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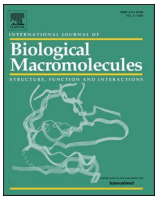

Comparative effects of phosphorylation and acetylation on glycolysis and myofibrillar proteins degradation in postmortem muscle

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ABSTRACT

The study investigated the different effects between protein phosphorylation and acetylation on glycolytic enzyme activity and myofibrillar protein degradation. Lamb *longissimus thoracis lumborum* muscles were homogenized and then inhibitors were added for incubation at 4 °C. Phosphatase inhibitor was added to produce a high phosphorylation level (PI group) and lysine deacetylase inhibitor was added to produce a high acetylation level (DI group). The lactate and ATP content in the PI group was inhibited compared with that in the DI group ($P < 0.05$). Phosphofructokinase (PFK) activity was negatively related with the phosphorylation level and was positively related with the acetylation level in the DI group ($P < 0.05$). The degradation of troponin T and desmin of the DI group were restrained when compared to that in the PI group ($P < 0.05$). Compared with initial PFK and desmin, the simulation of phosphorylation and acetylation of PFK and desmin showed different electrostatic potential at the surface and a more unstable structure. The phosphorylation level of the DI group was increased, suggesting that the changes of protein acetylation altered protein phosphorylation. In conclusion, compared with protein phosphorylation, protein acetylation had a greater effect on promoting glycolysis and inhibiting protein degradation.

1. Introduction

It has been demonstrated that protein posttranslational modification (PTM) is one of the ways to regulate meat quality development [1]. Protein phosphorylation and acetylation are two critical PTMs that are involved in different pathways in the postmortem muscle. The glycolytic enzymes and myofibrillar proteins are identified as the two largest target protein groups for phosphorylation and acetylation [2,3]. About 70 % phosphorylated proteins belong to the pathway of glycolysis and muscle contraction [4]. Phosphorylated glycolytic enzymes play also an important role in color stability in muscle [5]. In addition, 14 glycolytic enzymes and 18 myofibrillar proteins are identified as key acetylproteins in the postmortem porcine muscle, which are related to pH decline and rigor mortis [6]. The phosphorylated and acetylated proteins also interact with other proteins and jointly determine the final meat quality. Protein phosphorylation and acetylation play a very important role in the conversion from muscle to meat through the regulation of glycolysis and muscle contraction. However, whether protein phosphorylation and acetylation have different effects on important biological processes in postmortem meat has not been compared yet.

>600 types of PTMs have been identified [6]. The large amount of PTMs leads to possible interaction among them. The change of one PTM may prevent or prompt another PTMs, which calls PTM crosstalk or interaction [7]. The crosstalk between protein phosphorylation, acetylation, ubiquitination, methylation, S-nitrosylation, glycosylation, etc. has been demonstrated in previous studies [8,9]. PTM interaction regulates protein function more elaborately than a single PTM. Recent years, the importance of PTM interactions has been gradually focused in the field of meat science. Phosphoproteome and acetylome analyses, performed in pork with different preslaughter handling methods, showed that protein phosphorylation and acetylation jointly affect meat quality through different biochemical pathways [10]. The acetylation of myosin heavy chain and actin has seen to inhibit their phosphorylation, which sequentially promoted ATPase activity and then restrained

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actomyosin dissociation [11]. In addition, protein phosphorylation and acetylation interaction of hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PK) shows different co-regulation patterns in lamb with fast versus slow glycolysis rate [12]. Although the above studies did not investigate PTMs crosstalk very strictly, their results proved the possibility of PTMs crosstalk in postmortem meat. The co-regulation of protein phosphorylation and acetylation has become a new topic to explore the mechanism of meat quality formation.

The effect of a single PTM has been widely investigated in previous studies, but the different and cooperative effects between protein phosphorylation and acetylation need further study in meat science. The present study evaluated the effect of phosphorylation and acetylation on glycolytic enzymes activity and myofibrillar protein degradation at the same time to better research the important role of PTMs in meat quality development.

2. Materials and methods

2.1. Sampling and incubation

The carcasses of Small-tailed Han lamb were collected at Hebei Jinhong Halal Meat Co. LTD and the *longissimus thoracis lumborum* (LTL) muscle were collected from carcasses within 30 min after slaughter. The LTL muscle were quickly frozen in liquid nitrogen after removing visible connective tissue and fat and then they were transported to lab and stored at −80 °C. The frozen muscle samples were homogenized on ice with 0.2 mol/L phosphate buffer solution (pH 7.0) according to the ratio of 1:20 (muscle weight: solution volume). Protein concentration of muscle homogenization was adjusted to 3 μg/μL using a BCA assay (Thermo, Rockford, IL, USA). In order to change the protein phosphorylation and acetylation level of protein samples, three treatments were subjected as follows: 1) one table of phosphatase inhibitor (PhosStop, Roche, Germany) was added per 7 mL of muscle homogenization to produce high phosphorylation level (the PI group); 2) 35 μL of lysine deacetylase inhibitor (CUDC-101, Selleck, China) was added per 7 mL of muscle homogenization to produce high acetylation level (the DI group); 3) without adding any inhibitor (the CON group). The three treatments were incubated at 4 °C for 1 h, 3 h, 10 h and 24 h in a shaking bath with 600 rpm/min (Hangzhou Allsheng Instruments Co., Ltd., Hangzhou, China). All samples were collected on time and stored at −80 °C until analysis.

