosis as suggested by several studies is still highly debatable (For a review, see 1). Lysosome mediated apoptosis would result from a destabilization of the lysosomal membrane causing a release of cathepsins into the cytosol. These proteases would stimulate the mitochondrial pathway of apoptosis by contributing to the permeabilization of the outer mitochondrial membrane, perhaps as a result of Bid cleavage by these enzymes [2]. They could also trigger the cytosolic pathway, by activating effector caspases. However, several caspases, caspase-3 for example, seem to be poor substrates for lysosomal cathepsins «in vitro» [3]. The aim of our work was to investigate if lysosomes are involved in apoptosis of hepatocytes «in vivo» due to Fas ligation. The Fas apoptotic pathway was selected, because it has been extensively investigated as a model system for mammalian apoptosis. Moreover, Fas mediated cell death of hepatocytes is observed in several severe liver pathologies. Knowing whether lysosomal enzymes are involved «in vivo» in Fas apoptotic pathway may be of some help to explore specific therapeutic targets for cell death inhibition in these pathologies [4-6]. Until now, results published on the possible involvement of lysosomes in Fas induced apoptosis have been mostly obtained «ex vivo» and are controversial. While some suggest that lysosomes are involved [7-10] others suggest that they are not involved [11,12] in apoptotic death caused by agonistic antiFas. The work presented here aims at testing whether after an injection of anti Fas antibody (aFas) the lysosomal membrane is disrupted in mouse liver. Our method is simple and it involves determining the proportion of lysosomal enzymes found in high-speed supernatants of liver homogenates originating from controls and from animals injected with aFas. If lysosomes are involved in apoptosis after injection of aFas, one would expect that lysosomal enzymes would be released into the cytosol as a result of the injection and that an increase in proportion of unsedimentable lysosomal hydrolases in homogenates should definitely be observed, as it has been seen for example in ischaemic liver necrosis [13, 14]. Hepatocyte apoptosis was assessed by measuring the activity of the major executioner caspase, caspase-3, with a site-specific tetrapeptide substrate (DEVDase activity) and by determining the percentage of sulfite oxidase released in the high-speed supernatant of the homogenate. This enzyme is located in the intermembrane space of mitochondria [15] and is co-released with cytochrome c into the cytosol

during apoptosis [16]. The measurement of this enzymatic activity with cytochrome c as electron acceptor (sulfite cytochrome c reductase) allows quantification of the proportion of mitochondria whose outer membrane is permeabilized. This is easier than the determination of immunoprecipitated cytochrome c. In a limited number of experiments, the state of lysosomes was assessed by analyzing their behaviour in differential and isopycnic centrifugation. Experiments were first performed by injecting 10 μg aFas to animals. In these conditions, a rapid and massive apoptosis of the liver takes place as assessed by morphological and biochemical observations [17-19] and causes the death of most animal after one to two hours. Our results show that in that case, aFas injection causes a large and rapid increase of DEVDase activity and of unsedimentable sulfite cytochrome c reductase without modifying the proportion of unsedimentable activity of lysosomal enzymes. If the amount of aFas injected is lower (5 μg), the development of apoptosis is slower, the peak of DEVDase activity and of unsedimentable sulfite cytochrome c reductase is observed later and the animal can be kept in life for several hours. In these conditions, the percentage of unsedimentable lysosomal enzymes begins to increase, 2 to 3 hours after injection, indicating a progressive damage of lysosomes with time. For a sake of comparison, similar experiments were performed «ex vivo» with Jurkat cells. In these cells, involvement of lysosomes in Fas mediated apoptosis has been described [9]. Our results show that aFas treatment of these cells causes an increase of the unsedimentable activity of lysosomal enzymes but that takes place with a marked delay with respect to caspase-3 activation (DEVDase). Such increase almost coincides with a release into the culture medium of dipeptidylpeptidase III and DEVDase, two cytosolic enzymes.

EXPERIMENTAL

« In vivo » experiments

«In vivo» experiments were performed with female NMRI mice weighing 20-25 g. Animals were cared for using protocols approved by the FUNDP Commission d'éthique et en expérimentation animale. Mice were injected intravenously via tail vein with anti-Fas antibody (BD Biosciences Pharmingen) in a volume of 200 μl saline or with 200 μl saline for control experiments. Mice were sacrificed after selected times, the liver was removed and homogenized in ice-cold 0.25 M sucrose with a Potter & Elvehjem type homogenizer consisting of a smooth -walled glass tube fitted with a Teflon pestle (Arthur Thomas Co, Philadelphia, USA), rotating at 3000 rpm. Part of the homogenate was centrifuged at 35 000 rpm for 40 min in a 40 Beckman rotor to obtain an high-speed supernatant for determination of unsedimentable enzyme activity. Fractionation of the homogenate by differential centrifugation was achieved using the method of de Duve et al. [20]. A nuclear fraction N, a heavy mitochondrial fraction M, a light mitochondrial fraction L, a microsomal fraction P and a soluble fraction S were successively isolated by integrated forces, respectively of 10 000, 33 000, 250 000 and 3 000 000 g.min using the fractionation scheme described by these authors [16].

For isopycnic centrifugation, preformed linear density gradients of Percoll (27%-90%) with 0.25 M sucrose as a solvent were used and centrifugation was performed at 25 000 rpm in the SW 65 Beckman rotor for 60 min.

