Studies on the Swelling and Formation of Lipid Peroxide Induced by Ferrous Ion in Isolated Liver Mitochondria

I. Relation among Lipid Peroxidation, Swelling and Adenosinetriphosphatase in Isolated Mitochondria.

Shoji KAWASAKI and Ko SAKURAI

Department of Radiology,
Yamaguchi University School of Medicine,
(Director Prof. Ko SAKURAI)
(Received October 24, 1967)

INTRODUCTION

As we had previously reported (1, 2), the mitochondria which carries out an important part in metabolic processes in cell, especially in adenosine triphosphate (ATP) synthesis, showed the swelling by irradiation, and ferrous ion-induced swelling and lipid peroxide formation of liver mitochondria had been remarkably accelerated by whole body irradiation.

In vitro irradiation, on the other hand, had no effect on lipid peroxide formation and swelling of isolated mitochondria (3).

Hunter *et al.* (4, 5, 6, 7) demonstrated that ascorbate, glutathione and ferrous ion produced a swelling or lytic change and lipid peroxide formation in dilute suspensions of isolated liver mitochondria. We confirmed also that ferrous ion induced a swelling and lipid peroxide formation in isolated mitochondria. This structural changes of mitochondria shows not only similarities to but differences also from the phosphate-induced, electron transport dependent swelling.

This paper presents further studies on the nature of the energy requirement of ATP, the changes of adenosine triphosphatase activity during ferrous ion-induced swelling and lipid peroxide formation in dilute suspensions of isolated liver mitochondria.

MATERIALS AND METHODS

Mitochondrial preparation.

The procedure of isolation with 0.25 M sucrose previously described had been used to prepare mouse liver mitochondria (2).

Adenosine Triphosphatase Activity Measurement.

Incubation mixture was 0.02 M Tris-0.15 M KCl buffer (pH 7.4) containing 2 mM ATP, 40 μM $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ and 10^{-4}M 2,4-dinitrophenol(DNP),

or 10^{-3}M $MgCl_2$. They will be expressed as DNP-stimulated ATPase or Mg^{++}-stimulated ATPase, respectively in this report. Final volme was 20 ml. After the addition of the stock mitochondria into the reaction medium, released inorganic phosphate was measured through the method of Takahashi.

One ml of reaction mixtuie was poured into a test tube after every three minutes of incubation. One ml of 16 per cent perchloric acid was added to each sample. Each tube was placed in freezing box at −15°C for 40 minutes. Then, the mixture was centrifuged at 2000 r.p.m. for 10 minutes in a refrigerated room at 4°C. The supernatant was poured into a test tube containing one ml of 1.5 N H_2SO_4, one ml of 2 per cent ammonium molybdate and 4ml of isobutyl alcohol, and then the tube was shaken violently for 10 seconds. Two ml of the isobutyl alcohol layer of each sample was then poured into a test tube containing two ml of 0.5 per cent ascorbate and one ml of ethanol. The mixture was incubated at 37°C for 45 minutes and the color intensity of the mixture was then measured by the Shimazu Kotaki Photoelectric Colormeter using 710 mμ filter.

Lipid peroxide determination.

The lipid peroxide formation during the previous reaction was measured by the method of thiobarbituric acid (TBA) reaction as described by Hunter (4), or oxygen consumption described by Paul Hochstein and Lars Ernster, and the authors as well (2).

Swelling measurement.

The swelling of mitochondria induced by ferrous ions was measured by the method as before-mentioned (2).

RESULTS AND DISCUSSION

Effects of ferrous ion at the different concentration.

The lipid peroxide formation of mitochondria began after a lag period subsequent to the addition of ferrous ion (Fig. 1). The lag period and the amount of lipid peroxide formation was influenced by the concentration of ferrous ion. At high concentration, the amount of lipid peroxide formed increased and the lag period of lipid peroxide formation are prolonged.

The effect of mitochondrial protein at different concentration.

At the constant concentration of ferrous ion, when the concentration of protein has been increased, the lag period invariably remained, but the reaction velocity became moie rapid. The amount of lipid peroxide thus formed, however, was constant regardless to the quantity of mitochondrial protein (Fig. 2). It is clear from these results that the amount of lipid peroxide thus formed and the lag

period of its formation were influenced by ferrous ion, and that the reaction velocity of its formation was affected by mitochondrial protein.