2.2. Activity analysis of HK, PFK and PK

Three kinds of commercial kits were used to measure the activity of HK, PFK and PK in the three groups, respectively. Sample solutions were mixed according to the manufacturers' protocol (Solarbio Life Science, Beijing, China). The absorbances of 340 nm were evaluated to investigate the activity of HK, PFK and PK.

2.3. Measurement of lactate and ATP content

The collected solutions at 24 h of incubation were used for analyzing lactate and ATP content. Thirty microliter sample solutions were used to measure the lactate content using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and the absorbance at 530 nm was recorded for calculation. Another 30 μL sample solutions were used to detect ATP content using a commercial kit (Solarbio Life Science, Beijing, China) and the absorbance at 340 nm was documented for calculation.

2.4. Protein phosphorylation and acetylation level

Protein phosphorylation and acetylation levels were detected by fluorescence staining and western blotting [13]. Briefly, gels were stained by Pro-Q Diamond dye (Invitrogen, Eugene, OR, USA) and SYPRO Ruby dye (Invitrogen) to image phosphorylated protein and total protein, respectively. The ratio of band intensity of phosphorylated protein and total protein was regarded as relative protein phosphorylation level.

Western blots were run at 100 V for 200 min after electrophoresis to analyze protein acetylation level. Mouse anti-acetyllysine (dilution 1:1000, PTM-101, PTM, Hangzhou, China) was the primary antibody and HRP goat anti-mouse IgG (dilution 1:1000, 7076S, CST, Danvers, MA, USA) was the secondary antibody. The signals of acetylated protein in membranes were firstly visualized by ECL Substrate (Bio-Rad, Hercules, CA). Then, the membranes were stained with Coomassie blue to quantify total protein. The ratio of band intensity of acetylated protein and total protein was regarded as relative protein acetylation level.

2.5. Protein degradation and cleaved caspase 3

Sample solutions at 24 h of incubation were mixed with the same volume of loading buffer before electrophoresis. The western blot was performed as described by the previous study [12] with minor modifications. Briefly, 10 % of stain-free gels were used and western blotting was performed at 250 mA for 150 min. Total protein imaged by stain-free gel was regarded as the reference protein and used for quantification. Mouse anti-troponin T (dilution 1:1000, ab130003, Abcam, Cambridge, UK), rabbit anti-desmin (dilution 1:1000, A0699, ABclonal, Wuhan, China) and rabbit anti-caspase-3 (dilution 1:1000, A0214, ABclonal) were primary antibodies. HRP goat anti-mouse IgG (dilution 1:2000, AS003, ABclonal) and HRP goat anti-rabbit IgG (dilution 1:2000, AS014, ABclonal) were secondary antibodies.

2.6. Molecular dynamics simulation

The molecular dynamics simulation was performed in BIOVIA Discovery Studio 2019 (Neotrident Technology LTD., Beijing, China) to analyze the effect of PTMs on protein structure through referencing the method of Cao et al. [14]. Firstly, the sequences of PFK (W5QDD4) and desmin (W5QG29) were obtained from the UniprotKB database and homology modeling of their initial 3D structure was created. Secondly, the phosphorylated or acetylated PFK and desmin was created based on their initial model, respectively. The mimicking mutation of phosphorylation and acetylation were referenced by the previous studies [15,16]. Phosphate group is negatively charged, so the amino acid with negative charge was used to simulate phosphorylation. When the negative charged lysine is acetylated by acetyl group, it will transfer to a neutral charged lysine, so the amino acid with neutral charge was used to simulate acetylation. Specifically, serine (Ser) and threonine (Thr) were mutated to aspartic acid (Asp) and tyrosine (Tyr) was mutated to glutamic acid (Glu) to simulate phosphorylation state. Lysine (Lys) was mutated to glutamine (Gln) to simulate acetylation state. There were 8 phosphorylated sites (Ser 448, Ser 529, Ser 742, Thr 264, Thr 265, Thr 561, Thr 779, Tyr 530) and 17 acetylated sites (Lys 178, Lys 212, Lys 215, Lys 343, Lys 351, Lys 443, Lys 466, Lys 551, Lys 690, Lys 731, Lys 753, Lys 757, Lys 765, Lys 813, Lys 819, Lys 825, Lys 829) of PFK being mutated. There were 8 phosphorylated sites (Ser 358, Ser 361, Ser 301, Ser 28, Ser 31, Ser 32, Thr 59, Tyr 363) and 11 acetylated sites (Lys 109, Lys 43, Lys 125, Lys 378, Lys 240, Lys 241, Lys 297, Lys 299, Lys 318, Lys 309, Lys 449) of desmin being mutated. The selection of mutated sites of PFK and desmin in this study was based on the LC-MS/MS results which was from the unpublished data. Thirdly, initial PFK, phosphorylated PFK, acetylated PFK, initial desmin, phosphorylated desmin and acetylated desmin were proceeded by protein preparation, solvation and standard dynamics cascade. The targeted pH of protonation was set as 7.0 and temperature of equilibration was set as 277.15 K (4 °C), which were consistent with incubation system in the present study. Other parameters were set as default value.

