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Studies on the Development of Photoreceptor in the Nonchromatophore Organisms (V)
- Effects of FAD and $FADH_2$ on Light-Induced Mitochondrial ATPase and ATP Synthase in *Lentinus edodes* -

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무흡광색소 생물의 감광수용체 개발연구(V)
-표고버섯 중의 광감응성 Mitochondrial ATPase 및 ATP synthase에 대한 FAD 및 $FADH_2$의 효과 —

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ABSTRACT: Mitochondria in *L. edodes* were separated and purified by stepped sucrose density gradient centrifugation. In our previous work, we have found that the activation wavelengths of the mitochondrial ATPase and ATP synthase were 680 nm and 470 nm within the range of 400-700 nm, respectively.

The activities of the above enzymes with wavelengths of 300-400 nm region were investigated. The mitochondrial ATPase and ATP synthase were stimulated at 380 nm and 330 nm, respectively, for 30 min illumination compared with dark control group. They, however, were inhibited at 330 nm and 350 nm, respectively. The presence of FAD resulted in inhibition of the activity of the ATPase and stimulation of the activity of the ATP synthase by the activation and inhibition wavelengths. However, the activities of these enzymes were not changed by NADH for the above wavelengths. In the spectral properties, the oxidation of $FADH_2$ into FAD occurs in the presence of the enzymes for illumination of the activation and inhibition wavelengths. Therefore, we can predict that the mitochondrial ATPase and ATP synthase may function as oxidant in the redox reaction by the light illumination and that the light-induced pigment of the mitochondrial ATP synthase should be an oxidized form of a flavoprotein.

KEYWORDS: Mitochondrial ATPase, ATPsynthase

The ATPase located in a mitochondrial inner membrane is F_1-F_0 complex (Racker, 1976), containing two major components, F_1 and F_0 group. F_1-ATPase hydrolyzes ATP into ADP and phosphate ion(Pi) (Kielley, 1955) and F_1, F_0-ATPase (ATP synthase) synthesizes ATP from ADP and Pi (Okamoto *et al.*, 1977).

In our previous work, we have reported the purification and characterization of ATPase (Min *et al.*, 1986) and the activation of the light-induced mitochondrial ATPase (Min *et al.*, 1987[a], 1987[b]) and ATP synthase (Min *et al.*, 1989[a], 1989[b]) with the illumination of each wavelength of visible light in the mitochondria

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of *L. edodes*, a fungus which does not contain chromatophore such as chloroplast and bacterial chlorophyll. The activation wavelengths of the above two enzymes were 680 nm. and 470 nm, respectively.

There have been many studies on the light-induced phenomena and the photoreceptor for light in biological systems of plants (Leong and Briggs, 1981; Briggs and Iino, 1983) and microorganisms (Löser and Schäfer, 1980; Kumagai, 1982; Muñoz and Butler, 1975) using biochemical (Senger, 1982) and spectroscopic methods (Senger and Briggs, 1981). The photoreceptor for blue light in *Neurospora* (Muñoz and Butler, 1975), *Phycomyces* (Presti *et al.*, 1977) and corn coleoptile (Goldsmith *et al.*, 1980) is a flavoprotein which actually plays the role of photoreduction of a b-type cytochrome. De Fabo *et al.* (1976) and Harding and Shropshire (1980) have suggested that the photoreceptor from *Neurospora* is a carotenoprotein which causes the light-induced carotenoid biosynthesis.

The purpose of our present study is to identify the photoreceptor for the visible light, 680 nm and 470 nm, of the activation wavelengths of the mitochondrial ATPase and ATP synthase, respectively, and to observe the roles of two enzymes in the redox reaction in the mitochondria.

Therefore, we have tried to find; (1) the activation and inhibition wave-lengths of the enzymes for the illumination of 300-400 nm region containing the maximum absorption wavelengths of FAD and NADH; (b) the changes of activities of the two enzymes in the presence of FAD or NADH with the illumination of activation inhibition wavelengths; and (c) the absorbance change of $FADH_2$ in the presence of the enzymes with the illumination.

