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# The Effects of Visible Light on Iron Release from Ferritin Related to Lipid Peroxidation in the Retina

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We studied iron release from ferritin by irradiating the visible light, and then followed ferritin-mediated lipid peroxidation in the rod outer segment (ROS) fraction of the porcine retina. In the presence of several phosphorus compounds such as ADP and ATP, iron release from ferritin at pH 7.0 could be induced by irradiation of the visible light to the reaction mixtures. Furthermore, iron release from ferritin in the presence of ADP depended on the incubation time and the visible light irradiation. Moreover, we investigated lipid peroxidation level in the ROS fraction by two independent assay systems including the thiobarbituric acid (TBA) and ferrous oxidation/xylenol orange (FOX) methods. The visible light induced ferritin-mediated lipid peroxidation in the ROS fraction in time- and irradiance-dependent manners. In the dark condition, iron release and lipid peroxidation were not observed. Iron release from ferritin by irradiating the visible light may play an important role in the etiology of phototoxic injuries *in vivo*.

**Key words :** visible light; ferritin; ADP; lipid peroxidation; retina

## INTRODUCTION

Most of intracellular iron is stored in ferritin in the tissues. Ferritin can hold up to 4,500 iron atoms per molecule as a mineral core surrounded with the protein shell. Iron is incorporated into the ferritin as the form of $Fe^{2+}$ which is oxidized to $Fe^{3+}$, and is deposited in the mineral core as an insoluble hydrated ferric oxide. Iron release from ferritin is induced by various enzymes, including flavoprotein oxidases, xanthine oxidase and several dehydrogenases, and by low molecular weight-biological molecules, including urate, ascorbate, citrate, bicarbonate, lactate and apo-transferrin [1]. It has also been reported that the superoxide anion causes iron release from ferritin [2]. In addition, recent studies have shown that the irradiation of ultraviolet A causes iron release from ferritin and lipid peroxidation of low density lipoprotein in the presence of ferritin [3, 4].

In this study, we examined visible light-induced iron release from ferritin and ferritin-mediated lipid peroxidation of the porcine rod outer segment (ROS) fraction.

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## MATERIALS AND METHODS

*Ferritin incubation*

The solution of horse spleen ferritin (lot no. 90k7039, Sigma Chemical Co., St. Louis, MO, USA) in 20 mM tris-maleate buffer solution at various conditions of pH was placed into a well of a 24-well microplate and incubated for 0-60 min under the dark or lighting condition. Light irradiation was carried out using a white fluorescent light (27 W). The visible light at 4,000-17,000 *lx* was irradiated to ferritin solutions at 37 °C in a water bath.

For lipid peroxidation study, ferritin solution (0.5 mg/ml; 100 μl) including the porcine ROS fraction (0.5 mg protein/ml) was placed into a well of a 96-well microplate. This microplate was incubated under the same conditions as the experiments of iron release from ferritin.

*Quantitation of iron released from ferritin*

After the incubation, the reaction mixture was placed in the top of a 10 kDa cut-off filter (YM-10, Millipore Co., Bedford, MA, USA) and centrifuged at 13,000 rpm for 1 hr at 4 °C. The ferritin-free supernatant was divided into two solutions; one was added with desferrioxamine (1 mM at final concentration) and the other was added with the same volume of water for the blank absorbance. After 30 min at room temperature, the concentration of iron released in the

media was determined spectrophotometrically by subtracting the absorbance of the corresponding blank sample from that of the test sample at 430 nm due to ferrioxamine formation ($\varepsilon_{430\ nm}$ = 2,480 $M^{-1}$ $cm^{-1}$) [5].

*Measuring of lipid peroxidation of the porcine rod outer segment fraction*

The ROS fraction of the porcine retina was prepared by the procedure of Anderson [6]. To estimate lipid peroxidation level in the ROS fraction, we measured the concentration of malondialdehyde (MDA) by thiobarbituric acid (TBA) method [7] and that of lipid hydroperoxide (LOOH) by ferrous oxidation/xylenol orange (FOX) method [8].

## RESULTS

*Effects of pH*

Ferritin solutions (0.5 mg/ml) were incubated at 37 °C for 20 min under the dark (0 *lx*) or lighting condition (17,000 *lx*) at different pH conditions. Iron release from ferritin by irradiating the visible light was enhanced at acidic pH conditions (data not shown). On the other hand, iron release under the dark condition was not observed even at acidic pH conditions (data not shown).

*Effects of phosphorus compounds*

Iron release from ferritin by irradiating the visible light was also affected with the several phosphorus compounds added in the reaction mixture. Among six tested phosphorus compounds, ADP, ATP and pyrophosphate enhanced visible light-induced iron release, whereas phosphate, AMP and NADH did not show the enhancements (data not shown).

*Time course of iron release from ferritin*

In the presence of ADP, iron release level increased remarkably with the incubation time under the lighting condition. But, under the dark condition, only a slight degree of iron release was observed (data not shown).

When light irradiation was stopped at 10 min after starting, the rate of iron release was similar to that carried out under the dark condition (data not shown). When light irradiation was carried out again, the rate of iron release returned to that under the lighting condition (data not shown).

