EFFECT OF NITRIC OXIDE ON SUSCEPTIBILITY OF MYOFIBRILLAR PROTEINS TO CALPAIN-1 PROTEOLYSIS

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I. INTRODUCTION

Nitric oxide (NO) can be produced by the nitric oxide synthase activation in response to hypoxic/ischemia condition that also occurs in postmortem muscles [1]. Protein S-nitrosylation is formed by the attachment of nitric oxide to the sulfydryl of protein cysteine residue that could change protein conformation, activity and function. Many myofibrillar proteins including myosin, actin, desmin, titin and troponin were identified to be endogenously S-nitrosylated in skeletal muscle and showed a high reactivity with NO donor S-nitrosoglutathione (GSNO) [2]. In our recent research (submitted to Meat Science), S-nitrosylated titin and α-actinin 1 were identified in sarcoplasmic protein extracts at 3 d of postmortem aging in pork longissimus thoracis. Those detected myofibrillar proteins were presumed to be the degraded fragments. It is possible that nitric oxide and protein S-nitrosylation are involved in calpain-1-induced proteolysis of myofibrillar protein. Thus, the objective of this study was to investigate the effect of NO using endogenous GSNO as the donor on sensitivity of myofibrillar protein to calpain-1 proteolysis.

II. MATERIALS AND METHODS

Porcine semimembranosus (SM) within 45 min post-slaughter was obtained to purify the myofibrils and calpain-1. GSNO at 0, 20, 50, 250 and 1,000 μM were incubated with myofibrils (10 mg/ml) in 20 mM phosphate buffer (pH 7.0) at 37°C for 30 min. After reactions were completed, the myofibrillar proteins were centrifuged at 3,000 g for 10 min and washed with 20 mM phosphate buffer (pH 7.0) for 2 times. The pellet of each treatment was re-suspended in 20 mM phosphate buffer (pH 7.0) to detect sulfhydryl content [3], protein surface hydrophobicity [4], and S-nitrosylated protein [5]. The myofibrillar protein suspensions were adjusted to 6 mg/ml and then mixed with 4 μg calpain-1 (0.375 unit). The digestion was induced by 100 μM CaCl2. The reaction tubes were placed on a rocker with gentle agitation at 25°C. At each time point of 10, 30 and 60 min, 250 μl of myofibrils were removed from the tubes and added into 250 μl of 2.5% Sodium dodecyl sulfate (SDS) and 10 mM phosphate buffer (pH 7.0) to stop the reaction. The gel samples were made to detect myofibrillar protein degradation including desmin and troponin-T according to Carlson et al [6]. General linear model was used to determine the effect of GSNO concentration on protein thiol and protein hydrophobicity and mean differences were compared using Duncan's multiple-range test ($P < 0.05$).

III. RESULTS AND DISCUSSION

Protein thiol content of myofibrillar protein is presented in Table 1. Both the total thiol and free thiol were significantly decreased by the increments of GSNO concentration ($P<0.05$). The 20 and 50 μM GSNO treatment significantly reduced the thiol content compared to the control while there was no variation between them. As the GSNO concentration increased to 250 and 1,000 μM, total and free thiol content showed significant decrease ($P<0.05$). Protein surface hydrophobicity and concentration were not significantly influenced by the GSNO treatment (Table 1, $P>0.05$). S-nitrosylated protein bands in 20 μM and 50 μM GSNO treatments was greater than the control (Fig. 1A). As the GSNO concentration increased to 250 μM, the total S-nitrosylation intensity showed a rapid increase compared to that of 50 μM GSNO treatment. The 1,000 μM GSNO treatment exhibited most and greatest S-nitrosylated protein bands and intensity, respectively. Along with the measurement of total thiol, the protein thiol was effectively attached by the GSNO-released NO to form the protein S-nitrosylation.