Materials and Methods

Materials

The fresh mushroom, *L. edodes*, used in this study, was purchased from a local market. Adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP), bovine albumin (BA), flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide-reduced form (NADH) were purchased from Sigma Chemical Co. Ammonium molybdate (AM) and coomassie brillant blue G-250 (CBB G-250) were from Merck Co. Tris (hydroymethyl) aminoethane (Tris), sucrose, 1-amino-2-naphtol-4-sulfonic acid (ANSA), sodium dithionite and all other used reagents were from Wako Co.

Purification of mitochondria

Mitochondria were separated and purified according to the procedure described by Min *et al.* (1987[a]) who have modified the methods of Cooper and Beevers (1969) and Douce *et al.* (1972).

The purified mitochondria were identified by electron micrographs (Zeiss, EM-109).

Light illumination

Each wavelengths of 300-400 nm region and the activation wavelengths of two enzymes, 680 nm and 470 nm, were illuminated for 30 min on mitochondria left in the dark state for 10 min, respectively. The light was obtained from monochromator (Ristu Oyo Kogaku Co.) using 300W tungsten-halogen lamp.

The illuminated mitochondria were experimented by the method of Min *et al* (1987[a]).

The quantum yield of actinic light was 2.0×10^{14} photon/cm^2.

Assay of mitochondrial ATPase and ATP synthase with illumination

The activities of the two enzymes in the illuminated mitochondria were assayed by the method of Min *et al.* (1987[a], 1989[b]) who have slightly modified the method of Rorive and Kleinzeller (1972).

One unit of the mitochondrial ATPase activity was defined as 1μmole of Pi released from ATP per minute per mg of protein at 37°C and pH 7.5, and one unit of the mitochondrial ATP synthase activity was defined ad 1μmole Pi decreased from substrate, Pi, under the same conditions.

Protein was determined by the method of Sedmak (1977), where bovine albumin was used as a standard.

Effects of FAD and NADH with illumination

The mitochondrial ATPase and ATP synthase were illuminated with a various wavelength; (a) activation wavelengths, 680 nm

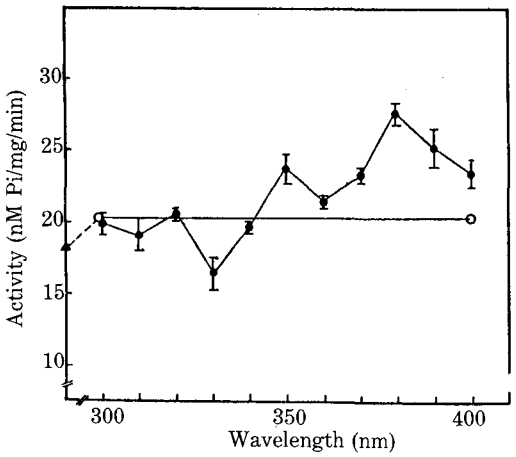

Fig. 1. The activity of mitochondrial ATPase after illuminated each wavelength (300-400 nm) for 30 min compared with control group. ●—●; 300-400 nm, ○—○; complex wavelength, ▲; dark state.

and 470 nm, (b) inhibition wavelenghts, 330 nm and 350 nm, of two enzymes, and (C) complex wavelength, in the measured by the method of Min *et al.* (1987[a], 1989[b]).

Spectrophotometric assays of $FADH_2$ with illumination

Absorbance on the illumination of the activation and inhibition wavelenghts with $FADH_2$ reduced by 0.02% dithionite were measure by UV-vis spectrophotometer (Shimadzu Co., M-240).

Two cuvettes containing 50 μM ATP, mitochondrial ATPase and 25 μM FAD, and the other two cuvettes containing 50 μM ATP, mitochondrial ATPase and 25 μM $FADH_2$ were illuminated with activation (680 nm) and inhibition (330 nm) wavelength with increasing time, respectively, for the measurement of absorbance.

For the mitochondrial ATP synthase, 48.8 μM ADP and Pi were used as substrate under the same conditions. The light path of cuvettes was 1 cm and the cuvettes were kept at 25°C.

Results and Discussion

Purification of mitochondria

Mitochondria were purified by stepped sucrose density gradient centrifugation.

