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Review of Postmortem Protein Oxidation in Skeletal Muscle and the Role of the Peroxiredoxin Family of Endogenous Antioxidants

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Abstract: The development of fresh meat quality is dictated by biochemical changes during the perimortem and postmortem period. Lipid and protein oxidation in postmortem skeletal muscle and meat products is detrimental to product quality. The mechanisms that influence lipid and protein oxidation in fresh meat remain unelucidated. Peroxiredoxins are thiol-specific antioxidant proteins that are highly reactive and abundant and may be involved in limiting oxidation early postmortem. This review aims to provide a background on oxidation in skeletal muscle, peroxiredoxins, a summary of proteomic experiments associating peroxiredoxins and meat quality, and the importance of context from proteomic methods and results. Additional controlled experiments considering the cellular conditions of postmortem skeletal muscle are necessary to further understand the contribution of peroxiredoxins to fresh meat quality development.

Key words: meat quality, oxidation, peroxiredoxin, tenderness, beef, pork
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Introduction

Fresh meat quality traits are affected by muscle composition, function, and the muscle tissue's response to the perimortem and early postmortem cellular environment. It is well documented that early postmortem metabolism influences the duration of the delay before the onset of rigor and the primary production of glycolytic intermediates and lactate. Importantly, the muscle cells and tissues lose the capability to remove or metabolize oxidation products that can affect protein function and thus impact meat color (Ramanathan et al., 2020), water-holding capacity (Lund et al., 2007), tenderness (Rowe et al., 2004b), and sensory quality (Soladoye et al., 2015). Oxidation of lipids generates oxidation products that cause a deterioration of flavor (Domínguez et al., 2019). Therefore, the oxidative environment in perimortem and postmortem muscle sets the stage for the development of fresh meat quality traits.

A key class of antioxidant proteins found in muscles are the thiol-specific antioxidant proteins called peroxiredoxins (Prdx). These proteins are an important part of the redox response in muscle and thus can be considered an indicator of past oxidative stress and resiliency in the face of oxidative stress (Rhee and Kil, 2017). Numerous proteomic experiments have determined that Prdx are associated with variation in fresh meat quality (Hwang et al., 2005; Jia et al., 2007; Carlson et al., 2017). However, the reactivity and complexity of Prdx isoforms and modifications make it necessary to consider the context of these results. The objective of this review was to define the influence of protein oxidation on fresh meat

quality and outline the chemistry of Prdx. Their function as antioxidant proteins in muscle's response to oxidative stress is important, specifically because it can influence the perimortem metabolism in muscle.

Reactive Oxygen Species

Reactive oxygen species (ROS) is a broad term that encompasses a wide array of molecules, which vary in reactivity, abundance, reactant specificity, products, and reaction mechanisms. Reactive oxygen species are classified as radical and non-radical molecules of oxygen. Radical molecules, such as superoxide, hydroxyl, alkoxyl, and peroxyl radicals, have one or more unpaired electrons in the valency shell and are generally highly reactive and unstable. Non-radical compounds, such as hydrogen peroxide (H_2O_2), singlet oxygen, and ozone, are chemically stable compounds and less reactive than free radicals; however, these non-radical compounds can be converted to more reactive radical species (Phaniendra et al., 2015). Two primary sources of endogenous ROS production include the leakage of electrons through the inner mitochondrial membrane during oxidative phosphorylation and oxygen-metabolizing reactions catalyzed by NADPH oxidases. Exogenously, ROS can also be introduced through sources including but not limited to osmotic stress, ultraviolet radiation, heavy metals, metabolic distress, and disease challenges. The diversity in ROS underscores the complexity surrounding the significance and relevance of oxidation reactions in cells. A single view of ROS may interpret these oxidation reactions as detrimental to a cell. Yet specific oxidation reactions have been attributed to cellular signaling events and are necessary for living cell function and growth.

The term oxidative stress was defined as "a disturbance in the prooxidant-antioxidant balance in favor of the former" (Sies, 1986; Sies et al., 2017). Specific ROS are necessary for redox signaling events and therefore cannot always be viewed as detrimental. The definition of oxidative stress has since been refined to be "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage" (Estevam et al., 2015). Oxidative stress can range from a mild physiological maintenance challenge, called oxidative eustress, to the toxic oxidative burden that damages biomolecules, called oxidative distress (Sies, 2020).

In postmortem skeletal muscle, numerous physiological changes occur and are well characterized. The oxidation products in postmortem skeletal muscle are commonly and routinely quantified. The term oxidative stress is often used to describe the conditions in postmortem skeletal muscle; however, these observations primarily draw on research conducted in living cells or model systems. The context of postmortem skeletal muscle is distinct from living tissue regarding pH, ionic strength, and availability of energy and substrate. Therefore, specific consideration and an appreciation for these differences are necessary to understand oxidative events in postmortem skeletal muscle. The quantification of oxidized protein and lipid components in postmortem skeletal muscle is better characterized. Meanwhile, there are substantial gaps in the understanding of oxidation reactions. While not intended to be an exhaustive list, unanswered questions about oxidation events in postmortem skeletal muscle are provided below.

1. Which antioxidant systems remain functional, and how do changes in pH, temperature, and ionic strength affect those systems? The decline in pH and temperature accompanied by increased ionic strength in postmortem skeletal muscle impact enzyme and protease functionality (Carlin et al., 2006; England et al., 2014). A similar change to antioxidant proteins' activity or function, particularly those that rely on thiol residues like Prdx, may occur due to postmortem biochemical changes. A better understanding of the impact of the postmortem biochemical changes on antioxidant proteins could aid in identifying mechanisms to promote or increase antioxidant protein activity to improve meat quality.
2. During the postmortem period, does the availability of reducing cofactors, such as NADPH, become limiting? The rate and extent of NADPH production in postmortem skeletal muscle are not well characterized. Limited success in identifying NADPH has been achieved in postmortem skeletal muscle in beef (Muroya et al., 2019) and pork (Muroya et al., 2014). Additional research is necessary to understand the functionality of the major enzymes that produce NADPH and other reducing cofactors in skeletal muscle and factors that influence the production and abundance of these cofactors under conditions mimicking postmortem skeletal muscle.
3. Which oxidants are produced in the greatest amount or are the most significant? The diversity of oxidants that can be produced in skeletal muscle is numerous. Hydrogen peroxide is one of the most abundant oxidants; however, hydrogen

peroxide can be converted into other, more reactive compounds (Winterbourn, 2018). The contribution or impact of specific oxidants in postmortem skeletal muscle toward lipid and protein oxidation is unclear. A greater understanding of the more impactful oxidants could help facilitate new research on mechanisms to control or limit extensive lipid and protein oxidation.