«Ex vivo» experiments

«Ex vivo» experiments were carried out with Jurkat cells (clone E 6-1) cultured in RPMI 1640 medium supplemented with 10% foetal calf serum, 10 mM Hepes, penicillin (100 U/ml), streptomycin (0.1 mg/ml) and L-glutamine (0.03%). For the experiments, incubation was performed at a density of $2 \cdot 10^6$ cells /ml, in absence or in presence of human aFas (clone CH-11, Bio Connect, Huissen, The Netherlands). For the preparation of homogenates, cells were collected by centrifugation, suspended in 0.25 M sucrose and sonicated for 5 sec (SONIC Vibra Cells, amplitude 40). The efficiency of homogenisation was checked by phase contrast microscopy. In addition, the percentage of dipeptidyl peptidase III, a cytosolic enzyme, recovered in high speed supernatants of homogenates was regularly measured; it was always greater than 90% of the homogenate total activity, indicating that most of the cells are disrupted by the homogenisation procedure. On the other hand, as shown in the results, only 10 to 15% of lysosomal enzyme activities were found in high speed supernatant of homogenates originating from control cells, illustrating that homogenisation did not disrupt the majority of normal lysosomes. High-speed supernatants were prepared as described for the liver

Enzyme measurements

To measure caspase-3 like activity (DEVDase activity), aliquots of homogenates were incubated at 37°C with 50 μ M Ac-DEVD-AMC (N-Acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin), 50 mM Tris HCl buffer, pH 7.5, 0.1 M NaCl, 0.1% Triton X-100 and 10 mM dithiothreitol in a volume of 0.2 ml. The reaction was stopped by adding 1.3 ml of 50 mM glycine buffer, pH 10.5, containing 5 mM EDTA and 0.5% Triton X 100. To assay β -glucuronidase activity, the medium contained 1 mM 4-methylumbelliferyl- β -D-glucuronide, 20 mM acetate buffer pH 5, 0.05% Triton X-100 in a volume of 0.2 ml. Incubation was performed at 37°C and the reaction stopped by adding 1.3 ml of 50 mM glycine buffer pH 10.5, containing 5 mM EDTA and 0.5% Triton X-100. Medium for the determination of cathepsin B contained 250 μ M Z-Arg-Arg-7-amido-4-methylcoumarin, 0.1 M phosphate buffer pH 6, 5 mM aminoethanethiol, 1 mM EDTA and 0.05% Triton X-100 in a volume of 0.5 ml. The reaction was stopped by adding 1.3 ml of 50 mM glycine buffer pH 10.5, containing 5 mM EDTA and 0.5% Triton X-100. For dipeptidylpeptidase III, incubation was carried out in a medium containing 0.25 mM Arg-Arg-naphthylamide and 25 mM Tris buffer, pH 8 in a volume of 0.2 ml. Reaction was stopped by adding 1.3 ml of 50 mM glycine buffer, pH 10.5, containing 5 mM EDTA and 0.5% Triton X 100. In each determination, the released fluorogenic group (aminomethyl-coumarin, 4-methyl umbelliferone or naphthylamine) was determined with a Versa Fluor Fluorometer, (Bio-Rad, Nazareth-Eke, Belgium), excitation filter 170-

2420, emission filter 170-2421. β -galactosidase and cathepsin C were measured as described by Lecocq et al. [21], sulfite cytochrome c reductase by the method of Wattiaux- De Coninck and Wattiaux [15] and cytochrome oxidase as described by de Duve et al.[20].

RESULTS

Initially, experiments were performed by injecting 10 μ g of aFas. Such an injection causes a massive apoptosis of hepatocytes as assessed by biochemical and morphological criteria [17-19] and the death of the animals 90 to 120 min after injection. To monitor liver apoptosis, we examined the chronological changes of DEVDase activity and of unsedimentable sulfite cytochrome c reductase taking place after aFas injection. The unsedimentable activity of β -galactosidase, a reference hydrolase of lysosomes was estimated to see whether there is a possible rupture of these organelles' membrane. As shown in Figure 1, injection of aFas causes first a rapid increase of homogenate DEVDase activity up to a maximum 60 to 80 min after injection. With time, the percentage of unsedimentable sulfite cytochrome c reductase increases and by about 80 min, it is up to 95% of homogenate activity after 80 min, indicating that at that time most of the mitochondria have a permeabilized outer membrane. The percentage of unsedimentable β -galactosidase is low before aFas injection (\pm 10%) and is not modified by such an injection. Even when apoptosis caused by aFas injection is stimulated by a fructose injection [22], one does not observe an increase of unsedimentable β -galactosidase activity. The total and unsedimentable activities of three other lysosomal hydrolases, β -glucuronidase, cathepsin C and cathepsin B were measured in the control homogenates and in the homogenates obtained 80 min after aFas injection. As shown in Figure 2A the proportion of these enzyme activities recovered in the homogenate supernatants is not increased by aFas injection, as observed for β -galactosidase. Moreover, the total liver activities of the four lysosomal hydrolases are not significantly decreased after the injection (Figure 2 B). This indicates that the low proportion of their unsedimentable activity does not result from a loss or an inhibition of the hydrolases after they would have been released into the cytosol of apoptotic cells.

