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The role of GSH peroxidase in protecting the membrane of rat liver mitochondria

GSH-induced high amplitude swelling of rat liver mitochondria is counteracted mainly by C-factor I (ref. 1) which is identical with GSH peroxidase[1,2]. Since this type of disintegration of the mitochondrial membranes is accompanied by accumulation of lipid peroxides[3] and since GSH peroxidase is able to reduce hydroperoxides of unsaturated lipids[4–6], it may be suggested that the C-factor activity of GSH peroxidase is attributable to its own peroxidatic action. The present investigation supplies further evidence for this concept.

Liver mitochondria were prepared from SIV-50 rats according to HOGEBOOM *et al.*[7], but in 0.25 M sucrose and 0.02 M Tris–acetate buffer of pH 7.4; the mitochondria were washed twice with the above solution and stored at 0° until used.

GSH-induced swelling and lipid peroxide formation of mitochondria were studied under following conditions: Exactly 3 h after the killing of the animals the mitochondria were suspended in 0.125 M KCl in 0.02 M Tris–acetate buffer of pH 7.5 containing 20 mM GSH to give an initial absorbance at 520 nm of approx. 0.5. GSH was adjusted to the pH of the incubation medium by 2 M NaOH. Swelling of the mitochondria was observed at 25° by absorbance readings at 520 nm according to LEHNINGER *et al.*[8]. Formation of lipid peroxides was determined in separate samples as described in ref. 9. In one set of experiments the initial concentration of GSH was modified, in another set the pH of the incubation medium was varied.

Glutathione peroxidase activity of the mitochondrial suspension was determined at 37° according to ref. 10 exactly 2.5 h after the killing of the animals. When intact mitochondria were investigated, only the fraction of GSH peroxidase was determined which was accessible to the GSH of the surrounding medium and which probably was bound to the mitochondrial surface[1,11] ("surface activity"). The "total activity" of the GSH peroxidase of mitochondria was determined in the presence of 0.1 % Triton X-100. The unit of activity (at 37°) was defined as the amount of enzyme (dissolved in 1 ml) which effects a $\Delta \log c_{GSH}$/min of 1 (ref. 12).

We were able to confirm the observation of LEHNINGER AND SCHNEIDER[13] that swelling of mitochondria is induced by GSH and increases with the amount of GSH added. In addition, the formation of lipid peroxides correlated with the degree of swelling, as has been demonstrated by HUNTER *et al.*[3] for GSH/GSSG-induced swelling.

Studies on GSH-induced swelling of mitochondria at different pH values allow one to challenge the hypothesis that lysis of membranes caused by peroxidation of the lipids is mainly prevented by GSH peroxidase, since the enzyme shows a pronounced pH dependency[10]. As spontaneous swelling of mitochondria occurs at extreme pH values, difference plots of spontaneous and GSH-induced swelling (Fig. 1A) are constructed to demonstrate more clearly the portion attributable to the influence of GSH itself. The curves become steeper and the lag phases significantly shorter with decreasing pH. These results are in fairly good agreement with those of LEHNINGER AND SCHNEIDER[13], although we were unable to show the decrease of the swelling rate below pH 6.5 found by these authors. At pH 6 the suspension of mitochondria tends

to aggregate in the Tris–acetate–KCl medium so that no reliable light-scattering measurements could be performed. The degree of swelling (or lysis[8]) of mitochondria is paralleled by the amount of lipid peroxides which accumulates during the incubation time (Fig. 1B). However, formation of thiobarbituric acid-positive material continues even after the swelling of mitochondria has reached its final state. Since we may accept that peroxidation of lipids is not prevented by alkaline pH, the extreme low values for lipid peroxides found at pH 8 are due to the higher reaction rate of the GSH peroxidase under these conditions. In addition, we may conclude that the products of the enzymatic reduction of lipid peroxides[5,6] do not disturb the architecture of the mitochondrial membrane, at least not morphologically, as the peroxides apparently do.

