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Investigation on Photochemical Reduction of NAD^+ to NADH in Liposomal Solution

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In the reaction system with AOH^+ as a photosensitizer, the generation of NADH stopped after 20 min irradiation at low level. On the other hand, when a photosensitizer was Chla, non-stop generation of NADH was observed after 80 min irradiation. This is because NADH generated was consumed by a side reaction in AOH^+-reaction system, whereas it does not occur in Chla-reaction system.

Recently, there are many papers about the development of the model system for photosynthesis,[1] but much of these systems were investigated using a sacrificial electron donor consumed during irradiation. In the most of such case, ΔG for the sum of reactions is negative or photoinduced reaction is irreversible. Lipid bilayer vesicles offer the reaction systems using non-sacrificial electron donor. When visible light-irradiation is carried out to the homogeneous solution containing ascorbate (Asc^-) as a non-sacrificial and regenerable electron donor, 1,1'-dimethyl-4,4'-dipyridinium (MV^{2+}) as an electron acceptor and photosensitizer, no accumulation of radical cation of MV^{2+} ($MV^{+\bullet}$) is not observed due to buck electron transfer between the radical pair. On the contrary, $MV^{+\bullet}$ is accumulated in liposomal system, in which lipid bilayer membrane act as a barrier for this back electron flow. We have already reported the up-hill electron transfer in the liposomal system from Asc^- to MV^{2+} with 3,6-bis-dimethylamino-acridine (AOH^+) as a photosensitizer and an electron carrier penetrating across the liposomal membrane.[2] In this report, photochemical reduction of NAD^+ to NADH, which has been often investigated in homogeneous systems with sacrificial donor,[3] was examined in the liposomal system. Photosensitizer used here is AOH^+ or chlorophyll a (Chla) which is embedded among the membrane, and we also report the difference in the generation of NADH between two type of photosensitizer. The special catalyst, such as natural enzyme or Rh polypyridine complex,[4] is necessary for regiospesific reduction of NAD^+ to 1,4-NADH. In this study, the enzyme, NADH : lipoamide oxidoreductase (LipDH), was used.

Liposome containing 1 mol dm^{-3} of Asc^- in the inner compartment was prepared by ultrasonic irradiation to the dispersion of egg phosphatidylcholine (egg PC) and Asc^- with or without Chla, and treat it by gel-filtration. Visible light was irradiated by 500 W Xe arc-lamp through cut-off glass filter at 20 °C to the deaerated liposomal reaction mixture adjusted to pH 7.5. The reaction mixture are composed of MV^{2+} (10 mmol dm^{-3}), NAD^+ (150 μmol dm^{-3}), LipDH (25units), AOH+ (5 μmol dm^{-3}) or Chla (4 of Chla per 1 liposome), and Asc^- in the inner compartment of liposome. Change in the concentration of $MV^{+\bullet}$ or NADH was determined by absorption spectrum and furthermore reversed-phase HPLC for NADH and NAD^+.[5]

The time course of NADH produced during irradiation with one of each photosensitizers, is shown in Fig. 1. The formation of NADH revealed the difference between photosensitizers clearly. The AOH^+-reaction

