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LIPID PEROXIDATION IN LIVER AND EHRLICH ASCITES CELL MITOCHONDRIA

GY. SZABADOS, L. TRETTER and I. HORVATH

2nd Institute of Biochemistry, Semmelweis University Medical School, Budapest, H-1444, POB. 262, Hungary

Ehrlich ascites cell mitochondria are highly resistant to lipid peroxidation as compared to liver mitochondria from host animals. Succinate protects mitochondria from peroxidative damage, proteins from cross-links, enzymes from inactivation of the enzymes and membranes from permeability changes. The sensitivity of Ehrlich ascites cell mitochondrial membranes to lipid peroxidation is significantly increased in sub-mitochondrial particles. Lipid peroxidation in tumour mitochondrial membranes can not be diminished by succinate as effectively as in liver mitochondria. Ascites cell mitochondria seems to be protected very efficiently from peroxidative damage by a glutathione-dependent mechanism.

KEY WORDS: Lipid peroxidation, mitochondria, tumour, succinate, glutathione.

INTRODUCTION

Lipid peroxidation seems to be associated with, and may even be necessary for, many physiological as well as pathological processes.[1–5] Many tumour cells peroxidize very much less than corresponding normal tissues.[6–8] A general hypothesis was put forward that an increased rate of cell division is associated with a decreased rate of lipid peroxidation.[9–11]

Membranes of mitochondria contain unsaturated fatty acids in high porportion,[12,13] so they are especially vulnerable to peroxidative attack. Therefore, the comparison of the lipid peroxidation of ascites cell mitochondria with that of non-transformed cell, in particular with liver mitochondria might help to find an explanation why certain tumour mitochondria are highly resistant to lipid peroxidation. Moreover, these studies might help to elucidate whether there exists a correlation between the low susceptibility to lipid peroxidation of ascites tumour mitochondria and the high mitotic rate of the tumour cells.

PROTECTION AGAINST LIPID PEROXIDATION IN LIVER MITOCHONDRIA

In the early 70's an enzymatic NADPH-dependent lipid peroxidation was described[14] in liver mitochondria. This process resulted in the oxidative cleavage of mitochondrial fatty acids, particularly of the highly unsaturated species. Concurrent with these alterations, the formation of malondialdehyde and the impairment of mitochondrial respiratory activity was observed. It was shown[15] that the respiratory substrates,

Correspondence should be addressed to Gy. Szabados.

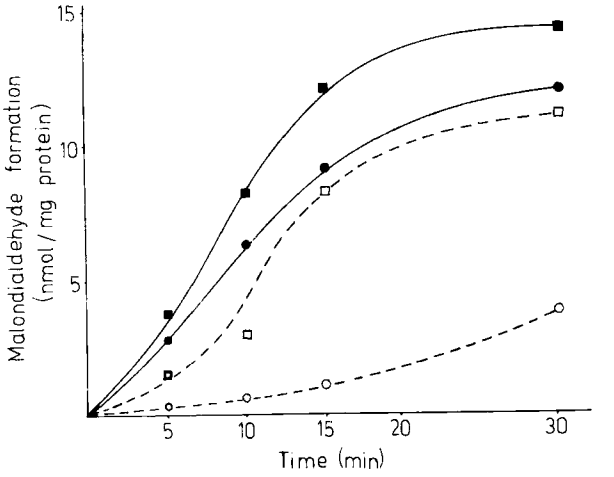

FIGURE 1 Effect of succinate on ADP/Fe/NADPH induced lipid peroxidation in mitochondria and mitoplasts. Malondialdehyde formed in the presence of (●—●) mitochondria, (■—■) mitoplasts, (○—○) mitochondria + succinate, (□—□) mitoplast + succinate. Data from ref. 17.

particularly succinate effectively inhibited the NADPH-dependent malondialdehyde formation in isolated liver mitochondria.

Figure 1 demonstrates that succinate exerts protection against malondialdehyde formation not only in mitochondria, but also in mitoplasts. We decided to study the lipid peroxidation in mitoplasts because, despite existing convincing evidence, some doubts have still remained that the lipid peroxidation in isolated mitochondria might be initiated by contaminating microsomes.