4. Which oxidation events are the most impactful to postmortem meat quality development? The perimortem and postmortem cellular environments and events that contribute to oxidative damage remain largely undefined. Mitochondrial disruption postmortem could be a source of oxidants and NADPH oxidases (Brand, 2016; Sinha et al., 2013). The interplay between lipid and protein oxidation also must be considered as both occur in the same environment (Estévez et al., 2020). The consequences of oxidation on meat quality are better characterized; however, refined research questions should address how oxidant sources, quantities, and oxidative events negatively influence meat quality postmortem.

This discussion aims to briefly discuss advances in redox signaling to describe how this may play a role in postmortem skeletal muscle and highlight the work related to antioxidant proteins that have been less studied and less characterized in postmortem skeletal muscle. A review of studies identifying relationships between antioxidant proteins and meat quality will be evaluated, and the importance of context in research and results will also be discussed.

Reactive oxygen species impact on living tissue

In living tissue, ROS were deemed a negative consequence of aerobic metabolism. It is increasingly clear that cells can elicit regulated responses to H_2O_2 and other ROS. Hydrogen peroxide has been recognized as the primary redox signaling molecule because it selectively reacts with cysteine thiol groups, and these oxidation reactions are reversible reactions via other cellular thiols (Antunes and Brito, 2017). Additional redox signaling pathways with nitric oxide and 4-hydroxy-2-nonenol exist; however, these pathways are less understood because of the complexity involved with these reaction mechanisms.

Cysteine oxidation initially yields a sulfenic acid (-SOH), which is unstable and highly reactive. The proximity of an additional thiol or glutathione group can promote the formation of an intramolecular or intermolecular disulfide bond. The sulfenic acid can be oxidized further to a sulfinic acid ($-SO_2H$) and, subsequently, a sulfonic acid ($-SO_3H$) by 1 or 2 additional H_2O_2 molecules, respectively (Roos and Messens, 2011). These oxidative modifications of cysteine are, in many cases, reversible reactions, in which biological reductants, mainly the thioredoxin system and glutaredoxin systems, reduce primarily protein disulfide bonds and glutathionylated proteins, respectively (Poole, 2015). In living tissue, NADPH supplies the electrons to reversibly reduce specific oxidized proteins, and the cell can produce NADPH through normal metabolism to meet the needs of the cell.

The research regarding thiol oxidation and redox signal transduction is relatively new, and the exact mechanisms that facilitate the recognition of the signal after thiol oxidation remain uncharacterized. There have been hypotheses developed to explain these events. The main hypotheses of redox signal transduction include the direct oxidation of the target protein, oxidation via a sensor protein, and regulation of the oxidation state of the target via a secondary product of the sensor such as thioredoxin. Current and future advancements in instrumentation, technology, and analyses can help us further study the role of H_2O_2 redox signaling and elicit the specific mechanisms for signal transduction in living cells.

Reactive oxygen species impact on postmortem muscle tissue

Protein oxidation caused by ROS can have major implications for protein function and animal health. Reactive oxygen species can lead to multiple protein oxidation issues affecting function, including decreases in protein solubility due to polymerization, enzyme activity loss, and reduced efficiency in carrying out functions (Stadtman and Oliver, 1991; Shindoh et al., 1992; Agarwal and Sohal, 1994).

Protein carbonylation is the addition of a carbonyl group, such as aldehydes or ketones, to a protein molecule. Carbonyl groups are characterized by a carbon atom attached to an oxygen molecule by a double bond (Barreiro and Hussain, 2010). Protein carbonyl content may be increased as a result of many oxidative reactions and is used as a primary marker for oxidative stress in living tissues. Amino acid side chains particularly susceptible to carbonylation include histidine, arginine, and lysine (Estévez, 2011). Carbonylation changes the protein structure and function and generally leads to the need for removal. The carbonylation

reaction is considered irreversible, though the inclusion of a reducing agent within a tissue sample has been seen to limit total carbonyl formation (Wong et al., 2013). Measuring protein carbonyl content relative to a given amount of protein is an accepted method of determining the degree of protein oxidation in a sample.

The 2,4-dinitrophenylhydrazine (DNPH) method (Reznick and Packer, 1994) is based on a reaction between carbonyl compounds and DNPH, which forms a 2, 4-dinitrophenyl that displays a maximum absorbance at 370 nm. The assay process determines protein and carbonyl content to account for protein lost through the assay. At completion, total carbonyl content per mg of protein can be determined (Estévez, 2011). Oliver et al. (1987) initially developed the DNPH method to measure differences in protein oxidation between biological samples. Since then, it has been widely used in determining protein oxidation in tissues. The DNPH method of carbonyl content analysis remains the primary method to measure protein oxidation in tissue samples.

Meat protein oxidation is detrimental to meat quality factors, including tenderness, color, and water-holding capacity. The changes to protein structure from oxidation reactions can alter protein folding, solubility, and reactivity. Meat proteins influence various fresh meat characteristics; thus, protein oxidation can directly affect meat quality and functionality. It is crucial to understand meat protein oxidation because of the adverse effects on meat eating quality and the shelf life of a meat product. Developing methods to limit or control protein oxidation will improve meat quality and the ultimate value and sustainability of meat products.

Protein oxidation has also been implicated in many pathological conditions and aging in human health, including Alzheimer's disease, Parkinson's syndrome, and muscle dystrophy (Soladoye et al., 2015). There is less understanding about the consumption of oxidized proteins and their contribution to pathological conditions and nutrient content and absorption. As a result of oxidation, protein structure and folding pattern are altered. These conformational changes are hypothesized to reduce the digestibility and bioavailability of proteins and increase the occurrence of certain diseases or medical conditions (Estévez and Luna, 2017). Meat protein oxidation is often only related to meat quality deterioration, and the consequences of the intake of oxidized proteins on human health and nutrition are often overlooked (Estévez and Xiong, 2019). A recent report using an *in vitro* model simulated digestion to determine the effects of oxidation on the digestibility of beef and pork patties. After 7 d of illuminated storage, significant indicators of protein oxidation were quantified. Oxidation of proteins in beef and pork patties resulted in fewer free amino groups, suggesting protein oxidation decreases proteolysis during digestion *in vitro* (Rysman et al., 2016). Further research is needed to comprehend the effects of protein oxidation on human health. The intake of oxidized proteins may contribute to the development of some diseases and limit meat proteins' digestibility.