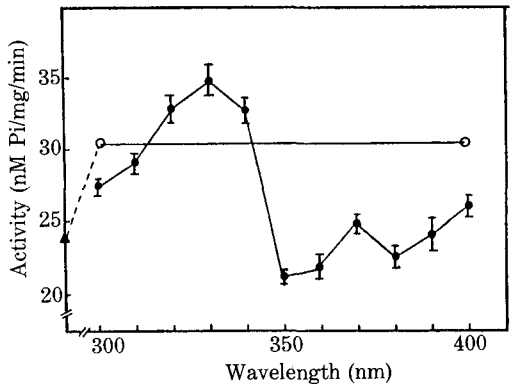

Fig. 2. The activity of mitochondrial ATP synthase after illuminated each wavelength (300-400 nm) for 30 min compared with control group. ●—●; 300-400 nm, ○—○; complex wavelength, ▲; dark state.

The purified mitochondria were identified with electron micrographs in our previous paper (1987[a]).

Activities of mitochondrial ATPase and ATP synthase with illumination

The change of activity of the mitochondrial ATPase illuminated with each wavelength of 300-400 nm region for 30 min is shown in Fig. 1. As shown in Fig. 1, the activity of the enzyme was increased 1.5 times by 380 nm and inhibited by 330 nm compared with the dark control group. The activity of the mitochondrial ATP synthase under the same conditions was increased 1.45 times by 330 nm and inhibited by 350 nm as shown in Fig. 2.

Therefore, on the illumination of visible light region (400-700 nm), the mitochondrial ATPase and ATP synthase were activated at 680 nm (Min *et al.*, 1987[a]) and 470 nm (Min *et al.*, 1989[a]), respectively, and or near UV light (300-400 nm), the above two enzymes were activated at 380 nm and 330 nm, and inhibited by 330 nm and 350 nm, respectively.

From the above results, the mitochondrial ATPase seemed to be activated at longer wavelength, and the mitochondrial ATP synthase was activated at shorter wavelength.

Effect of FAD on light-induced mitochondrial ATPase ATP synthase

To elucidate the role of FAD as photoreceptor that induces the activations of the mitochondrial ATPase and ATP synthase, the activities

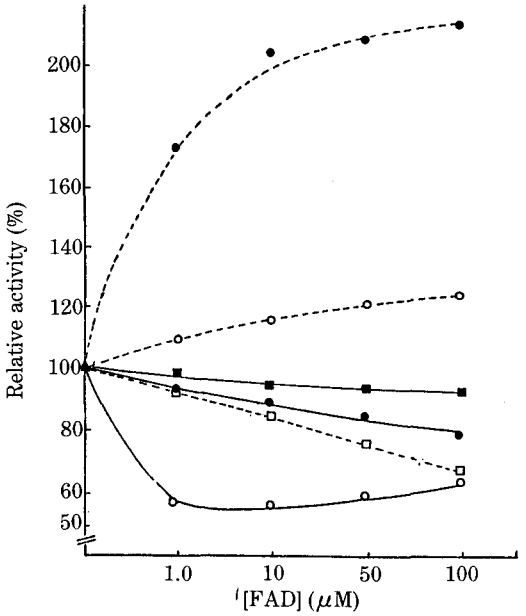

Fig. 3. Effect of FAD on the activity of mitochondrial ATPase and ATP synthase illuminated at each wavelength for 30 min. ●---●; activity of mitochondrial ATP synthase at 350 nm, ○---○; activity of mitochondrial ATP synthase at 470 nm, □---□; activity of mitochondrial ATP synthase at complex wavelength, ●—●; activity of mitochondrial ATPase at 330 nm, ○—○; activity of mitochondrial ATPase at 680 nm, ■—■; activity of mitochondrial ATPase at complex wavelength, ▲; control group.

of the two enzymes illuminated with the activation and inhibition wavelengths in the presence of FAD were measured. The results are summarized in Fig. 3.

The activity of the mitochondrial ATPase with the illumination of complex wavelength was decreased with increasing the concentration of FAD, and it was decreased 38% and 21% with activation (680 nm, Min *et al.*, 1987[a]) and inhibition (330 nm) wavelengths compared with the abscence of FAD. However, for the mitochondrial ATP synthase, while the illumination of complex wavelength decreased the activity by 33%, that of activation (470 nm, Min *et al.*, 1989[a]) and inhibition (350 nm) wavelengths showed the increase of activity by 24% and 114%, respectively.