The rate of malondialdehyde formation was found to be at least as high or even higher in mitoplasts than in mitochondria. This observation, together with the results of other groups,[14,16] strongly suggests that the NADPH-dependent lipid peroxidation in mitochondrial preparates is really a mitochondrial event, and can not be ascribed to microsomal contamination. This peroxidising system seems to be associated with the inner membranes of mitochondria.

Succinate inhibits not only malondialdehyde formation but also protects mitochondrial proteins from the consequences of the free radical attack. The lipid peroxidation-induced changes in mitochondrial proteins were analysed by SDS polyacrylamide gradient gel electrophoresis.

Lanes 1–5 in Figure 2 show the protein bands of the samples from mitochondria that were not exposed to free radical attack during incubation for different times from 0 to 40 minutes. It can be seen that when lipid peroxidation was not induced, the electrophoretic pattern of the mitochondrial proteins was not altered during 40 min of incubation.

Lanes 6–9 represent mitochondria in which lipid peroxidation was induced for 10, 20, 30 and 40 minutes, respectively. In these samples the loss of some bands and accumulation of high molecular weight proteins can be detected at the top of the gel. The most likely explanation of these observations is that malondialdehyde generates cross-links between polypeptide chains of disappeared bands and the protein aggregates are seen as densely stained areas at the top of the gel. Lanes 10–13 show the

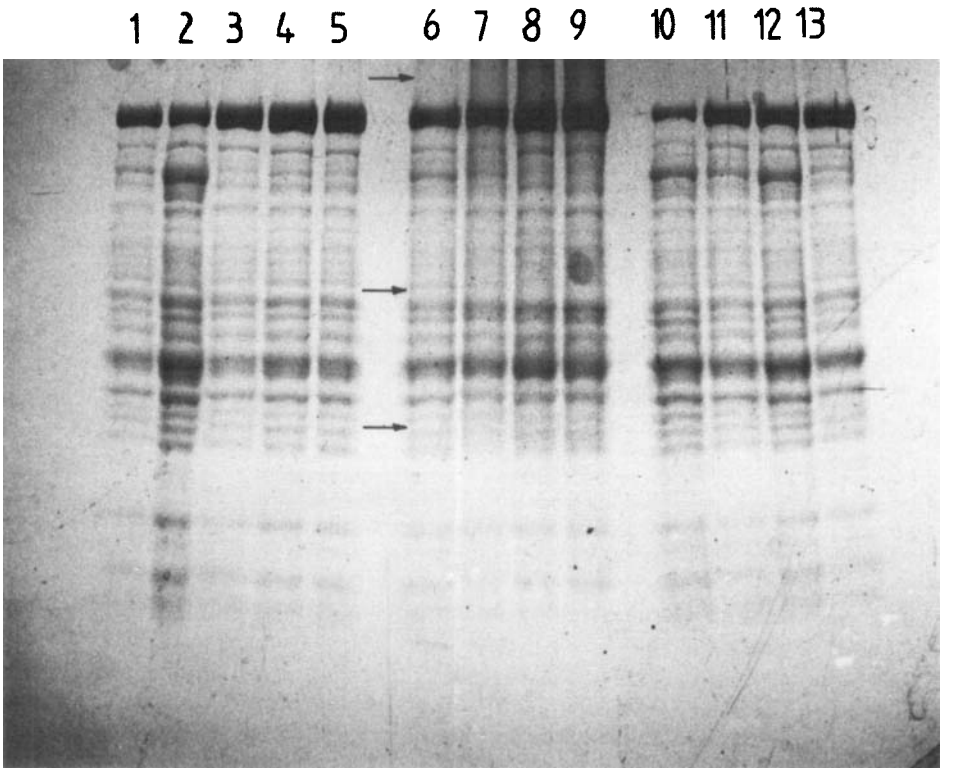

FIGURE 2 Lipid peroxidation induced changes in mitochondrial proteins. Lanes 1–5 represent samples from control, lanes 6–9 lipid peroxidised, and lanes 10–13 succinate treated lipid peroxidised mitochondria. Incubation times at 37°C 0 min lane 1; 10, 20, 30, 40 min lanes 2–5, 6–9, and 10–13, respectively. The arrows indicate the disappeared bands and the accumulated protein aggregates. For details see ref. 18.

striking effect of succinate. Succinate prevented the loss of the bands and appearance of the densely stained areas.

