Photochemical Reduction of NADP+ by Zinc Protoporphyrin Reconstituted Myoglobin as a Simple Model of Photosystem I

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Photoinduced electron transfer between zinc protoporphyrin reconstituted myoglobin (Zn-Mb) and NADP+ functions as a model of photosystem I by forming NADPH. The reduction efficiency of NADP+ depended strongly on the solution pH, which was explained by the difference in the redox potential and/or the static interaction between Zn-Mb and a sacrificial donor triethanolamine (TEA).

The driving force and distance between an electron donor and an acceptor are important factors controlling the rates of photoinduced electron transfer.[1] Metalloproteins with metalloporphyrin redox centers have been widely studied, because the distance between donor and acceptor can be controlled by choosing the position of a specific amino acid that is modified with a sensitizer.[2] Zinc or magnesium porphyrins (Zn-P or Mg-P) have longer lifetimes for the excited triplet state compared with other metalloporphyrins.[3-11] Zn-P or Mg-P reconstituted metalloproteins are therefore useful for studying the role of an apoprotein in photoinduced energy or electron transfer reactions in biological systems.[3-11] Hoffman and Ratner[12] proposed that conformational changes of metalloproteins (a gating mechanism) influences the rate of photoinduced electron transfer. Barboy and Feitelson[6] and Tsukahara et al.[11] confirmed the gating mechanism by using Zn-Mb and/or Mg-Mb. Examples of photoinduced electron transfer using metalloprotein to store free energy in the product are limited. Vernon reported that nicotinamide adenine dinucleotide (NADH) was formed using chlorophyll as a sensitizer and ascorbic acid as a sacrificial donor using NAD reductase as an enzyme.[13] NAD reductase was necessary to trap the NAD radicals.

Reduced nicotinamide adenine dinucleotide phosphate (NADPH) is produced in photosystem I and it delivers electrons and/or protons to many enzymatic systems. Many useful reactions can be driven using the corresponding enzyme and photochemically produced NADPH mimicking photosynthesis.

We report that photochemical reduction of nicotinamide adenine dinucleotide phosphate (NADP+) using zinc protoporphyrin (Zn-PP) reconstituted myoglobin (Zn-Mb) functions as a sensitizer and an enzyme, with triethanolamine (TEA) as a sacrificial donor. This system is a simple model of photosystem I, where the importance of apoprotein as a reaction site is presented for the first time.

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. Apomyoglobin (apoMb) [14-16] and Zn-Mb[6, 10, 17] were prepared according to the literature. The concentration of Zn-Mb was determined by the absorbance at 280 nm (ε = 1.58 x 10^4 M^{-1} cm^{-1}).[10] All other reagents were G.R. grade. The UV-visible and circular dichrosim (CD) spectra were recorded with a Shimadzu UV-3100 spectrophotometer and a JASCO J-720 spectropolarimeter, respectively. Photoirradiation 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 light intensity at the cell was 0.7 mW / cm^2.

The absorption and CD spectra of Zn-Mb (λmax = 428 nm, ε_{428} = 1.57 x 10^5) prepared in the present study were identical as those reported previously[3,10], and Zn-Mb showed no structural change in the pH region studied (pH 6-9).

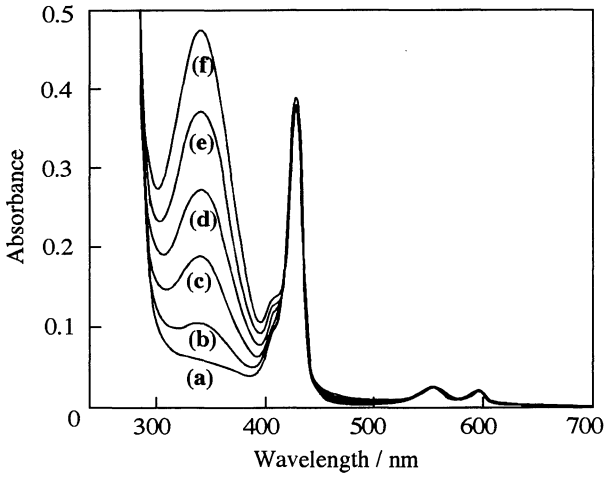

Figure 1. Change in UV– visible absorption spectra for 1 M TEA, 20 μM Zn-Mb, and 50 mM NADP+ in a 100 mM of phosphate buffer solution (pH 9.2) on photoirradiation($\lambda > 390$ nm). Irradiation time was (a); 0, (b); 0.5, (c); 1.5, (d); 2.5, (d); 3.5, (e); 4.5, (f); 5.5 h.

Figure 1 shows the visible spectral change of a pH 8.5 phosphate buffer solution containing 1 M TEA, 20 μM Zn-Mb and 50 mM NADP+ on photoirradiation (>390 nm). An increase in the absorbance at 340 nm, attributed to NADPH formation, occurred. The Soret band remained constant during photoirradiation, suggesting that Zn-Mb acted as a photocatalyst. Although the quantum efficiency of the reaction has not been estimated, ca. 40% of NADP+ was converted to NADPH in 5.5 hours. The yield of NADPH depended strongly on the concentration of TEA. Without TEA no NADPH were obtained in the present conditions. It should be emphasized that direct photoreduction of NADP+ without using ferredoxin-NADP+-reductase (FNR) was achieved using this simple model of photosystem I.

