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Review

Contribution of nitric oxide and protein *S*-nitrosylation to variation in fresh meat quality

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ABSTRACT

As a primary source of reactive nitrogen species, nitric oxide (NO) is a signaling molecule playing multiple roles in physiological processes. NO exerts these pleiotropic effects mainly through the covalent attachment to the sulfhydryl group of protein cysteines to form *S*-nitrosothiol (protein *S*-nitrosylation). It has been two decades since NO was first investigated for its role in meat tenderization. Progress has been made, including studies by manipulating the NO levels in muscle cells, suggesting possible effects in the pre-slaughter and post-slaughter environment. NO has potential effects on the meat quality of beef, lamb, chicken and pork muscles. However, it has been difficult to determine the exact mechanism(s) of NO action as it has variable effects on meat quality including tenderness, water holding capacity and color. It is speculated that NO and protein *S*-nitrosylation may be involved in muscle to meat conversion through the regulation of postmortem biochemical pathways including glycolysis, Ca^{2+} release, proteolysis and apoptosis.

1. Introduction

The main aim of the meat industry is to achieve consistent fresh meat quality with acceptable organoleptic properties of tenderness, color, juiciness and flavor to meet consumers demands. However, the variability in meat quality has consistently been reported within various animal species, muscle types and even position within a cut (Lefaucheur, 2010). There can also be significant variation in meat quality and biochemical traits in different locations within one muscle (Rhee, Wheeler, Shackelford, & Koohmaraie, 2004) resulting in each piece of meat being specific and unique. Many studies have focused on genetics, nutrition, pre-slaughter stress and post-slaughter processing to account for the differences (Ferguson & Warner, 2008; Warner, Greenwood, Pethick, & Ferguson, 2010), but the causes of large variations in meat quality are still not fully understood. Determination of meat quality is a result events occurring throughout the animal's life and also the post-slaughter period including the conversion of muscle-to-meat (Lana & Zolla, 2016). The conversion of muscle-to-meat involves integration of many biochemical and biological changes in postmortem muscle that can be affected by multiple factors in the animal. Thus, the extent of postmortem muscle biochemical events such as glycolysis, state of muscle contraction, stress reactions, apoptosis and proteolysis are fundamental for the determination of mechanisms determining meat quality as a consequence of the conversion of muscle-to-meat.

Nitric oxide (NO) is produced in skeletal muscle and acts as a secondary messenger in signal transduction in a wide range of physiological processes (Kaminski & Andrade, 2001; Pacher, Beckman, & Liaudet, 2007). NO exerts its effect in biological systems mainly through the cyclic guanosine monophosphate (cGMP) pathway and the cGMP-independent pathway, called protein *S*-nitrosylation (Ziolo, 2008). Protein *S*-nitrosylation can be involved in regulating protein activity, localization, stability and their interactions, thus potentially determining critical post-translational modifications (Hess, Matsumoto, Kim, Marshall, & Stamler, 2005). Many skeletal muscle functions are regulated by NO and protein *S*-nitrosylation including muscle contraction, myocyte differentiation and glucose homeostasis (Stamler & Meissner, 2001). The role of NO and NO-induced protein *S*-nitrosylation in determination of meat quality was first investigated by Cook, Scott, and Devine (1998). Since then, although process has been made

Abbreviations: ALDO, aldolase; ENO, enolase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GP, glycogen phosphorylase; GPI, glucose-6-phosphate isomerase; LDH, lactate dehydrogenase; PKM, pyruvate kinase; PFK, phosphofructokinase; PGAM, phosphoglycerate mutase; PGM, phosphoglucose mutase; PGK, phosphoglycerokinase; PYGM, glycogen phosphorylase; TPI, triose phosphate isomerase