2.7. Statistical analysis

Data were reported as means ± standard deviation and analyzed by one-way ANOVA in SPSS Statistic 22.0 (SPSS Inc., Chicago, IL, USA). Differences between comparisons were performed using the Duncan's multiple range test ($P < 0.05$). The images of electrophoresis and western blotting were quantified using Quantity One 4.6.2 (Bio-Rad). Principal component analysis (PCA) and Pearson's correlation analysis were analyzed by Origin 2021 (OriginLab Co., USA).

3. Results

3.1. Phosphorylation and acetylation level of total protein in three treatments

Phosphatase inhibitor and lysine deacetylase inhibitor were added to incubation systems for changing protein phosphorylation and acetylation levels of homogenated muscle samples. The protein phosphorylation level of the PI group was significantly increased at 1 h, 3 h and 24 h compared with that of the CON group ($P < 0.05$, Fig. 1B). The protein acetylation level of the DI group was higher than that of the CON group at 3 h and 24 h ($P < 0.05$, Fig. 1D). It was suggested that adding inhibitors effectively adjusted protein phosphorylation and acetylation status which was the basis of the present study to investigate their effects. In addition, the results of Zhang et al. [11] suggested that protein phosphorylation and acetylation might influence each other. In the present study, thereby, the protein acetylation level in the PI group and protein phosphorylation level in the DI group were also measured respectively to investigate whether interactive effects of protein phosphorylation and acetylation exist. Protein phosphorylation level of the DI group was increased in 1 h and 3 h after adding lysine deacetylase inhibitor ($P < 0.05$, Fig. 1B), suggesting that the deacetylation inhibition possibly prompted phosphorylation. However, when adding phosphatase inhibitor to raise protein phosphorylation level in PI group, its acetylation level was insignificant different from the CON group ($P > 0.05$, Fig. 1D). The results showed a potential interactive effect between protein phosphorylation and acetylation under external modulation.

3.2. The effects of protein phosphorylation and acetylation on glycolysis

The lactate content, ATP content and activity of three glycolytic rate-limiting enzymes were analyzed to evaluate the effects of protein phosphorylation and acetylation on glycolysis. The lactate content was highest in the DI group and lowest in the PI group at 24 h ($P < 0.05$, Fig. 2A), showing an opposite effect of protein phosphorylation and acetylation on final glycolysis. Compared with the CON and DI group, the ATP content in the PI group was lower at 24 h ($P < 0.05$, Fig. 2B). HK displayed a repressed activity in the PI group when compared to that in the DI group at 3 h and 24 h ($P < 0.05$, Fig. 3A). The activity of PFK in the PI group was raised at the beginning of the incubation period at 1 h

