O-06-02

S-Nitrosylation Of RyR1 And SERCA1 In PSE snd RFN Pork (#470)

Ying Y. Wang, Wan G. Zhang, Guang H. Zhou

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

Introduction

Protein S-nitrosylation is defined as the covalent attachment between NO and the sulfhydryl group of protein cysteines that can regulate protein structure, activity and function [1]. Ryanodine receptor type 1 (RyR1) plays a crucial role in calcium release from sarcoplasmic reticulum in cycles of muscle relaxation and contraction, while sarcoplasmic reticulum calcium ATPase 1 (SERCA1) is responsible for the calcium re-uptake process. Several cysteine residues of RyR1 and SERCA1 are easily modified by NO to form S-nitrosothiols. Many researches have shown that protein S-nitrosylation can regulate sarcoplasmic calcium concentration by increasing RyR1 activity and inhibiting SERCA1 activity [2-5]. Excessive calcium release in the early stage of postmortem can induce extreme muscle contraction and then accelerate muscle metabolism. In our current study, protein S-nitrosylation levels of RyR1 and SERCA1 were detected at 1 h postmortem in pork longissimus thoracis. It is postulated that abnormally high S-nitrosylation level of RyR1 and SERCA1 can disorder the balance of sarcoplasmic calcium leading to accelerated pH decline and the development of PSE meat. Thus, the aim of this study was to compare of the S-nitrosylation levels of RyR1 and SERCA1 between PSE and RFN pork to explore the underlying mechanism in the development of PSE meat.

Methods

Porcine longissimus thoracis (LT) was obtained at 1 h postmortem to analyze the quality and biochemical indexes. Seven pale, soft and exudative (PSE) and seven red, firm and non-exudative (RFN) meat were selected according to the following criteria [6]: PSE, pH < 6.0, L* > 50 at 1 h postmortem, drip loss > 10% at 24 h postmortem; RFN, pH > 6.0, L* < 50 at 1 h postmortem, drip loss < 10% at 24 h postmortem. Shear force was detected using the modified method of Thompson et al [7]. Sarcoplasmic calcium concentration was determined following the method described by Parrish et al [8]. S-nitrosylation levels of RyR1 and SERCA1 were detected by biotin switch combined with the immune-blotting method. Protein samples were prepared following the direction of S-Nitrosylation Western Blot kit. The S-nitrosylated sample was enriched by immobilized anti-TMT resin for S-nitrosylated protein analysis. The protein S-nitrosylation level was expressed by the ratio of enriched S-nitrosylated proteins and unenriched total proteins. S-Nitrosylated proteins and unenriched total proteins were determined using western blotting proposed by Zhang et al [9]. All data were analyzed by one-way analysis of variance (ANOVA) using SAS version 9.1and mean differences were compared by Fisher's protected least significant difference (LSD) test ($P < 0.05$). Mean values were expressed as mean ± standard error and significance was reported at $P < 0.05$.

Results

As shown in Table 1, the pH, the color at 1 and 24 h and the drip loss at 24 h postmortem were significantly different between PSE and RFN samples ($P < 0.05$). In addition, higher shear force was detected in PSE meat than in RFN meat at 24 h postmortem ($P < 0.05$). The results were consistent with previous study which reported PSE meat possessed inferior quality including pale color, high water loss and tough texture after cooking [10]. Sarcoplasmic calcium concentration is presented in Fig. 1. PSE meat possessed a higher sarcoplasmic calcium concentration than RFN meat at 1 h postmortem (228.33 μM for PSE vs 38.89 μM for RFN, $P < 0.05$). Excessive calcium concentration in sarcoplasm can considerably accelerate the rate of muscle metabolism, lactic acid production and pH decline [11]. An abnormally high sarcoplasmic calcium concentration in the early stage of postmortem was thought to be the primary cause of muscle contraction and then induced increased glycolysis [12]. The dysregulation of RyR1 and SERCA1 may be responsible for the disorder of sarcoplasmic calcium concentration.

As presented in Fig. 2, higher S-nitrosylation levels of RyR1 and SERCA1 were observed in PSE meat than in RFN meat ($P < 0.05$). Increased RyR1 S-nitrosylation in PSE meat can promote RyR1 to release calcium that can accelerate muscle contraction. Furthermore, a higher S-nitrosylation level of SERCA1 in PSE meat may inactivate SERCA activity, leading to less calcium re-uptake from sarcoplasm. Consequently, the muscle relaxation cycle was inhibited, and muscle contraction was accelerated, which led to a high glycolysis rate. Thus, different S-nitrosylation levels of RyR1 and SERCA1 could explain the variation of sarcoplasmic calcium concentration between PSE meat and RFN meat. Excessive calcium could induce intense skeletal muscle contraction, lactic acid accumulation and faster rate of pH decline, leading to the occurrence of PSE meat with the deteriorated feature.

