Photo-triggered Chemical Reduction of $NADP^+$ by Zn Reconstituted Myoglobin and Cytochrome c

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We reported that a simple model for photosystem I which NADPH was produced by photochemical reduction using zinc protoporphyrin (Zn-PP) reconstituted myoglobin (Zn-Mb) as a sensitizer and an enzyme. Since, we have studied the system to clear the reduction process or important factors to control the interaction between the protein and $NADP^+$. Recently, we have found that the nicotinamide adenine dinucleotide phosphate ($NADP^+$) was reduced under dark in a solution containing Zn-Mb and TEA after the solution had been irradiated for hours, i.e., the reduction of $NADP^+$ was reduced chemically under dark. In the previous work, the light intensity at the cell was ca. 0.7 mW. Under this relatively weak light intensity conditions, the so-ret band absorption of the Zn-Mb was almost constant during photo-irradiation and the reduction of $NADP^+$ did not take place under dark after the photo-irradiation was stopped. However, under experimental conditions described in the present paper, the chemical reduction of $NADP^+$ by a photoproduct or photodegradated product of the Zn-Mb produced by photo-irradiation with ca. 2.8 mW did take place.

Myoglobin from horse skeletal muscle (Mb) was obtained from Sigma, and was used without further purification. Zinc protoporphyrin IX (Zn-PP) was purchased from Aldrich and used without further purification. Photo-irradiation was carried out under N_2 atmosphere at room temperature using a 100 W Xe lamp (Hamamatsu Photonics, Japan). A water filter and a sharp cut filter (UV-39 or Y-45 glass filter, Toshiba) were used to cut IR and UV light, respectively. The absorption and CD spectra of Zn-Mb (λmax = 428 nm, ε = 1.57 x 10^5) prepared in the present study were identical as those reported previously, and Zn-Mb showed no structural change in the pH region studied (pH 6-9). Product was identified with a HPLC system (JASCO).

Figure 1 shows the visible spectral change of a pH 9.0 phosphate buffer solution containing 1 M TEA, 10 M Zn-Mb and 1 mM $NADP^+$ on photo-irradiation (> 390 nm) and in the dark. Before irradiation clear Soret band was observed at 428 nm and no absorbance around 340 nm (curve a). When visible light (> 390 nm) was irradiated for 3.5 hours, an increase in the absorbance at 340 nm, attributed to NADPH formation occurred with the decrease in the Soret band (curve b). The peak wavelength of the soret band was shifted to ca. 420 nm and the absorbance was decreased drastically. Under these conditions, NADPH was produced photochemically to some extent and also degradation of Zn-Mb took place. Further photo-irradiation induced the structural change in Zn-Mb.

Interestingly, even after the photo-irradiation was stopped, the absorbance at 340 nm was increased with an increase of time (curve c-g). These results show that $NADP^+$ was reduced not photochemically but chemically after photo-irradiation to the solution. The absorbance at 340 nm increased with time even in the dark. After 7 days, ca. 70% of $NADP^+$ was converted to NADPH chemically.

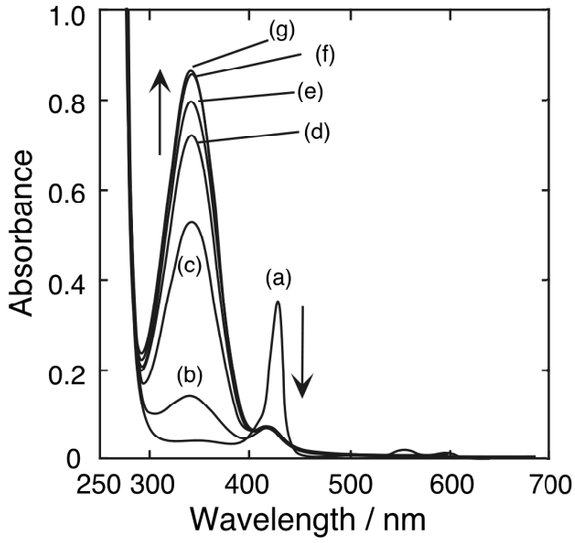

Figure 1. UV-visible spectral change for 10 µM of Zn-Mb, 1 M of TEA, and 1 mM of $NADP^+$ in 10 mM of phosphate buffer (pH 9.0). (a) before irradiation, (b) after irradiation with visible light (> 390 nm) with the light intensity of 2.8 mW for 3.5 hours. (c) 1, (d) 2, (e) 3, (f) 6, and (g) 9 days after curve (b) in the dark under N_2 atmosphere.