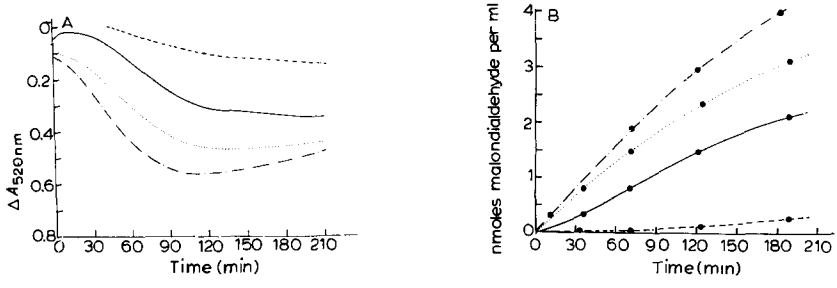

Fig. 1. Swelling and accumulation of lipid peroxides of mitochondria at different pH's. Mitochondria prepared from two female rats of 130 g (44 days old) were suspended in 0.125 M KCl–0.02 M Tris–acetate medium (1.8 mg protein per ml). The pH's of the medium were: –·—·–, 6.5; ·····, 7.0; ———, 7.5; – – –, 8.0. A. Decrease in absorbance in the presence of 20 mM GSH. The curves were corrected for spontaneous swelling in the absence of GSH. B. Accumulation of lipid peroxides during incubation period (nmoles malondialdehyde per ml of suspension) in the presence of 20 mM GSH. No significant amount of thiobarbituric acid-positive material was observed when mitochondria were incubated in the absence of GSH.

The interpretation of the above results remains somewhat ambigous since it is difficult to measure GSH-induced swelling of mitochondria at a pH beyond 7.1–7.6. We thus looked for a possible way of investigating mitochondria under identical conditions but with different levels of intrinsic GSH peroxidase. Mitochondria which fulfill this requirement are available, since the activity of the GSH peroxidase of the rat liver mitochondria depends on age and sex of the animals, in the same manner as described for rat liver supernatant by PINTO AND BARTLEY[14], the dependency on age being more pronounced in females than in males (see legends of Figs. 2 and 3). As we expected from the above experiments, GSH-induced swelling and accumulation of lipid peroxides of the mitochondria are inversely related to their GSH peroxidase activity (Figs. 2A, 2B, 3A, 3B). In males, short lag phases and high rates of swelling are observed, so that the negative correlation to peroxidase activity does not reach the significance of the results obtained with females. The relation of GSH peroxidase activity and peroxide accumulation is significant in any case, as the latter continues over a longer period. However, there are some discrepancies if mitochondria containing equal amounts of GSH peroxidase of male and female rats are compared. In liver mitochondria of older female rats lipid peroxides are found in higher accumulation than expected by their GSH peroxidase activity (compare Figs. 2B and 3B).

This may be partially due to differences in protein concentration of the samples. But it is more likely that sex differences in lipid metabolism are to be considered[15].

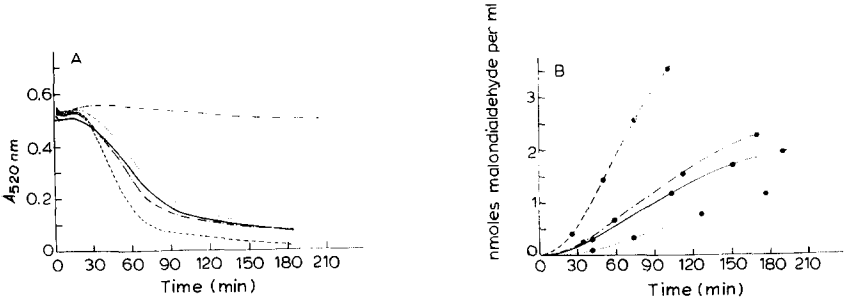

Fig. 2. GSH-induced swelling and accumulation of lipid peroxides of liver mitochondria prepared from male rats of different age at 20 mM GSH. – – – –, animals aged 22 days (40 g); "total" GSH peroxidase activity: 0.055 unit/mg mitochondrial protein; 2.17 mg protein per ml suspension. –·—·–, animals aged 38 days (120 g); GSH peroxidase activity: 0.090 unit/mg; 1.93 mg protein per ml. ———, animals aged 48 days (180 g); GSH peroxidase activity: 0.096 unit/mg; 1.86 mg protein per ml. ·····, animals aged 78 days (300 g); GSH peroxidase activity: 0.134 unit/mg; 1.7 mg protein per ml. The "surface activity" amounts to approximately one half of the "total activity". A. Swelling curves. Since spontaneous swelling did not differ significantly between the mitochondria of the respective groups of animals during the period of observation, only one representative curve is given (—·····—). B. Accumulation of lipid peroxides given in nmoles malondialdehyde per ml suspension. No significant amounts of lipid peroxides could be detected without addition of GSH.