The previous results strongly suggest that liver lysosomes remain intact until the animal death, during apoptosis resulting from an injection 10 μ g of aFas. To obtain additional information on the state of lysosomes under these conditions, we have determined the behaviour of these organelles when they are subjected to differential and isopycnic centrifugation in isoosmotic sucrose. Such an experimental approach allows to determine if the size and the density of the particles are modified after aFas injection. Figure 3 illustrates the distribution of β -galactosidase taken as a reference enzyme for lysosomes, after differential centrifugation in 0.25 M sucrose performed according to de Duve et al [20]. This was done on a liver homogenate originating from a control mouse and from a mouse injected with aFas sacrificed 60 min after injection. Obviously, both distribution patterns are similar, β -galactosidase is mainly recovered in M and L fractions and present in low amount in the S

fraction. This is in contrast to the distribution of sulfite cytochrome c reductase, as aFas injection strongly increases the percentage of that enzyme associated with the unsedimentable S fraction to the detriment of the N and M sedimentable fractions. These results indicate that aFas injection does not modify the average sedimentation coefficient of lysosomes in isoosmotic sucrose and therefore does probably not cause a swelling of these organelles as it is seen in the change from apoptosis to necrosis during cell death [23]. That the liver lysosomes are not swollen after aFas injection is supported by the results of isopycnic centrifugation reported in Figure 4. M+L fractions isolated from the liver of a control and from an aFas injected mouse sacrificed 60 min after injection, were subjected to an isopycnic centrifugation in a Percoll gradient with 0.25 M sucrose as a solvent. This procedure allows one to determine the density of a particle under iso-osmotic conditions. The distribution curve of β -galactosidase is the same whether the M+L fraction originates from the control or the aFas injected mouse, indicating that the lysosome mean density in isoosmotic conditions, is not affected by the injection. This is contrary to the behavior of mitochondria as ascertained by the distribution curve of reference enzyme cytochrome oxidase. Such curve is markedly shifted towards the low-density zones of the gradient after aFas injection, probably as a result of the swelling of the mitochondria that takes place during apoptosis [16]. It is also worthwhile to mention that these results show that sedimentable lysosomal enzymes, present in the liver homogenates after aFas injection, are truly associated with lysosomes. They could not be an artefact arising due to readsorption after their release on sedimentable structures such as mitochondria, fragments of endoplasmic reticulum, Golgi or plasma membrane. In summary, our results show that an injection of 10 μ g aFas to mice that causes a massive apoptosis of the liver leading to a quick death of the animal does not cause a leakage of lysosomes.

In a second set of experiments, we investigated the possible influence of the agonist dose on liver apoptosis caused by aFas injection. We found that apoptosis was delayed when the amount of injected aFas was decreased along with an increase in the duration of life of treated animals. As illustrated in Fig 5, after an injection of 5 μ g of aFas, the increase of DEVDase and of unsedimentable sulfite cytochrome c reductase with time is slower than after a 10 μ g injection, a maximum value is obtained only after 4 h and is followed by a marked decrease in activity for both enzymes. Unsedimentable activity of the lysosomal hydrolases, β -galactosidase, cathepsin C and β glucuronidase do not change until about two hours after injection. Later, a steady increase occurs indicating a progressive leakage of lysosomes.

The cell death pathway triggered by Fas ligation in Jurkat cells is a prototypic model of apoptosis «ex vivo». An involvement of lysosomes has been described in that process [9]. This is why we were interested in seeing if a leakage of lysosomes can be observed with our methodology in Jurkat cells treated with aFas. We used homogenates originating from Jurkat cells that had been cultured in presence of aFas (50 ng/ml) for increasing times. Apoptosis was monitored by

measuring DEVDase, leakage of lysosomes was assessed by determining the percentage of unsedimentable β -galactosidase and cathepsin C. In addition, permeabilisation of plasma membrane was appraised by measuring in the culture medium, two cytosolic enzymes dipeptidyl peptidase III [24] and DEVDase [25]. Results are presented in Fig 6. An increase of DEVDase activity is observed after aFas addition to the medium and about two hours are required to obtain a maximum value. On the other hand, an increase of unsedimentable lysosomal hydrolases occurs in the homogenate but with a delay with respect to DEVDase increase. Interestingly, in parallel, an increase of dipeptidyl peptidase III activity is seen in the culture medium. The same is true for DEVDase, an enzymatic activity that can only originate from cells in which caspase-3 activation -therefore apoptosis- occurred. These results strongly suggest that the leakage of lysosomes we observed after aFas treatment is associated with a permeabilisation of the plasma membrane. Similar results (not shown) were obtained in a more limited number of experiments by treating Jurkat cells with aFas at a concentration of 20 ng and 200 ng/ml.

DISCUSSION

Our results show that if the injection to mice of aFas (10 μ g/ animal) induces a fast and massive apoptosis of the liver leading to a rapid death of the animals, liver lysosomes remain intact, their enzymes are not required to cause death of hepatocytes. If the dose of aFas is lower (5 μ g/ animal) and leads to a slower development of apoptosis as well as a prolonged survival of animals, an increase of unsedimentable activity of lysosomal enzymes in the homogenates can be observed 2 or 3 h after the injection, indicating a disruption of lysosomes inside the cells or the organelles becoming more fragile making them more susceptible to homogenisation procedure. On the other hand, after 4h, DEVDase activity decreases. That could result from an inactivation of the enzyme in dead cells [26] or due to a release from liver cells owing to a plasma membrane leakage. A similar explanation could be used to explain the decrease of unsedimentable sulfite cytochrome c reductase activity. In Jurkat cells, aFas causes an increase of the unsedimentable activity of lysosomal hydrolases but that begins later than the caspase-3 activation (DEVDase).