Conclusion

The current study showed that the sarcoplasmic calcium concentration in PSE meat was six times higher compared to RFN meat at 1 h postmortem. The S-nitrosylation level of RyR1 and SERCA1 in PSE meat was significantly higher than in RFN meat. Abnormally high S-nitrosylation level of RyR1 and SERCA1 can putatively induce increased sarcoplasmic calcium. Our study provides a new perspective to explain the development of PSE meat in the early stage of postmortem muscle.

Notes

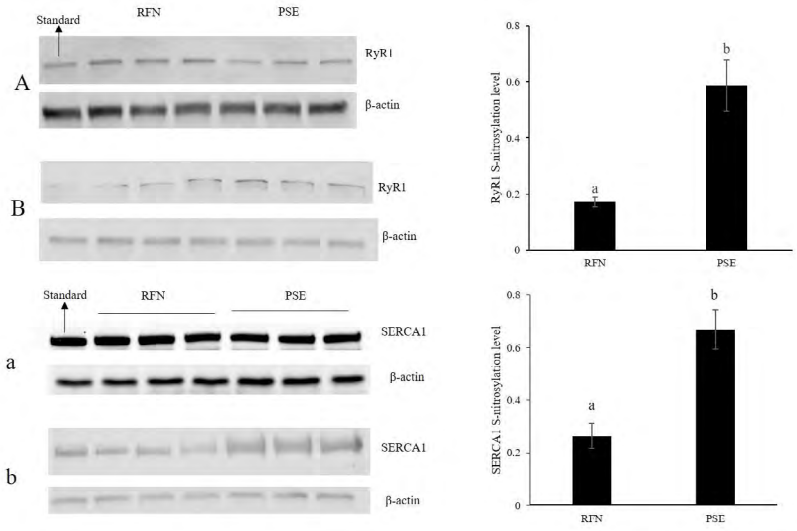

Fig. 2 RyR1 and SERCA1 S-nitrosylation level at 1 h postmortem of RFN and PSE pork

A. The RyR1 content before enrichment. B. The S-nitrosylated RyR1. RyR1 S-nitrosylation level was expressed as the ratio of quantification of B/A. a. The SERCA1 content before enrichment. b. The S-nitrosylated SERCA1 content. SERCA1 S-nitrosylation level was expressed as the ratio of quantification of b/a. Standard was one of the samples in postmortem muscle with clear and stable band in trial test and was loaded into each gel. Means with different superscripts differ significantly ($P < 0.05$, $n = 7$).

Notes

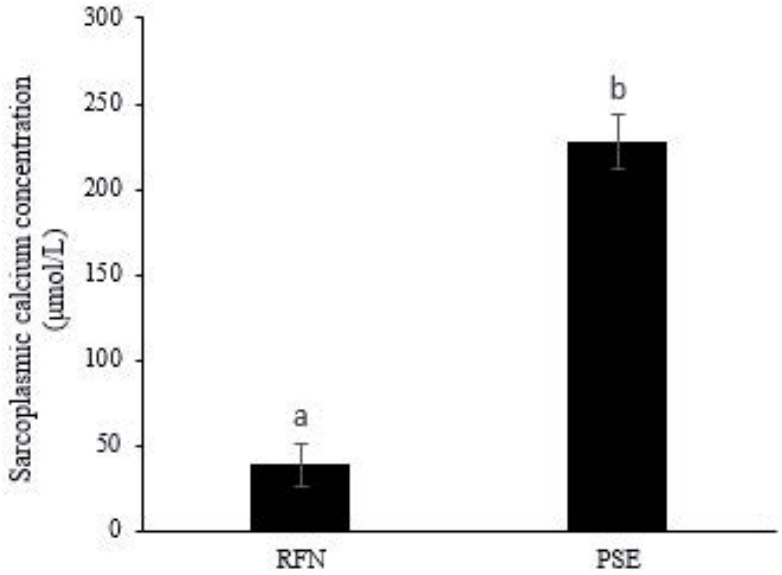

Fig. 1 Sarcoplasmic calcium concentration at 1 h postmortem of RFN and PSE pork
Means with different superscripts differ significantly ($P < 0.05$, $n = 7$).

Index	Time (h)	RFN	PSE
pH	1	6.08±0.14^{a}	5.62±0.18^{b}
	24	5.60±0.07^{a}	5.48±0.08^{b}
L*	1	39.65±0.93^{b}	50.67±4.26^{a}
	24	43.80±0.67^{b}	53.27±2.12^{a}
Drip loss (%)	24	5.09±3.29^{b}	12.85±5.90^{a}
Shear force (N)	24	48.51±10.95^{b}	64.73±8.72^{a}

Table 1. pH, color, drip loss and shear force in PSE and RFN pork
Means within the same row with different letters are significantly different ($P < 0.05$, $n = 7$).

Notes