CROSSTALK BETWEEN PHOSPHORYLATION AND ACETYLATION OF PYRUVATE KINASE REGULATE ITS ACTIVITY *IN VITRO*

Xiaolan Huang, Xin Li, Li Chen*, and Dequan Zhang

Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences/Key Laboratory of Agro-Products Processing, Ministry of Agriculture and Rural Affairs

*Corresponding author email: chenliwork@126.com

I. INTRODUCTION

Glycolysis is the main energy metabolism in the post-mortem meat. Pyruvate kinase is one of the rate-limiting enzymes of glycolysis pathway, and its activity affects the glycolytic rate [1]. Excessive glycolytic rate leads to PSE (pale, soft, exudative) meat, and insufficient glycolysis rate leads to DFD (dark, firm and dry) meat, which will cause serious economic losses [2]. Early research have found that the crosstalk of protein post-translational modifications (PTMs) can affect energy metabolism, muscle contraction, and stress response and meat quality by changing the structure and function of proteins [3]. The activity of pyruvate kinase was influenced by the acetylation and phosphorylation crosstalk, but the regulatory mechanism is not well known. This study aimed to investigate the effect of crosstalk between phosphorylation and acetylation of pyruvate kinase on its activity by constructing an *in vitro* model containing pyruvate kinase with different levels of phosphorylation and acetylation.

II. MATERIALS AND METHODS

The rabbit muscle pyruvate kinase was used to construct treatment groups with different levels of phosphorylation and acetylation: 1) group P: exogenous kinase inhibitors and deacetylation inhibitors, 2) group A: exogenous protein kinase A and acetyltransferase inhibitors, and 3) group C: control. The amount of ATP and acetyl-coA was 2 μM/100 μg protein and 1 mM in each group. The final volume was adjusted to 5 mL with incubation bufferr (10 mM $MgCl_2$, 10 mM KCl, 10 mM DL-ditiothreitol (DTT), 50 mM Tris, pH 6.8).The three groups were incubated at 30 ℃ for 60 min. The samples were collected during incubation at 15 and 60 min, respectively. The phosphorylation levels of pyruvate kinase were measured by SDS-PAGE and fluorescent staining. The acetylation levels of pyruvate kinase were measured by Western blotting. The activities of pyruvate kinase were measured using a commercial kit (BC0545, Beijing Solaibao Technology Co., LTD, China). The data were analyzed by SPSS Statistic 21.0 (IBM Corporation, Armonk, USA). The data were exhibited as the means ± standard deviations. The significance of the difference was set as $P<0.05$.

III. RESULTS AND DISCUSSION

The results of pyruvate kinase phosphorylation and acetylation are shown in Figure 1.The results of Pro-Q Diamond staining showed that the gray values of protein bands in the group P gradually decreased during incubation *in vitro*, while the gray values of protein bands in the group A gradually increased, indicating that acetylation could inhibit pyruvate kinase phosphorylation. However, there was no significant difference in the phosphorylation level of pyruvate kinase between the P and A groups. With the increase of incubation time, there was no significant change in pyruvate kinase activity in the P and A groups, but it was significantly increased in the control group ($P<0.05$), as shown in Figure 2. The activity of pyruvate kinase in the control group was significantly higher than that in P and A groups at the same incubation time ($P<0.05$). The results indicated that the crosstalk between phosphorylation and acetylation in the system inhibited the activity of pyruvate kinase. It may be that the interaction between phosphorylation and acetylation changes the force between Mg^{2+} and K^{+} and the active site of pyruvate kinase, which changes the active conformation of the enzyme, resulting a decreasing in enzyme activity [4].

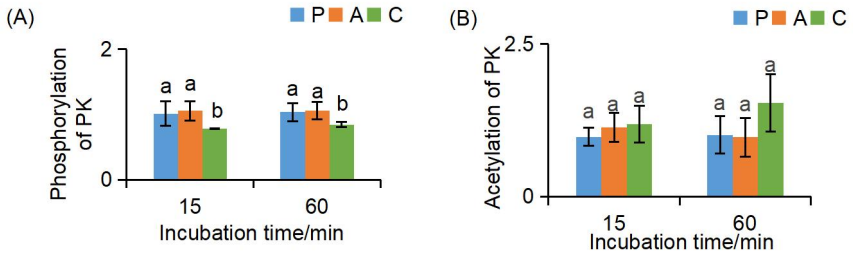

Figure 1. Relative phosphorylation andacetylation level of pyruvate kinase with different phosphorylation and acetylation levels groups during incubation *in vitro*. P: exogenous kinase inhibitors and deacetylation inhibitors group. A: exogenous protein kinase A and acetyltransferase inhibitors group. C: control. a-b: different letters at the same time points are significantly different between groups (P<0.05).

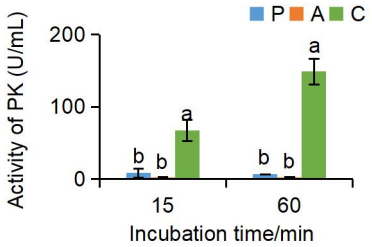

Figure 2. Activity of pyruvate kinase with different phosphorylation and acetylation levels groups during incubation *in vitro*. P: exogenous kinase inhibitors and deacetylation inhibitors group. A: exogenous protein kinase A and acetyltransferase inhibitors group. C: control. a-b:different letters at the same time points are significantly different between groups (P<0.05).

IV. CONCLUSION

The crosstalk between phosphorylation and acetylation can inhibit pyruvate kinase activity. This study provides a theoretical basis for the development of precise control technology of fresh meat quality, so as to reduce the economic loss caused by meat quality deterioration.

ACKNOWLEDGEMENTS

This study was financially supported by the General Program from National Natural Science Foundation of China (32072144).

REFERENCES

1. Schormann, N., Hayden K. L., Lee, P., Banerjee, S. & Chattopadhyay, D. (2019). An overview of structure, function, and regulation of pyruvate kinases. Protein Science 28(10):1771-1784.
2. Chauhan, S. S. & England, E. M. (2018). Postmortem glycolysis and glycogenolysis: insights from species comparisons[J]. Meat Science 144: 118-126.
3. Li, X., Zhang, D., Ren, C., Bai, Y., Ijaz, M., Hou, C. & Chen, L. (2021). Effects of protein posttranslational modifications on meat quality: A review. Comprehensive Reviews in Food Science and Food Safety 20(1): 289-331.
4. Leticia, R. S., Gloria, H. A., Carlos, G. C., Martin, G. A., A. R. R., Annia, R. H., Alan, L. M., Paul, G. C., Cristina, R. M. & Alicia V S. (2022). The K^+-Dependent and -Independent Pyruvate Kinases Acquire the Active Conformation by Different Mechanisms. International Journal of Molecular Sciences 23(3): 1347.