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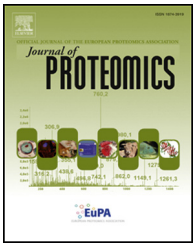

Quantitative phosphoproteomic analysis of porcine muscle within 24 h postmortem

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ABSTRACT

Protein phosphorylation can regulate most of the important processes in muscle, such as metabolism and contraction. The postmortem (PM) metabolism and rigor mortis have essential effects on meat quality. In order to identify and characterize the protein phosphorylation events involved in meat quality development, a quantitative mass spectrometry-based phosphoproteomic study was performed to analyze the porcine muscle within 24 h PM using dimethyl labeling combined with the TiSH phosphopeptide enrichment strategy. In total 305 unique proteins were identified, including 160 phosphoproteins with 784 phosphorylation sites. Among these, 184 phosphorylation sites on 93 proteins had their phosphorylation levels significantly changed. The proteins involved in glucose metabolism and muscle contraction were the two largest clusters of phosphoproteins with significantly changed phosphorylation levels in muscle within 24 h PM. The high phosphorylation level of heat shock proteins (HSPs) in early PM may be an adaptive response to slaughter stress and protect muscle cell from apoptosis, as observed in the serine 84 of HSP27. This work indicated that PM muscle proteins underwent significant changes at the phosphorylation level but were relatively stable at the total protein level, suggesting that protein phosphorylation may have important roles in meat quality development through the regulation of proteins involved in glucose metabolism and muscle contraction, thereby affecting glycolysis and rigor mortis development in PM muscle.

Biological significance
The manuscript describes the characterization of postmortem (PM) porcine muscle within 24 h postmortem from the perspective of protein phosphorylation using advanced

Abbreviations: PM, postmortem; PSE meat, pale, soft, and exudative meat; LC–MS/MS, liquid chromatography tandem mass spectrometry; TiSH, titanium dioxide (TiO_2)–sequential elution from immobilized metal affinity chromatography (SIMAC)–hydrophilic interaction liquid chromatography (HILIC); H/L and M/L, heavy/light and medium/light; ALDOC, fructose-bisphosphate aldolase C; GP, glycogen phosphorylase; GS, glycogen synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDHA, L-lactate dehydrogenase A; PGM, phosphoglucomutase; PK, pyruvate kinase; TPI, triosephosphate isomerase; TPM, tropomyosin; PHKB, phosphorylase b kinase subunit beta; PGAM, phosphoglycerate mutase; MYH, myosin heavy chain; MYL2, myosin regulatory light chain 2; HSP, heat shock protein.

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phosphoproteomic techniques. In the study, the authors employed the dimethyl labeling combined with the TiSH phosphopeptide enrichment and LC–MS/MS strategy.
This was the first high-throughput quantitative phosphoproteomic study in PM muscle of farm animals. In the work, both the proteome and phosphoproteome were analyzed, and the large number of identified peptides, phosphopeptides and phosphorylation sites can greatly enrich the current farm animal protein database.
The proteins involved in glycometabolism, muscle contraction and heat shock proteins (HSPs) showed significantly changed phosphorylation levels during PM meat development. This work indicated that PM muscle proteins underwent significant changes at phosphorylation level but were relatively stable at the total protein level, suggesting that protein phosphorylation may have important roles in meat development through the regulation of proteins involved in metabolism and muscle contraction, thereby affecting glycolysis and rigor mortis development in PM muscle.
The work can promote the understanding of PM muscle metabolism and meat quality development, and be helpful for future meat quality control.

1. Introduction

During the conversion of muscle into meat, the postmortem (PM) metabolism in muscle within 24 h PM undergoes substantial changes, which fundamentally determines most of the important qualities of raw meat, such as ultimate pH, tenderness, color and water-holding capacity [1]. Shortly after slaughter, in response to the lack of oxygen supply and the high rate of ATP consumption, muscle glycogen is degraded (glycogenolysis) and metabolized in anaerobic glycolysis to replenish ATP, and prevent the formation of actomyosin crossbridge. This process also produces lactate, H^+ and heat, leading to the pH decline. As the consumption of ATP exceeds its synthesis from glycolysis, the irreversible formation of actomyosin crossbridges increases muscle tension, and such muscle contraction cannot be released due to the lack of ATP to remove the Ca^{2+} from sarcoplasm, indicating the onset of rigor mortis, and rigor mortis is complete with the exhaustion of ATP [1]. The onset of rigor mortis at elevated temperature (39–42 °C) and low pH causes the denaturation of myofibrillar and sarcoplasmic proteins, which results in the pale pork color and reduced water holding capacity [2,3]. Together with glycolysis and rigor mortis in PM muscle within 24 h PM, meat researchers also proposed that programmed cell death or apoptosis before rigor mortis could also regulate the proteolysis and affect the meat tenderness [4,5]. The proteins involved in these biochemical processes are subjected to complicated regulations that significantly contribute to the meat quality development.

Reversible protein phosphorylation plays essential roles in the regulation of critical biological processes including metabolism, signaling transduction, proliferation and differentiation [6,7]. In recent years, large scale phosphoproteomic studies have been performed in different muscle systems and revealed that protein phosphorylation can regulate most of the important processes in muscle, such as metabolism and contraction [8–10]. In PM muscle, numerous studies targeting one or several proteins have revealed that many enzymes and myofibrillar proteins can be regulated by phosphorylation, such as pyruvate kinase [11], AMP-activated protein kinase (AMPK) [12], acetyl-CoA carboxylase (ACC) [13] and myosin regulatory light chain 2 (MYL2) [14], and phosphorylation of these proteins can regulate metabolism and rigor mortis development in PM muscle, thereby affecting the final meat quality. Recently, a gel-based phosphoproteomic strategy was employed by us to analyze the phosphorylation change of sarcoplasmic proteins and myofibrillar proteins [15,16]. The results demonstrated that sarcoplasmic proteins from PM porcine muscle with different pH decline rates showed diverse changes at the phosphorylation level. The phosphorylation changes of several glycolytic enzymes were related to both PM pH decline rate and PM time [15]. However, the phosphorylation pattern of myofibrillar proteins was mainly changed with PM time [16]. The protein phosphorylation has also been found to be related to genetic variation and electrical stimulation in pork and beef by our group [17,18]. Phosphoproteomic studies were also performed on beef with different tenderness [19]. Overall, it is speculated that protein phosphorylation may be involved in the meat quality development through the regulation of metabolic enzyme activity and rigor mortis development.

In this work, with the aim to identify and characterize the phosphorylation sites that change in PM muscle, we performed the quantitative MS-based phosphoproteomic analysis of PM porcine muscle within 24 h PM (1 h, 6 h and 24 h PM) using dimethyl labeling of peptides [20,21] combined with the TiSH strategy for phosphopeptide enrichment and fractionation [22]. The resulting data were analyzed by different bioinformatics tools to explore the phosphorylation events and characterize the phosphoproteins involved in PM meat quality development. To our knowledge, this was the first quantitative phosphoproteomic study in PM muscle of farm animals. Overall, the results suggest a complex change in the pattern of protein phosphorylation in PM porcine muscle. The proteins involved in glucose metabolism and muscle contraction were the two largest clusters of phosphoproteins with significantly altered phosphorylation levels, suggesting that the protein phosphorylation significantly affects glucose metabolism and rigor mortis in PM muscle. The slaughter stress may result in altered phosphorylation level of HSP proteins in early PM. The present findings can promote the understanding of PM muscle metabolism and meat quality development, and be helpful for future meat quality control.