As shown in Fig. 1, the degradation of desmin and troponin-T showed opposite tendency by the GSNO treatments. There was no significant difference of original desmin band intensity at 10 and 30 min of

incubation while more degradation of desmin was presented in 1,000 μM GSNO treatment group compared to other groups at 60 min incubation (Fig. 1B). As for troponin-T, 1,000 μM GSNO treatment group had less degradation than other groups at 30 min, whereas the significant difference was found at 250 and 1,000 μM GSNO treatment groups in comparison to the other three groups at 60 min (Fig. 1C). It was derived that a biphasic response of different myofibrillar protein to proteolysis emerged in GSNO treatment model. Our findings in the current study have significance in elucidating the involvements of nitric oxide and protein S-nitrosylation during the postmortem aging.

Table 1 Protein concentration (mg/ml), total thoil (nmol/mgpro), free thiol (nmol/mgpro) and protein hydrophobicity (μg) of myofibrillar proteins treated by different concentration of GSNO.

Index	C	G1	G2	G3	G4	P-value
Protein concentration	10.46±0.28	10.53±0.42	10.47±0.14	10.84±0.32	11.12±0.17	0.437
Total thoil	87.80±1.36^{a}	81.07±3.66^{b}	76.79±1.20^{b}	67.98±2.10^{c}	56.38±1.49^{d}	< 0.001
Free thiol	46.66±0.56^{a}	43.60±1.24^{b}	42.76±1.00^{b}	35.18±0.81^{c}	26.77±0.86^{d}	< 0.001
Protein hydrophobicity	4.62±0.20	4.37±0.04	4.34±0.27	4.08±0.16	4.17±0.09	0.294

Note: “C,G1,G2,G3, and G4” represent 0, 20, 50, 250 and 1000 uM GSNO treatment group, respectively. Values were presented as means±standard deviation, “a-d” different letters differ among treatment (P<0.05)

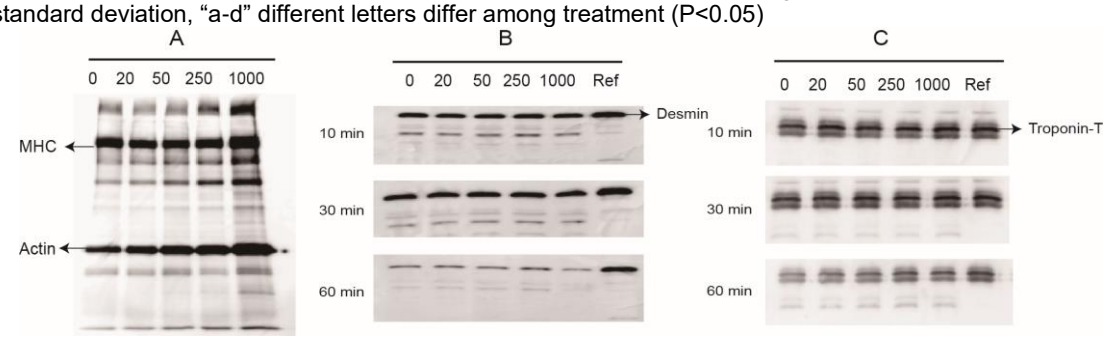

Figure 1. Representative western-blot images of S-nitrosylated myofibrillar proteins (A), desmin (B) and troponin-T (C) degradation at 10, 30, and 60 min of calpain-1 incubation. Note: MHC indicates myosin heavy chain. “Ref” indicates myofibrillar proteins without GSNO treatment and calpain-1 incubation.

IV. CONCLUSION

The current investigation showed that increasing concentration of GSNO significantly decreased the thiol content of myofibrillar protein and caused the accumulation of S-nitrosylated protein. The GSNO treatment also changed the sensitivity of myofibrillar proteins to calpain-1 proteolysis with the biphasic effects. Our results give a better understanding of the previous reports concerning the effect of nitric oxide and protein S-nitrosylation on the postmortem biochemistry and the quality of meat.

ACKNOWLEDGEMENTS

This project was partially funded by the Iowa Agricultural and Home Economics Experiment Station project number 3721, National Natural Science Foundation of China (Grant No: 31571853), and Joint PhD Study Fellowship from the China Scholarship Council (Grant: 201606850028). The authors thank Dr. Ed Steadham for technical assistance.

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