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MORPHOLOGICAL STUDY ON THE FORMATION OF ZINC PROTOPORPHYRIN IX IN PARMA HAM

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I. INTRODUCTION

While the demand for natural additive-free meat products is increasing, nitrite/nitrate-free meat products generally have an inferior color. In contrast, Parma ham (*Prosciutto di Parma*), a traditional Italian dry-cured ham, appears bright red without the addition of nitrite/nitrate. Zinc protoporphyrin IX (ZnPP) contributes to this redness [1]. ZnPP is endogenously formed during the Parma ham maturation. Therefore, accelerating ZnPP formation would be a novel technique for improving the color of nitrite/nitrate-free meat products.

The formation mechanism of ZnPP in Parma ham has been studied for a couple of decades. Recently, it was suggested that ZnPP is formed from heme dissociated from myoglobin and hemoglobin. Specifically, the iron is removed from the heme to form protoporphyrin (PPIX), which is then zinc inserted to form ZnPP [2]. Ferrochelatase (FECH) is thought to be responsible for this metal substitution.

However, considering the location of the enzyme and the substrates involved in the proposed ZnPP formation mechanism in Parma ham, the question remains as to how they interact to form ZnPP. In the living body of mammals, FECH is localized in the mitochondrial inner membrane, whereas myoglobin and hemoglobin, which are the heme donors in the proposed ZnPP formation mechanism, exist in the myoplasm and vessels, respectively. Thus, under the presumption of localization in the living body, the enzyme and the substrates of ZnPP formation are separated by the mitochondrial membranes, plasma membrane, and blood vessel walls. One of the most reasonable mechanisms of contact between FECH and heme is that the mitochondrial membrane structure becomes fragile during ham maturation, exposing FECH to the cytoplasm. However, the mechanism by which FECH and heme interact to form ZnPP, and how mitochondrial morphology changes during the maturation in Parma ham have not yet been investigated.

Therefore, in this study, we aimed to verify the proposed mechanism of ZnPP formation from the morphological perspective, and we investigated the distribution of ZnPP and the mitochondria morphology in Parma ham by various microscopic observations.

II. MATERIALS AND METHODS

The *rectus femoris* (RF), *biceps femoris* (BF), *semimembranosus* (SM), and *semitendinosus* (ST) muscles were excised from three Parma hams (maturation periods: 14–18 months). Frozen sections were prepared from these hams and observed by a confocal laser scanning microscopy. The distribution of ZnPP was evaluated by observing its autofluorescence (Ex/Em: 405/570–610 nm), and the distribution of slow-twitch muscle fibers and mitochondria was evaluated by immunostaining with anti-slow-twitch myosin and anti-COX4 antibodies, respectively. The ultrastructure of the RF was observed by cryo-scanning electron microscopy (cryo-SEM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

III. RESULTS AND DISCUSSION

ZnPP autofluorescence was observed in muscle fibers of Parma ham. After rinsing with phosphate-buffered saline, the fluorescence intensity decreased, indicating that ZnPP exists in a soluble state in the cytoplasm. Muscle fibers were divided into two types depending on the intensity of ZnPP autofluorescence. The fibers with stronger ZnPP autofluorescence coincided with the distribution of slow-twitch muscle fibers. This indicates that ZnPP formed more intensely in slow-twitch muscle fibers.

Focusing on the difference between the muscles, RF and BF showed higher ZnPP autofluorescence compared with SM and ST (Fig.1). Moreover, in RF and BF, ZnPP autofluorescence at the periphery of the muscle fibers was observed in a granular form (Fig.1, arrows). Because RF and BF are rich in slow-twitch muscle fibers that show high ZnPP formation in this study, as reported by Wakamatsu et al. 2019 [3], this granular structure was expected to play an important role in the formation of ZnPP. The diameter of the granular was 1–2 μm, and it was significantly larger

and more numerous in slow-twitch muscle fibers than in fast-twitch muscle fibers. Since the granular size is larger than the mitochondria or many other organelles and could not be identified, we further investigated its microstructure. In cryo-SEM and SEM, the holes of almost equivalent size to those of the fluorescent granules in Parma ham were observed, but not in the fresh meat. Although this hole could not be identified as a granular structure that showed high ZnPP autofluorescence, it was speculated to be involved in the ZnPP formation.

We also examined the mitochondria in Parma ham to reveal the relationship with ZnPP formation. As reported in many studies, mitochondria are distributed in the periphery of muscle fibers. In addition, ZnPP partially co-localized with the mitochondria, suggesting that it is formed near the mitochondria. In the TEM images, myofibril structures, especially Z lines, were observed even in Parma ham matured for at least one year. Mitochondria-like structures were also observed, but their cristae structure was unclear compared with that of fresh meat. These results suggest that ZnPP is formed near the mitochondria, where membrane structures are vulnerable during Parma ham processing.

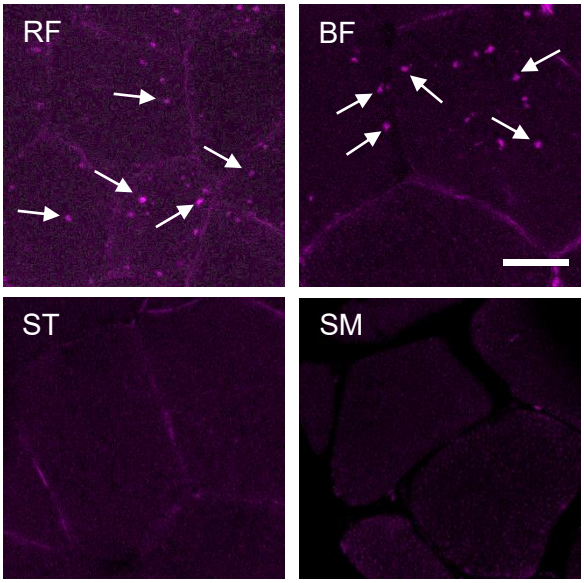


Fig.1. ZnPP autofluorescence images of different muscles from Parma ham (Ex/Em: 405/570–610 nm).
Arrows: granular structures with high ZnPP autofluorescence. Bar: 25 μm.

IV. CONCLUSION

In the Parma ham, ZnPP was observed in the cytoplasm, and granules, especially in the QF and BF. Some of these were observed in the vicinity of the mitochondria. In addition, a fragile mitochondria membrane structure was observed in Parma ham. These results support the possibility that FECH can be exposed to the cytoplasm from weakened mitochondria and contact heme to form ZnPP. Moreover, this study revealed the involvement of granule structure in ZnPP formation, especially in slow-twitch muscle fibers. The identification of this structure needs to be addressed in future studies.

ACKNOWLEDGEMENTS

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