Ferritin, a physiological iron donor for microsomal lipid peroxidation

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In the process of lipid peroxidation of microsomes induced either by oxygen radicals generated by xanthine oxidase or by NADPH, ferritin is able to donate the necessary iron. The amount of ferritin necessary to catalyze the process of lipid peroxidation is in the physiological range. In contrast to the finding with phospholipid liposomes, catalase hardly stimulates the lipid peroxidation of microsomes.

Ferritin *Iron* *Lipid peroxidation* *Microsome*

1. INTRODUCTION

Superoxide (O_2^-) is associated with the cellular damage occurring under various pathological conditions [1,2]. One of the main targets is the phospholipids of the membrane [3]. Aqueous O_2^- is rather unreactive and the deleterious effects have been ascribed to more reactive intermediates [4]. The formation of these reactive radicals is dependent on the presence of a transition metal [3], of which iron is probably the most important. Although the final answer as to whether the radicals are hydroxyl radicals ($OH^\cdot$) or complexes between iron and superoxide is not known, the role of iron as a catalyst is well accepted.

The amount of free iron in the cell is negligible and practically all the cellular iron is located in ferritin. The ferritin molecule is able to store up to 4500 ions of iron, but under normal conditions about 2200 ions of iron per molecule of ferritin. The iron is stored in the ferric form but released as ferrous ions. Therefore, for the mobilization of iron a reductive step is involved, however the exact mechanism is unknown, as are the natural cellular reductants. O_2^- derived from stimulated granulocytes [5] or generated by xanthine oxidase [6,7] is able to mobilize iron from ferritin. With xanthine oxidase there is also an O_2^--independent mobilization of iron, which can be important under ischaemic conditions [7].

Recently it has been shown with phospholipid liposome models that ferritin is able to donate the necessary iron for the process of lipid peroxidation [6,8,9]. The purpose of this report is to show that ferritin in the physiological range is able to catalyze the lipid peroxidation of native microsomes.

2. MATERIALS AND METHODS

Liver microsomes were isolated from the liver of Wistar rats as described [10]. Microsomes were finally taken up in 0.125 M KCl. Lipid peroxidation was performed in 0.1 M Tris-HCl (pH 6.8), 3 mM ADP and 1 mg/ml microsomes (final concentrations in 3 ml). Lipid peroxidation was induced either by dialyzed xanthine oxidase and xanthine (both at the indicated concentrations) or by NADPH (0.4 mM). Lipid peroxidation was followed by measuring the thiobarbituric acid-reacting products. At the indicated times, samples (0.5 ml) were withdrawn and mixed with 0.15 ml trichloroacetic acid (20%), 0.05 ml butylated hydroxytoluene (0.2%) and 0.15 ml thiobarbituric

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acid (0.05 M). The mixture was centrifuged, the supernatant boiled for 10 min and after cooling the absorbance at 535 nm measured. An extinction coefficient of 156000 $M^{-1} \cdot cm^{-1}$ was used.

Iron mobilization from ferritin was measured with 1 mM bathophenanthroline, using an extinction coefficient of 22140 $M^{-1} \cdot cm^{-1}$ at 530 nm. Mobilization of iron from ferritin was performed in the same buffer as lipid peroxidation.

Xanthine oxidase, xanthine, NADPH, ADP, catalase and superoxide dismutase were obtained from Boehringer, Mannheim; bathophenanthroline from Merck, Darmstadt. Cadmium-free ferritin from horse spleen (22% iron; 50% saturation) was purchased from Boehringer and was gel filtered on Sephadex G-50 (Pharmacia, Uppsala) to exclude interference by non-specific bound iron.

3. RESULTS

The dependence of microsomal lipid peroxidation on the amount of xanthine oxidase with ferritin as iron source is shown in fig.1. With a xanthine concentration of 0.33 mM lipid peroxidation is lowered beyond a xanthine oxidase concentration of about 10 mU/ml. Increasing the substrate concentration to 0.66 mM, the decrease in lipid peroxidation begins at a higher concentration of xanthine oxidase. Furthermore, these results show that ferritin can supply the necessary iron. Fig.2 exhibits the time dependence of the microsomal lipid peroxidation induced by xanthine oxidase and xanthine with various ferritin concentrations. Also in the physiological range of liver ferritin, it can supply iron ions to catalyze lipid peroxidation. This is substantiated in fig.3, which shows the dependence of lipid peroxidation on the amount of ferritin present and the amount of iron which is mobilized during the same period as measurement of lipid peroxidation. The extent of lipid peroxidation is clearly correlated with the amount of iron mobilized. Catalase hardly shows any stimulatory effect on lipid peroxidation of microsomes, which contrasts with findings with the phospholipid model [6].

