EFFECT OF HEMOGLOBIN DEGRADATION ON THE ZINC PROTOPORPHYRIN IX FORMATION IN PORK LIVER HOMOGENATE

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I. INTRODUCTION

Zinc protoporphyrin IX (ZnPP) contributes to the bright red color of Parma ham, a traditional Italian dry-cured ham prepared without nitrite/nitrate [1]. ZnPP is endogenously formed in meat and internal organs. Among the different internal organs, the liver shows an outstanding ZnPP-forming capacity [2]; thus, it could be applied for color improvement of nitrite/nitrate-free meat products. However, the reason for the high ZnPP-forming capacity in the liver remains unclear. The optimum pH of ZnPP formation in the liver was reported to be relatively low, around 4.5–5.0 [2,3]. In addition, some skeletal muscles rich in slow-twitch muscle fibers show high ZnPP formation and low optimum pH (4.75) [4]. Because heme is the precursor of ZnPP, the release of heme induced by myoglobin (Mb) degradation at a lower pH is suggested to contribute to high ZnPP formation in these muscles. Thus, it can be hypothesized that hemoglobin (Hb), the main heme protein in the liver, is degraded during ZnPP formation, contributing to its high ZnPP-forming capacity. Therefore, this study aimed to elucidate the effect of Hb degradation on ZnPP formation in liver homogenate.

II. MATERIALS AND METHODS

As a model experimental system for ZnPP formation, 20% liver homogenate was supplemented with antibiotics, adjusted to pH 4.5, and then incubated anaerobically at 25°C for 3 days. For quantification, heme and ZnPP were extracted using an ethyl acetate-acetic acid mixture (4:1) and separated using reversed-phase high-performance liquid chromatography. In addition, the amount of ZnPP formed under various conditions was evaluated based on the fluorescence intensity (excitation/emission: 420/590 nm) of the 75% acetone extract of the model homogenate. EDTA, carbon monoxide (CO), and pepstatin A were added to the model homogenate as a divalent metal ion chelator, a ligand that stabilized heme iron, and a protease inhibitor, respectively. Hb degradation was evaluated by western blotting (WB) method. Statistical analysis was performed using a one-way analysis of variance with Tukey's honest significant difference test. $P < 0.05$ was considered as statistically significant.

III. RESULTS AND DISCUSSION

The ZnPP formation pathway was investigated in liver homogenates. During incubation, the heme levels decreased with an increase in ZnPP. The addition of EDTA inhibited ZnPP formation and led to the accumulation of protoporphyrin IX (PPIX). These results indicate that ZnPP in the liver homogenate is formed from heme via PPIX in the same way as in the skeletal muscle [5]. In addition, CO significantly inhibited ZnPP formation, suggesting that the release of heme from heme proteins is essential for ZnPP formation. Because Hb is the most abundant heme protein in the liver, it is assumed to contribute to ZnPP formation as a heme donor.

Next, Hb degradation during the incubation was evaluated using WB. On day 1, the band density of the Hb β chain drastically decreased (Fig. 1A), indicating that a large part of Hb was degraded at an early period of incubation. In addition, Hb degradation was vigorous in the lower pH, compared to the

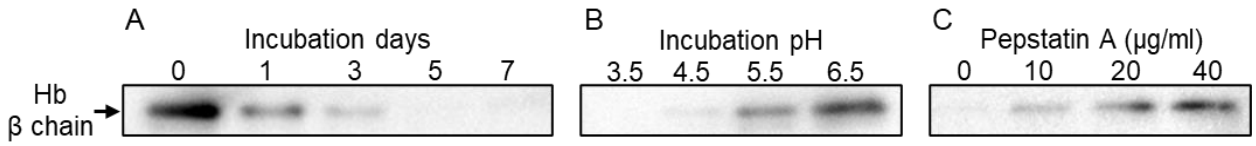

Figure 1. WB images of the model homogenate with different incubation periods (A), pH (B), and concentration of pepstatin A added (C). A and C were incubated at pH 4.5, and B and C were incubated for 3 days.

neutral pH (Fig. 1B), suggesting the involvement of acid proteases. Moreover, pepstatin A, which is the competitive inhibitor of the aspartic proteases, inhibited the Hb degradation dose-dependently (Fig. 1C). Thus, the aspartic proteases such as cathepsin D and E were suggested to degrade Hb during the incubation, especially in the early period. However, pepstatin A did not affect the ZnPP formation (Fig. 2). For the reason, the high ability of Hb to dissociate heme without degradation, which is reported to contribute fast ZnPP formation in meat products [6] could be considered. Consequently, in the liver at low pH, Hb was degraded during the incubation and thus in turn lead to heme release. However, this degradation would not be essential for the high ZnPP formation amount of the liver homogenate.

Conversely, nevertheless its high ZnPP content, the appearance of the liver homogenate after ZnPP formed is inferior to that of the skeletal muscle. Because ZnPP formed in skeletal muscle binds to apo-Mb and apo-Hb for solubilization [5], Hb degradation in the liver homogenate might affect the solubility of ZnPP and, its appearance. In this study, pepstatin A addition improved the appearance of the homogenate compared to the group without pepstatin A (Fig. 3), suggesting that Hb degradation caused an undesirable color.

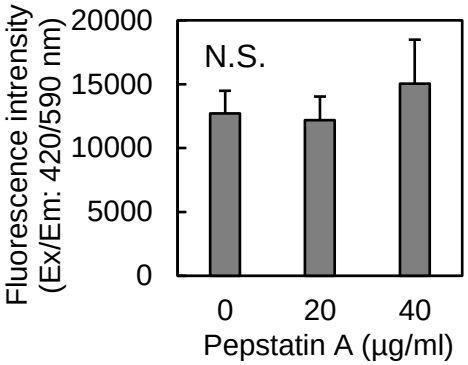

Figure 2. The effect of the addition of pepstatin A on the ZnPP formation in the liver homogenate. Bars: standard errors (n = 6). N.S.: no significant difference among the groups.

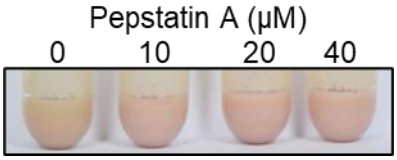

Figure 3. The appearance of the liver homogenate after incubated with different concentrations of pepstatin A for 3 days.

IV. CONCLUSION

It has been suggested that ZnPP is formed from heme dissociated from heme proteins, mainly Hb, in the liver, as in the skeletal muscles. Hb degradation during incubation was confirmed but was not essential for high ZnPP formation. Moreover, Hb degradation has been suggested to result in an undesirable appearance. Therefore, the prevention of Hb degradation was assumed to improve the coloring effect of the liver homogenate without compromising its high ZnPP-forming capacity.

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