*Visible light and ferritin-induced lipid peroxidation*

When the ROS solution containing ferritin was irradiated by the visible light, the level of MDA (Fig. 1) in the ROS increased with the incubation time. The increase of MDA level was dependent on the irradiance of the visible

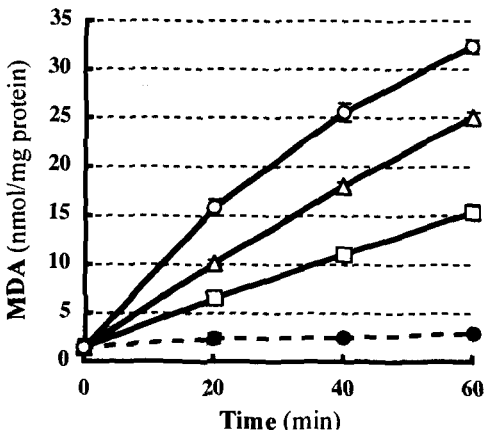


Figure 1. Time course of light-induced MDA production of ROS fractions in the presence of ferritin. ROS fraction (0.5 mg protein/ml) containing ferritin (0.5 mg/ml) was incubated at 37 °C. Solid lines show the values under the light irradiation (open circle, 17,000 *lx*; open triangle, 8,500 *lx*; open square, 4,000 *lx*). Dash lines show the values under dark conditions (closed circle). The incubated samples were subjected to TBA measurement. Values presented are arithmetic means ± S.D. of triplicates for MDA concentration.

light, while under the dark condition it was not produced until at least 60 min. LOOH level increased in the same dependences as that of the MDA level (Fig. 2).

## DISCUSSION

Iron release from ferritin was significantly increased by irradiating the visible light in the presence of several phosphorus compounds such as ADP, ATP and pyrophosphate (data not shown). However, ADP itself did not cause iron release because the reduction potential of Fe(III)-ADP/Fe(II)-ADP (+0.10 V) is higher than that of Fe(III)-ferritin/Fe(II)-ferritin (-0.19 V) [9]. These results may be consistent with the previous report that ADP is capable of oxidizing $Fe^{2+}$ to $Fe^{3+}$ [10]. ADP would contribute to the chelation of free $Fe^{2+}$ and/or the autoxidation of $Fe^{2+}$ to $Fe^{3+}$, leading to the prevention of re-incorporation of the $Fe^{2+}$ released.

Among 6 tested phosphorus compounds, ADP, ATP and pyrophosphate enhanced visible light-induced iron release, but phosphate, AMP and NADH did not enhance iron release (data not shown). Thus, it is quite reasonable to consider that visible light-induced iron release from ferritin might be enhanced by compounds containing di/triphosphate terminal but not adenine and ribose portions.

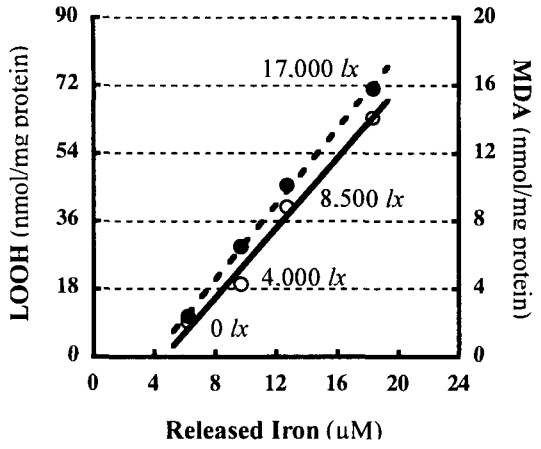


Figure 2. Correlation between released iron concentration in the presence of 10 mM ADP and the amounts of LOOH and MDA products. The values of iron released from ferritin and the values of LOOH and MDA formations of ROS after 20 min incubation under the various irradiation were plotted. The solid line is for the correlation of released iron with LOOH products (open circle); The dash line is for the correlation of released iron with MDA products (closed circle).

The other nucleoside di/triphosphate might also enhance visible light-induced iron release as well as ADP and ATP.

Furthermore, we investigated whether iron released from ferritin by irradiating the visible light can participate in lipid peroxidation of the ROS fraction. There was a significant correlation between the levels of visible light-induced iron release from ferritin and the amounts of MDA and LOOH in the ROS fraction produced by irradiating the visible light in the presence of ferritin (Fig. 2), suggesting that the productions of MDA and LOOH might be attributable to iron release from ferritin.

There was a complete correlation between light-induced iron release from ferritin and the amount of LOOH and MDA productions by ferritin and light in ROS fraction (Fig. 2). It means that the productions of the latters might be attributable to iron released from ferritin. In the absence of ADP iron released from ferritin by visible light can participate in lipid peroxidation of ROS fraction, suggesting that polar phospholipids such as phosphatidic acid, phosphatidyl serine and phosphatidyl ethanolamine may work as iron chelators similar to ADP.

In conclusion, we confirmed that the visible light induced iron release from ferritin in the presence of several phosphorus compounds, and that the visible light can induce lipid peroxidation in the presence of ferritin. This study pointed out the possibility that iron released from ferritin might cause lipid peroxidation in the cells and tissues when the strong visible light irradiated. Iron release from ferritin by irradiating the visible light may play an important role in the etiology of phototoxic injuries *in vivo*.

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