Effects of phosphorylation of myosin heavy chain and actin on their acetylation and coregulation on actomyosin dissociation in ovine muscle

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Objectives: Myosin and actin are the two most abundant cytoskeletal proteins, which form actomyosin and take charge of actomyo- sin dissociation. Substantial evidence have suggested the important role of actomyosin dissociation on postmortem meat tenderiza- tion (Li et al., 2012). The phosphorylation and acetylation sites of myosin heavy chain and actin were identified indicating the pos- sible regulation on actomyosin dissociation (Zhou et al., 2019; Cao et al., 2020). Protein posttranslation modifications (PTMs) crosstalk defines the negative or positive interaction of various PTMs which coregulate protein traits in a more complex manner (Hunter, 2007). But whether there are crosstalk of phosphorylation and acetylation on actomyosin dissociation regulation is still unknown. Thus, the objective of this study was to investigate the effects of myosin heavy chain and actin phosphorylation on their acetylation and the possible mechanism of protein phosphorylation and acetylation on coregulation of actomyosin dissociation.

Materials and Methods: The *Longissimus thoracis lumborum* muscle from twelve Small Tail Han sheep × local sheep were collect- ed. About 3 g frozen meat were homogenized with 75 mL of ice-cold buffer containing 0.6 M KCl, 0.01 M Na_2CO_3 and 0.04 M NaHCO3 (pH 7.2). The homogenate samples were incubated with alkaline phosphatase inhibitor (inhibiting dephosphorylation) and protein kinase inhibitor (inhibiting phosphorylation) 4℃ for 0, 0.5, 4, 12, 24, 48, and 72 h. The protein phosphorylation levels of myosin and actin were measured by SDS-PAGE and fluorescent staining. The acetylation levels and myosin heavy chain and ac- tin, and actomyosin dissociation degree were measured by Western blotting. The influence of myosin heavy chain and actin phos- phorylation on the structure of actomyosin was analyzed by molecular dynamics simulation.

Results and Discussion: The phosphorylation level of myosin heavy chain and actin in the alkaline phosphatase inhibitor treatment group was significantly higher than that in the control and protein kinase inhibitor treatment groups at 4, 12, and 72 h of incubation ($P < 0.05$). The acetylation level of actin in the alkaline phosphatase inhibitor treatment group was significantly lower than that in the protein kinase inhibitor treatment group after incubation for 4, 12, 24, 48, and 72 h ($P < 0.05$), and the acetylation level of myo- sin heavy chain changed irregularly. The results indicated that the phosphorylation of actin inhibited its acetylation, while the phos- phorylation of myosin heavy chain had no obvious regularity on its acetylation. The degree of actomyosin dissociation was always higher in the phosphoatase inhibitor treatment group than that other two groups during 0-72 h incubation ($P < 0.05$). The results of previous studies showed that the electrostatic binding of tropomyosin and myosin would be changed after the phosphorylation of actin S325 and acetylation of actin K328, which then affected the combination of myosin and actin (Liu et al., 2018; Schmidt et al., 2019). The results of molecular dynamics showed that the phosphorylation of myosin heavy chain S2, S3 and S54 and actin S54 and Y55 increased the total energy, potential energy, and kinetic energy of actomyosin. However, the bond energy of actomyosin was reduced, which caused the unstable structure of actomyosin. It might due to the negative charge of phosphate group decreased the structure stability of actomyosin and then caused actomyosin dissociation.

Conclusions: The phosphorylation of myosin heavy chain and actin made the structure of actomyosin unstable. The phosphorylation of actin antagonized its acetylation and then co-promoted actomyosin dissociation.

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Key words: Phosphorylation, Acetylation, Myosin heavy chain, Actin, Dissociation