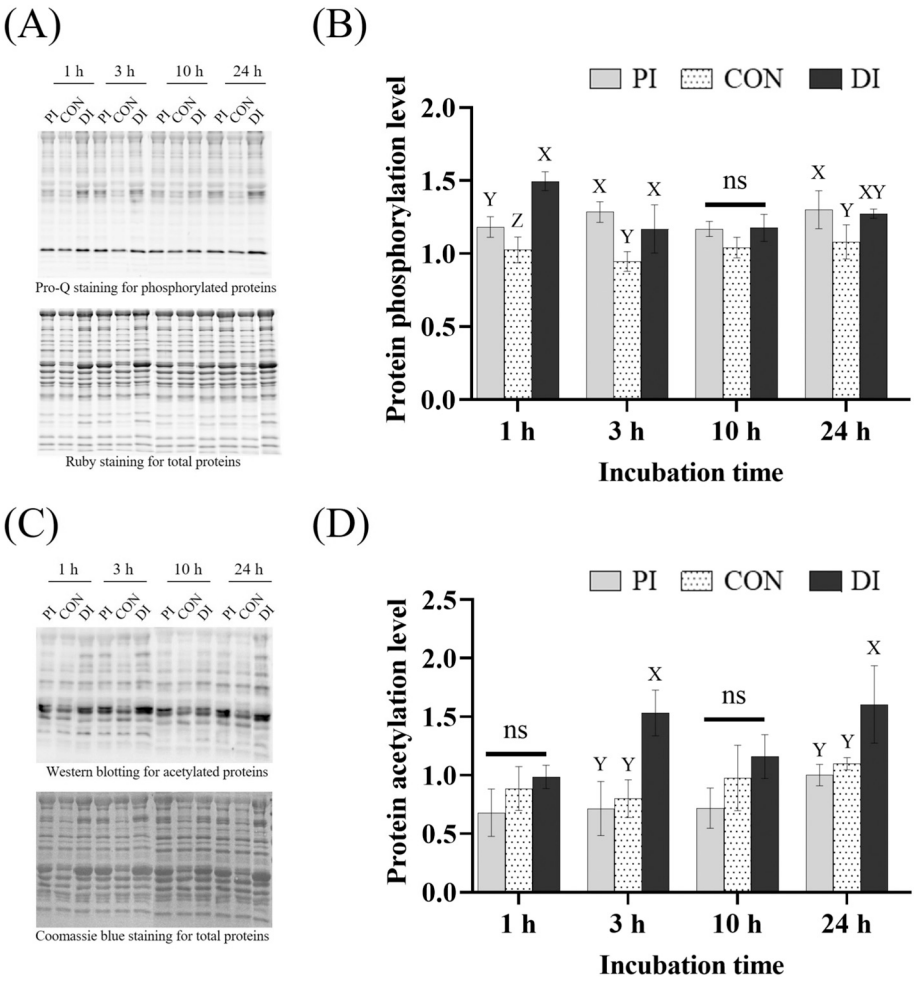


Fig. 1. Phosphorylation and acetylation level of muscle proteins in the three groups during incubation. (A) The images of pro-Q staining for phosphorylated proteins and ruby staining for total proteins. (B) Protein phosphorylation level of muscle proteins. (C) The images of western blotting for acetylated proteins and Coomassie blue staining for total proteins. (D) Protein acetylation level of muscle proteins. X-Z means significant differences at the same incubation time between three groups ($P < 0.05$). "ns" $P > 0.05$. PI: phosphatase inhibitor group. CON: control group. DI: deacetylase inhibitor group.

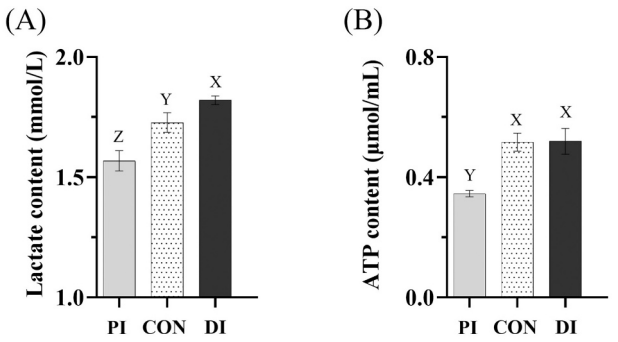


Fig. 2. The lactate content (A) and ATP content (B) after adding phosphatase inhibitor and deacetylase inhibitor in the three groups at 24 h incubation. X-Z means significant differences at the same incubation time between three groups ($P < 0.05$). PI: phosphatase inhibitor group. CON: control group. DI: deacetylase inhibitor group.

and 3 h ($P < 0.05$, Fig. 3B) and gradually decreased to the lowest value at 24 h. The changes of PFK activity in the CON and DI group were relatively stable compared to that in the PI group (Fig. 3B). However, PK activity in the PI group was lower in 1 h, 3 h and 10 h compared to that in the CON and DI groups ($P < 0.05$, Fig. 3C), and there were no significant differences between three groups at 24 h ($P > 0.05$, Fig. 3C).

A correlation analysis was performed to better know the relationship between enzyme activity and protein phosphorylation and acetylation. PFK activity in the PI group indicated a negative relationship with protein phosphorylation level ($P < 0.05$, Fig. 4A). There was a positive relationship between PK activity and phosphorylation level ($P < 0.05$, Fig. 4B). HK activity was positive correlated with protein acetylation level in the DI group ($P < 0.05$, Fig. 4C). There was a negative correlation between PFK activity and protein phosphorylation level but a positive correlation between PFK activity and protein acetylation level ($P < 0.05$, Fig. 4C). Thus, various relationship patterns could be identified due to the different adjusted PTM levels in the three groups.