From these results, we found that FAD acts

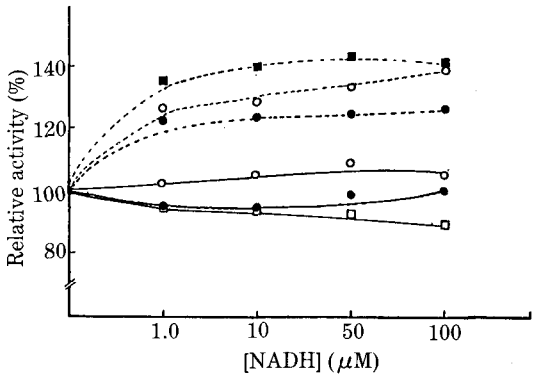

Fig. 4. Effect of NADH on the activity of mitochondrial ATPase and ATP synthase illuminated at each wavelength for 30 min. ■---■; activity of mitochondrial ATP synthase at complex wavelength, ○---○; activity of mitochondrial ATP synthase at 350nm, ●---●; activity of mitochondrial ATP synthase at 470 nm, ○—○; activity of mitochondrial ATPase at 330 nm, ●—●; activity of mitochondrial ATPase at 680 nm, □—□; activity of mitochondrial ATPase at complex wavelength, ▲; control group.

as enzyme inhibitor independent of illumination of activation and inhibition wavelengths for the mitochondrial ATPase, whereas FAD plays the role of photoreceptor inducible activation by 470 nm for the mitochondrial ATP synthase which contains in the form of flavoprotein binding to protein.

Therefore, we suggest that the photoreceptor pigment with light on the mitochondrial ATP synthase may be a flavoprotein.

This result is similar to that of previous studies, in which a flavoproten is the photoreceptor for near UV and blue light in *Neurospora* (Muñoz and Butler, 1975), *Phycomyces* (Presti *et al.*, 1977) and corn coleoptile (Goldsmith *et al.*, 1980).

Effect of NADH on light-induced mitochondrial ATPase and ATP synthase

In the presence of NADH, the changes of activities on the illumination of the activation and inhibition wavelengths of the two enzymes are summarized in Fig. 4.

The activity of the mitochondrial ATPase illuminated with complex wavelength was decreased by 10% with increasing concentration of NADH compared with that in the absence of

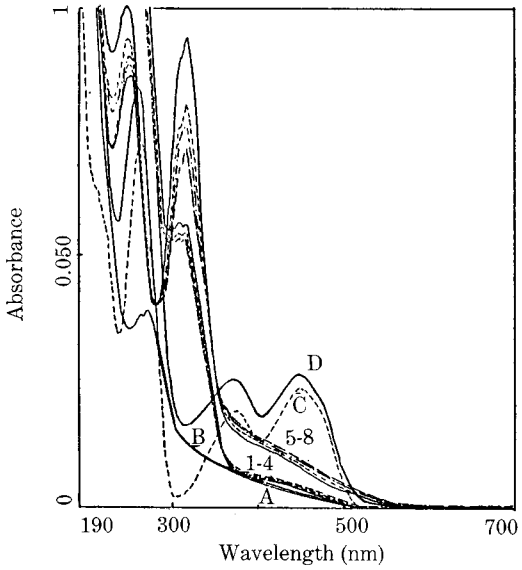

Fig. 5. The change of absorption spectra of $FADH_2$ by mitochondrial ATPase during various illumination times under 680 nm. The absorption spectra were measured with the increase of 1, 3, 5 and 10 min illumination. (A) mitochondria, (B) mitochondria with ATP under 680 nm, (C) FAD, (D) mitochondria with ATP and FAD under 680 nm, (1-4) the change of absorbance of $FADH_2$ under 680 nm, (5-8) the increase of absorbance of $FADH_2$ by mitochondria, ATP and $FADH_2$ under 680 nm.