Concurrent with the above events or independently from them, significant changes in oxidative capacity of mitochondria were observed during NADPH-dependent lipid peroxidation.[14,18]

Succinate dehydrogenase proved to be highly sensitive to peroxidative damage (Figure 3). After 30 min exposure to peroxidative stimuli 60% of the succinate dehydrogenase activity was lost. Inactivation of succinate dehydrogenase was efficiently abolished by succinate added to peroxidizing media. Since this enzyme is known to contain essential sulfhydryl groups, it may be supposed, that oxygen free radicals and/or lipid hydroperoxides inactivate this enzyme by blocking its reactive thiol groups.

Interestingly, the matrix enzyme glutamate dehydrogenase was insensitive to peroxidative attack, but leaked from mitochondria after peroxidation (Table 1). After 20 min incubation more than 50% of dehydrogenase activity was found in the postmitochondrial supernatant fraction. The increase in permeability and the progression of malondialdehyde formation showed close correlation. Succinate protected

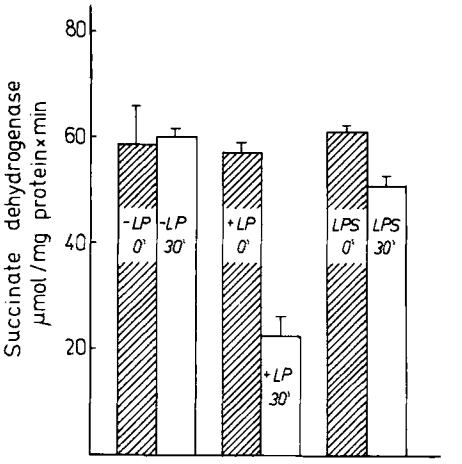

FIGURE 3 Effect of lipid peroxidation on succinate dehydrogenase activity of mitochondria. The − LP, + LP, and LPS represent control, lipid peroxidised, and succinate treated lipid peroxidised mitochondria, respectively. From ref. 18.

against permeability changes of the membranes proportionally to the protection against lipid peroxidation.

Succinate protection against ADP/Fe/NADPH-dependent lipid peroxidation is related to the reduction of coenzyme Q. In ubiquinone-depleted submitochondrial particles the protection by succinate was almost completely abolished when lipid peroxidation is induced by ADP/Fe/NADPH. In the same preparations significant inhibition by succinate was found during cumene hydroperoxide induction.[19] Reduction of cytochrome P-450 was suggested to explain the mechanism of succinate effect

TABLE 1
Membrane permeability changes after lipid peroxidation
A) The recovery of glutamate dehydrogenase activity from the postmitochondrial supernatant fraction

	Time of incubation			
	0 min	10 min	20 min	30 min
Media	Glutamate dehydrogenase activity (mU)			
Control	25 ± 14	30 ± 17	46 ± 42	46 ± 38
LP	32 ± 16	187 ± 102	452 ± 208	968 ± 226
LPS	33 ± 20	47 ± 18	78 ± 62	492 ± 216

B)The kinetics of malondialdehyde formation

	Time of incubation			
	0 min	10 min	20 min	30 min
Media	Malondialdehyde (nmol/mg protein)			
LP	0	7.6 ± 2.4	12.4 ± 1.9	16.0 ± 1.2
LPS	0	2.7 ± 2.6	7.0 ± 4.0	9.5 ± 3.0

LP medium contained in addition to control ones: NADPH and ADP/Fe^{3+}, LPS medium is the same as LP, succinate added. For details see ref. 18.

effect on cumene hydroperoxide dependent lipid peroxidation, since the reduced enzyme can act as a peroxidase in the cumene hydroperoxide system.[19]

The succinate-mediated protective mechanism operates not only *in vitro*, but its existence was shown in animals exposed to gamma-irradiation.[20] Succinate, administered to rats and mice prior to irradiation protected irradiated animals from elevated mitochondrial lipid peroxidation. Simultaneously, increased survival was seen in succinate treated groups.

Thus, it may be concluded that succinate is involved in the protection of mitochondria from the uncontrolled radical reactions, which can be initiated by different agents.

