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Changes Of Nitric Oxide Synthase In Postmortem Beef (#458)

Qin Hou, Wan G. Zhang, Guang H. Zhou

Nanjing Agricultural University, College of Food Science and Technology, Nanjing, China

Introduction

Nitric oxide synthase (NOS) catalyzes the five electrons transfer of arginine to produce arginine and nitric oxide (NO) which is considered to be the main source of endogenous NO in postmortem muscles [1]. This reaction requires a range of cofactors, including oxygen molecules, reduced coenzyme II (nicotinamide adenine dinucleotide phosphate), flavin mononucleotide, flavin adenine dinucleotide, calmodulin, tetrahydrobiopterin and heme [2]. The endogenous generated NO can modify the sulfhydryl sites of protein cysteine resulting in protein S-nitrosylation [3]. NO and NO-mediated Protein S-nitrosylation are possibly involved in postmortem aging by regulating energy metabolism, calpain system, calcium release and apoptosis [2]. In order to further study the contribution of NO and protein S-nitrosylation to the fresh beef quality, it is necessary to study the biochemical changes of NOS in postmortem beef. The activity of NOS has been detected in various animals including chicken, turkey, trout, and pork [4]. However, studies on NOS changes in postmortem beef have not been reported. Therefore, this study aimed to investigate the activity, the expression and the location of NOS in bovine semimembranosus muscle (SM) during postmortem aging.

Methods

Five bovine SM muscles were obtained from the right side of carcasses within 45 min post-slaughter and aged for 14 d at 4 °C under vacuum packaging. At 0 (45 minutes post-slaughter), 1, 3, 7 and 14 d, the myofibrillar proteins were extracted to determine the activity and expression of NOS. The activity of NOS in bovine SM was measured according to the procedure of NOS activity assay kit (Nanjing Jiancheng Company, Nanjing, China). Then the protein concentration of myofibrillar proteins was determined and adjusted to 8 mg/ml with the buffer (0.1 M NaCl, 0.1% CHAPS, and 5 mM HEPES, pH 6.5). Western blotting was employed in the measurement of neuronal NOS (nNOS) content following the report of Liu et al. [5]. In addition, five SM samples (0.5 cm×0.5 cm×1 cm) were cut parallel to the muscle fiber and immediately frozen in liquid nitrogen. The 8 μm slices were obtained perpendicular to the myofiber and fixed by 4% paraformaldehyde for 4 h. Then SABC-Cy3 immunohistochemical staining kit (SA1074, Wuhan Boster Company, Wuhan, China) was used to assay the distribution of nNOS. The data analysis was conducted by One-way analysis of variance (ANOVA) and Duncan's multiple-range test ($P<0.05$).

Results

The activity of NOS in bovine SM myofibrillar protein is presented in Table 1. The results showed that bovine SM muscle retained NOS activity throughout the 14 d of postmortem storage. The activity of NOS at 0 d was 0.8 nmol/mg protein and then decreased significantly at 1, 3 and 7 d ($P<0.05$). No significant differences were observed between 7 d and 14 d ($P>0.05$). This finding differed from the earlier reports that NOS activity in pork, chicken and trout was only detected at the early period of storage [4]. Differences in animal species were possibly responsible for the inconsistent results. In addition, it is inferred that the differences in NOS activity might result in the varied NO production which potentially affects postmortem biochemistry to regulate fresh meat quality.

The nNOS is regarded as the major isoform of NOS in skeletal muscle [6]. As shown in Figure 1, a 160 kDa band of nNOS was detected in bovine SM muscle. Compared to 0 d, the relative content of nNOS in SM muscle decreased significantly at 1, 3, and 7 d ($P<0.05$). Especially at 7 d, the content of nNOS decreased by approximately 64% compared to 0 d. There were no significant differences in nNOS content between 7 d and 14 d ($P>0.05$).