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in clarifying the effects of NO on meat quality, many challenges remain. NO can be detected in postmortem beef longissimus lumborum soon after slaughter and has been reported to remain for several days post-mortem (Cook et al., 1998). Also *S*-nitrosylated proteins have been observed in beef muscles for 7 days post-mortem (Zhang, 2009). Across the study, meat quality, especially tenderness, was affected by different levels of NO in postmortem muscle cells, however, the results were inconsistent across animal species and muscle types (Cook et al., 1998; Cottrell, McDonagh, Dunshea, & Warner, 2008; Cottrell, Ponnampalam, Dunshea, & Warner, 2015; Zhang, Marwan, Samaraweera, Lee, & Ahn, 2013). The primary source of NO is from the conversion from L-arginine to L-citrulline, catalyzed by nitric oxide synthase (NOS). In general, the extent of protein *S*-nitrosylation is dependent on the localization and the expression of NOS (Iwakiri et al., 2006). In postmortem muscle, the concentration and activity of NOS differs among muscle types (Liu et al., 2015) and animal species (Brannan & Decker, 2002). Thus, it is conceivable that different amounts of NO and NO-induced protein *S*-nitrosylation would influence the diverse biochemical processes during the conversion of muscle to meat, and thus possibly have a role in the underlying mechanism of meat quality variation. In this review, we present the basic knowledge of NO and protein *S*-nitrosylation, and then review the effect of NO and protein *S*-nitrosylation on meat quality, and their involvement in potential biochemical pathways of glycolysis, Ca^{2+} release, apoptosis and proteolysis.

2. The basics of NO

NO is a free radical which is freely diffusible across cell membranes and structures acting as a second messenger in signaling transduction (Derakhshan, Hao, & Gross, 2007). In mammalian cells, it could be biologically synthesized by three isoforms of NOS including neuronal NOS (nNOS), macrophage (immune)/inducible NOS (iNOS) and endothelial NOS (eNOS) (Stamler & Meissner, 2001). Generally, the NOS molecule is comprised of a Zn^{2+} binding region, a N-terminal oxygenase domain of heme, L-Arg and H_4B, a CaM-binding region and a C-terminal reductase domain of FMN, FAD and NADPH (Fig. 1A). As NOSs are determined from the different cells or systems, the variations in the primary structure of individual NOS are responsible for their broad and abundant localizations throughout the tissues (Fig. 1A). All three isoforms of NOS can simultaneously be expressed in skeletal muscle, while nNOS is the predominant isoform and undertakes the major NOS activity in skeletal muscle. In addition, the reduction of circulating nitrate and nitrite in blood and tissue is considered as the complementary pathway to form NO (Lundberg, Weitzberg, & Gladwin, 2008). In normal physiological conditions, the concentration of nitrate and nitrite in plasma is approximately 20–40 μM and 50–300 nM, respectively (Gladwin et al., 2000; Lundberg & Govoni, 2004). Plasma nitrate and nitrite serve as pools of NO that can potentially be converted back to NO to complement NO production when oxygen supply is limited (Rassaf, Feelisch, & Kelm, 2004). The generation of NO by these pathways is upregulated by hypoxia and acidosis, situations that decrease the oxygen-dependent NOS enzyme activities to produce NO (Østergaard et al., 2007).

With a single electron in the 2p-π anti-bonding orbit, NO can either donate the electron to form a nitrosonium cation (NO^+) or accept an electron for pairing to form a nitroxyl anion (NO^-) (Stamler, Singel, & Loscalzo, 1992). NO readily reacts with reactive oxygen species (ROS) to form more reactive derivatives (Fig 1B). Particularly, peroxynitrite ($ONOO^-$) is generated by the reaction of accumulated NO and superoxide anion ($O_2\cdot^-$) to be potentially cytotoxic. The oxidized NO-derived species (NO_x) including $ONOO^-$, NO_2 and N_2O_3, are involved in nitrosative stress that surpasses the physiological requirement and scavenging capacity. They readily react with biological macromolecule including proteins, lipids and DNA to form a specific and stable end product, regarded as biomarkers (Fig. 1C). As for proteins, *S*-nitrosothiol/N-nitrosamines and 3-nitrotyrosine are the main products for the respective nitrosation and nitration modification of protein amino acid residues. N_2O_3 has a high affinity for thiol-containing peptides such as glutathione (GSH), generating *S*-nitrosoglutathione (GSNO, Fig 1C) which acts as a pool of *S*-nitrosothiol in biological systems. The reaction of NO and myoglobin to form nitrosylmyoglobin (Fig. 1C) is regarded as a NO scavenger in skeletal and cardiac muscles. Protein 3-nitrotyrosine is generated by the substitution of a hydrogen with a nitro group ($-NO_2$) of the tyrosine residue provoked by the $ONOO^-$ and $NO_2\cdot$ (Fig. 1C) (Radi, 2004). Protein 3-nitrotyrosine is commonly detected under many pathological conditions and has been proposed to have functional and biological effects on disease development (Souza, Peluffo, & Radi, 2008). In addition to protein, DNA can also be attacked by NO_x and $ONOO^-$resulting in single-strand breakage.