Cysteine is highly reactive and susceptible to oxidation and disulfide bond formation. The cross-linking of proteins, such as myosin heavy chain isoforms, has been associated with changes to meat quality characteristics, such as tenderness or water-holding capacity. Lund et al. (2007) and Kim et al. (2010) stored cuts of pork and beef in high-oxygen packages and vacuum packages, typical methods of storing meat for consumers. Under these conditions and extended postmortem storage conditions of 14 and 9 d, both authors reported increased myosin disulfide cross-linking on non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels for meat stored in high-oxygen packaging compared with vacuum packaging. The authors also noted differences in instrumental star probe (Kim et al., 2010) and sensory tenderness and juiciness scores (Lund et al., 2007; Kim et al., 2010), where the high-oxygen packaged meat was tougher compared with the vacuum-packaged meat. Interestingly, Kim et al. (2010) showed that despite changes in proteolysis factors, such as degradation of desmin or troponin, or the autolysis of calpain-1, these changes were not responsible for decreases in tenderness or juiciness. It is unclear whether myosin cross-linking alone is responsible for decreases in meat tenderness and juiciness or whether other biochemical factors are involved.

Xiong et al. (2009) used different oxidizing systems to determine the effect of oxidation on myofibrillar proteins extracted from pork *serratus ventralis* muscle. Three systems—an iron-catalyzed, linoleic acid, and metmyoglobin-oxidizing system—were employed, because these systems generate different ROS, such as hydroxyl radicals, ferryl [iron(IV)-oxy] species, and hydroperoxide or peroxyl radicals, respectively (Xiong et al., 2009). Hydroxyl radicals and ferryl oxygen species induced greater cross-linking and aggregation of myosin heavy chain and actin protein than the other oxidizing systems. The oxidation-induced protein cross-linking alters protein functionality in processed meat products, where high protein oxidation levels reduce water-holding capacity, gelling strength, and emulsification properties of proteins (Xiong and Guo, 2020).

Protein oxidation is also known to occur in fresh meat, and one mechanism to study protein oxidation has involved the use of irradiation on fresh beef. Proteins in beef strip loins that were irradiated were more oxidized than non-irradiated steaks, as evidenced by increased myofibrillar and sarcoplasmic protein carbonyl content and western blots of oxidized myofibrillar and sarcoplasmic proteins (Rowe et al., 2004a). Protein carbonyl content was positively correlated with Warner-Bratzler shear force (WBSF), where greater carbonylation was associated with greater WBSF values or more tough steaks. Calpain-1 autolysis was slower in irradiated steaks than in non-irradiated steaks, indicating that calpain-1 was not as active early postmortem. Irradiation decreased troponin-T and desmin degradation at 3, 7, and 14 d post-irradiation (Rowe et al., 2004b). Steers fed the vitamin E–supplemented diet had a greater amount of α-tocopherol in the *longissimus lumborum* (LL). When steaks from vitamin E–supplemented steers were irradiated, there was less oxidation of sarcoplasmic proteins, indicating that supplementation of vitamin E can limit sarcoplasmic protein oxidation (Rowe et al., 2004a). The activity of calpastatin was greater in irradiated steaks at day 0, 3, and 14 post-irradiation than in non-irradiated steaks. Additionally, steaks from cattle supplemented with vitamin E had lower calpastatin activity than steaks not supplemented with vitamin E (Rowe et al., 2004b). While irradiation induced extreme oxidative conditions, these studies demonstrate that oxidative environments are detrimental to meat quality by suppressing calpain-1 autolysis and activity and increasing calpastatin activity early postmortem, yielding tougher meat even at extended postmortem aging times.

Calpain oxidation has been associated with increased meat toughness by sensory and instrumental measurements. The oxidation of calpain by H_2O_2 decreases the activity of calpain-1. The oxidation of calpastatin by H_2O_2 did not affect calpastatin activity (Carlin et al., 2006); thus, the compounding effect of calpain oxidation and calpastatin's ability to inhibit calpain activity may further restrict postmortem proteolysis. However, oxidation of the calpain-1/calpastatin complex by H_2O_2 at pH 6.5 and 7.5 increased calpain-1 activity and desmin degradation, but not at pH 6.0 (Carlin et al., 2006). In another study, calpain-1 activity and autolysis were decreased after exposure to an oxidative environment of 200 μM H_2O_2. The oxidation of calpain-1 resulted in a disulfide bond between the active site cysteine[115] and an adjacent cysteine[108] (Lametsch et al., 2008). The disulfide bond was the primary oxidation product of calpain-1, as no sulfenic acid was detected at the active site.

The oxidation of myofibrillar and sarcoplasmic proteins is detrimental to meat quality, including tenderness, water-holding capacity, and color. Including antioxidants in meat animal diets can limit protein oxidation and influence meat quality development. The intake of oxidized proteins may affect disease development and nutrient availability during digestion. The role of naturally occurring antioxidant proteins, such as Prdx, is hypothesized to limit protein oxidation and contribute to meat quality development. The function of the family of Prdx in a postmortem system has not been evaluated; therefore, further research is needed to determine whether Prdx play a guardian role against oxidation postmortem.

Peroxiredoxin

Peroxiredoxins are a class of thiol-specific antioxidant proteins ubiquitously expressed across many cell types and organelles. Structures of over 100 Prdx are known, ranging from 160 to 220 amino acids in length, with larger variations containing additional secondary structures (Karplus, 2015). All known Prdx share a recognizable structural core containing 7 central β-strands surrounded by 5 α-helices, yielding a high-affinity binding site with a highly conserved cysteine residue termed the peroxidatic cysteine (C_P). This peroxide binding site is not present in other antioxidant enzymes, such as glutathione peroxidase or catalase. The peroxide binding site allows Prdx to have similar second-order rate constants with hydrogen peroxide and other hydroperoxides compared with glutathione peroxidase or catalase, despite Prdx's lack of a metal cofactor or selenocysteine (Rhee and Kil, 2017). Because they represent 0.1% to 0.8% of soluble proteins in mammalian cells, Prdx are a significant component of redox response (Wood et al., 2003). The abundance of Prdx was estimated to be 50- and 500-fold greater in yeast cells than glutathione peroxidase and catalase, respectively (Karplus, 2015). The relatively high expression and high reaction rate constants suggest that Prdx serve as the primary mechanism to reduce hydroperoxides.

Structure and classification

Peroxiredoxins can be classified either by sequence or reaction mechanism similarities. Peroxiredoxins are grouped into 6 subfamilies—Prdx1, Prdx5, Prdx6, thioredoxin peroxidase (Tpx), bacterioferritin comigratory

protein (BCP), and alkyl hydroperoxide reductase E (AhpE)—based on sequence similarity, regardless of species (Nelson et al., 2011). The isoforms of Prdx in the Tpx, BCP, and AphE subfamilies are not expressed in mammals. In mammals, 6 isoforms of Prdx (Prdx1–6) have been identified, where mammalian Prdx1–4 are members of the Prdx1 subfamily and Prdx5 and Prdx6 are members of the Prdx5 and Prdx6 subfamilies, respectively (Rhee and Kil, 2017).