Immunofluorescence results showed that relatively strong and uniform nNOS fluorescence was observed along with the cell membrane of bovine SM (Figure 2). A recent study indicated that the distribution of nNOS with the sarcolemma was essential for functional nNOS signaling in dystrophic muscles [7]. Mislocalization of nNOS in skeletal muscle could lead to fibrosis and muscular dystrophy [1]. Very few fluorescent spots were observed in the cytoplasm of bovine SM. This cytoplasmic distribution promotes the interaction of NO with S-nitrosylation-related proteins [8]. In addition, higher fluorescence intensity was observed in the cell membrane at 1 d of postmortem storage (Figure 2A) compared to the negative control (Figure 2D). The fluorescence intensity decreased at 3 d (Figure 2B) and almost disappeared at 7 d (Figure 2C). These results indicate that nNOS in bovine SM is degraded gradually during postmortem aging.

Conclusion

The current study demonstrated for the first time that bovine SM muscle retained NOS activity during 14 d of postmortem storage. NOS activity and nNOS content decreased gradually within 7 d of storage. In addition, the distribution of nNOS in the sarcolemma and cytoplasm suggests that NO may be involved in muscle metabolism and play a potential role in the regulation of meat quality.

Notes

References

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Postmortem aging time (d)	0	1	3	7	14
NOS activity (nmol/mg protein)	0.800±0.034^{a}	0.665±0.026^{b}	0.526±0.007^{c}	0.396±0.007^{d}	0.382±0.005^{d}

Table 1 Nitric oxide synthase (NOS) activity in beef semimembranosus muscle during postmortem aging
Note: Values are expressed as mean ± standard error. Different letters indicate significant difference at $P<0.05$ ($n=5$).

Notes

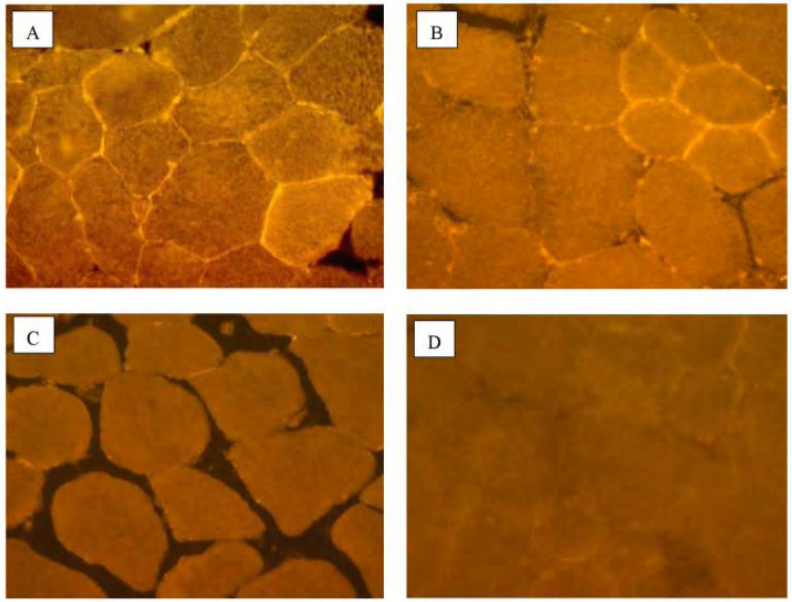
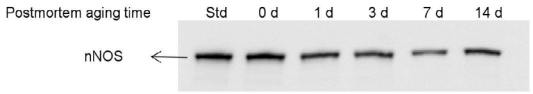


Figure 2 Localization of nNOS in beef semimembranosus muscle during postmortem aging

Note: "A-C" shows immunostaining of nNOS at 1, 3, and 7 d of storage while "D" is the negative control; The magnification of images is 400×.



Postmortem aging time (d)	0	1	3	7	14
Relative content of nNOS	1.013±0.030[a]	0.738±0.036[b]	0.600±0.021[c]	0.468±0.015[d]	0.460±0.022[d]

Figure 1 Representative graph of western blot of nNOS and its relative content in postmortem beef

Note: Values are expressed as mean ± standard error. Different letters indicate significant difference at $P<0.05$ ($n=5$).

Notes