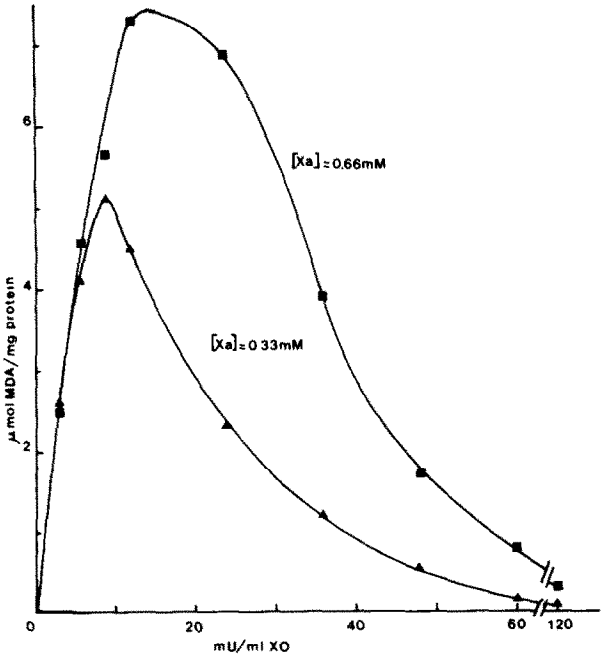

Fig.1. Lipid peroxidation of microsomes vs the amount of xanthine oxidase at two different xanthine concentrations. (▲—▲) 0.33 mM xanthine, (■—■) 0.66 mM xanthine. 570 μg ferritin/ml and 1 mg microsomes/ml.

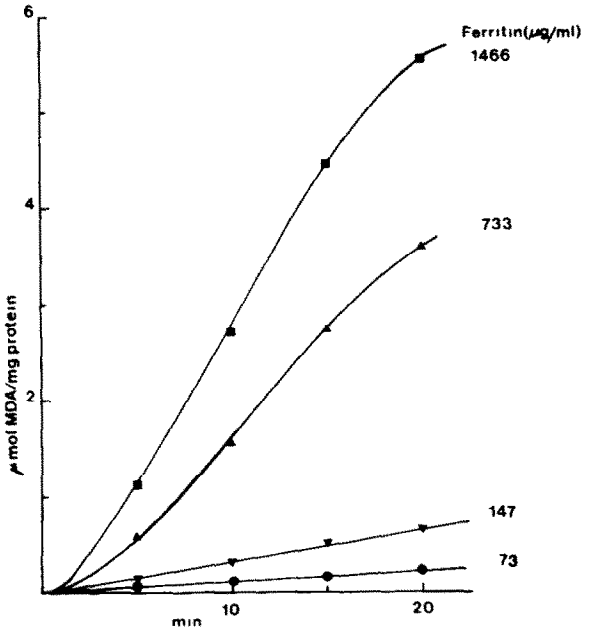

Fig.2. Lipid peroxidation of microsomes induced by xanthine oxidase and xanthine at various ferritin concentrations. (●—●) 73 μg/ml, (▼—▼) 147 μg/ml; (▲—▲) 733 μg/ml and (■—■) 1466 μg/ml. 10 mU xanthine oxidase/ml, 0.33 mM xanthine and 1 mg microsomes/ml.

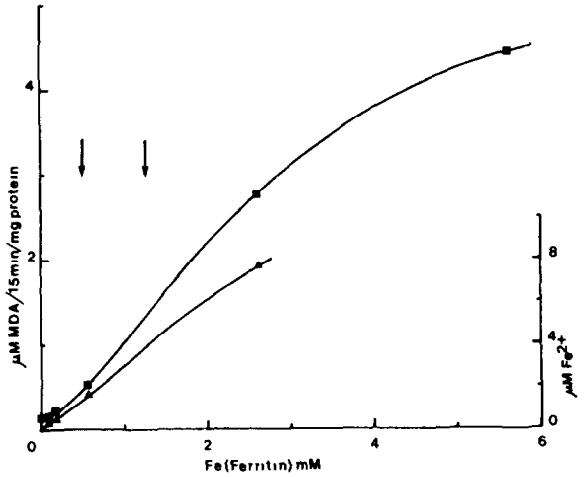

Fig.3. The ferritin dependence of lipid peroxidation of microsomes, induced by xanthine oxidase and xanthine. (■—■) MDA production and (▲—▲) iron mobilisation from ferritin under identical conditions. 10 mU xanthine oxidase/ml; 0.33 mM xanthine and 1 mg microsomes/ml.

