TWO OPTIMUM pH FOR ZINC PROTOPORPHYRIN IX FORMATION EXIST IN PORK

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I. INTRODUCTION

Zinc protoporphyrin IX (ZnPP) is a characteristic red pigment in meat products that are produced without the addition of nitrate or nitrite [1]. But, the formation mechanism of the red pigment in meat products has not been elucidated yet. If the ZnPP formation mechanism is elucidated, the color of meat products without nitrate or nitrite can be improved. ZnPP and protoporphyrin IX (PPIX) formation at optimum pH 5.5 was discovered using *longissimus* muscle in which ZnPP was derived from PPIX whose precursor was not heme [2]. Recently, it was found that ZnPP is able to be formed at pH higher than 4.9 after an extensive drying period up to 177 days, indicating that both pH and production times are crucial factors for its formation in nitrate-free porcine dry fermented sausages [3]. The optimum pH for ZnPP formation differs depending on various internal organs and those of porcine heart, liver and kidney were 5.0–5.5, 4.5 and 5.5–6.0, respectively [4]. On the other hand, it has been suggested that substitution of iron by zinc could be occurred to form ZnPP [5]. Although it has been assumed that myoglobin, heme and ferrochelatase plays a major role, ZnPP formation mechanism is quite indistinct. The ultimate pH of fresh meat is considered as a potentially important factor for ferrochelatase activity to form ZnPP. To elucidate the mechanism, we hypothesized that plural ZnPP formation mechanisms with different optimum pH exist in pork. Therefore, the purpose of this study was to clarify the optimum pH for ZnPP formation in various skeletal pig muscles. In addition, the mechanism by which ZnPP is formed in another optimum pH discovered in pork and the effect of myoglobin on ZnPP formation were examined.

II. MATERIALS AND METHODS

Model Experiment

Model experiments were prepared as described by Wakamatsu *et al.* [2] with minor modifications. After adjusting pH of pork homogenate to 4.0~7.5, antibiotics were added to the model solutions and incubated at 25°C in darkness for 5 days anaerobically. For further experiments, pork homogenate (*infraspinatus* muscle) was adjusted to new optimum pH of ZnPP formation and incubated at 37°C. The formation of PPIX was investigated using model solution containing 0.5 mM EDTA. ZnPP and PPIX were detected by fluorescence intensity Ex/Em: 420/590 and Ex/Em: 410/630 nm, respectively.

SDS-PAGE and Western Blotting analysis

Protein samples were loaded onto 20% polyacrylamide gel to conduct SDS-PAGE. The western blotting was conducted using anti-myoglobin polyclonal antibody, horseradish peroxidase-conjugated anti-IgG antibody and chemiluminescence detection reagent.

Quantitative analysis of ZnPP, PPIX and heme

The contents of ZnPP, PPIX and heme were determined by HPLC as described by Wakamatsu *et al.* [2].

III. RESULTS AND DISCUSSION

a. Effects of various skeletal muscles and pH on ZnPP formation in pork

We investigated the effect of pH on ZnPP formation in 20 kinds of porcine skeletal muscle. ZnPP formation pattern due to the pH was different among skeletal muscles and was classified into three large groups (Fig. 1). ZnPP formation optimum pH in porcine skeletal muscle was 5.5 as well as 4.75. The ability of ZnPP formation at pH 4.75 seemed to be higher than that of pH 5.5.

b. Effects of various factors on ZnPP and PPIX formation at pH 4.75

Oxygen, nitrite and ferrochelatase inhibitor significantly inhibited the ZnPP and PPIX formation at pH 4.75. These findings were similar to the formation at pH 5.5 [2]. However, optimum temperature for ZnPP

formation differed between at pH 4.75 and 5.5. Thus, ZnPP formation mechanisms in two different optimum pH are different. Then, the behavior of PPIX formation at pH 4.75 was almost similar to those of ZnPP, suggesting that ZnPP formation at pH 4.75 depends on PPIX formation.

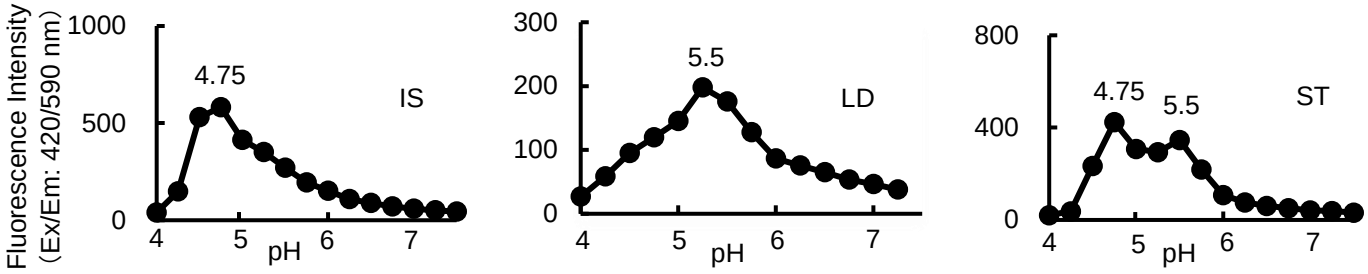

Figure 1. Typical three patterns of optimum pH for ZnPP formation among twenty muscles
IS: *Infraspinatus* muscle, LD: *Longissimus dorsi* muscle, ST: *Semitendinosus* muscle

c. Role of myoglobin on ZnPP formation at pH 4.75

In order to check the role of myoglobin in ZnPP formation, we examined the degradation of myoglobin at pH 4.75 after incubation. After 10 days of incubation, a significant degradation of myoglobin was observed. When the myoglobin is subjected to a partial decomposition, substitution reaction of iron and zinc by ferrochelatase to be promoted [6]. Therefore, a large amount of ZnPP formation at pH 4.75 may be attributed to the degradation of myoglobin.

d. Quantitative determination of heme, ZnPP and PPIX at pH 4.75

In order to verify whether ZnPP and PPIX were formed from heme at pH 4.75, we determined the amounts of ZnPP, PPIX and heme in the model solutions. Although ZnPP formation was significantly increased after 5 and 10-days of incubation, the amount of heme was not reduced. Addition of EDTA into a model solution almost inhibited ZnPP formation and increased PPIX formation significantly, but there was no change in the heme content. Thus, decrease of heme, corresponded to the increase of ZnPP and PPIX at pH 4.75, was not observed. Therefore, ZnPP and PPIX formation at pH 4.75 was not caused by Fe–Zn substitution in heme and was independently formed in the model solution during incubation. Mitochondria have the ability to form PPIX from oxymyoglobin, and therefore, are directly related to the release of Fe^{2+} from porphyrin ring in myoglobin [7]. The formation of ZnPP and PPIX at pH 4.75 was not derived from heme that was derived from myoglobin though the precursor is still unclear.

IV. CONCLUSIONS

Two optimum pH at 5.5 and 4.75 for ZnPP formation exist in pork. In case of optimum pH at 4.75, ZnPP was derived from PPIX, but its precursor was not heme. Furthermore, the degradation of myoglobin was observed but the decrease of heme, corresponded to the formation of ZnPP and PPIX, was not observed at pH 4.75. This study will be worthwhile to elucidate the ZnPP formation mechanism in meat products.

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