EFFECT OF MONOCHROMATIC ULTRAVIOLET LIGHT ON LIPOPEROXIDE FORMATION AND ON THE FUNCTION OF ISOLATED RAT LIVER MITOCHONDRIA

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(Received for publication October 1, 1977)

The exposure of mitochondrial suspension to a broad spectrum ultraviolet light produced a lipoperoxide which was directly proportional radiant energy. The oxygen consumption of state 4 in mitochondrial respiration increased gradually, the ADP/O ratio and respiratory control ratio were decreased with an increasing dose of radiant energy. These photobiochemical changes in mitochondria were produced by ultraviolet light in the range of 260 - 300 nm in wavelength.

INTRODUCTION

Mitochondria contains about 25 % lipids consisting mainly of unsaturated fatty acid. Ottolenghi et al. (1955) early studied that ultraviolet light initiated lipid peroxidation in the isolated rat liver mitochondria. Lipid peroxidation is a source of free radicals which can react at random in the mitochondria and therefore should be damaging to the mitochondrial enzymes. We decided to see if lipoperoxides formed by photooxidation produce the biological effect to the respiratory function of mitochondria, and find the wavelength areas of effectiveness for the photobiochemical changes.

MATERIAL AND METHODS

Rat liver mitochondria was prepared

according to the method of differential centrifugation (Schneider, 1948) Twenty ml of the mitochondrial suspension (approximately 5 mg of protein per ml) was exposed to ultraviolet light as described in our previous paper (Noda, 1975). The ultraviolet exposure was performed by a broad spectrum ultraviolet lamp (Toshiba H400-P), or a high pressure xenon arc grating monochromator (Japan Spectroscopic Co. Ltd, CRM-FA). The lipoperoxide content was determined by a thiobarbituric acid reaction (Masugi and Nakamura, 1977), and was expressed as n mole of malonaldehyde per mg protein. Oxygen utilization by mitochondria was determined polarographically with a Clark type oxygen electrode, and was shown as $\text{m}\mu$ atoms per minute per mg protein (Noda, 1975). In the present paper, succinoxidation system was subjected as an index of

mitochondrial function. The consumptive state (state 1, 2, 3 and 4) of conditions limiting the rate of respiration were defined as described by Chance and Williams (1955). Respiratory control ratio (state 3/state 4) and ADP/O ratio were calculated from respiring response shown in the chart of oxygen consumption.

RESULTS AND DISCUSSION

In normal rat mitochondria, the lipoperoxide content was 0.59 ± 0.03 nmol per mg protein. The exposure of mitochondrial suspension to a broad spectrum ultraviolet (UV) light produced a lipoperoxide which was directly proportional to the radiant energy as shown in Fig. 1.

Fig. 1 Amount of lipid peroxidation formed in mitochondria following UV exposure.

However, UV irradiation through window glass did not generate any effect on the lipoperoxide formation. To determine the wavelengths responsible for lipoperoxide formation, monochromatic wavelength between 240 and 320 nm at intervals of 20 nm were used with 11.2×10^6 erg/cm² of exposure level. The change of lipoperoxide content in expos-

ed mitochondria was expressed as percentage deviation from unexposed control. The wavelengths having the greatest efficiency for producing lipoperoxide in ultraviolet light were found between 260 nm and 300 nm, as shown in Fig. 2.

Fig. 2 Effect of monochromatic wavelengths of ultraviolet light on the lipoperoxide content and respiratory control of mitochondria.

Fig. 3 Effect of ultraviolet light exposure to the mitochondrial oxygen consumption.

Noda (1975) in our laboratory reported the photobiochemical changes of mitochondrial function exposed to a broad ultraviolet light. The oxygen consumption of state 4 increased gradually with moderate dose of ultraviolet light. Both of respiratory control ratio (RCR) and ADP/O ratio decreased with radiated energy, as shown in Fig. 3. Addition of DNP had no effect, and there was no respiratory control by ADP. ATPase activity increased after exposure. However, ultraviolet light irradiation through window glass was found to generate non-effect on oxygen consumption of mitochondrial suspension. From these results, it was concluded that the oxidative phosphorylation was uncoupled with electron transport system of mitochondria exposed to UV light. In the present experiment, the effectiveness of monochromatic wavelength exposure to the mitochondrial respiratory system was determined. State 4 oxygen consumption, RCR and ADP/O ratio in the exposed mitochondria were expressed as percentage deviation from each unexposed control, as shown in Fig. 2.

The curve illustrating the photoxidative change of State 4 consumption is similar to the action spectrum pattern of lipoperoxide formation. The RCR and ADP/O ratio decrease significantly following exposure with wavelengths under the range of 300 nm. For these studies it becomes apparent that the exposure of mid-wavelength ultraviolet light results in a photobiological changes of mitochondrial function.

In a present time, it is not clear if the respiratory chain is directly affected by lipoperoxide formed by ultraviolet exposure. However in our separate ex-

periment (Sayanagi, 1977.), the addition of antioxidant such as Vitamin B₂, α -tocopherol and glutathione could inhibit the lipoperoxidation and restore the respiratory changes of mitochondria. These results suggest that the formation of lipoperoxide results in an inability of mitochondrial function as observed in this paper. In interpreting the biological effects of ultraviolet irradiation, consideration must be given to as certain the wavelength areas of effectiveness.

Our studies with ultraviolet light monochromator indicated that lipoperoxide can be produced in mitochondria by mid-ultraviolet light. Short and long ultraviolet light was less effective in this regard than mid-ultraviolet light.

REFERENCES

- CHANCE, B. and WILLIAMS, G. R. (1955). Respiratory enzymes in oxidative phosphorylation. *J. biol. Chem.*, **217**, 383-393.
- MASUGI, F. and NAKAMURA, T. (1977). Measurement of thiobarbituric acid valute in liver homogenate solubilized with sodium dodecylsulphate and variation of the values affected by vitamin E and drugs. *Vitamin*, **51**, 21-29.
- NODA, K. (1975). Effect of ultraviolet exposure on mitochondrial respiration system. *J. Kurume Med. Assoc.*, **38**, 879-896.
- OTTOLENGHI, A., BERNHEIM, F. and WILBUR, K. M. (1955). The inhibition of certain mitochondrial enzymes by fatty acids oxidized by ultraviolet light or ascorbic acid. *Archs. Biochem. Biophys.*, **56**, 157-164.
- SAYANAGI, H. (1977). The effect of ultraviolet light on swelling and function of rat liver mitochondria. *J. Kurume Med. Assoc.* (in press).
- SCHNEIDER, W. C. (1948). Intracellular distribution of enzymes. *J. biol. Chem.*, **176**, 259-262.