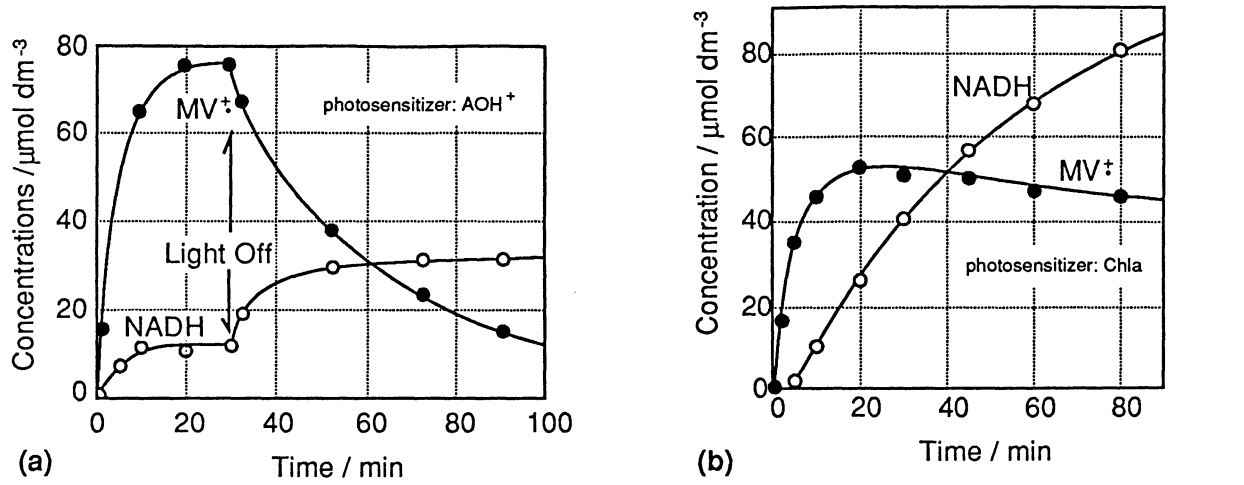

Fig.1. The time course of [NADH] and [MV^{+}•] during irradiation at 20 °C. (a); AOH^{+}-reaction system. Initial conditions: [AOH^{+}] = 5 μmol dm^{-3}, [MV^{2+}] = 10 mmol dm^{-3}, [NAD^{+}] = 150 μmol dm^{-3}, 25 units of LipDH, 1 mol dm^{-3} of Asc- in the inner compartment of liposome, [egg PC] = 2.5 mmol dm^{-3}. The reaction mixture was irradiated by visible light (460 - 750 nm) for 30 minutes and was stood afterword in the durk condition. (b); Chla-reaction system. Initial conditions are equal to those of (a) except 4 of Chla per 1 liposome. The range of irradiating light is from 580 to 750 nm.

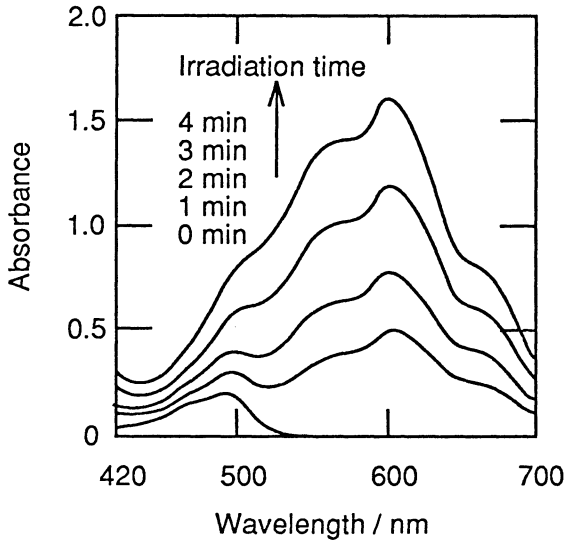

Fig. 2. Absorption spectral changes during irradiation to the deaerated mixture containing NADH (250 μmol dm^{-3}), AOH^{+} (5 μmol dm^{-3}), and MV^{2+} (10 mmol dm^{-3}) adjusted to pH 7.5 at 20 °C.

showed maximal formation of NADH after 20 min irradiation but the formation of NADH was observed once again when irradiation was stopped after that, whereas the Chla-reaction continued to produce further NADH even after 80 min irradiation. This result shows that NADH produced was consumed remarkably by a side reaction in AOH^{+}-reaction system during irradiation. There are some reports about photochemical redox systems using NADH as an electron donor.[6] So it is probable that NADH produced acts as an electron donor to $^{3}AOH^{+}$* or AOH^{2+}•, and that was confirmed as follows.

Figure 2 shows Changes in absorption spectra when aqueous solution containing NADH (250 μmol dm^{-3}), AOH^{+} (5 μmol dm^{-3}), MV^{2+} (10 mmol dm^{-3}), was exposed to visible light (>420 nm). The spectral changes in about 500 - 700 nm region, characteristic of MV^{+}•, indicate the occurrence of photoinduced reduction of MV^{2+}. The results of the analysis of the reaction mixture by HPLC are shown in Fig. 3, and Fig. 4 presents changes in the concentration of MV^{2+}, NAD^{+}, and NADH during

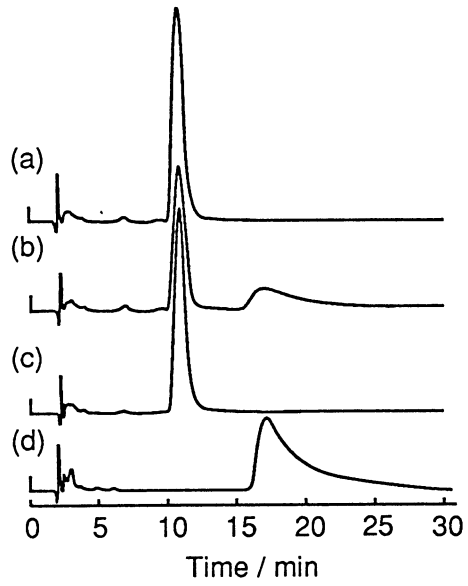

Fig. 3. Reverse-phase HPLC analysis of (a) the deaerated reaction mixture described in Fig. 2 before irradiation; (b) the same mixture after 4 min irradiation; (c) NADH buffer solution; (d) NAD^+ buffer solution. Experimental conditions of (a) and (b) are as in Fig. 2. Column: radial PAK cartridge C-18, mobile phase: H_2O- 0.1 mol dm^{-3} NH_4HCO_3-MeOH (30 : 69 : 1), flow rate: 1.5 ml min^{-1}. The eluent was monitored at 250 nm.