Alternatively, 1-Cys, typical 2-Cys, and atypical 2-Cys Prdx can distinguish the number of reactive cysteines and the reaction mechanism of Prdx (Hall et al., 2011). Mammalian Prdx1–4 are classified as typical 2-Cys, Prdx5 is an atypical 2-Cys, and Prdx6 is a 1-Cys. The 1-Cys Prdx only has a C_P, whereas 2-Cys Prdx contain a resolving cysteine (C_R) that participates in a condensation reaction after oxidation of the C_P, ultimately forming a disulfide bond. Typical 2-Cys Prdx function as a homodimer and maintain C_P and C_R on adjacent subunits, whereas atypical 2-Cys Prdx function monomerically and have C_P and C_R within the same subunit (Wood et al., 2003).

Fully folded and locally unfolded conformations

Peroxiredoxin molecules utilize a unique conformation change in their catalytic cycle to carry out their peroxidase activity. This conformational change was first described by Wood et al. (2002) and involves converting a fully folded active site to a locally unfolded state. In the fully folded state, the C_P rests at the bottom of the substrate-binding pocket, where it can react with a hydroperoxide molecule. Still, it is limited in reacting with other thiol groups or other reagents (Karplus, 2015). In this binding pocket, the thiolate group (C_P-S-) of the C_P is stabilized through a network of polar interactions with arginine, threonine, and proline residues—often termed the catalytic triad. The stabilization of the catalytic triad lowers the pKa and makes approximately 90% of the thiolate available at physiological pH (Zeida et al., 2019). After binding with a peroxide molecule, the active site will be converted to the locally unfolded form. This shift in active site conformation exposes the C_P side chain and facilitates the reaction with other cysteine groups to further the catalytic cycle.

Catalytic cycle of peroxiredoxin

The initial peroxidation step in the Prdx catalytic cycle maintains the active site in the fully folded conformational state. The C_P-S- is located at the bottom of the active site when a hydroperoxide enters the binding pocket. The peroxidation step of all Prdx originates similarly where the C_P-S- initiates an S_N2 nucleophilic attack on hydroperoxide, resulting in a peroxidatic sulfenic acid (C_P-SOH) and water.

The proceeding resolution step involves the reaction of the C_P-SOH with a second thiol group. For 2-Cys Prdx, the C_R will act as the second thiol group within the same or adjacent subunit. A conformational change must occur first as the active site binding pocket sterically limits disulfide bond formation (Karplus, 2015). After the conformational change occurs, the C_P-SOH is accessible for attack by the C_R, resulting in either an inter-subunit or intra-subunit disulfide bond for typical and atypical 2-Cys Prdx, respectively. For 1-Cys Prdx, no disulfide bonds are formed due to the absence of an adjacent C_R-SH. Thus, an alternative reducing system is required, but the literature does not do a complete job of fully characterizing the alternative reducing system. Potentially, glutathione can reduce 1-Cys Prdx, catalyzed by glutathione transferase π; however, the efficiency of this system *in vivo* is unclear (Zeida et al., 2019).

The reduction or recycling step of 2-Cys Prdx typically involves the physiological reductant thioredoxin. The N-terminal cysteine of thioredoxin first attacks the 2-Cys disulfide bond at the C_R, which results in a thiolate of C_P and a mixed disulfide. The C-terminal cysteine of thioredoxin attacks the N-terminal cysteine, forming a disulfide bond within the thioredoxin molecule while releasing C_R as a thiol (Zeida et al., 2019). The thioredoxin disulfide bond is reduced by thioredoxin reductase using electrons derived from NADPH (Netto and Antunes, 2016). The 2-Cys Prdx then undergoes a conformational shift back to a fully folded state to prepare for subsequent reactions with hydroperoxides.

A competitive reaction with the C_P-SOH can also occur if the disulfide bond resolution step is slow enough to allow the reaction of C_P-SOH with a second hydroperoxide molecule. The hyperoxidized product is a peroxidatic sulfinic acid (C_P-SO_2H) (Rhee and Kil, 2017). Unlike the C_P-SOH, the C_P-SO_2H cannot be reduced by the thioredoxin or glutathione systems. However, sulfiredoxin, an ATP-dependent protein, catalyzes the reduction of C_P-SO_2H to C_P-SOH (Rhee et al., 2007). The reduction to C_P-SOH by sulfiredoxin allows the Prdx to re-enter the catalytic cycle. Alternatively, the C_P-SO_2H can be oxidized further to a sulfonic acid (C_P-SO_3H), an unreactive end product. There are no known mechanisms to recover the C_P-SO_3H. The sensitivity of Prdx to reversible

hyperoxidation has been proposed to be an evolutionary adaptation to H_2O_2 signaling, to couple circadian rhythm to Prdx function, or for Prdx to serve as a protein chaperone (Rhee, 2016; Rhee and Kil, 2017). The physiological relevance of hyperoxidation is not known; however, hyperoxidation may have a role in signal transduction.

Quaternary structure of peroxiredoxins

Typical 2-Cys Prdx can assume a variety of oligomeric conformations based on localized redox state and necessary function. All Prdx found in animal tissues are obligate dimers (Barranco-Medina et al., 2008). As discussed previously, this dimer may form disulfide bonds or exist in the reduced state depending on its stage in the catalytic cycle. These dimers may form A (alternative)-type or B (β-sheet)-type interfaces based on β-strand interactions (Karplus, 2015). B-type dimers (also known as parallel dimers) associate through the edges of their β-strands to form a 14-stranded β-sheet. These dimers have been seen to be more stable than the A-type form. A-type (or perpendicular) Prdx dimers associate through loops at the C-terminal ends of β-strands 3 through 7 (Wood et al., 2002).

The first report of Prdx dimers forming a larger oligomeric structure was in 1969, when transmission electron microscopy analysis of torin isolated from erythrocytes revealed ring complexes with apparent 10-fold symmetry (Harris, 1969). Some ring complexes stack into columns of varying lengths forming higher molecular weight conformations. Since these original observations, more extensive studies have characterized the circumstances of Prdx decamer formation. B-type dimers may come together to form the decamer structure through interactions at the A-type interface (Karplus, 2015). Biological factors that have been documented to increase decamer formation include high (Kitano et al., 1999) or low (Kato et al., 1985; Chauhan and Mande, 2001) ionic strength, low pH (Kristensen et al., 1999), and high mineral content, including magnesium (Kato et al., 1985) and calcium (Allen and Cadman, 1979; Plishker et al., 1992). Peroxiredoxin concentration influences decamer formation, with formation threshold levels ranging from 1 to 2 μM (Barranco-Medina et al., 2008).

