ROLE OF CYTOCHROME C IN GLUTATHIONE INDUCED SWELLING AND LIPID PEROXIDATION IN LIVER MITOCHONDRIA

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There is considerable evidence that glutathione induced swelling and lipid peroxidation in rat liver mitochondria do not require active electron transport from substrate, a sharp contrast to the situation with phosphate induced swelling. (Hunter, et al., 1962, 1963a, 1963b). However, since four inhibitors (NaCN, antimycin A, SN 5949, NHQNO) which do react with the electron transport chain prevent both the swelling and the lipid peroxidation, we investigated the effect of glutathione on mitochondria from which electron transport carriers such as DPN and cytochrome C had been removed. Investigation of the possible role of cytochrome C was especially important, since Tappel and Zalkin (1959) have shown that cytochrome C and other heme compounds can catalyze peroxidation of unsaturated fatty acids.

Mitochondria were prepared in .33 M sucrose as previously described (Hunter et al., 1963). Our standard mitochondrial preparation was depleted of cytochrome C by the procedure described by Jacobs and Sanadi (1960). DPN was removed by preincubation with phosphate (Hunter, 1956). Swelling and lipid peroxidation experiments were carried out in dilute suspensions of mitochondria (protein = 100 - 150 μg/ml) in 0.175 M KCl plus 0.025 M Tris, pH 7.4 at 25°C. Swelling was measured in a Baush

and Lomb Spectronic 20 as a decrease in turbidity (D_{520}). Lipid peroxide was measured by the thiobarbituric acid color reaction (TBA - A_{532}) (Ottolenghi, 1959).

When dilute suspensions of intact mitochondria are exposed to a mixture of 5 mM GSSG and 1 mM GSH there is a fairly prompt swelling that is closely correlated with the appearance of lipid peroxides. Both GSSG and GSH must be present (Neubert and Lehninger, 1962; Hoffsten et al., 1962). However, when cytochrome C depleted mitochondria were used, the rate of glutathione induced swelling and lipid peroxidation was greatly reduced (Fig. 1) in exactly parallel fashion. As would be expected, PO_4 + β-hydroxybutyrate induced swelling, which is absolutely dependent on electron transport and generation of high energy intermediates, was virtually completely eliminated by removal of cytochrome C.

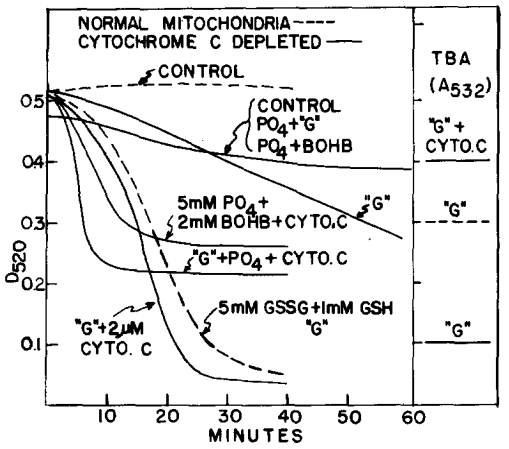

Fig. 1. The effect of cytochrome C depletion on phosphate induced swelling and on glutathione induced swelling-lysis and lipid peroxidation. Comparison of normal and depleted mitochondria. 0.175 M KCl + 0.025 M Tris pH 7.4 medium, 24°C.

Addition of 0.25 - 1.0 μM cytochrome C in experiments with depleted mitochondria restored both the typical swelling-lysis curve and lipid peroxidation. Higher concentrations of added

cytochrome C appeared to have little additional effect (Fig. 2). In normal mitochondria the addition of cytochrome C, even in concentrations as high as 10 μM, had very little effect on swelling-lysis, but the lipid peroxide values were sometimes higher with added cytochrome C. From these results it would appear that lipid peroxidation responsible for or associated with glutathione induced swelling occurs primarily at the site for cytochrome C binding in the mitochondria. Large amounts of added cytochrome C produce some generalized lipid peroxidation, but small amounts of endogenous cytochrome C and of added cytochrome C which becomes rebound to the mitochondria are much better catalysts for the glutathione induced swelling.