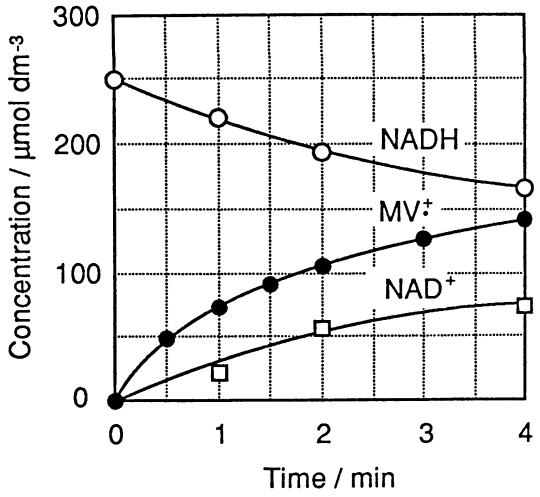

Fig. 4. The time course of [NADH], [$MV^{+\bullet}$], and [NAD^+] when the solution as in Fig 2 was irradiated.

irradiation. It is obvious that NAD^+ was produced after irradiation with the concomitant decrease of NADH (Fig. 3 (a), (b), and 4). These results demonstrate that NADH acts as an electron donor to generate $MV^{+\bullet}$, reductive product, and NAD^+, oxidative product and overall reaction is Eq. 1. On the other hand, only the degradation of Chla was

$$NADH + 2\,MV^{2+} \longrightarrow NAD^+ + 2MV^{+\bullet} + H^+ \qquad (1)$$

observed instead of the generation of $MV^{+\bullet}$ when irradiation was carried out to the liposomal solution containing Chla, MV^{2+} and NADH without Asc^- (Fig. 5). In this case, NADH did not have the ability of an electron donor. Porphyrin ring of Chla is expected to locate at a short distance from the surface of the bilayer membrane,[7] and so it is likely that NADH is difficult to contact with Chla within the limit of the electron transfer. There is one question about the oxidizing process of NADH. The oxidation of NADH proceeds through three steps (Eq. 2). The intermediate, NAD•, undertakes the dimerization to generate NAD_2 [8] besides

$$NADH \xrightarrow{e^-} NADH^{+\bullet} \xrightarrow{H^+} NAD\bullet \xrightarrow{e^-} NAD^+ \qquad (2)$$

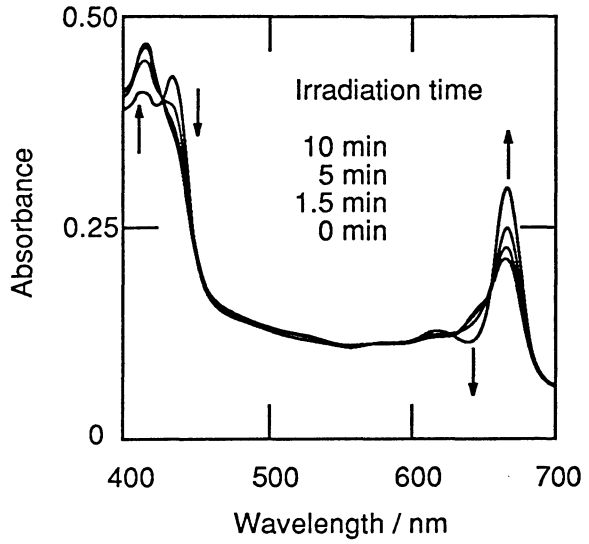

Fig. 5. Absorption spectral changes during irradiation at 20 °C to the deaerated liposomal solution containing NADH (250 μmol dm^{-3}), MV^{2+} (10 mmol dm^{-3}) in bulk solution, and Chla (4 Chla per 1 liposome) among the liposomal membrane. No Asc^- is in the inner compartment of liposome.

the oxidative generation of NAD^+. Although NAD_2 can be detected by the HPLC measurement,[5] no significant peak in the HPLC chart did not appear except NAD^+ or NADH peak, indicating that NAD_2 was not produced or was beyond the range of HPLC measurement. It is expected that NAD• reduces MV^{2+} before the coupling of NAD• to NAD_2, since NAD• is a strongly reducing agent[9] and the concentration of MV^{2+} is much larger than that of NAD•.

Thus, NADH-consuming reaction, which makes the yield of NADH lower, takes place in the AOH^+-reaction system, whereas such an unfavorable reaction does not occur in the Chla-reaction system, so that the effective reduction of NADH was observed. Furthermore, in the reaction system using 3,6-bis-(dimethylamino)-10-octadecyl-acridine (AO^+-$C_{18}H_{37}$), where reaction conditions were same as those of Chla-reaction system, NADH-formation rate was similar to that in Chla-reaction system (data not shown) though some obscurities about AO^+-$C_{18}H_{37}$-reaction system remain. The details of AO^+-$C_{18}H_{37}$-reaction system in addition to AOH^+- or Chla-reaction system are currently under investigation.

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