Peroxiredoxin oligomer formation is also strongly influenced by the Prdx redox state. The reduction of Prdx's C_P is the primary factor contributing to the stabilization of the Prdx decamer, with multiple experiments using different methods confirming this theory (Wood et al., 2003). However, some reduced Prdx will generally exist in the dimer form. An increase in the presence of the hyperoxidized form of Prdx can lead to decamer formation and stacked decamer rings, thus affecting cell signaling (Schröder et al., 2000; Dietz et al., 2006). Oxidized Prdx preferentially exists in the dimer form due to conformational changes in the active site related to the catalytic cycle of Prdx. The fully folded active site structure present in all catalytic states, excluding the oxidized disulfide, buttresses the decamer building A-type interface. In contrast, oxidation destabilizes the decamer by the locally unfolded active site state. This suggests that as Prdx is active in its catalytic cycle, it continuously switches from dimer to decamer conformation (Wood et al., 2002; Flohe and Harris, 2007).

Gel-based methods on measuring peroxiredoxins

The form and oxidation state of Prdx is known to vary. The variation in molecular weight and oxidation state of Prdx can be assessed with SDS-PAGE (Poynton and Hampton, 2014). The inclusion or exclusion of reducing agents, such as 2-mercaptoethanol, in the sample medium will affect which form or oxidation state is visualized with SDS-PAGE and immunoblotting. Total Prdx content may be analyzed by utilizing a reducing agent in the sample preparation (Feng et al., 2014). To visualize Prdx disulfide bonds and other quaternary structures, samples should be prepared without reducing agents (Cox et al., 2010). Multiple commercial antibodies are available to measure different Prdx isoforms through immunoblot analysis. Antibodies have also been developed for hyperoxidized Prdx by recognizing the amino acid sequence of the C_P active site for the sulfinic and sulfonic acid forms (Woo et al., 2003). These various methods allow for the interpretation of different forms and oxidation states of Prdx. Thus, research results should be considered in the context of the sample preparation and the methods utilized.

Post-translational modifications of peroxiredoxins

Post-translational modifications (PTM) are covalent processing events that change a protein's properties by proteolytic cleavage or adding a modifying group to one or more amino acids. A protein's activity, localization, turnover, and interaction with other proteins can be affected and modulated by one or more PTM (Mann and Jensen, 2003). Regarding Prdx, PTM have been shown to modulate the peroxidase activity, which may help explain the signaling capabilities of Prdx.

A summary of known PTM of Prdx1 and Prdx2 has been published (Rhee and Woo, 2020). A total of 11 and 7 amino acids for Prdx1 and Prdx2, respectively, are known to be modified by one or more PTM. The modifications related to Prdx1 and Prdx2 include hyperoxidation, phosphorylation, acetylation, glutathionylation, and s-nitrosylation. Peroxiredoxin-1 and Prdx2 share approximately 78% sequence identity and more than 90% sequence similarity, yet the differences in PTM appear to promote distinct functions and regulatory mechanisms (Rhee and Woo, 2020). Hyperoxidation has already been discussed to inactivate the peroxidase activity of Prdx by oxidation of C_P. Almost all of the PTM that have been identified for Prdx1 and Prdx2 decrease their peroxidase activity. Conversely, the phosphorylation of serine^{32} in Prdx1 and acetylation of lysine^{197} and lysine^{196} of Prdx1 and Prdx2, respectively, increase the peroxidase activity (Rhee and Woo, 2020). The significance of the modifications of Prdx in mammalian tissue is not well understood. The extent to which PTM may regulate Prdx function or activity is not well characterized. Future work needs to document differences in Prdx protein abundance and consider differences in PTM, because these modifications can affect the activity and function of Prdx.

Peroxiredoxin and Meat Quality

Protein functionality influences fresh meat quality. There has been a significant effort to determine undefined sources of meat quality variation using many protein chemistry techniques. The application of 2D SDS-PAGE, liquid chromatography and mass spectrometry (LC-MS), and western blotting have allowed detecting and quantifying a wide variety of proteins. Generally, reducing agents are included during protein extraction to reduce disulfide bonds present in the sample or that may randomly occur during sample preparation. The reduction and alkylation of free cysteines are necessary with complex protein mixtures to aid during the focusing and resolving stages of 2D and 1D SDS-PAGE and during protein digestion of LC-MS analysis. However, the inclusion of reducing agents may remove important modifications or disulfide bonds of some proteins that may provide further insight into the mechanisms of meat quality development. Without these protein modifications and disulfide bonds, the context in which the proteins are analyzed changes the results and interpretation of the data and may further confound these studies. One group of proteins that would be affected by the inclusion of reducing agents during sample preparation is the Prdx family of proteins. An appreciation for the change that reducing agents impart on certain proteins, such as Prdx, has often been ignored when discussing protein results in meat quality studies. Table 1 outlines studies that have identified associations between Prdx abundance and meat quality traits.

Tenderness

Tenderness of meat is a critical quality trait of meat products. Consumers prefer and are willing to pay more for meat products deemed tender; however, the consistent production of tender meat products remains elusive. Therefore, an understanding of factors that influence meat tenderness development is necessary. During postmortem aging of pork, Hwang et al. (2005) evaluated the change in protein profile over 7 d of postmortem aging. The abundance of Prdx2 decreased numerically during 7 d of postmortem aging in pork LL samples, and Prdx2 was positively correlated with WBSF values. The authors do not discuss this decrease in abundance, yet based on the evidence, a reasonable hypothesis is that Prdx2 is degraded postmortem.

Jia et al. (2007) evaluated proteome changes occurring immediately postmortem in Norwegian Red bulls. *Longissimus thoracis* (LT) samples were collected at 1, 2, 3, 6, 10, and 24 h postmortem, and proteins soluble at low ionic strength were extracted and resolved using two-dimensional SDS-PAGE. The abundance of Prdx2 and antioxidant protein 2 (Prdx6) increased up to 24 h postmortem, whereas Sp-22 (Prdx3) remained unchanged over that same time. In a subsequent study, Jia et al. (2009) classified LL steaks from Norwegian Red bulls as tough or tender based on 7 d postmortem WBSF values. Muscle biopsies of the live animal and samples at 1 h postmortem were collected. Proteins soluble at low ionic strength were extracted, separated, and resolved with 2D-DIGE, and Prdx6 was detected using immunoblot techniques. Three different spots of Prdx6 separated by isoelectric point were identified by immunoblotting. The abundance of Prdx6 spot 1, the most acidic, was greater in LL muscle biopsy samples from cattle classified as tender versus tough based on 7 d postmortem WBSF values. The abundance of Prdx6 spot 1 and spot 2 was greater in the 1 h postmortem LL samples classified as tender than tough. A greater abundance of the more acidic Prdx6 spot 1 may indicate that a greater portion of the Prdx6 protein population was oxidized; however, the specific protein modifications of the 3 spots of Prdx6 remain uncharacterized.