Pharmacological donors of NO and inhibitors of NOS are widely used in biological research and clinical trials (Mukherjee, Cinelli, Kang, & Silverman, 2014; Wegener & Volke, 2010). The most widely used NOS inhibitors are the L-arginine analogues that compete with L-arginine at the NOS binding site in the NOS molecule, although the inhibition is reversible and poorly selective (Table 1). To address this issue, selective NOS inhibitors have been designed using the differences in the electronic and steric effects of the binding site region of the crystal structure of NOS (Mukherjee et al., 2014). For example, 7-Nitroindazole (7-NI), which belongs to the indazole derivates, shows high nNOS selectivity *in vivo,* but less selectivity *in vitro*. Moreover, 1-[2-(trifluoromethylphenyl)imidazole (TRIM) was designated to be a more selective inhibitor for nNOS and showed selectivity both *in vitro* and *in vivo* based on its binding of cofactor BH_4 and the BH_4 availability in the tissue. As for NO donors, sodium nitro-prusside (SNP) is widely used to liberate 1 mol of NO per SNP. *S*-nitrosothiols (general formula "R-SNO") contain a single bond between the sulfydryl group and the NO moiety and is considered as a donor of NO^+. Two typical *S*-nitrosothiols are widely utilized as NO donors which are GSNO and *S*-nitroso-N-acetylpenicillamine (SNAP, Table 1). A wide range of factors can influence the release of NO from *S*-nitrosothiols including light, heat, superoxide, transition metals and enzymes (Miller & Megson, 2007). Diazeniumdiolates (NONOates) with a diolate group [$N(O^-)N{=}O$)] linking to the nucleophile adduct via a nitrogen atom are designated to have different half-lives from seconds to hours (Table 1). In summary, when conducting biological experiments, it is critical to optimize NO donors while considering many factors including NO release rate, reaction conditions and related by-products.

3. Protein nitrosylation

The most extensively elucidated mechanism for NO signaling is that of binding and alteration of the function of heme proteins, including soluble guanylyl cyclase (sGC) (Thomas et al., 2003) and cytochrome *c* oxidase (Martínez-Ruiz, Cadenas, & Lamas, 2011). However, abundant evidence suggests that NO exerts a biological effect in a heme-independent manner, mostly through *S*-nitrosylation. *S*-nitrosylation refers to the covalent attachment of an NO group to the sulfhydryl moiety of protein cysteines, resulting in the generation the *S*-nitrosothiol (R−S−N=O). Protein *S*-nitrosylation is a reversible and post-translational modification recognized as the regulator of protein activity, localization, stability and interaction (Foster, Hess, & Stamler, 2009; Marozkina & Gaston, 2012).

There are several pathways for the formation of *S*-NO bond, including oxidative *S*-nitrosylation by NO_x, catalysis by the metalloproteins and trans-nitrosylation (Kovacs & Lindermayr, 2013). As shown in Fig. 2, the oxidative pathway largely depends on the concentration of reactive nitrogen species such as NO_2, N_2O_3 and $ONOO^-$. Metalloproteins generally contain redox-active metal ions such as iron and copper that can rapidly scavenge NO radicals. The metal-containing proteins promote SNO formation either through the one-electron oxidation of thiols to $RS\cdot$ or by the formation of metal-nitrosyl complex intermediates (Stubauer, Giuffrè, & Sarti, 1999; Vanin, Malenkova, &