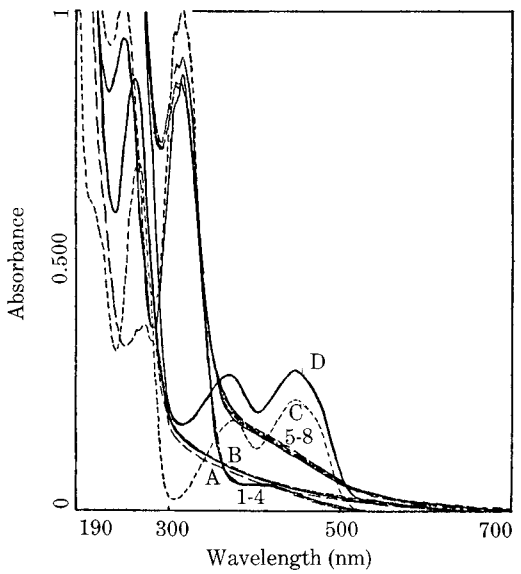

Fig. 6. The change of absorption spectra of $FADH_2$ by mitochondrial ATPase during various illumination times under 330 nm. The absorption spectra were measured with the increase of 1, 3, 5 and 10 min illumination. (A) mitochondria, (B) mitochondria with ATP under 330 nm, (C) FAD, (D) mitochondria with ATP and FAD under 680 nm, (1-4) the change of absorbance of $FADH_2$ under 330 nm, (5-8) the increase of absorbance of $FADH_2$ by mitochondria, ATP and $FADH_2$ under 330 nm.

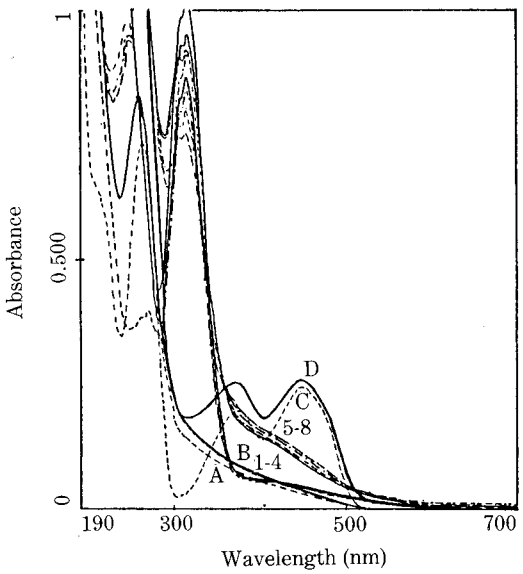

Fig. 7. The change of absorption spectra of $FADH_2$ by mitochondrial ATP synthase during various illumination times under 470 nm. The absorption spectra were measured with the increase of 1, 3, 5 and 10 min illumination. (A) mitochondria, (B) mitochondria with ATP and inorganic phosphate (Pi) under 470 nm, (C) FAD, (D) mitochondria with ADP, Pi and FAD under 470 nm, (1-4) the change of absorbance of $FADH_2$ under 470 nm, (5-8) the increase of absorbance of $FADH_2$ by mitochondria, ADP, Pi and $FADH_2$ under 350 nm.

NADH. However, the activity was not changed by the illumination of 680 nm and 330 nm. The activity of the mitochondrial ATP synthase with complex wavelength was increased up to 42%, and showed the similar increase with 470 nm and 350 nm.

Therefore, NADH is independent of the activity change in the two enzymes with the illumination.