Table 1. A summary of previous muscle biology or meat quality research in which isoforms of peroxiredoxins were quantified or identified

Species/ Muscle	*N*	Muscle Fraction	Method	Category	Prdx	Abundance Change	Reference
Beef: LTL	18	Total protein, reducing	1D/ LC-MS	Tenderness	Prdx3	More abundant in 1 h postmortem sample of steaks classified as tender vs. tough based on 14 d postmortem WBSF values.	Zhu et al., 2021
Beef: LT	50	Low ionic extract, reducing	2D pH 3–10	Tenderness	Prdx1	Two spots, less abundant in 24 h postmortem sample of steaks classified as tender vs. tough based on 24 h postmortem WBSF values.	Malheiros et al., 2021
					Prdx2	Less abundant in tender vs. tough.	
					Prdx6	Less abundant in tender vs. tough.	
Beef: LL	15	Total protein, reducing	2D-DIGE Carbonyl Cy-Dye pH 3–10	Tenderness	Prdx1	More carbonylated at 48 h postmortem in steaks classified as tender vs. intermediate and tough based on 3 d postmortem WBSF values.	Malheiros et al., 2019
					Prdx2	More carbonylated in tender vs. intermediate and tough.	
Beef: SM	8	Isolated mitochondria, reducing	2D pH 4–7	Tenderness	Prdx3	Two spots. More abundant in isolated mitochondria 2.5 h postmortem from SM classified as tender vs. tough based on 21 d postmortem WBSF values.	Grabež et al., 2015
Beef: LT	A1: 26 A2: 16 B: 14	Low ionic extract, reducing	2D-DIGE pH 5–8 and 2D western blot	Tenderness	Prdx6	A1: Spot 1 was more abundant in muscle biopsies from cattle classified as tender vs. tough based on 7 d postmortem WBSF values. A2: Spots 1 and 2 were more abundant in 1 h postmortem muscle samples from cattle classified as tender vs. tough based on 7 d postmortem WBSF values. B: No differences.	Jia et al., 2009
Pork: LL	12	Low ionic extract, reducing	2D-DIGE pH 4–7, western blot	Tenderness	Prdx2	More abundant in pork chops at 8 and 21 d postmortem classified as HSP vs. LSP based on 21 d postmortem star probe values.	Schulte et al., 2020
Pork: LL	24	Low ionic extract, reducing	2D-DIGE pH 4–7, western blot	Tenderness	Prdx2	Two spots in DIGE were more abundant in aged chops classified as HSP vs. LSP based on 11–16 d postmortem star probe value. Western blot also showed more abundance in HSP vs. LSP.	Carlson et al., 2017
					Prdx6	More abundant in aged pork chops classified as HSP vs. LSP.	
Pork: LL	20	Total protein, reducing	2D pH 3–10	Postmortem storage	Prdx2	Decrease in abundance at 3 and 7 d aging compared with 0 d postmortem sample. Prdx2 was significantly correlated with WBSF.	Hwang et al., 2005
Beef: LT	8	Low ionic extract, reducing	2D pH 4–7	Postmortem storage	Prdx2	Increased in abundance up to 24 h postmortem.	Jia et al., 2007
					Prdx3	No change over 24 h postmortem.	
					Prdx6	Increased in abundance up to 24 h postmortem.	
Pork: LL	12	Muscle exudate, reducing	2D pH 4–7	Postmortem storage	Prdx2	More abundant in muscle exudate samples at 3 vs. 7 d postmortem.	Di Luca et al., 2013
					Prdx6	More abundant in muscle exudate samples at 1 vs. 3 d postmortem.	
Pork: LL	12	Muscle exudate, reducing	2D-DIGE pH 4–7	Drip loss	Prdx2	Two spots, more abundant in muscle exudate samples, classified as low and intermediate vs. high drip loss when analyzed regardless of day postmortem.	Di Luca et al., 2016
Pork: LD	20	Total protein, reducing	2D pH 3–10	24 h pH	Prdx6	More abundant at 0 h postmortem muscle of pork chops classified as high vs. low based on 24 h postmortem pH values.	Subramaniyan et al., 2017
Pork: LL	20	Low ionic extract, reducing	Western blot	14 d pH	Prdx2	More abundant at 45 min in chops classified as normal vs. low based on 14 d postmortem pH values.	Zuber et al., 2021
Pork: LL	12	Low ionic extract, reducing	2D pH 3–10	Lipid content	Prdx6	More abundant at 20 min postmortem in chops classified as high vs. low based on total lipid content at 36 h postmortem.	Liu et al. 2009

Table 1. (*Continued*)