When the experiment of Figure 1 was carried out at pH 6.8,

the Soret band decreased with irradiation time, indicating that degradation of Zn-PP in Zn-Mb took place. In addition, when longer wavelengths ($\lambda > 430$ nm) were used to excite only the Q bands of Zn-Mb, the degradation of Zn-Mb decreased, but no significant amount of NADPH was formed. This is probably because the redox potential of TEA at pH 6.8 (0.94 V[18]) is more positive than the acceptor level of Zn-Mb* (0.85 V[18]). Therefore, the electron transfer from TEA to Zn-Mb* became uphill reaction. The excited porphyrin ring in Zn-Mb could be attacked by H_2O or OH^- at pH 6.8.

When Zn-PP was used as a sensitizer instead of Zn-Mb at pH 8.5, no significant NADPH formation was observed. The excited triplet lifetime of Zn-Mb has been reported to be 13.7 ms, whereas that of Zn-PP was 0.7 ms.[6] The difference in the lifetime for these excited states may cause the change in efficiency of NADPH production. Also, the interaction between Zn-PP and NADPH was not observed by CD spectroscopy.

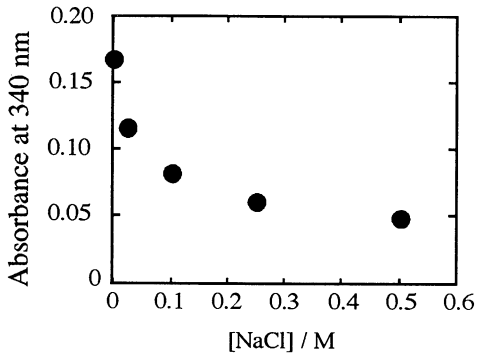

Figure 2. Dependence of NaCl concentration on NADPH formation. The solution conditions were the same as those of Figure 1. The absorbace was measured after irradiating light ($\lambda > 390$ nm) for 3 h.

The electrostatic interaction between Zn-Mb and $NADP^+$ is also a factor in $NADP^+$ reduction. Figure 2 shows the dependence of the rate of NADPH formation on the concentration of NaCl. The amount of NADPH formed decreased with an increase in the concentration of NaCl, indicating that an electrostatic interaction between Zn-Mb and $NADP^+$ or Zn-Mb and TEA plays an important role in the photoreduction of $NADP^+$. In a CD spectrum of NADPH and Zn-Mb in a phosphate buffer solution at pH 8.5, the induced CD band at 340 nm was clearly observed, indicating an interaction between NADPH and Zn-Mb under these conditions. The intensity of the CD band at 340 nm did not change on addition of 1 M NaCl, suggesting strong interaction between Zn-Mb and NADPH. This means that NADPH complexed with Zn-Mb and the distance between NADPH and the redox center of Zn-Mb may be fixed by the size of apoMb. When the same experiment was carried out at pH 6.8, the CD spectrum was identical to that at 8.5. The interaction between Zn-Mb and NADPH was independent of pH and ionic strength within the ranges studied. The formation of NADPH by electron transfer from Zn-Mb* to $NADP^+$ is independent of ionic strength. The reason for ionic strength dependent NADPH formation can not be explained by an electrostatic interaction between NADPH and Zn-Mb.

On the other hand, the isoelectric point of Mb (from horse heart skeletal muscle) was reported to be 6.8.[18] Zn-Mb is reconstituted from apoMb and Zn-PP, so it is reasonable that the isoelectric point of Zn-Mb is the same as Mb. In addition, the pKa of TEA is known to be 7.76.[19] At pH < 6.8 both Zn-Mb and TEA are positively charged. Therefore, electrostatic repulsion may cause the rate of electron transfer to be slower than at pH 8.5, where Zn-Mb is charged negatively and most TEA molecules are neutral.

In conclusion, Zn-Mb photocatalyzes the reduction of $NADP^+$ with TEA serving as a sacrificial electron donor. By irradiating visible light ($\lambda > 390$ nm) for 5.5 h at pH 8.5, ca. 40% of $NADP^+$ was converted to NADPH. This system serves as a simple model of photosystem I. The reduction efficiency of $NADP^+$ depended on the pH of the solution due to the potential difference and electrostatic interaction between TEA and Zn-Mb.

References and Notes

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18 Cyclic voltammograms of TEA and Zn-Mb were recorded at an indium oxide electrode in a phosphate buffer solution of pH 6.8. TEA and Zn-Mb showed oxidation peaks at 0.94 and 0.85 V, respectively. Although the electrode reactions were not reversible, the oxidation peak potentials were assumed to be the electron levels of interest, for comparison.
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