THE BIFUNCTIONAL EXPLORATION OF PYRUVATE KINASE IN POSTMORTEM MEAT

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I. INTRODUCTION

Pyruvate kinase (PK) acts as one of the glycolytic rate-limiting enzymes and involves in meat quality regulation. Recent studies have reported that some metabolic enzymes can also be regarded as protein kinase in live tissues [1]. A variety of proteins in mitochondria, cytoplasm and nucleus can be phosphorylated by PK [2]. The role of PK as a glycolytic enzyme or a protein kinase appears to participate in the development of meat quality. The objectives of the present study was to evaluate the bifunctional properties of PK in postmortem meat.

II. MATERIALS AND METHODS

This study includes two parts. First, PK inhibitor (shikonin) was added to lamb meat to evaluate the glycolytic activity of PK through measuring the lactate content and physicochemical traits of meat quality. Second, An vitro system was performed to investigate the kinase activity of PK. PK was mixed with myofibrillar protein in buffer (1 mM PEP, 100 mM KCl, 50 mM MgCl2, 1 mM DTT, 1 mM NaVO4, 5% glycerin, 30 mM HEPES, pH 7.5) and incubated at 4, 25 and 37℃. Protein phosphorylation induced by PK was evaluated. The comparisons between different groups were analyzed by one-way analysis of variance (Duncan's multiple range test) and a t-test ($P < 0.05$).

III. RESULTS AND DISCUSSION

The linear fit was performed to better understand the relationship between the lactate content and PK activity. An insignificant pearson'r of -0.20 showed between lactate content and PK activity (Figure 1A), but the myofibrillar fragmentation index was significantly decreased in 1 d and 5d ($P < 0.05$, Figure, 1B). When stored at 4 h, the absorbance of the PKI group at 540 nm and 580 nm were reduced (Figure 1C). Thus, it was speculated that PK might be involved in meat quality regulation through other pathways besides its glycolytic activity.

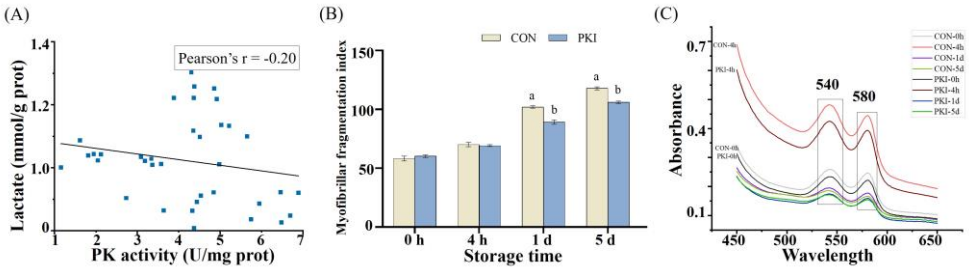

Figure 1. The effect of PK on lactate (A), myofibrillar fragmentation index (B) and myoglobin spectrum (C). a-b shows significant differences ($P < 0.05$). The absorbance at 540 nm and 580 nm represents the oxymyoglobin status.

The kinase assay of PK was conducted to demonstrate whether PK could induce protein phosphorylation. Compared with the control group, the global phosphorylation level of myofibrillar proteins in the PK group was significantly higher at 4℃ for 1 h incubation ($P < 0.05$, Figure 2A)., there was a relative stable change between incubation times ($P > 0.05$, Figure 2B). However, It was

significantly different between the PK group and the control group for 0.5 h and 2 h when incubated at 25℃ ($P < 0.05$, Figure 2B). During the whole incubation at 37℃, the global phosphorylation level of myofibrillar proteins in the PK group was significantly higher compared to that in the control group ($P < 0.05$, Figure 2C). The kinase activity of PK was sensitive to temperature. The findings of a previous study demonstrated that the phosphorylation of myofibrillar proteins inhibited their degradation [3]. Thus, the degradation and phosphorylation of actin and desmin were evaluated (Figure 3). The results showed that both desmin and actin were substrates that could be phosphorylated by PK, especially at the incubation temperature of 37℃. In addition, the actin and desmin phosphorylation induced by PK might take part in their degradation.

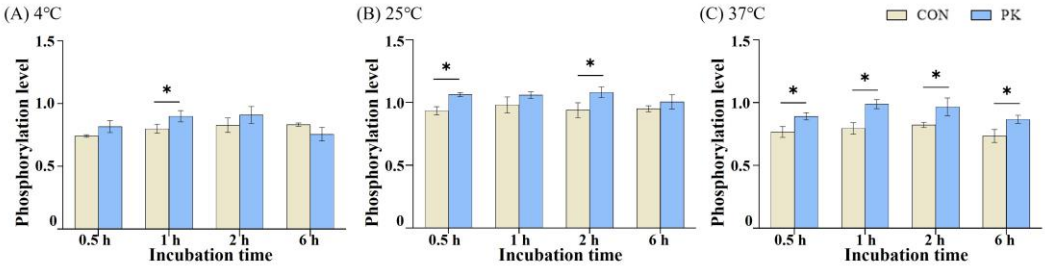

Figure 2. Effect of PK on phosphorylating myofibrillar proteins at 4℃ (A), 25℃ (B) and 37℃ (C) in the control and PK groups. * $P < 0.05$.

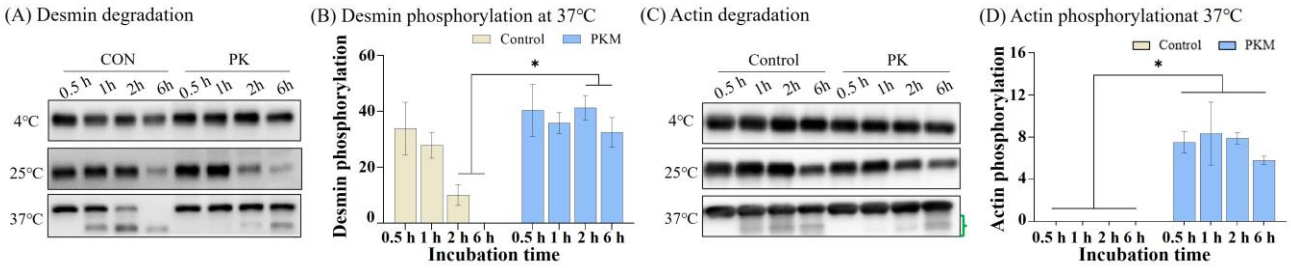

Figure 3. The desmin degradation (A), desmin phosphorylation (B), actin degradation (C) and actin phosphorylation (D) during incubation. The green label in images of (A) and (C) represents the degradation bands. * $P < 0.05$.

IV. CONCLUSION

The role of PK as a glycolytic enzyme and a kinase activity both had effect on meat quality regulation. Especially, the phosphorylation of desmin and actin was increased and the degradation of desmin and actin was decreased after myofibrillar proteins incubated with PK at 37℃. PK induced phosphorylation might be another potential pathway of meat tenderness formation through protein degradation regulation.

ACKNOWLEDGEMENTS

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