Phototriggered Chemical Reduction of $NADP^+$ by Zn-reconstituted Myoglobin and Triethanolamine as a Sacrificial Donor

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The phototriggered chemical reduction of $NADP^+$ was accomplished in the dark by photoirradiated zinc protoporphyrin-reconstituted myoglobin (Zn–Mb) and triethanolamine (TEA) as a sacrificial donor. The reduction efficiency of $NADP^+$ in the dark depended strongly on the concentration of TEA and the duration of photoirradiation.

Metalloproteins with metalloporphyrin redox centers have been widely studied because the distance between a donor and an acceptor can be controlled by choosing the position of the specific amino acid that is modified with a sensitizer.[1–5]

Reduced nicotinamide adenine dinucleotide phosphate (NADPH) is produced in photosystem I, and it delivers electrons and/or protons to many enzymatic systems. We reported that a simple model for photosystem I, wherein NADPH was produced by photochemical reduction using zinc protoporphyrin (Zn–PP), reconstituted myoglobin (Zn–Mb) as a sensitizer and enzyme. Since then, we have studied the system to elucidate the reduction process and to determine the key factors for controlling the interaction between the protein and $NADP^+$. Recently, we found that nicotinamide adenine dinucleotide phosphate ($NADP^+$) is reduced in the dark in a solution containing Zn–Mb and TEA after the solution had been irradiated for several hours; in other words, $NADP^+$ can be chemically reduced in the dark. In our previous work,[6] the light intensity at the cell was approximately 0.7 mW. Under this relatively low intensity of light, the Soret band absorption of Zn–Mb was almost constant during photoirradiation, and the reduction of $NADP^+$ did not take place in the dark after termination of the photoirradiation. However, under the experimental conditions described in the present report, the chemical reduction of $NADP^+$ was mediated by a photoproduct or photodegrated product of Zn–Mb produced by photoirradiation at approximately 2.8 mW.

Myoglobin (Mb), apomyoglobin (apo Mb) and Zn–Mb were prepared as previously described.[6] All other reagents were G.R. grade. The UV–visible and circular dichroism (CD) spectra were recorded with a Shimadzu UV-3100 spectrophotometer and a JASCO J-720 spectropolarimeter, respectively. Photoirradiation was carried out under an atmosphere of N_2 at room temperature using a 100-W Xe lamp (Hamamatsu Photonics). A water filter and a sharp-cut filter (UV-39 or Y-45 glass filter, Toshiba) were used for IR and UV light, respectively. The light intensity at the cell was 2.8 mW/cm^2.

The absorption and CD spectra of Zn–Mb ($\lambda_{max} = 428$ nm, $\varepsilon_{428} = 1.57 \times 10^5$) prepared in the present study were identical to those reported previously,[3,5] and Zn–Mb showed no structural change in the pH range studied (pH 6–9). Photo products were analyzed with a HPLC system (Bio CAD 700E-KT, Applied Biosystems).

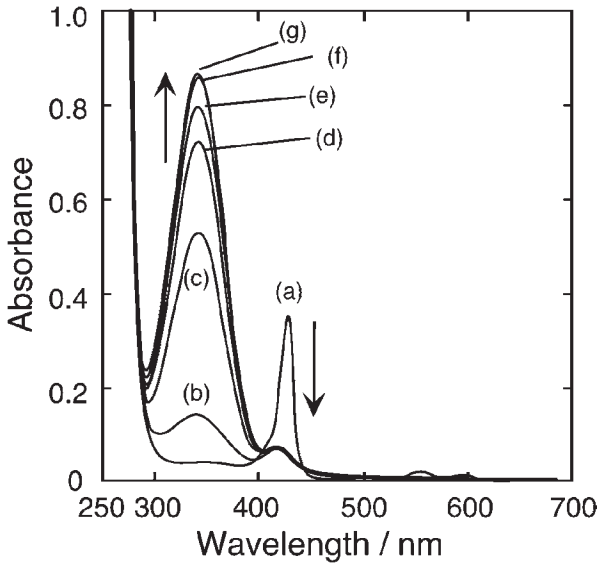

Figure 1. UV–visible spectral change for 10 μM of Zn–Mb, 1 M of TEA, and 1 mM of $NADP^+$ in 10 mM of phosphate buffer (pH 9.0). (a) Before irradiation. (b) After 3.5 h of irradiation with visible light ($\lambda > 390$ nm) at an intensity of 2.8 mW. Following the irradiation, spectra were measured after 1 (c), 2 (d), 3 (e), 6 (f), and 9 (g) days in the dark under an atmosphere of N_2.

Figure 1 shows the visible spectral change in a pH 9.0 phosphate buffer solution containing 1 M of TEA, 10 μM of Zn–Mb, and 1 mM of $NADP^+$ upon photoirradiation ($\lambda > 390$ nm) and in the dark. Before irradiation, a clear Soret band was observed at 428 nm, with no absorbance around 340 nm (curve a). Upon irradiation with visible light ($\lambda > 390$ nm) for 3.5 h, there was an increase in the absorbance at 340 nm, which is attributed to NADPH formation, along with a decrease in the Soret band (curve b). The peak wavelength of the Soret band was shifted to approximately 420 nm, and the absorbance was dramatically decreased. Under these conditions, there was some extent of photochemical production of NADPH in addition to degradation of Zn–Mb. Further photoirradiation induced a structural change in Zn–Mb.

Interestingly, even after the photoirradiation was stopped, the absorbance at 340 nm increased with time (curves c–g). These results show that $NADP^+$ was reduced not photochemically but rather chemically following photoirradiation of the solution. Furthermore, the absorbance at 340 nm increased with time even in the dark. After 7 days, approximately 70% of $NADP^+$ was chemically converted to NADPH. The yield of NADPH depended strongly on the duration of photoirradiation.