3.3. The effects of protein phosphorylation and acetylation on protein degradation and apoptosis

The abundance of troponin T, desmin and caspase 3 at 24 h in three groups was measured. The relative abundance of troponin T in the DI group was higher than that in the PI and CON group ($P < 0.05$, Fig. 5A), showing that troponin T degradation was inhibited in the DI group. The relative abundance of desmin was lowest in the PI group and highest in the DI group ($P < 0.05$, Fig. 5B), which suggested a possible accelerated degradation in high phosphorylation level and restrained degradation in high acetylation level. The abundance of cleaved caspase 3 determines its effect on apoptosis. However, no significant differences existed among three groups in the present study ($P > 0.05$, Fig. 5C).

3.4. The effects of protein phosphorylation and acetylation on PFK and desmin based on molecular dynamics simulation

The structural changes of phosphorylated and acetylated PFK and desmin were simulated, respectively. The electrostatic surface potential was markedly different between phosphorylated and acetylated PFK (Fig. 6A). Compared with phosphorylation, the acetylation of PFK in the III area led to the negatively charged surface potential, but the acetylation of PFK in the IV area led to the positively charged surface potential (Fig. 6A). The total energy of phosphorylated and acetylated PFK was higher than that of initial PFK (Fig. 6B). The percentage of helix of initial PFK was 44 %, while it decreased to 41 % and 42 % respectively after phosphorylated and acetylated (Fig. 6B). The phosphorylation of desmin in the I area turned to the negatively charged surface potential, and the acetylation of desmin in the IV area turned to the negatively charged surface potential (Fig. 6C). When desmin was phosphorylated and acetylated, its total energy decreased compared with that of initial desmin (Fig. 6D). The content of helix, sheet and loop changed ±1 % between initial desmin and phosphorylated or acetylated desmin (Fig. 6D).

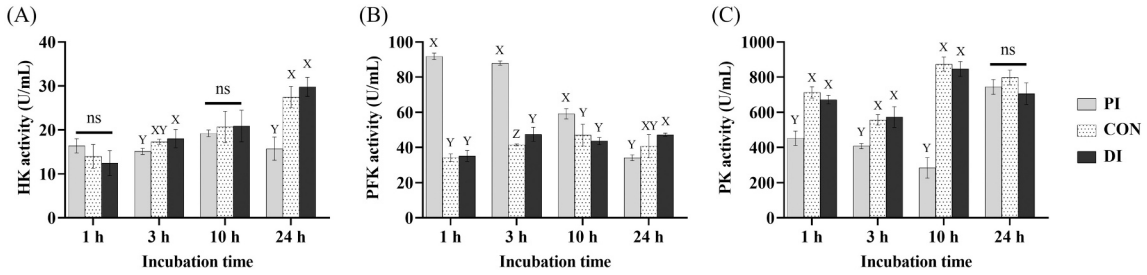


Fig. 3. The activity of HK (A), PFK (B) and PK (C) after adding phosphatase inhibitor and deacetylase inhibitor in the three groups during incubation. X-Z means significant differences at the same incubation time between three groups ($P < 0.05$). "ns" $P > 0.05$. HK: hexokinase. PFK: phosphofructokinase. PK: pyruvate kinase. PI: phosphatase inhibitor group. CON: control group. DI: deacetylase inhibitor group.

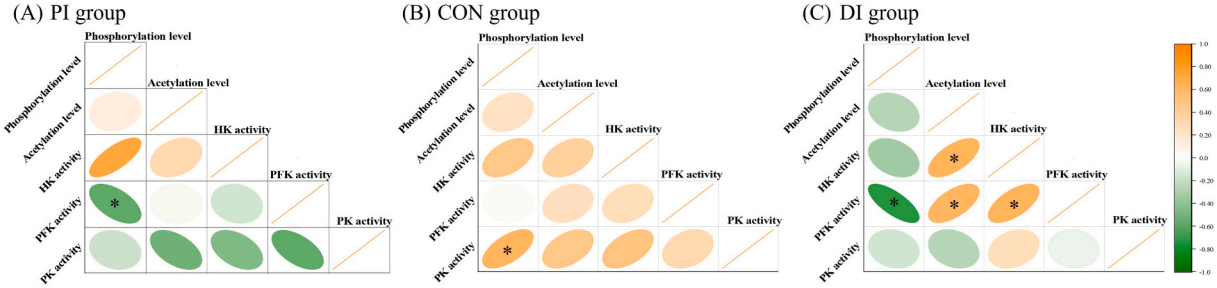


Fig. 4. Correlation analysis between phosphorylation level, acetylation level, HK activity, PFK activity and PK activity in PI group (A), CON group (B) and DI group (C). The bigger the ellipse, the larger the correlation coefficient. The orange color represents a positive correlation, the green color represents a negative correlation. "*" $P < 0.05$. HK: hexokinase. PFK: phosphofructokinase. PK: pyruvate kinase. PI: phosphatase inhibitor group. CON: control group. DI: deacetylase inhibitor group.