Our results, «in vivo» and «ex vivo» could be explained by supposing that aFas can aggress cells by two processes, one that does not involve lysosomes and begins relatively early and a second one that takes place later and imply lysosomes. According to previous biochemical and morphological observations [17-19] and our observations, it is probable that the first process corresponds to apoptosis. In Jurkat cells, the second process goes with a rupture of plasma membrane as illustrated by the release of cytosolic enzymes into the medium. That strongly suggests that it is a necrotic one and could explain the leakage of lysosomes, a phenomenon frequently observed in cell necrosis (1, 13, 14, 23, 27) The striking similarity that we observe between the time courses of cytosolic enzyme release and the lysosome leakage is in agreement with the recent observations that plasma membrane

disruption and necrosis are linked to the susceptibility of lysosomes to rupture [23]. A same reasoning can probably be applied to liver. Leist et al. [28] have shown that after aFas injection to mice, apoptosis of hepatocytes, characterized by DNA fragmentation, largely precedes lysis of these cells, monitored by serum alanine aminotransferase level. Our interpretation is strongly supported by several observations suggesting the presence of a necrotic death pathway in Fas receptor signalling. Matsumura et al [29] have reported that in Jurkat cells, both apoptotic and necrotic death pathways are activated through the Fas receptor. As shown by Vercaemmen et al. [26], Fas triggering in fibrosarcoma cells leads first to a rapid and strong apoptotic response followed after 3h by a necrotic process, these two death pathways co-existing in the same cell. According to Kawahara et al [30], Fas associated protein with death domain (FADD) can cause a necrotic death of Jurkat cells. Leist et al. [28] have shown that activation of Fas «in vivo» and «ex vivo» causes first apoptosis of hepatocytes followed by their necrosis. That we do not observe a leakage of lysosomes in liver after an injection of 10 μ g aFas is probably explained by the fact that in these conditions, the massive apoptosis of hepatocytes induces a rapid death of the animal due to a fulminant liver failure, before the development of the slower necrotic process could occur.

It is to be noted that the increase of undedimentable lysosomal enzyme after aFas treatment is relatively modest. The value found in the control liver and Jurkat cells homogenates is more or less 10 to 12 % of the total activity. After aFas treatment, it reaches 30 to 35%. If we refer to the postulate of biochemical homogeneity of subcellular structures [20], that would mean that 20 to 25% of the lysosomes (on a mass basis) become fragile with the treatment. This could originate from the fact that on an average 20-25% of lysosomes are leaky in each cell or that all the lysosomes in 20-25% of the cells have become fragile

What causes the leakage of lysosomes induced by Fas triggering ? There are probably several factors that are involved. First, activated caspases could destabilize lysosomes by proteolysis of their membrane. Many years ago, it was shown that proteolytic enzymes could disrupt lysosomal membrane «in vitro» [31]. More recently, it has been reported that incubation of lysosomes with caspase-8 makes the lysosomal membrane more fragile [11]. A second possibility is that reactive oxygen radicals that accumulate in cells after stimulation of Fas receptor [26] disrupt lysosomal membrane as observed «in vitro» [32]. A third factor that could play a role is the strong decrease of cellular ATP concentration. aFas treatment brings about a progressive depletion of cytochrome c in mitochondria with consequently a mitochondrial respiratory dysfunction [18] and a decrease of ATP production. As shown by Leist et al. [33], ATP depletion induces a change from apoptosis to necrosis. Such depletion is associated with an increase of lysosome size, making these organelles fragile and favouring their rupture [23]

Most of the studies aimed at investigating whether lysosomes are involved in Fas mediated apoptosis have been done on isolated cells. Our observations allow to compare results obtained «in

vivo» and «ex vivo». They show in both cases, the importance of taking into account the time existing between the stimulation of Fas and the determination of the possible leakage of lysosomes. Such leakage is a late phenomenon that we suggest is associated not with apoptosis but with the slow necrotic process induced by Fas

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LEGENDS

Figure 1

Caspase-3-like protease (DEVDase) activation (A) and unsedimentable activities (B) of sulfite cytochrome c reductase (open squares) and β -galactosidase (open triangles) at increasing time after injection of anti Fas antibody (10 μ g/animal). Determinations were performed on total homogenate for DEVDase, on total homogenate and on high speed supernatant (35 000rpm for 40 min, Beckman rotor type 40) for sulfite cytochrome c reductase and β -galactosidase. DEVDase activity was given in nmoles methylumbelliferone released/ min/ liver. The unsedimentable activity was expressed as percentage of the total activity present in the homogenate. Error bars represent the standard error of the mean for at least three animals. Closed symbols give the values obtained after

stimulation of apoptosis by an intraperitoneal injection of fructose (250 mg) 15 min before aFas injection .

Figure 2

Unsedimentable (A) and total activities (B) of sulfite cytochrome c reductase (a), β -galactosidase (b), β -glucuronidase (c), cathepsin C (d) and cathepsin B (e) present in liver homogenates originating from mice injected with saline (open bars) or with antiFas antibody (10 μ g/animal) (closed bars) and sacrificed 80 min after injection. Unsedimentable activity was expressed as percentage of the activity present in the homogenate. For sulfite cytochrome c reductase and β -galactosidase, values indicated in Figure 1 have been used. Values for unsedimentable activity of sulfite cytochrome c reductase of treated versus control mice are significantly different ($P < 0.01$) but not for unsedimentable lysosomal hydrolases ($P > 0.05$). To uniformize the presentation, total activity measured in the aFas injected mice liver homogenates is given as percentage of the activity found in control mice liver homogenates supposed to be equal to 100. Error bars represent the standard error of the mean for at least three animals. Values found in homogenates of injected and control mice do not significantly differ ($P > 0.05$).

Figure 3

Distribution of β -galactosidase (A) and of sulfite cytochrome c reductase (B) after differential centrifugation in 0.25M sucrose. N, nuclear fraction, M, heavy mitochondrial fraction, L, light mitochondrial fraction, P, microsomal fraction, S, soluble fraction. Liver originates from a saline injected mouse (open bars) or from a aFas injected mouse killed 60 min after injection (closed bars) DEVDase activities of homogenates were respectively of 0.1 nmole/min/liver for the control and 90.1 nmoles/min/liver for aFas injected mouse.