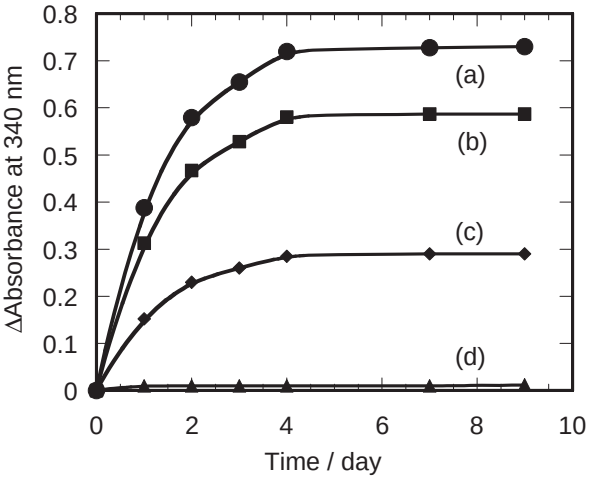

Figure 2. Absorbance change in the solution containing 10 μM of Zn–Mb, 1 M of TEA, and (a) 1.0, (b) 0.7, (c) 0.3, and (d) 0.1 M $NADP^+$ in the dark under an atmosphere of N_2. The solution was irradiated with visible light ($\lambda > 390$ nm) for 3.5 h before measurement.

When the photoirradiation time was 1 h, approximately 20% of the NADP was converted after 9 days in the dark. In addition, when longer wavelengths ($\lambda > 430$ nm) were used to excite only the Q bands of Zn–Mb, the degradation of Zn–Mb was suppressed, and there was no significant formation of NADPH. Moreover, without photoirradiation, NADPH was never obtained under the current conditions. It should be emphasized that our system achieved chemical reduction of $NADP^+$ in the dark without the use of ferredoxin-$NADP^+$-reductase.

The reduction efficiency also depended on the concentration of TEA used as a sacrificial donor. To determine the effect of the TEA, we performed similar experiments as in Figure 1 but changed the concentration of TEA. Figure 2 shows the change in absorbance at 340 nm vs time. When 1 M of TEA was used, as shown in Figure 2a, the absorbance at 340 nm increased gradually and finally reached a steady state. The amount of NADPH formed was reduced with decreasing TEA concentrations. When 0.7 M of TEA was used, the absorbance change after 7 days was approximately 70% of that obtained with 1 M of TEA. When 0.3 M of TEA was used (curve c), the change after 7 days was approximately 45% of that of curve (a). In addition, without TEA, photochemical and chemical reductions were not observed. Therefore, the reduction in the dark requires an electron donor. Finally, when triethylamine was used as instead of TEA, the yield was drastically decreased.

To elucidate the mechanism of the chemical reduction in the dark, we examined several kinds of reaction conditions. When only TEA was added to the photoirradiated Zn–Mb and $NADP^+$ solution in the dark, chemical reduction was not observed. Similarly, reaction was not observed when both TEA and $NADP^+$ were added to the photoirradiated Zn–Mb solution. On the other hand, when only $NADP^+$ was added to the photoirradiated TEA and Zn–Mb solution, reduction of $NADP^+$ occurred with the same efficiency as with the TEA/Zn–Mb/$NADP^+$ system. These results indicate that the photoreaction of Zn–Mb and TEA plays an important role in the reduction.

CD spectra of the solution were recorded to observe the structural change in Zn–Mb upon photoirradiation. Figure 3 shows the CD spectra for a mixture of 1 M of TEA, 10 μM of Zn–Mb, and 1 mM of $NADP^+$ before and after photoirradiation. Other conditions were the same as in Figure 1. Following irradiation, we observed a clear Soret band at 428 nm attributed to the Zn–Mb (curve a). After photoirradiation for 3.5 h, the Soret band disappeared, and a new CD band around 340 nm appeared. The appearance of this band may be due to a relatively strong interaction between Zn–Mb or apo Mb and the NADPH formed under these conditions.

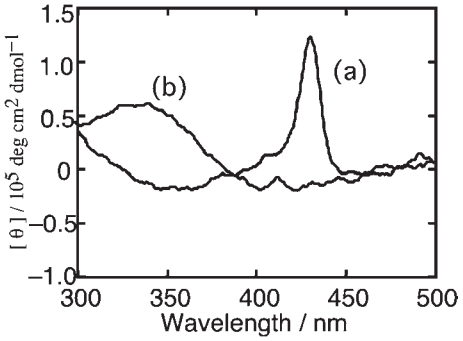

Figure 3. CD spectra for 10 μM of Zn–Mb, 1 M of TEA, and 1 mM of $NADP^+$ in 10 mM of phosphate buffer (pH 9.0). (a) Before irradiation. (b) After 3.5 h of irradiation with visible light ($\lambda > 390$ nm) at an intensity of 2.8 mW.

The reduced product produced by the reaction of Zn–Mb and TEA was applied for the malic enzyme system (50 μM of the reduced product, 3.45 unit of malic enzyme, and 500 mM pyruvic acid in Atkins–Pantin buffer (pH 6.7)) in order to confirm whether the product acts as a co-factor for the reaction. The amount of the produced maic acid using the product in this system as a co-factor was over 95% of the amount when β-NADPH (Sigma) was used as a co-factor. This means the reduced product functions as the co-factor (β-NADPH) for the malic enzyme system.

In conclusion, we found that chemical reduction of $NADP^+$ occurs in the presence of Zn–Mb and TEA under irradiation with visible light ($\lambda > 390$ nm). The structure of the compound that acted as a catalyst is not clear, but, after 7 days in the dark, approximately 70% of the $NADP^+$ was converted to NADPH.

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