LIPID PEROXIDATION IN EHRLICH ASCITES CELL MITOCHONDRIA

Ascites tumour mitochondria seem to be protected very efficiently from peroxidative damage by a different mechanism.

In previous studies it was shown that cells, which undergo rapid cell division are somewhat more resistant to lipid peroxidation than corresponding normal tissues.[7,16,21-23] Moreover, the peroxidative activity seems to be inversely related to the mitotic activity of the tumours.[24] In particular it was found with ascites cell mitochondria that ascorbate[25] or ferrous ions[6] are not able to produce a significant rate of malondialdehyde formation.

As shown in Figure 4, significantly less malondialdehyde was produced by the Ehrlich ascites cell mitochondria than by the liver mitochondria of host animals. The effect was independent of the nature of the pro-oxidant stimuli: cumene hydroperoxide, ADP/Fe/ascorbate, or ADP/Fe/NADPH elicited similar responses.

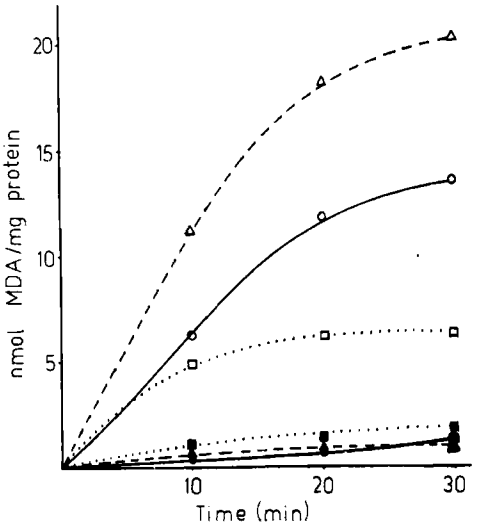

FIGURE 4 Lipid peroxidation in liver and ascites cell mitochondria. Malondialdehyde formation of mitochondria in the presence of ADP/Fe/NADPH (△—△) liver, (▲—▲) ascites cell; ADP/Fe/NADPH (○—○) liver, (●—●) ascites cell; and cumene hydroperoxide (□—□) liver, (■—■) ascites cell. Data from ref. 26.

Owing to the low rate of lipid peroxidation in ascites cell mitochondria no measurable inhibitory effect of succinate was detectable.

The insensitivity of tumour cell constituents, especially mitochondria to lipid peroxidation might be due either to the alteration of pro-oxidant/antioxidant balance, or to changes in membrane lipid composition.

The diminished sensitivity of the ascites cell mitochondria to oxidative stress might be explained if we suppose that the activity of the enzymes responsible for elimination of the reactive oxygen species is higher in tumour cells. In contrast, several tumours, which exhibit strongly inhibited malondialdehyde formation upon oxidative stress seem to be deficient in defensive enzymes against oxygen radicals.[27–29] Particularly, in Ehrlich ascites cell mitochondria no Mn-containing mitochondrial SOD activity was found,[30] although superoxide generating capacity was unaltered.[31] In line with this it seems to be very likely as it was suggested,[6] that the low sensitivity of ascites cell mitochondria to lipid peroxidation is due to the diminished content of polyunsaturated fatty acids in their membranes. Several experimental evidence supported this view. It was reported, that the amount of polyenoic acids was significantly decreased in mitochondria from the poorly differentiated Morris hepatoma 7777.[32] These findings together with other results[27,33,34] could lead to the general conclusion, that in tumour membranes the rate limiting factor for peroxidation might be the low PUFA availability.[8]

In contrast, our results do not support this view, at least this does not seem to be

