ROLE OF MITOCHONDRIA IN LIGHT-INDUCED OXIDATION OF OXYMYOGLOBIN IN MEAT

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I. INTRODUCTION

Consumers rely heavily on fresh meat color as a purchase indicator. Discoloration of meat is caused by the oxidation of cherry-red oxymyoglobin (OxyMb) to brown metmyoglobin (MetMb). Oxidation of OxyMb has been studied in terms of the NADH-dependent cytochrome b5 myoglobin reduction system and autoxidation [1]. However, light-induced oxidation of OxyMb has not been well studied because of its low photosensitivity [2].

We hypothesized that the light-induced discoloration observed in meat could not be explained by the low photosensitivity of OxyMb. In this study, we analyzed the wavelengths responsible for light-induced discoloration and estimated the photoreceptors. In addition, we investigated the cytochrome a in complex IV of mitochondria as a light receptor for light-induced meat discoloration.

II. MATERIALS AND METHODS

Test 1: 3x distilled water was added to 3 mm minced bovine *Biceps femoris* and homogenized. The supernatant obtained by the centrifugation of the homogenate at 10,000 × g for 10 min was used as the sample. The sample was irradiated with light of various wavelengths using a spectral irradiator (NIJI-2, Bunkoukeiki, Tokyo, Japan), and absorbance spectra were measured at 350-750 nm over time. The MetMb ratio was determined using Tang's equation [3] to evaluate the oxidation rate of Mb at each wavelength. Test 2: To evaluate the effect of cytochrome c on Mb oxidation rate, OxyMb (prepared from equine Mb) was mixed with oxidized cytochrome c and the absorbance spectra at 450-750 nm were measured over time. Test 3: The effect of the heated supernatant (equivalent to 100 mg/ml of *Biceps femoris*; heated to 75°C) on the oxidation rate of Mb during light exposure was evaluated. The reaction solution comprised 0.71, 0.1, and 0.067 mg/ml of OxyMb, mitochondria, and cytochrome c (oxidized form), respectively. Test 4: The effects of mineral salts (18.3 mmol/l NaCl, 81.8 mmol/l KCl, 0.7mM $CaCO_3$, 9.0 mmol/l $MgCl_2$, 54.8 mmol/l PO_4(Na), 0.4 mmol/l Fe-gluconate, and 0.6 mmol/l Zn-gluconate) on the oxidation rate of Mb during light exposure were evaluated. The reaction solution comprised 0.71, 0.1, and 0.060 mg/ml of OxyMb, mitochondria, and cytochrome c (oxidized form), respectively, and mineral salt. Test 5: The effect of zinc concentration on the oxidation rate of Mb during light exposure was evaluated. The reaction solution comprised 0.59, 0.1, and 0.063 mg/ml of OxyMb, mitochondria, and cytochrome c (oxidized form), respectively, and 0, 3, 6, 15, or 30 µmol/l Zn-gluconate. Test 6: The effect of zinc on the cytochrome c reduction rate during light exposure was evaluated. The reaction solution comprised 0.1 mg/ml mitochondria, 0.23 mg/ml cytochrome c (reduced form), and 10 µmol/l Zn-gluconate.

Statistical analysis: Student's t-test was used to compare two groups, and Scheffé's F-test, following a one-factor ANOVA, was used for multiple comparisons.

III. RESULTS AND DISCUSSION

If light-induced discoloration of meat is due to the photoexcitation of Mb, the wavelength-inducing oxidation would be similar to the absorption spectrum of Mb. However, when the oxidation rates were

plotted for each irradiation wavelength, the spectra differed from those of Mb derivatives (Fig. 1). The photoreceptor in light-induced discoloration was not Mb, but cytochrome a, which has absorption peaks at approximately 450 and 600 nm. Cytochrome a is a pigment present in complex IV of the mitochondrial electron transfer system; complex IV is a well-known cytochrome c oxidase. When oxidized cytochrome c was mixed with OxyMb, cytochrome c was reduced, and MetMb was formed. Approximately 58.6% of Mb was oxidized immediately. This indicated that the activation of complex IV could be responsible for the oxidation of OxyMb via cytochrome c.

Light-induced oxidation was not clearly observed in the control containing Mb, mitochondria, or cytochrome c. In contrast, it was evident upon the addition of the heated supernatant (Table 1, Test 3). The mineral salts imparted photosensitivity to the oxidation reaction (Table 1, Test 4), and Zn was found to increase the photosensitivity (data omitted) from salt screening. In the presence of Zn, complex IV activity increased upon light irradiation (P<0.05) and the photooxidation rate of Mb increased with increasing Zn concentration (Figure 2).

These results suggest that Zn regulates the electron transfer system in mitochondria but regulated only partially when exposed to light. In summary, the light-induced oxidation of OxyMb in meat is attributed to the following mechanism. Light incident on the tissue excites cytochrome a in mitochondrial complex IV, which is regulated by Zn. This activates cytochrome c oxidase. Oxidized cytochrome c then removes electrons from Mb, thereby turning the meat brown.

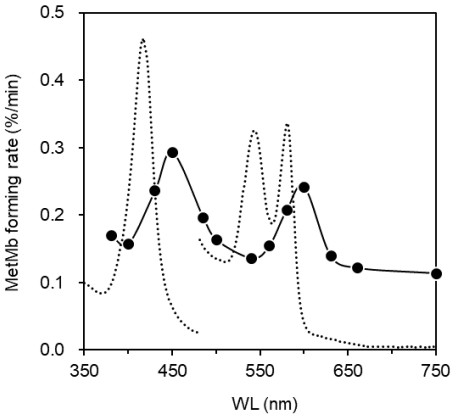

Figure 1. Light-induced oxidation
dotted line: absorption spectra of OxyMb
solid line: spectra of MetMb forming rate

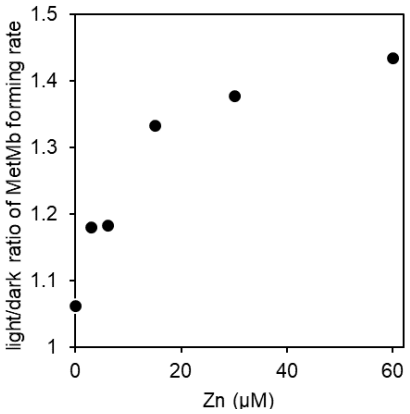

Figure 2. Effect of Zn on light-induced oxidation

Table 1 MetMb forming rate (%/min) and light/dark ratio

Item	Control			+supernatant			+mineral salts		
	dark	light	ratio	dark	light	ratio	dark	light	ratio
Test 3	0.94	0.97	1.04[a]	0.94	1.12	1.19[b]			
Test 4	0.95	1.04	1.10[x]				0.29	0.54	1.88[y]

a-b: P<0.05; x-y: P<0.05

IV. CONCLUSION

Cytochrome a could be the photoreceptor of light-induced discoloration. The reactions shown in this study are likely to occur at different rates, depending on the location of the tissue. This is because the concentrations of cytochrome c and Zn are not constant in a tissue. Aging may reduce the imbalance in these concentrations in tissues. Understanding the variation in light-reaction behavior with tissue location and/or aging time will be studied in future.

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