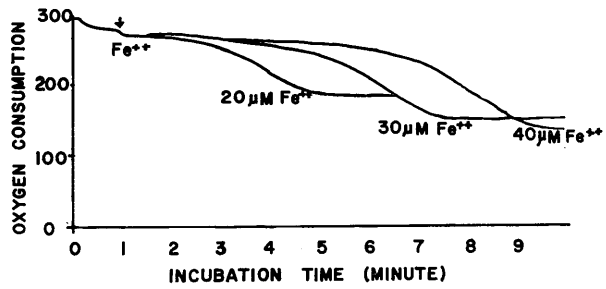

Fig. 1. The effect of concentration of ferrous ion on ferrous ion induced lipid peroxidation. Lipid peroxidation of mouse liver mitochondria measured by oxymeter.
To basic madia consisted of a 0.15 M KCl-0.02 M Tris- HCl buffer added 20 μM, 30 μM, or 40 μM ferrous ion respectively.

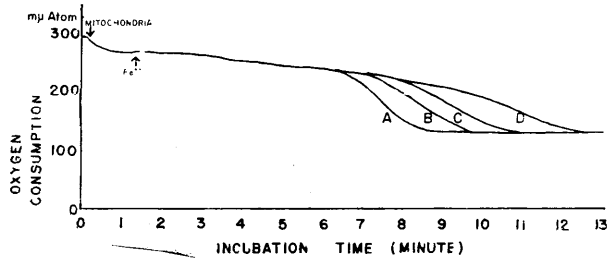

Fig. 2. The effect of concentration of mitochondrial protein on ferrous ion-induced lipid peroxidation.
A. unit concentration of mitochondrial protein
B. 2 units of protein
C. 3 units of protein
D. 4 units of protein

The Relationship of ATPase Activity of Isolated Mitochondria to Lipid Peroxide Formation and Swelling.

Mitochondrial ATPase were divided into DNP-stimulated ATPase (concerned with the maintenance of mitochondrial function), Mg^{++}-stimulated ATPase (concerned with the maintenance of mitochondrial structure), and endogeneous ATP-ase which appeared in non stimulant medium. The Mg^{++}-stimulated ATP-ase activity as shown in Fig. 3 became apparent after the on-set of a decrease of activity of DNP-stimulated ATPase. The activity of endogenous ATPase appeared when the Mg^{++}-stimulated ATPase activity decreased. It will be clear from these results that, when mitochondrial swelling was occurred by force of ferrous ion in Tris-KCI buffer solution, the lipid peroxide was formed

firstly, and mitochondrial function decreased after that (defined as a decrease of DNP-stimulated ATPase activity), and subsequently there is the change in mitochondrial structure (as Mg^{++}-stimulated ATPase appeared and mitochondrial swelling began). Then, endogenous ATP-ase of mitochondria appeared at the end of the swelling, Mg^{++}-stimulated ATPase activity at the same time decreased (Fig. 4). It is suggested that the energy of ATP was consumed violently during the mitochondrial lysis or disintegration at the end of swelling as was postulated by Hunter *et al.* (4, 5, 6, 7). From these results and previous paper (1, 2, 3), mitochondria of mouse liver were a swollen state in the cell after irradiation, and it showed the increase of ATP-ase activity for these structural changes. The swelling and lipid peroxidation of mitochondrial membrene which was described by Hunter *et al.* and the authroes, might occured in the cell after irradiation.

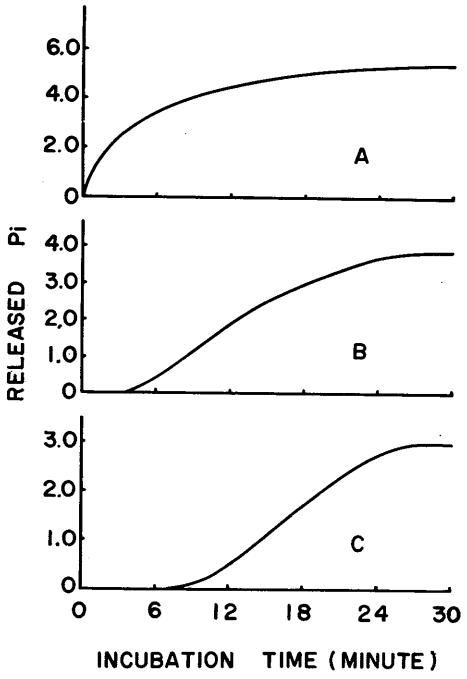

Fig. 3. Changes of the activity of ATPase during lipid peroxidation of mouse liver mitochondria induced by ferrous ion.
A. DNP-stimulated ATPase
B. Mg^{++}-stimulated ATPase
C. endogenous ATPase