Species/ Muscle	*N*	Muscle Fraction	Method	Category	Prdx	Abundance Change	Reference
Pork: LL	24	Low ionic, reducing and non-reducing	Western blot	RFI and health status	Prdx2	Total Prdx2 content (with reducing agent) was greater immediately postmortem in LL from pigs classified as HRFI vs. LRFI.Prdx2 decamer (absent reducing agent) was greater immediately postmortem in LL from pigs classified as HRFI vs. LRFI.Prdx2 2nd, the faster-migrating band (absent reducing agent) was greater immediately postmortem in LL from pigs classified as HRFI vs. LRFI.	Patterson et al., 2021
Pork: LL	24	Total protein, reducing	2D pH 3–11	RFI	Prdx6	More abundant at 20 min postmortem muscle of pigs classified as high vs. low RFI.	Vincent et al., 2015
Pork: ST (red and white)	24	Low ionic extract, reducing	2D-DIGE pH 3–10	Heat stress	Prdx1	Less abundant immediately postmortem in the white portion of ST from heat-stressed vs. thermal-neutral pigs.	Cruzen et al., 2015
					Prdx2	Two spots. Both were less abundant immediately postmortem in red and white ST from heat-stressed vs. thermal-neutral pigs.	
					Prdx6	Three spots. One spot was greater in the red portion of ST in heat-stressed vs. thermal-neutral and pair-fed thermal-neutral pigs. A different spot was less abundant in red ST from heat-stressed vs. pair-fed thermal-neutral pigs.	
Pork: LD	16	Total protein, reducing	2D pH 4–7	Heat stress	Prdx6	More abundant at 15 min postmortem in LD muscle from heat-stressed vs. control pigs.	Cui et al., 2018
Pork: ST (red and white)	24	Low ionic extract, reducing	2D-DIGE pH 3–10	Heat stress	Prdx2	Two spots. Both were less abundant immediately postmortem in red ST from heat-stressed vs. thermal-neutral pigs.	Cruzen et al., 2017
Beef: ST	4	Low ionic extract, reducing	2D pH 3–10	Color	Prdx2	More abundant in ST steaks at 0 vs. 15 d storage.	Wu et al., 2015
					Prdx6	Less abundant in ST steaks at 0 vs. 10 d storage. Significantly correlated with a^* value.	
Beef: LT	23	Low ionic extract, reducing	2D pH 3–10	Color	Prdx1	Less abundant at 96 h postmortem in steaks from carcasses classified as dark cutter vs. non-dark cutter.	Mahmood et al., 2018
					Prdx3	More abundant at 96 h postmortem in steaks from carcasses classified as a-typical dark cutter vs. non-dark cutter.	
Beef: LL, PM	7	Low ionic extract, reducing	2D pH 5–8	Color	Prdx2	More abundant in LL vs. PM at 24 h postmortem. Significantly correlated with surface color stability.	Joseph et al., 2012
Beef: LL	8	Low ionic extract, reducing	2D pH 3–10	Color	Prdx2	One spot was less abundant in steaks from the LL at 10 d storage packaged in HiOx vs. CO packaging. Two spots were less abundant in steaks from the LL at 15 d storage packaged in HiOx vs. CO packaging.	Yang et al., 2018
					Prdx3	One spot was less abundant in steaks from the LL at 15 d storage packaged in HiOx vs. CO packaging.	
					Prdx6	One spot was less abundant in steaks from the LL at 15 d storage packaged in HiOx vs. CO packaging.	
Beef: LL, PM	4	Low ionic extract, reducing	2D pH 3–10	Color	Prdx1	One spot was less abundant in steaks from the LL at 10 and 15 vs. 0 d storage.	Wu et al., 2016
					Prdx2	One spot was less abundant in steaks from the LL at 15 vs. 0 d storage, whereas 2 were more abundant in steaks from the PM at 15 vs. 0 d storage.	
					Prdx3	One spot was greater in abundance in steaks from the PM at 15 vs. 0 d storage.	
					Prdx6	One spot was greater in abundance in steaks from the PM at 10 and 15 vs. 0 d storage.	

DIGE = Difference in gel electrophoresis; HiOx = high-oxygen; HRFI = high residual feed intake; HSP = high star probe; LC-MS = liquid chromatography–mass spectrometry; LD = *longissimus dorsi*; LL = *longissimus lumborum*; LRFI = low residual feed intake; LSP = low star probe; LT = *longissimus thoracis*; PM = *psoas major*; Prdx = peroxiredoxins; RFI = residual feed intake; ST = *semitendinosus*; WBSF = Warner-Bratzler shear force; CO = carbon monoxide.

Similarly, Grabež et al. (2015) isolated mitochondria at 2.5 h postmortem from the *semimembranosus* (SM) of Norwegian Red cattle. The WBSF of 21 d aged SM samples was measured, and 2 groups—tender WBSF (2.33 ± 0.22 kg, $n = 4$) and tough WBSF (7.45 ± 0.73 kg, $n = 4$)—were used for two-dimensional SDS-PAGE analysis. Two spots of thioredoxin-dependent peroxide reductase (Prdx3) were greater in abundance in isolated mitochondria for SM steaks classified as tender versus tough based on 21 d WBSF values. In beef LT classified as tender (3.38 ± 0.33 kg, $n = 9$) and tough (6.53 ± 0.41 kg, $n = 9$) based on 14 d postmortem WBSF values, Zhu et al. (2021) solubilized total proteins (resolved briefly on 1D SDS-PAGE gels) and excised, identified, and quantified proteins with LC-MS. Peroxiredoxin-3 was more abundant in 1 h postmortem samples of steaks classified as tender versus tough based on 14 d postmortem WBSF values. These studies show that Prdx3 abundance is greater in early postmortem samples from steaks classified as tender versus tough based on aged WBSF values. Peroxiredoxin-3 is expressed primarily in the matrix of mitochondria. It could be hypothesized that the greater abundance of Prdx3 in the early postmortem samples of tender steaks helps maintain a reducing environment for a longer period postmortem compared with the tough steaks. The specific form of Prdx3 and the extent to which Prdx3 is active in postmortem muscle are unknown.

Malheiros et al. (2019) classified LL steaks from Angus cattle as tender (3.90 ± 0.29 kg, $n = 5$) and tough (7.60 ± 0.80 kg, $n = 5$) based on WBSF at 3 d postmortem. Total proteins were extracted and labeled with Carbonyl Cy-Dye for 2D-DIGE analysis. One spot of both Prdx1 and Prdx2 was more carbonylated at 2 d postmortem in steaks classified as tender versus tough. In another study, LT were collected from Nellore cattle at 24 h postmortem (Malheiros et al., 2021). Steaks were categorized into tender (3.9 ± 0.7 kg, $n = 15$), moderately tough (5.6 ± 0.7 kg, $n = 20$), and tough (7.9 ± 1.4 kg, $n = 15$). Proteins soluble at low ionic strength were solubilized and resolved with 2D SDS-PAGE. The abundance of Prdx1, Prdx2, and Prdx6 was greater at 24 h postmortem in steaks classified as moderately tough and tough versus tender. From these studies, Prdx1 and Prdx2 were more carbonylated early postmortem in steaks categorized as tender versus tough, and the abundance of Prdx1, Prdx2, and Prdx6 was greater in steaks classified as tough versus tender.

In another study, pork loin chops were separated into low (LSP; 4.95 ± 0.07 kg, $n = 12$) and high (HSP; 7.75 ± 0.21 kg, $n = 12$) on star probe texture at 14 d postmortem. In aged pork at 14 d postmortem, Prdx2 and Prdx6 were less abundant in LSP than in HSP classification (Carlson et al., 2017). In a separate study, pork loin chops were separated into LSP (5.72 ± 0.57 kg, $n = 6$) and HSP (8.76 ± 0.60 kg, $n = 6$) based on star probe texture at 21 d postmortem. At 8 and 21 d postmortem, Prdx2 abundance was less in LSP than HSP based on immunoblot analysis (Schulte et al., 2020). These results may imply that Prdx2 and Prdx6 are greater in abundance perimortem or immediately postmortem in response to oxidative stress conditions. However, it is unclear whether all Prdx are active in maintaining reducing conditions to promote meat tenderness development by protecting calpains from oxidation. Between different isoforms of Prdx, the direction of the change in abundance is not always the same. Some isoforms of Prdx may be more influential in developing meat tenderness than others; however, it remains unclear what significance each isoform has for meat tenderness. The abundance and form of Prdx might simply give insight into oxidative stress perimortem. Alternatively, Prdx may be a readily available substrate for calpains, and other factors may cause variation in meat tenderness. The degradation of Prdx could explain their lesser abundance in more tender meat. Additional experiments are necessary to better characterize the conditions that influence Prdx form and abundance under conditions that mimic perimortem and postmortem skeletal muscle conditions.