Figure 4

Distribution of β -galactosidase (A) and cytochrome oxidase (B) after isopycnic centrifugation in a Percoll gradient, with 0.25 M sucrose as a solvent, of a total mitochondrial fraction (M+L) isolated from the liver of a saline injected mouse (open squares) or of a aFas injected mouse killed 60 min after injection (closed squares). M+L fraction was layered at the top of the gradient. Centrifugation was performed at 25 000 rpm for 60 min in the Beckman rotor SW 65. Fractions are numbered from the top to the bottom of the gradient. DEVDase activities of homogenates were respectively of 0 nmoles/min/liver for the contrôle and 0 nmoles/min/liver and 66.1 nmoles /min/liver for aFas injected mouse

Figure 5

Caspase-3- like protease (DEVDase) activation (A) and unsedimentable activities of sulfite cytochrome c reductase (B), β -galactosidase (C), cathepsin C (D) and β -glucuronidase (E) at increasing time after injection of anti Fas antibody ($5 \mu\text{g/ animal}$). Determinations were performed on total homogenate for DEVDase, on total homogenate and on high speed supernatant (35 000rpm for 40 min, Beckman rotor type 40) for sulfite cytochrome c reductase and acid hydrolases. DEVDase activity was given in nmoles methylumbelliferone released/ min/ liver. The unsedimentable activity was expressed as percentage of the total activity present in the homogenate. Error bars represent the standard error of the mean for at least three animals. The last values were obtained in a single experiment.

Figure 6

Caspase-3-like protease (DEVDase) activation in cells (A), unsedimentable activities of β -galactosidase (B) and of cathepsin C (C) in cell homogenates, activities of dipeptidylpeptidase III (D) and of DEVDase (E) in culture medium, at increasing times after addition of aFas (50 ng/ml) to Jurkat cells culture medium. Determinations were performed on total homogenate of Jurkat cells and on the culture medium for DEVDase, on total homogenate and on high speed supernatant for β -galactosidase and cathepsin C. DEVDase activity was given in nmoles methylumbelliferone released/ min/ 2×10^6 .cells or ml of culture medium. Dipeptidylpeptidase III activity is given in nmoles naphthylamine released/min/ ml of culture medium. The unsedimentable activity was expressed as percentage of the total activity present in the homogenate. Error bars represent the standard error of the mean for five cells preparations. (F) Outline of (A), (B), and (E) to compare the time courses of apoptosis (A) lysosome leakage (B) and cell lysis (E).

ACKNOWLEDGEMENTS

This work was supported by the Fonds de la Recherche Fondamentale Collective (FRFC). MC Gasingirwa holds a fellowship from Coopération Universitaire au Développement (CUD). Authors are indebted to Sandra Misquith for helping in the preparation of the manuscript and Michel Savels for the artwork.

Figure 1

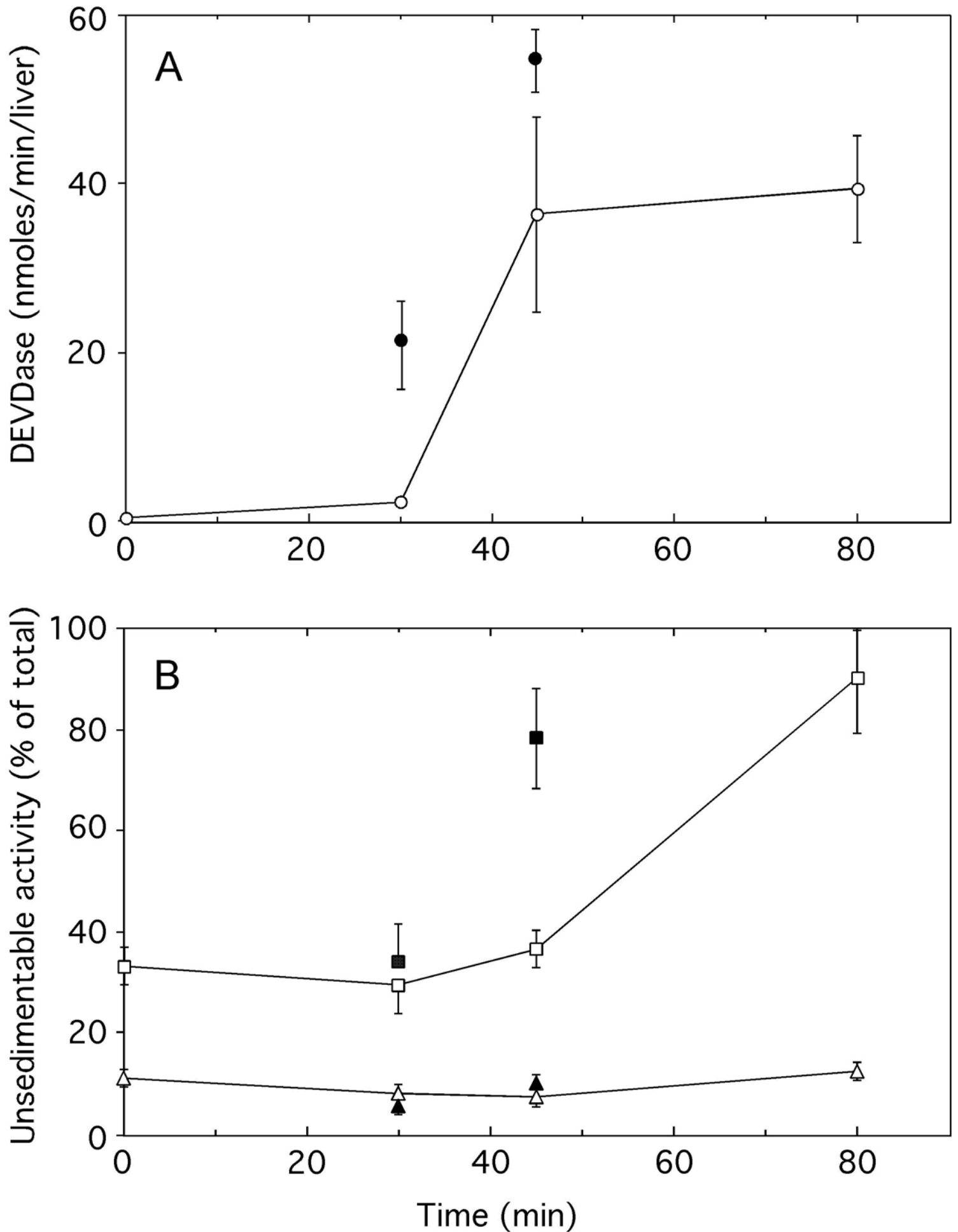


Figure 2

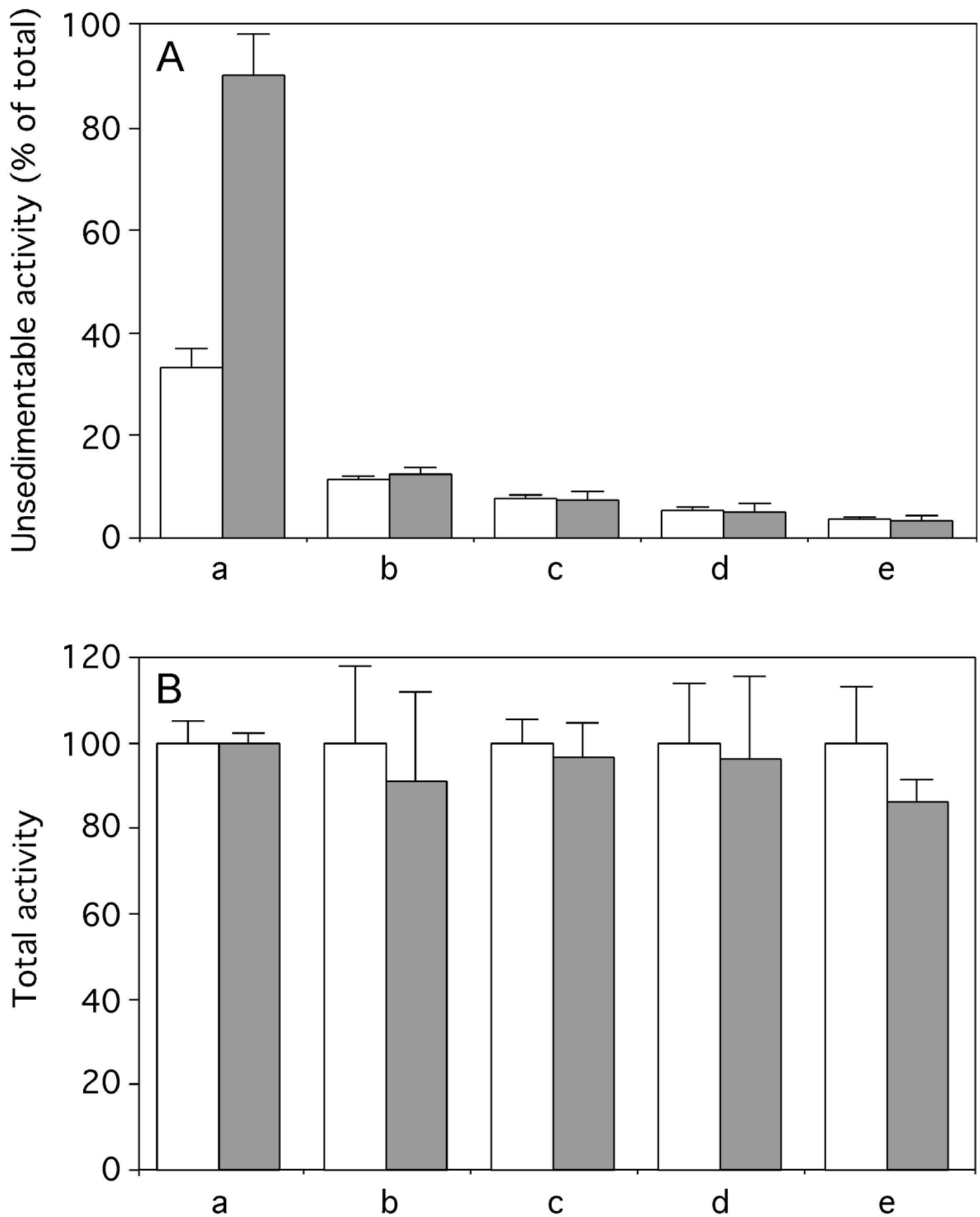


Figure 3

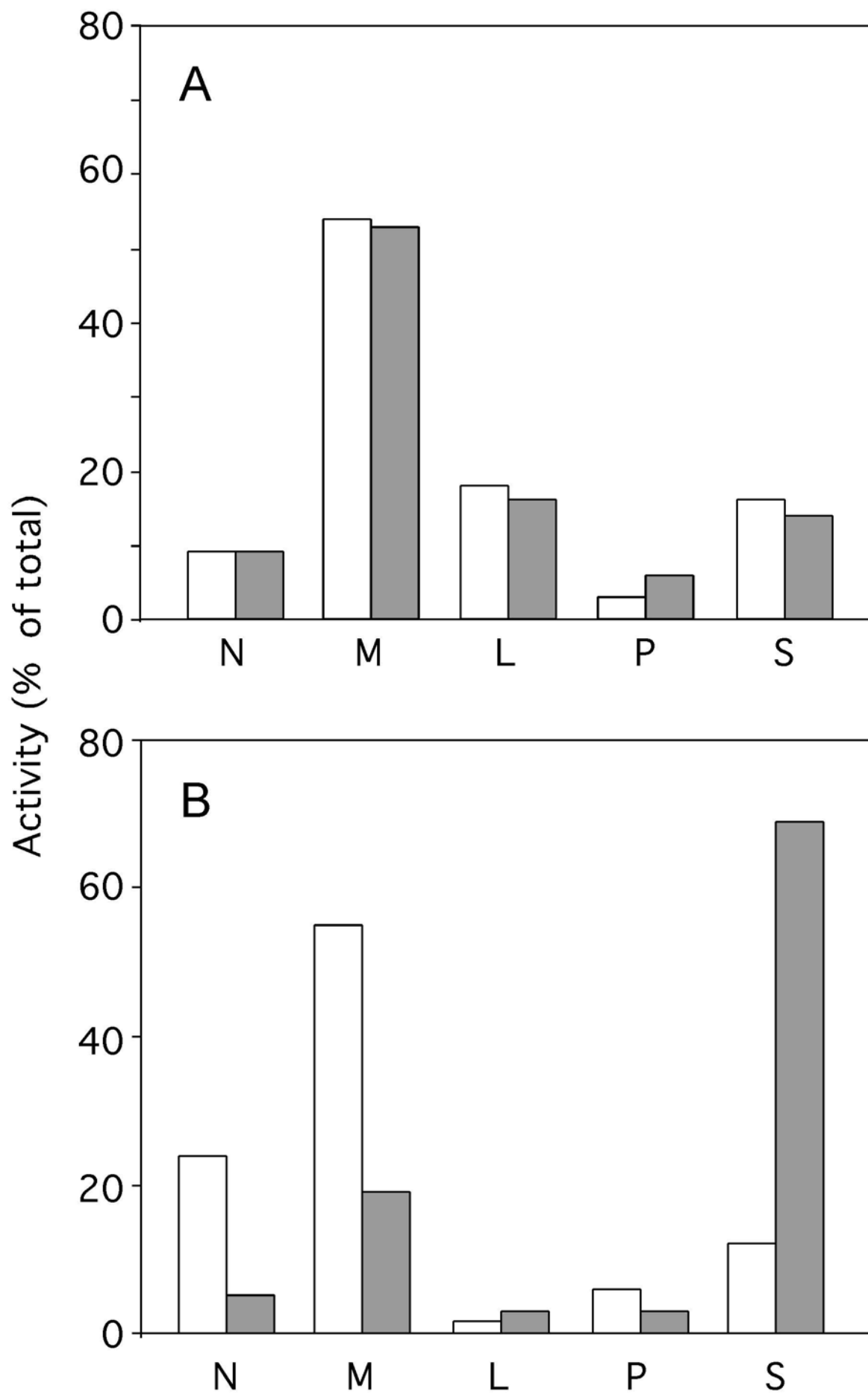


Figure 4