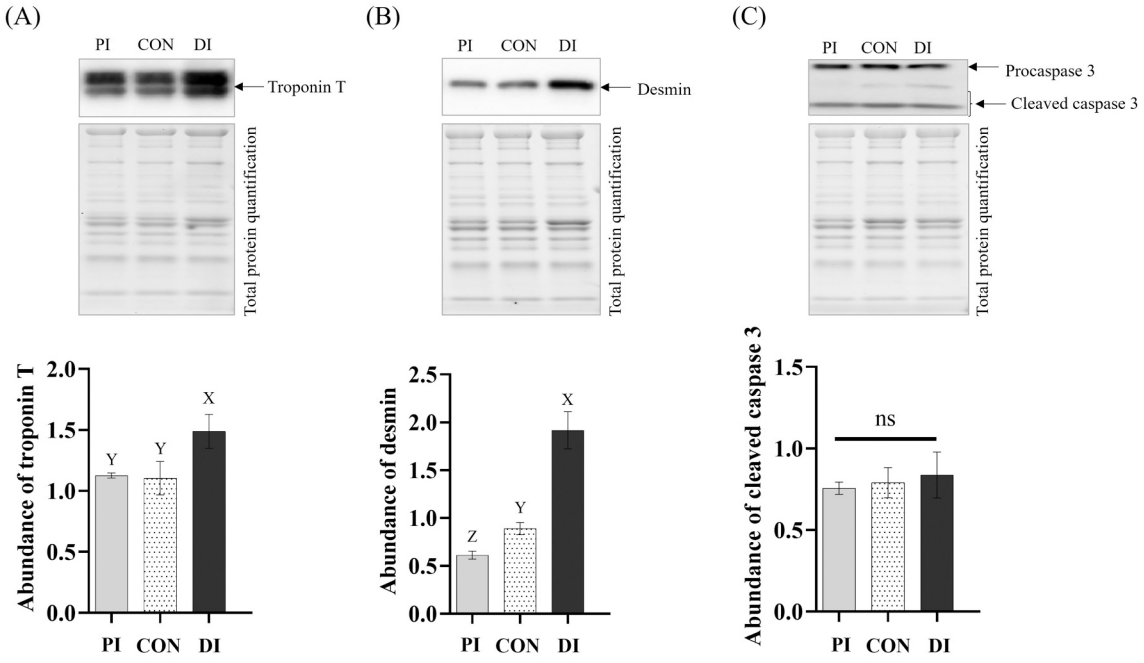
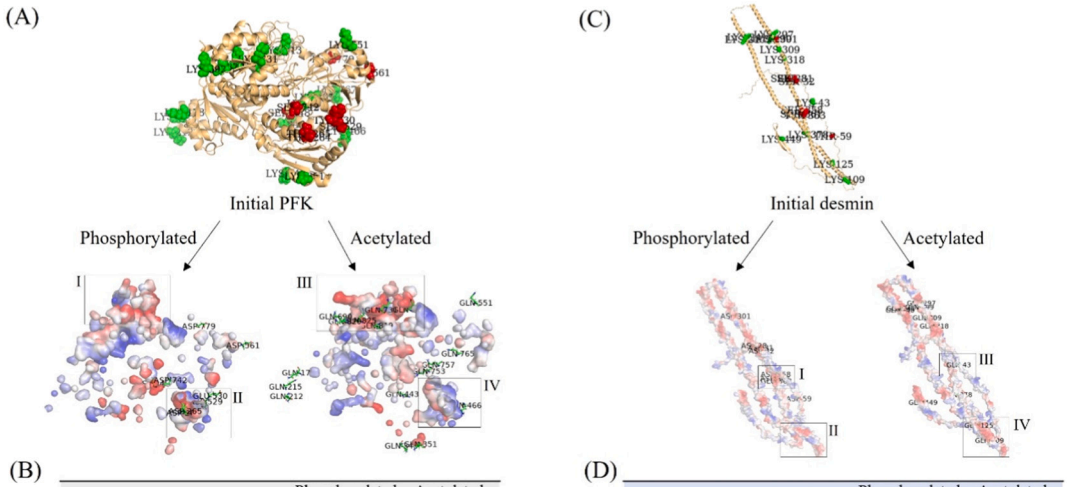


Fig. 5. The relative abundance of troponin T (A), desmin (B) and cleaved caspase 3 (C) after adding phosphatase inhibitor and deacetylase inhibitor in thee three groups at 24 h incubation. Total protein was regarded as the reference protein and used for quantification. X-Z means significant differences at the same incubation time between three groups ($P < 0.05$). "ns" $P > 0.05$. HK: hexokinase. PFK: phosphofructokinase. PK: pyruvate kinase. PI: phosphatase inhibitor group. CON: control group. DI: deacetylase inhibitor group.