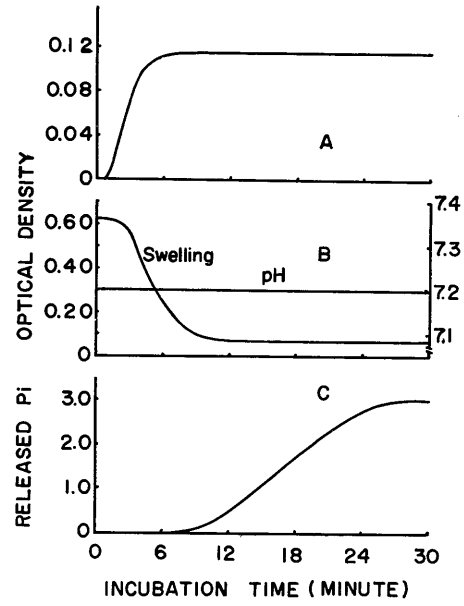

Fig. 4. Changes of endogenous ATPase activity (C), pH of medium and swelling (B) during lipid peroxiidation (A) of mouse liver mitochondria induced by ferrous ion.

CONCLUSION

In Tris-KCl buffer solution (pH 7.4), mitochondria retained the slow oxygen consumption at the start after the addition of ferrous ion, and after a lag period the extremely rapid oxygen consumption (lipid peroxide formation) of mitochondria occurred.

The lag period and the amount of lipid peroxide formed were affected by the concentration of ferrous ion, and the formation velocity was determined by the concentration of mitochondrial protein.

The mitochondrial function for the formation of lipid peroxide (activity of DNP-stimulated ATPase) decreased gradually. The increase of Mg^{++}-stimulated ATPase activity and the high grade of swelling of mitochondria appeared at the end of the formation of lipid peroxide, and then, at the end of the above phenomena, the mitochondria showed the remarkable energy requirement of ATP and was brought into lysis and disintegration.

The Scientific Research Fund (Irie group) of the Ministry of Education, together with the Cancer Investigation Fund of the Welfare Ministry (Tsukamoto group) were awarded to this investigation and research.

REFERENCES

1) Kawasaki, S.: Effect of Whole Body X-Irradiation on the Swelling of Rat Liver Mitochondria. *J. Rad. Res.*, **6**: 111, 1965.
2) Sakurai, K. and Kawasaki, S.: Effect of Irradiation on the Structure and Function of Mouse Liver Mitochondria. *An. Rep. Lab. Protein Chem. Yamaguchi Med. Sch.*, **2**: 40-49, 1967.
3) Sakurai, K. and Kawasaki, S.: The Effects of Radiation *in vitro* on Oxidative Phosphorylation, Adenosine Triphosphatase, Swelling and Lipid Peroxide Formation of Mouse Liver Mitochondria, *Yamaguchi Igaku*, **16**: 163, 1967.
4) Hunter F. E. *et al.*: Swelling and Lysis of Rat Liver Mitochondria Induced by Ferrous Ions. *J. Biol. Chem.*, **238**: 193, 1963.
5) Hunter F. E. *et al.* Studies on the Mechanism of Swelling, Lysis, and Distingration of Isolated Liver Mitochondria Exposed to Mixtures of Oxidized and Reduced Glutathione. *J. Biol. Chem.*, **239**: 614, 1964.
6) Hunter F. E. *et al.* Studies on the Mechanism of Ascorbate induced Swelling and Lysis of Isolated Liver Mitochondria. *J. Biol. Chem.*, **239**: 604, 1964.
7) Hunter F. E. *et al.* Effects of Phosphate, Arsenate and other Substances on Swelling and Lipid Peroxide Formation when Mitochondria are treated with oxidized and reduced Glutathione. *J. Biol. Chem.*, **239**: 622, 1964.
8) Paul Hochstein and Lars Ernster.: ADP activated Lipid Peroxidation coupled to the TPNH Oxidase System of Microsomes. *Biochem. Biophys. Res. Commun.*, **12**: 388, 1963.