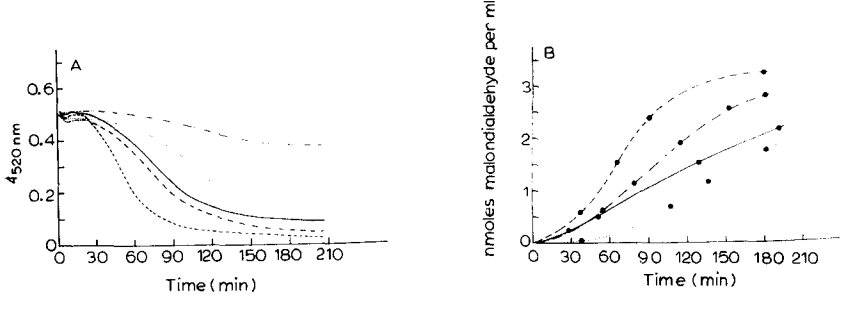

Fig. 3. GSH-induced swelling and accumulation of lipid peroxides of liver mitochondria prepared from female rats of different age at 20 mM GSH. – – – –, animals aged 22 days (40 g); GSH peroxidase activity: 0.066 unit/mg mitochondrial protein; 2.6 mg protein per ml suspension. –·—·–, animals aged 38 days (108 g); GSH peroxidase activity: 0.124 unit/mg; 2.18 mg protein per ml. ———, animals aged 48 days (140 g); GSH peroxidase activity: 0.156 unit/mg; 2.90 mg protein per ml. ·····, animals aged 78 days (210 g); GSH peroxidase activity: 0.210 unit/mg; 2.03 mg protein per ml. A. Swelling curves. See legend Fig. 2A. B. Accumulation of lipid peroxides. See legend Fig. 2B.

Summarizing, we may briefly recall the main facts. (1) By addition of GSH to washed rat liver mitochondria *in vitro* GSH peroxidase is detached from the mitochondrial surface[1,13]. This results in "high amplitude" swelling (lysis) and accumulation of lipid peroxides. (2) Swelling and lipid peroxide accumulation of mitochondria are inversely related to the pH dependency of GSH peroxidase reaction rate. (3) Stability of rat liver mitochondria and the rate of lipid peroxide destruction is proportional to their intrinsic GSH peroxidase activity. Thus it is evident that GSH per-

oxidase is the essential factor in preventing accumulation of lipid peroxides and lysis of mitochondrial membranes *in vitro*. We should like to assume that *in vivo*, too, the enzyme plays a major part in maintaining the morphological and functional integrity of the mitochondria.

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1 D. NEUBERT, A. B. WOJTCZAK AND A. L. LEHNINGER, *Proc. Natl. Sci. U.S.*, 48 (1962) 1651.
2 L. FLOHÉ, W. SCHLEGEL AND E. SCHAICH, *Z. Klin. Chem. Klin. Biochem.*, 8 (1970) 149.
3 F.E. HUNTER, JR., A. SCOTT, P.E. HOFFSTEN, J.M. GEBICKI, J. WEINSTEIN AND A. SCHNEIDER, *J. Biol. Chem.*, 239 (1964) 614.
4 C. LITTLE AND P. J. O'BRIEN, *Biochem. Biophys. Res. Commun.*, 31 (1968) 145.
5 B. O. CHRISTOPHERSEN, *Biochim. Biophys. Acta*, 164 (1968) 35.
6 B. O. CHRISTOPHERSEN, *Biochim. Biophys. Acta*, 176 (1969) 463.
7 G. H. HOGEBOOM, W. C. SCHNEIDER AND G. E. PALLADE, *J. Biol. Chem.*, 172 (1948) 619.
8 A. L. LEHNINGER, B. L. RAY AND M. SCHNEIDER, *J. Biophys. Biochem. Cytol.*, 5 (1959) 97.
9 R. L. HEATH AND L. PACKER, *Biochem. Biophys. Res. Commun.*, 19 (1965) 716.
10 F. SCHNEIDER AND L. FLOHÉ, *Z. Physiol. Chem.*, 348 (1967) 540.
11 W. SCHLEGEL, Biochemische Diplomarbeit, Tübingen, 1969.
12 L. FLOHÉ AND I. BRAND, *Z. Klin. Chem. Klin. Biochem.*, 8 (1970) 156.
13 A. L. LEHNINGER AND M. SCHNEIDER, *J. Biophys. Biochem. Cytol.*, 5 (1959) 109.
14 R. E. PINTO AND W. BARTLEY, *Biochem. J.*, 112 (1969) 109.
15 R. E. PINTO AND W. BARTLEY, *Biochem. J.*, 115 (1969) 449.

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