(B)

	Initial PFK	Phosphorylated PFK	Acetylated PFK
Total Energy (kcal/mol)	-212578	-213090	-212961
Helix (%)	44	41	42
Sheet (%)	14	14	13
Loop (%)	42	45	45

(D)

	Initial desmin	Phosphorylated desmin	Acetylated desmin
Total Energy (kcal/mol)	-585361	-587649	-586135
Helix (%)	66	67	67
Sheet (%)	5	4	5
Loop (%)	29	29	28

Fig. 6. Molecular dynamics simulation of phosphorylated and acetylated PFK and desmin. (A) The electrostatic potential in surfaces of phosphorylated and acetylated PFK. (B) The total energy, helix (%), sheet (%) and loop (%) of different states PFK. (C) The electrostatic potential in surfaces of phosphorylated and acetylated desmin. (D) The total energy, helix (%), sheet (%) and loop (%) of different states desmin. The 3D structures of initial PFK and desmin were displayed in light orange. Mutated phosphorylated sites in initial PFK and desmin were displayed with red spheres. Mutated acetylated sites in initial PFK and desmin were displayed with green spheres. Significant changed area of surfaces electrostatic potential between phosphorylated PFK and acetylated PFK, phosphorylated desmin and acetylated desmin were marked with boxes. Mutated sites were displayed with sticks in phosphorylated and acetylated PFK and desmin. Molecular dynamics simulation was performed by BIOVIA Discovery Studio 2019. Visualization of surfaces electrostatic potential was processed by Pymol 2.5. PFK: phosphofructokinase.

4. Discussion

Protein phosphorylation and acetylation influence meat quality. Phosphorylation of sarcoplasmic proteins has been found to be related with pH decline, color stability and tenderness [1]. Myofibrillar protein phosphorylation is greatly linked with muscle contraction and

tenderization [17]. Protein acetylation regulates water holding capacity and other quality attributes during early postmortem [18]. However, previous studies mainly evaluated the effects of a single PTM on meat quality, the comparative effects of protein phosphorylation and acetylation have not been studied. In the present study, protein acetylation had greater effect on promoting glycolysis and inhibiting myofibrillar protein degradation when compared to protein phosphorylation. Protein acetylation is a well-known PTM which is dominant in regulating energy metabolism [19]. Energy metabolism converts aerobic glycolysis to anaerobic glycolysis after slaughter of animals. The changes of energy metabolism are most dramatic and fast during early period of postmortem. Thus, protein acetylation might have influence on meat quality development though energy metabolism regulation.

Recently, a few studies started focusing on the interaction of protein phosphorylation and acetylation in meat science. For example, lysine deacetylase inhibitors (for inducing high acetylation level) and lysine acetyltransferase inhibitors (for inducing low acetylation level) were respectively added to postmortem muscle samples, then phosphorylation level of myosin heavy and actin were also measured [11]. The consequent results suggested that the inhibition of protein phosphorylation might increase the acetylation of myosin heavy chain and actin. Thus, the adjustment of one kind of PTM through adding inhibitors might affect another kind of PTM in the same incubation system. The protein acetylation level of the PI group and phosphorylation level of the DI group were also measured in the present study to investigate the possible interactive effect between these two PTMs. The acetylation level in the PI group was not different after adding phosphatase inhibitor for regulating the high phosphorylation level (Fig. 1D). However, it was unexpected that the phosphorylation level in the DI group was also increased in the present study (Fig. 1B). The results of a previous study showed that acetylation of renal tubular epithelial cells was required for its subsequent phosphorylation [20], which might explain the increasing phosphorylation level in the DI group in the present study. However, another previous study displayed that the increased acetylation caused by CUDC-101 inhibited EGFR (epidermal growth factor receptor) phosphorylation in tumor cells [21], which was opposite with our result. There were two possible reasons for the different results: 1) the EGFR phosphorylation caused by CUDC-101 was in a dose-dependent way; 2) only EGFR phosphorylation level was detected but the total phosphorylation level was not evaluated in the previous study. The result of the present study showed that the change in protein acetylation might alter protein phosphorylation but is limited to infer the PTMs crosstalk yet.