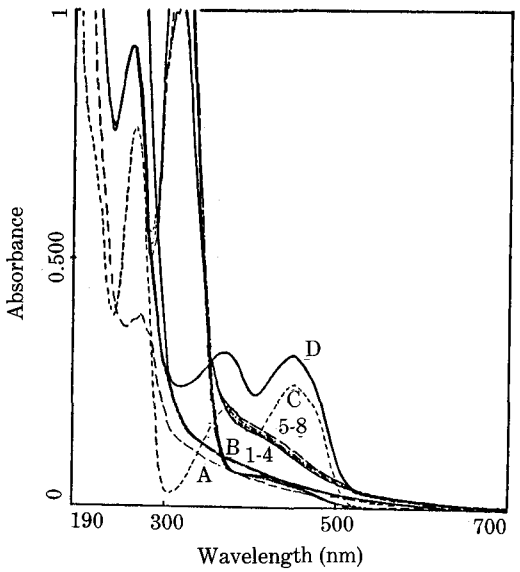

Fig. 8. The change of absorption spectra of $FADH_2$ by mitochondrial ATP synthase during various illumination times under 350 nm. The absorption spectra were measured with the increase of 1, 3, 5 and 10 min illumination. (A) mitochondria, (B) mitochondria with ADP and Pi under 350 nm, (C) FAD, (D) mitochondria with ADP, Pi and FAD under 350 nm, (1-4) the change of $FADH_2$ under 350 nm, (5-8) the increase of $FADH_2$ absorbance of $FADH_2$ by mitochondria, ADP, Pi and $FADH_2$ under 350 nm.

Absorption spectra of $FADH_2$ with light illumination

To understand the relationship between the changes of the activities the two enzymes with the illumination and the function of the flavoprotein as the photoreceptor, absorbance changes of FAD and $FADH_2$ were measured in the presence of the enzymes with illumination. The results are shown in Fig. 5, 6, 7 and 8

The oxidation of $FADH_2$ into FAD was increased with increasing time by illumination of the activation and inhibition wavelengths of the two enzymes in the presence of $FADH_2$. Whereas, in the presence of FAD, there is no changes in absorbance.

From the above results, we can predict that the two enzymes on the redox reaction in the mitochondria of *L. edodes* can act as an oxidant for the oxidation of $FADH_2$ into FAD by the illumination, and especially, the flavoprotein of the mitochondrial ATP synthase may participate in the redox system with photoreduction by the above light illumination.

Conclusions

1. The activity of the mitochondrial ATPase was increased up to 1.5times at 380 nm and inhibited at 330 nm in the illumination of 300-400 nm region.

2. The activity of the mitochondrial ATP synthase was increased by 1.45times at 330 nm and inhibited at 350 nm under the above same conditions.

3. FAD acts as an enzyme inhibitor independent of illumination of activation (680 nm) and inhibition (330 nm) wavelengths for the light-induced mitochondrial ATPase.

4. FAD plays a role of photoreceptor pigment for the light-induced mitochondrial ATP synthase containing a flavoprotein binding to protein.

5. The activities of the two enzymes were not changed in the presence of NADH with the above illumination.

6. Both enzymes resulted in the oxidation from $FADH_2$ into FAD by the illumination of the activation and inhibition wavelengths, respectively.

7. The mitochondrial ATPase and ATP synthase are considered to be an oxidant in the redox reaction of mitochondria when illuminated, and especially, the flavoprotein in the mitochondrial ATP synthase may be related to the redox system together with photoreduction when illuminated.

摘 要

표고버섯(*L. edodes*) 중의 mitochondria는 설탕밀도단계기울기법에 따라 분리정제 하였다.

앞서 보고한 바와 같이, 각 파장별 빛조사(400-700 nm)에 따른 mitochondrial ATPase와 ATP synthase의 활성도는 680 nm와 470 nm에서 각각 활성화되었다.

본 연구에서, 400 nm 이하의 파장별 빛조사에 따른 mitochondrial ATPase 및 ATP

synthase의 활성도는 380 nm와 330 nm에서 각각 활성화되었으며, 330 nm 및 350에서 각각 억제되었다. FAD의 존재하에서, mitochondrial ATP synthase는 활성화 파장 및 억제 파장의 조사에 의하여 활성도가 각각 증가된 반면, mitochondrial ATPase의 활성도는 감소되었다. 그러나, NADH의 존재하에서, 이들 파장의 조사에 의한 효소의 활성도는 변화가 없었다. 또한, 두 효소는 각각의 활성화 파장 및 억제 파장이 조사됨에 따라 $FADH_2$를 FAD로 산화시키는 spectrum을 보였다.

이로써, 이 두 효소는 빛 조사에 의하여 생체내의 산화 환원반응의 산화제로 작용하였으며, 특히 mitochondrial ATP synthase의 활성화에 따른 광유발물질은 mitochondria 중에 존재하는 flavin 또는 flavoprotein으로 추정된다.

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