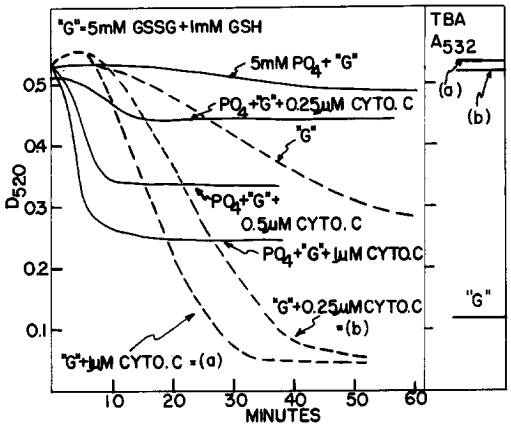

Fig. 2. The effect of various concentrations of added cytochrome C on restoring phosphate + substrate induced swelling and on glutathione induced swelling-lysis and lipid peroxidation. Fresh cytochrome C depleted mitochondria, KCl-Tris medium, 24°C.

Phosphate swelling is poorly restored by adding cytochrome C, probably because the level of endogenous substrate is very low in the depleted mitochondria. With the addition of β-hydroxybutyrate as substrate typical phosphate swelling occurs. It is important to note that 1 mM or even 5 mM GSH does not act as a substrate for PO_4 induced swelling even with added cytochrome C.

Yet, a mixture of 5 mM GSSG + 1 mM GSH with phosphate and cytochrome C does appear to feed electrons into the electron transport chain and produce a marked, extensive phosphate type swelling.

While 0.25 μM cytochrome C appears to restore glutathione swelling and lipid peroxidation, 1 μM appears to be the more optimal concentration for phosphate plus substrate or phosphate plus glutathione induced swelling (Fig. 2). With low amounts of cytochrome C the initial swelling with phosphate still occurs readily but the curves stop at considerably higher plateaus than with the addition of 1 μM cytochrome C or in normal mitochondria.

The removal of another electron transport carrier, DPN, appears to make no difference in the typical, rapid glutathione swelling-lysis, nor is there any apparent change in the amount of lipid peroxide formed (Fig. 3). The characteristic swelling with 5 mM phosphate or phosphate plus glutathione is markedly decreased in DPN depleted mitochondria.

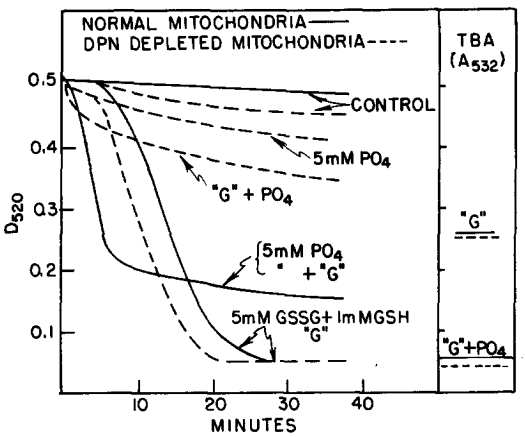

Fig. 3. A comparison of phosphate induced swelling and of glutathione induced swelling-lysis and lipid peroxidation in normal and in DPN depleted mitochondria. Fresh mitochondria, KCl-Tris medium, pH 7.4, 24°C.

These findings suggest that the lipid peroxidation and the swelling induced by glutathione in liver mitochondria require

not only both GSSG and GSH, but also are greatly dependent on cytochrome C at its normal locus in the mitochondria. The residual swelling and peroxidation seen in cytochrome C depleted mitochondria may be due to some cytochrome C not removed or to other cytochromes or non-heme iron components of the electron transport chain.

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