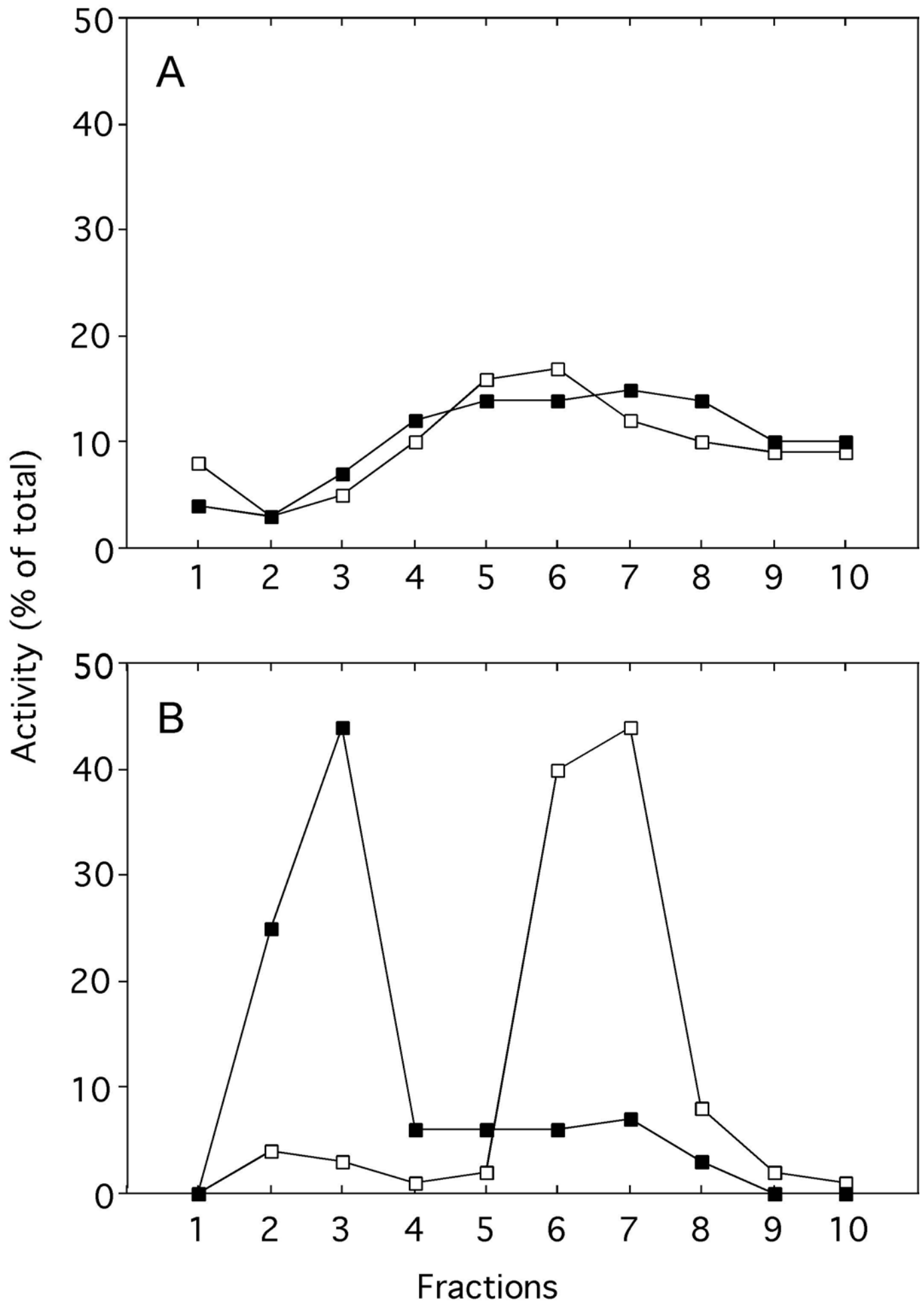


Figure 5

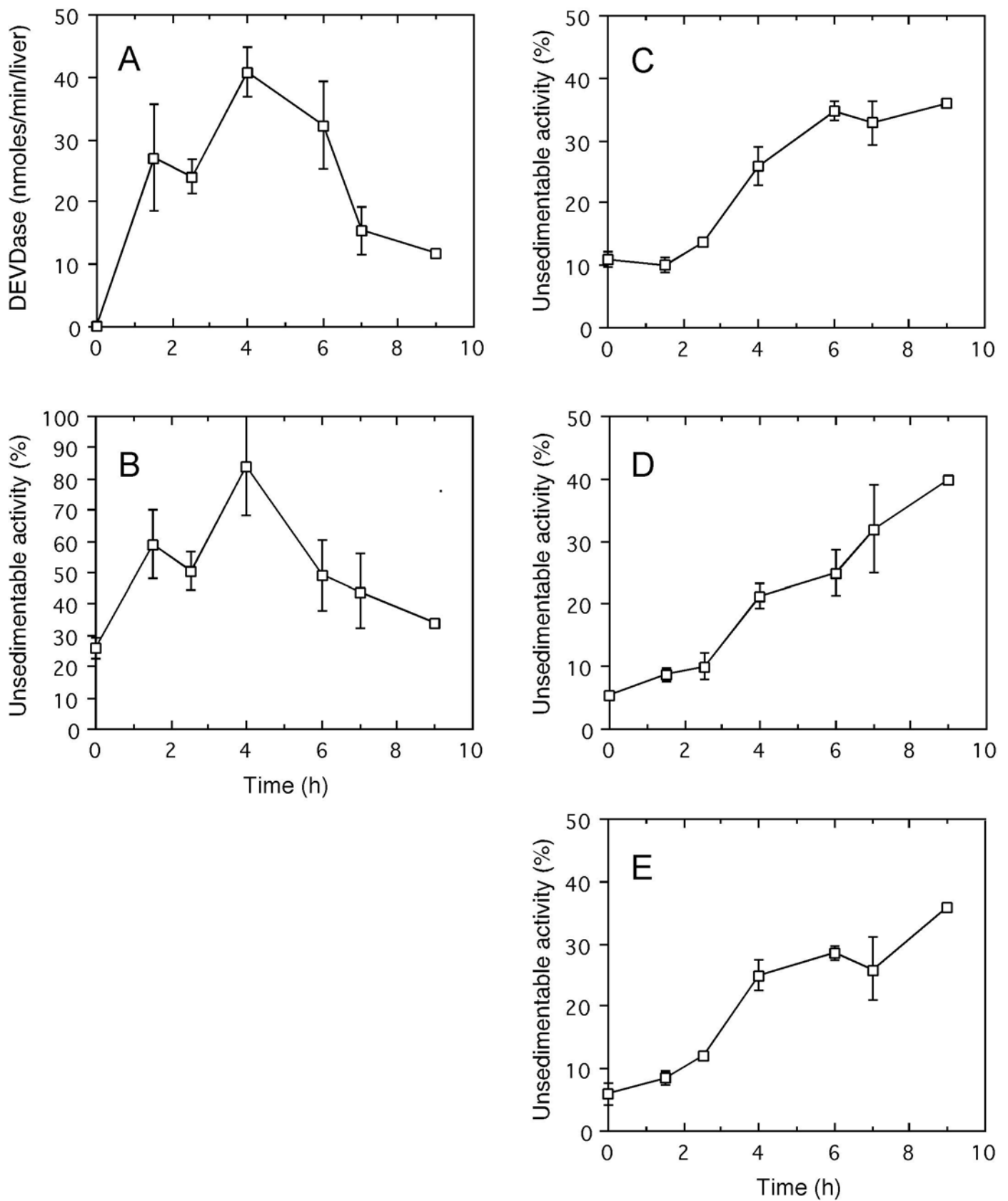


Figure 6