Color

Several proteomic experiments (as cited in Table 1) on postmortem meat retail storage have been conducted, and Prdx isoforms have been identified as differentially abundant. The following experiments packaged and stored steaks from Chinese Luxi yellow cattle for 0, 5, 10, or 15 d. Wu et al. (2015) extracted proteins soluble at low ionic strength from ST steaks and resolved proteins with 2D SDS-PAGE. The abundance of Prdx2 was greater at 0 d versus 15 d storage. The abundance of Prdx6 was greater at 10 d versus 0 d storage and negatively correlated with a^* value. Wu et al. (2016) compared LL and *psoas major* (PM) steaks. Peroxiredoxin-1 abundance was less at 10 d and 15 d versus 0 d storage, whereas Prdx2 was less abundant at 15 d versus 0 d storage in LL steaks. In the PM, 2 spots of Prdx2 and one spot of both Prdx3 and Prdx6 were greater in abundance at 15 d versus 0 d storage. Yang et al. (2018) packaged steaks in either high-oxygen (HiOx) or carbon monoxide (CO)-modified atmosphere packaging and extracted proteins soluble at low ionic strength. One spot of Prdx2 was less abundant from

LL steaks packaged in HiOx versus CO at 10 d storage, whereas 2 spots of Prdx2 were less abundant at 15 d storage. One spot of both Prdx3 and Prdx6 was less abundant in HiOx versus CO packaged steaks from the LL at 15 d storage. Joseph et al. (2012) compared beef LL and PM steaks at 1 d postmortem. Proteins soluble at low ionic strength were extracted, and 2D SDS-PAGE was conducted. Peroxiredoxin-2 abundance was greater in the LL than the PM, which the authors suggest contributed to the LL being more color stable over 9 d of retail display (Joseph et al., 2012).

These results highlight muscle differences regarding Prdx abundance related to meat color and muscle. The difference in abundance may be related to muscle fiber type, or Prdx abundance may be greater peri- or early postmortem to respond to oxidative stress. Skeletal muscles that rely on oxidative metabolism have a greater abundance of mitochondria, which produce a greater abundance of ROS (Glancy and Balaban, 2011; Brand, 2016). However, this assumption that Prdx are more abundant in more oxidative muscles conflicts with some of the aforementioned studies. It could be reasonably hypothesized that a greater abundance of Prdx would help promote a reducing environment that sustains meat color and limits myoglobin oxidation. Therefore, future research is needed to better characterize the differences of Prdx isoforms between muscles of different fiber types to allow us to better understand the relationship with meat color.

Water-holding capacity

Di Luca et al. (2013) utilized pork chops with an intermediate drip loss (3.91% ± 0.38%) and found that Prdx2 and Prdx6 decreased in abundance in muscle exudate between 1 d and 7 d storage. In pork chops classified as high (6.1% ± 1.4%), intermediate (3.9% ± 0.4%), or low (2.5% ± 0.3%) on drip loss, Di Luca et al. (2016) reported that 2 spots of Prdx2 were greater in abundance in muscle exudate from the low versus high drip loss chops. These studies report that Prdx2 and Prdx6 abundance in muscle exudate decreases with postmortem storage and the abundance of Prdx2 was greater in pork chops with higher water-holding capacity. The oxidation state of these Prdx was not reported, but Prdx may limit the oxidation of proteins, lipids, or other cellular components resulting in improved water-holding capacity.

Postmortem pH

Mahmood et al. (2018) compared LT steaks from normal and dark-cutting cattle at 4 d postmortem. Proteins soluble at low ionic strength were extracted and resolved with 2D SDS-PAGE. Peroxiredoxin-1 abundance was greater in normal versus dark-cutting beef, whereas Prdx3 abundance was greater in dark-cutting beef than normal beef. Dark-cutting beef exhibits an undesirable dark color and a higher ultimate pH. It is unknown whether the differences in Prdx1 and Prdx3 abundance are caused by antemortem or postmortem conditions.

The *longissimus dorsi* (LD) of Berkshire pigs was classified into high-pH (HpH; 5.92 ± 0.02; $n = 10$) or low pH (LpH; 5.55 ± 0.03; $n = 10$) groups based on 24 h postmortem pH. The abundance of Prdx6 was greater at 0 h postmortem in the HpH compared with the LpH group (Subramaniyan et al., 2017). When LL chops were categorized as normal pH (NpH; 5.59 ± 0.01; $n = 10$) and LpH (5.42 ± 0.01; $n = 10$) based on 14 d postmortem pH, Zuber et al. (2021) reported a greater abundance of Prdx2 at 45 min postmortem in NpH compared with LpH groups.

Antemortem conditions

Heat stress has been another model to study changes in the skeletal muscle protein in response to induced stress. Cruzen et al. (2015) compared pigs raised in thermal-neutral, pair-fed thermal-neutral, and heat-stress conditions for 12 h. The ST exhibits a red and white portion, allowing for muscle fiber–type comparisons within a single muscle. Proteins soluble at low ionic strength were extracted, and 2D-DIGE was conducted. Regardless of muscle portion, 2 spots of Prdx2 were less abundant immediately postmortem in ST from heat stress pigs versus thermal-neutral pigs. One spot of Prdx1 and Prdx6 was greater in the white and red portions, respectively. Cruzen et al. (2017) exposed pigs to 0, 2, 4, or 6 h of heat stress to study more acute heat stress. The ST was collected immediately postmortem, and proteins soluble at low ionic strength were extracted and resolved with 2D-DIGE. Again, 2 spots of Prdx2 were less abundant in the red portion of ST from heat stress pigs compared with thermal-neutral pigs.

In another heat stress experiment, Cui et al. (2018) exposed pigs to 3 wk of chronic heat stress. Total proteins were extracted from LD samples and resolved with 2D SDS-PAGE. In LD samples at 15 min postmortem, heat-stressed pigs had a greater abundance of Prdx6 than control pigs. These heat stress experiments suggest that Prdx isoforms respond differently to heat stress. Also, the abundance of Prdx isoforms may be altered differently between muscles

of different muscle fiber types. