AMP is a more potent activator of phosphofructokinase-1 activity than fructose 2,6-bisphospate in porcine skeletal muscle under simulated postmortem conditions

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Abstract - Phosphofructokinase-1 (PFK-1) is a key regulatory enzyme of postmortem glycolysis. PFK-1's activity is regulated antemortem by a number of compounds including adenosine monophosphate (AMP) and fructose 2,6-bisphosphate (F-2,6-BP). However, PFK-1's postmortem regulation by AMP and F-2,6-BP is still unclear. Therefore, a study was conducted where porcine *longissimus lumborum* samples were collected to determine PFK-1 activity as affected by various concentrations of AMP and F-2,6-BP at buffered pH. Both compounds increased PFK-1 activity. However, at physiological concentrations, 50 and 150 µM AMP increased PFK-1 activity compared to 1 and 2 µM F-2,6-BP. Thus, AMP may play a greater role in dictating the rate and extent of postmortem glycolysis and pH decline than F-2,6-BP.

Key Words – enzyme**,** glycolysis, pH

I. INTRODUCTION

After an animal is harvested for meat, the skeletal muscle attempts to maintain ATP through glycogenolysis and glycolysis [1]. The regulation of these biochemical processes are critical in determining the quality of meat as rapid and excessive glycolysis leads to the development of inferior meat resulting in significant economic loss to the meat industry [2]. Phosphofructokinase-1 (PFK-1) is the most important enzyme controlling glycolysis in living skeletal muscle and is the most likely candidate for regulation of postmortem glycolysis [3]. Therefore, understanding PFK-1's regulation is critical to understand the underlying mechanism responsible for the development of meat quality attributes. PFK-1's activity is regulated antemortem by a number of compounds including adenosine monophosphate (AMP) and fructose 2,6-bisphosphate (F-2,6-BP). A previous report indicated that in rapidly glycolysing postmortem porcine skeletal muscle, both AMP and F-2,6-BP were increased resulting in accelerated and extended postmortem glycolysis and pH decline [4]. However, it was unclear if PFK-1 was regulated postmortem by one or both compounds. Therefore, the present study was conducted to elucidate the regulation of PFK-1 activity by F-2,6-BP and AMP under simulated postmortem conditions in porcine skeletal muscle.

II. MATERIALS AND METHODS

Six market weight pigs (100–125 kg) were harvested at The Ohio State Meat Center under USDA-FSIS inspection. Samples were excised from the *longissimus lumborum* at 5 min post-slaughter, snap frozen in liquid nitrogen, and stored at −80°C. Phosphofructokinase activity was assayed as previously reported [3] in the presence of AMP and F-2,6-BP at buffered pH. Specifically, increasing concentrations of F-2,6-BP (1 µM, 2 µM, 10 µM, and 50 µM) or AMP (1 µM, 2 µM, 10 µM, 50 µM, and 150 µM) were added to the buffer adjusted to different pH 5.5, 6.0, 6.5 and 7.0. Enzymatic activity was measured in triplicate spectrophotometrically at 340 nm and reported as a relative activity increase (%) compared to baseline PFK-1 activity with no activator present. For the in vitro muscle glycolysis study, *longissimus lumborum* samples were powdered in liquid nitrogen and homogenized at a 1:10 ratio (wt/vol) in muscle glycolysing buffer [3, 5]. F-2,6-BP was added to the buffer to achieve an initial concentration of 0 µM, 1 µM, and 2 µM in order to replicate previously reported levels in postmortem porcine skeletal muscle [4]. Reaction vessels were placed at 25°C on a heating block, and aliquots were removed at 0, 30, 60, 120, 240, and 1440 min for pH analysis. In both studies, data were analyzed with a mixed model in JMP (SAS Institute Inc., Cary, NC). Each animal was recognized as an experimental unit and data were analyzed with a split-plot design. The least-squares means were evaluated using a Student's t-test and considered significant at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Muscle pH had a significant ($P < 0.001$) effect on PFK-1 activity such that activity increased with increasing buffer pH from 5.5 to 7.0 at every concentration of F-2,6-BP and AMP used in this study. Irrespective of buffer pH, there was a significant ($P < 0.001$) increase in PFK-1 activity when 50 µM F-2,6-BP was compared to all other treatments including all concentrations of AMP. However, 50 µM F-2,6-BP is very high compared to the physiological concentration in porcine skeletal muscle (1-2 µM) [4]. While F-2,6-BP levels in other porcine tissues are unknown, F-2,6-BP was approximately 10 µM in the liver, brain, testes, and spleen of mice [5]. There was no significant difference in the PFK-1 activity between 1 µM and 2 µM F-2,6-BP at pH 6.0 ($P = 0.82$) and pH 6.5 ($P = 0.78$). Recall, these concentrations were consistent with those found in normal (1 µM) and rapidly glycolysing (2 µM) porcine skeletal muscle [4]. We further investigated the effect of 0 µM, 1 µM, and 2 µM F-2,6-BP on glycolysis by utilizing an in vitro muscle glycolytic buffer system and found no difference of increased F-2,6-BP concentration on the rate of pH decline or ultimate pH of *longissimus lumborum* homogenates. Thus, increased F-2,6-BP at physiological concentrations in postmortem skeletal muscle may not significantly increase PFK-1 activity.

AMP also increased PFK-1 activity with increasing concentrations. At pH 6.5, AMP increased PFK activity at both 50 µM ($P \leq 0.0018$) and 150 µM ($P \leq 0.0053$) compared to the lower concentrations of AMP. A similar increase ($P \leq 0.024$) in activity was evident at pH 6.0. These data also agree with our previous investigation using the AMP deaminase inhibitor pentostatin (2-deoxycoformycin) to increase AMP concentrations [6]. In that study, increased levels of AMP accelerated and extended postmortem pH decline. Thus, AMP is an important activator of postmortem PFK-1 activity.

IV. CONCLUSION

F-2,6-BP and AMP both increased PFK-1 activity under simulated postmortem conditions. Additionally, F-2,6-BP was a more potent activator of PFK-1 compared to AMP at the same concentration (50 µM). However, at physiological concentrations, AMP increased PFK-1 activity at pH 6.0 and 6.5 compared to F-2,6-BP. Thus, AMP may be the more important regulator of postmortem PFK-1 activity in porcine skeletal muscle.

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