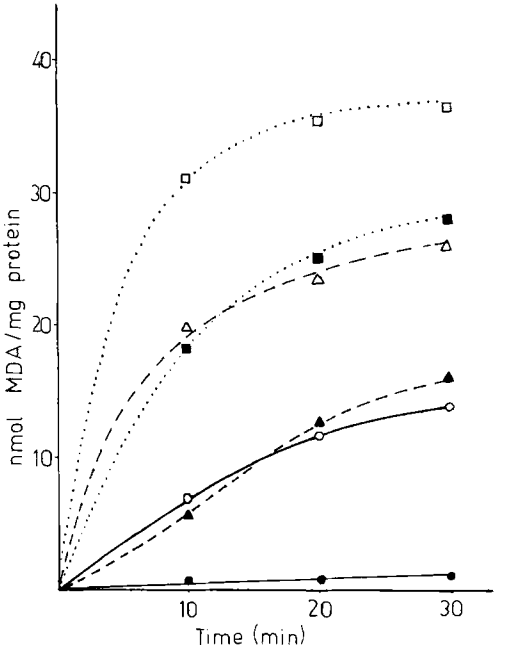

FIGURE 5 Lipid peroxidation of intact mitochondria and mitochondrial inner membrane preparates. Malondialdehyde formation of intact mitochondria in the presence of ADP/Fe/NADPH (○—○) liver, (●—●) ascites cell. Malondialdehyde formation of inner membrane preparates in the presence of ADP/Fe/NADPH (△—△) liver, (▲—▲) ascites mitochondria; ADP/Fe/ascorbate (□—□) liver, (■—■) ascites cell mitochondria. From ref. 26.

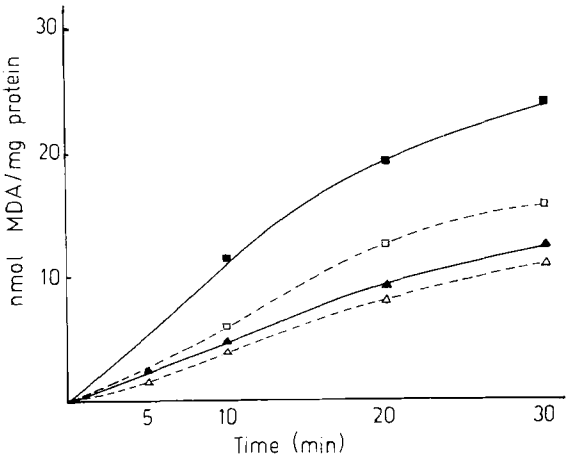

FIGURE 6 The effect of succinate on ascites mitochondrial membrane lipid peroxidation. Malondialdehyde formation of mitochondrial inner membranes in the absence (■—■) liver, (▲—▲) ascites membranes, and in the presence of succinate (□—□) liver, (△—△) ascites membranes. Data from ref. 26.

the case with Ehrlich ascites cell mitochondria (Figure 5). We observed that contrary to the very low lipid peroxidative activity of intact mitochondria from ascites cells, a much higher NADPH-dependent peroxidative activity was detected in the isolated inner membranes. The rate of malondialdehyde formation in ascites cell mitochondrial inner membranes was quite comparable to that, found in normal intact liver mitochondria.

An even higher lipid peroxidating capacity of ascites cell inner membranes was found in the presence of ADP/Fe/ascorbate (Figure 5), which might reflect more exactly the degree of unsaturation of the membrane lipids, than the NADPH-dependent process.

Since it is unlikely that the amount of highly unsaturated fatty acids would be increased during the isolation process of the inner membranes the insensitivity of intact ascites mitochondria to peroxidation can not be explained by the low availability of PUFA-s in the membranes. Therefore, we suggested the existence of an effective protective mechanism against peroxidative stimuli in these organelles. This mechanism should involve one or more matrix constituent(s), which are lost during isolation of the inner membrane.

As shown in Figure 6 succinate does not seem to be this matrix constituent.

Although succinate inhibited lipid peroxidation in ascites cell inner membranes, it

TABLE 2
Glutathione content, and glutathione-dependent enzyme activities of liver and ascites cell mitochondria

	GSH nmol/mg protein	GSH peroxidase nmol/mg protein	GSH reductase nmol/mg protein
Liver	3.4 ± 1.6	28.5 ± 7.0	37.5 ± 4.9
Ascites	6.1 ± 2.2	10.0 ± 2.3	10.2 ± 2.3

Results are expressed as mean ±S.D. n = 5.
For experimental details see ref. 26.

TABLE 3
Changes of glutathione content during incubation of isolated mitochondria

	Liver			Ascites		
	Time of incubation					
nmol/mg protein	0 min	15 min	30 min	0 min	15 min	30 min
Malondialdehyde	n.d.	11.9	16.0	n.d.	0.5	1.7
Glutathione	4.4	n.d.	n.d.	7.7	5.5	5.2

Data from ref. 26.

could not diminish the rate of malondialdehyde formation to the rate seen in intact ascites mitochondria.