Glycolysis is one of the most vital biochemical pathways in meat quality development [22]. HK, PFK and PK are three rate-limiting enzymes, whose activities are affected by protein phosphorylation and acetylation and subsequently determine final glycolysis. PFK is regarded as the most important regulator among these three enzymes. The PFK gene is highly involved in meat color, marbling, intramuscular fat and water moisture [23]. In this study, we show that there is a negative effect of total protein phosphorylation level and a positive effect of total acetylation level on PFK activity. From the results of previous studies, it was found that the activity of PFK was determined by its phosphorylation and acetylation [24,25]. PK is responsible for the last step of glycolysis, which also has a great impact on meat quality. Protein phosphorylation of PK isoform 3 displayed very high activity in PSE (pale, soft and exudative) meat which accelerated pH decline and meat quality deterioration [26]. A previous study determined the phosphorylation level of glycolytic rate-limiting enzymes after adding kinase and phosphatase inhibitors to postmortem meat [27]. The results of the phosphorylation level of glycolytic rate-limiting enzymes were consistent with the global phosphorylation. Thus, adding inhibitors not only changed the global PTM level, but also altered the specific protein PTM level. In addition, the incubation system was complex in the present study, leading to a limitation to clarify that the changes in enzyme activity come from the direct phosphorylation or acetylation or a by-product of upstream or downstream regulators. In further study, the inhibitors will be incubated with HK, PFK and PK respectively to change the phosphorylation and acetylation of them straightly to investigate the effect of PTM on enzyme activity in the further study. The technology of LC-MS/MS will be applied to identified the effective phosphorylated and acetylated sited of HK, PFK and PK.

Protein degradation is very closely related with meat tenderness. The degradation of troponin T and desmin is regarded as marker for tenderization [17]. The result of a previous study showed that phosphorylation of myofibrillar proteins restrained their degradation through decreasing proteolysis by μ-calpain [28]. In another study, serine phosphorylation of desmin increased its susceptibility to proteolysis and induced its greater degradation [29]. Compared with protein acetylation in the present study, protein phosphorylation promoted the degradation of troponin T and desmin. It was speculated that the addition of different phosphorylation inhibitors might affect diverse sites of targeted proteins and then changed their specific function. Sumandea et al. [30] investigated four main mutation sites in troponin T and they found that only Thr206 phosphorylation functionally regulated myofilament status. Compared with the wide studies of protein phosphorylation, the effect of protein acetylation on troponin T and desmin was less investigated. Although some acetylated sites of troponin T and desmin were identified [2], their functional roles have not been revealed completely. In the present study, it was found that protein acetylation of troponin T and desmin inhibited their degradation. It might be a possible reason that protein acetylation reduced the recognition of troponin T and desmin to proteolytic systems like μ-calpain and the proteasome. In addition, protein half-life is a better indicator to demonstrate protein degradation process, which reflects the protein stability [31]. The determination of protein half-life is supposed to be performed in the further study.

Apoptosis is an emerging topic about meat quality regulation in recent years. Apoptosis has great effect on many meat quality traits such as color, tenderness and water holding capacity [32]. Caspase 3 is a critical endogenous enzyme regulating apoptosis process in postmortem muscle. A previous study reported that high protein phosphorylation level was related to low caspase 3 activity [33]. Protein acetylation also affects the cleavage of caspase 3 [34]. However, the abundance of cleaved caspase 3 displayed an insignificant difference in the present study (Fig. 5C). The effect of PTM crosstalk on caspase 3 is different with that of a single PTM on caspase 3.

Some studies have demonstrated that PTMs affect protein properties through the technology of molecular simulation [11,14,35]. The charge distribution is important for interactions of targeted proteins with others, which determines the processes of crucial biochemical pathways [36]. The different charge distribution between phosphorylated and acetylated PFK might lead to the various activities in the PI and DI group. The total energy is an indicator to represent the structure stability. The results showed that phosphorylation and acetylation of PFK made their structure unstable. Compared with initial PFK, the ratio of the helix of phosphorylated and acetylated PFK was decreased, also suggesting a less stable structure of PFK. The total energy of phosphorylated and acetylated desmin was higher than that of initial desmin, but there was a subtle change of the secondary structure, which suggests the unimportant difference between the effect of phosphorylation and acetylation on secondary structure of desmin. Moreover, phosphorylation increased negative charge and acetylation changed to neutral charge, which might affect the identification of proteases on desmin and thus impacted its degradation. The previous studies changed the PTMs level of titin or μ-calpain first and then identified their PTMs sites, which is worthy to be referenced [37,38]. A further vitro study should be performed to change the PTMs level of PFK and desmin respectively and subsequently identify the PTMs sites of them, thus to better understanding the effect of PTMs on PFK and desmin.

5. Conclusion

The phosphorylation and acetylation levels of muscle proteins were effectively regulated by adding inhibitors. This study showed a different effect of phosphorylation and acetylation on glycolysis and myofibrillar proteins degradation. Compared with protein phosphorylation, protein acetylation increased the glycolysis process and inhibited the degradation of troponin T and desmin. Besides, the changes of protein acetylation affected protein phosphorylation of muscle proteins. The possible PTMs crosstalk in postmortem meat may complexly regulate the biochemical pathways.

CRediT authorship contribution statement

Chi Ren: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Li Chen:** Funding acquisition, Project administration, Writing – review & editing. **Yuqiang Bai:** Formal analysis. **Chengli Hou:** Writing – review & editing. **Xin Li:** Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing – review & editing. **Martine Schroyen:** Writing – review & editing. **Dequan Zhang:** Conceptualization, Data curation, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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