Lipid peroxidation of microsomes induced by NADPH is also catalysed by ferritin (fig.4). This lipid peroxidation is also hardly stimulated by catalase and scarcely inhibited by superoxide dismutase (Cu-enzyme). Fig.4 also shows the amount of iron which is released from ferritin during the time course, under conditions identical to those of lipid peroxidation.

4. DISCUSSION

Our data show the ability of ferritin to supply iron ions to promote lipid peroxidation in microsomes. This is in agreement with the finding in [6,8,9] performed with phospholipid liposomes. However, with microsomes no stimulating effect of catalase is seen, which contrasts with the results of Thomas et al. [6]. However, it should be borne in mind that microsomal preparations are contaminated with catalase, which is difficult to remove. The dependence of the extent of lipid peroxidation on the amount of xanthine oxidase is similar for microsomes and phospholipid liposomes. For microsomes we found that the amount of xanthine oxidase beyond which lipid peroxidation declines is dependent on the xanthine concentration. It is difficult to explain these results and the physiological meaning is rather obscure.

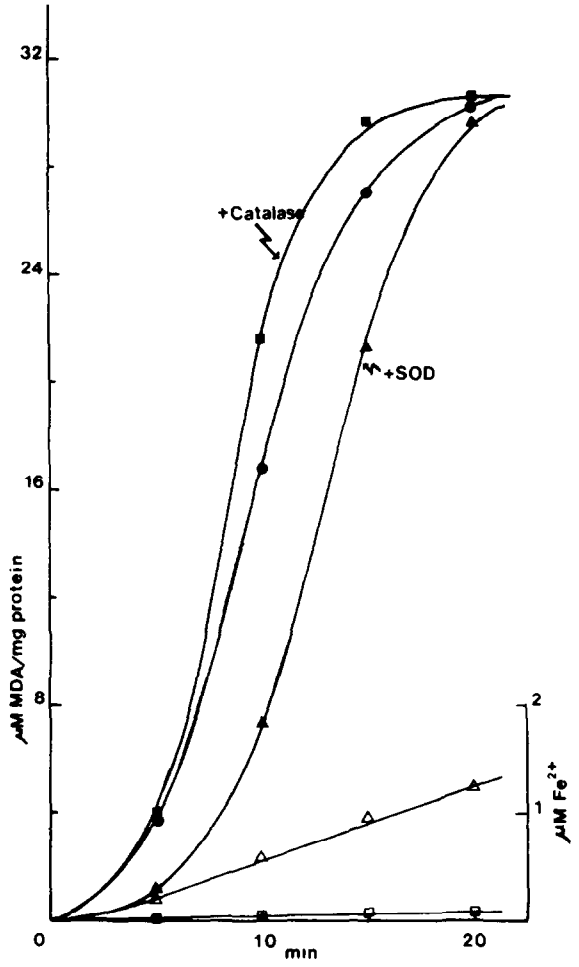

Fig.4. Lipid peroxidation of microsomes induced by NADPH (0.4 mM) and ferritin (540 μg/ml) and the effect of catalase (200 U/ml) and superoxide dismutase (20 μg/ml). (●—●) Control, (■—■) control + catalase, (▲—▲) control + superoxide dismutase (20 μg/ml); (□—□) control without ferritin and (△—△) Fe mobilization.

The capacity of ferritin to donate iron for the promotion of lipid peroxidation at a physiological pH is very important because most of the intracellular iron is located in the ferritin molecule. This implies that iron is always available for the promotion of lipid peroxidation, especially in cases of infiltration of granulocytes and macrophages or in reperfusion of ischaemic tissues. In the latter case it has been shown that conversion of xanthine dehydrogenase to oxidase occurs [11]. However, under this condition we have also found that in ad-

dition to the O_2^--dependent mechanism xanthine oxidase is also able to mobilize iron from ferritin in an O_2^--independent pathway [7].

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