The other matrix constituent, which might be involved in the protective events, can be glutathione.

In ascites cells the glutathione content of mitochondria was about 50% higher whilst the glutathione peroxidase and glutathione reductase activities were lower than in normal liver mitochondria (Table 2).

These data are in agreement with the results of others[36–40] on liver mitochondria. But the diminished glutathione peroxidase and reductase activities of ascites cell mitochondria are not consistent with the reduced lipid peroxidative activity of tumour organelles. In view of the high glutathione content of ascites cell mitochondria experiments were performed to see whether the glutathione was lost during incubation.

The results listed in Table 3 show that the glutathione concentration in ascites mitochondria after 30 min incubation is approximately as high as it is in liver mitochondria at the beginning of the incubation. In contrast, the glutathione content of liver mitochondria falls rapidly as lipid peroxidation proceeds. This loss might explain the increasing sensitivity of liver mitochondria to peroxidation, but the possibility that

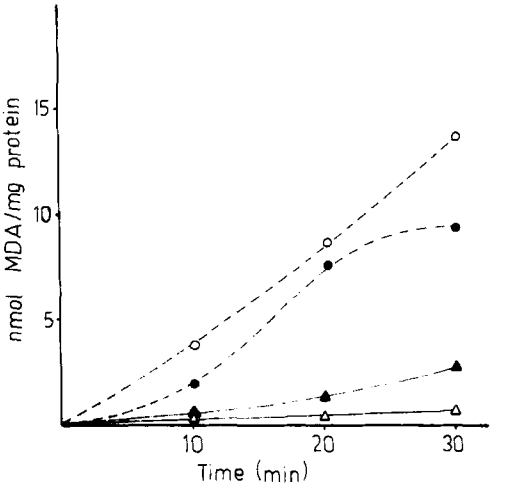

FIGURE 7 Effect of glutathione on lipid peroxidation of mitochondrial inner membranes. Malondialdehyde formation of mitochondrial inner membranes in the absence (○—○) liver, (●—●) ascites membranes; and in the presence of 5 mM glutathione (△—△) liver, (▲—▲) ascites membranes. For details see ref. 26.

the leakage of other matrix constituents might lead to the elevated malondialdehyde formation can not be ruled out.

In order to prove that in ascites cell mitochondria the glutathione is significant in protection against peroxidative events, experiments were designed to test if glutathione administration could inhibit lipid peroxidation in isolated membranes. Figure 7 shows the rate of the NADPH-dependent malondialdehyde formation in liver and ascites cell mitochondrial membranes both in the absence and in the presence of 5 mM glutathione. Glutathione diminished the rate of lipid peroxidation in both systems. Peroxidative activity of ascites mitochondrial membranes in the presence of glutathione was similar to that found in intact ascites cell mitochondria.

In general, in addition to other factors operating in hepatomas[7] a glutathione-dependent protective mechanism should be at least partially responsible for reduced rate of lipid peroxidation in intact mitochondria of Ehrlich ascites cells, because

1. the glutathione content of ascites cell mitochondria is higher than that of liver mitochondria.
2. the glutathione level was not altered significantly during incubation in ascites cell mitochondria contrary to the effect seen with liver mitochondria, in which the loss of glutathione was observed concomitantly with an increased malondialdehyde formation.
3. the difference in lipid peroxidative capacity of liver and ascites cell inner membranes, where a glutathione dependent protective mechanism may not operate, was much less pronounced than in intact mitochondria.
4. the lipid peroxidation was diminished in inner membrane preparations in the presence of glutathione.

It is suggested that in addition to other factors[7] one reason for the insensitivity to peroxidation of ascites mitochondria is the high resistance of their membranes to permeability changes. This results in the maintenance of the glutathione content at the original level during incubation. In turn this prevents mitochondria from peroxidative damage. Although this mechanism might operate in intact cells as well, it needs further investigation to decide whether there is any correlation between this feature of ascites cell mitochondria and the high mitotic rate of the tumour.

Acknowledgement

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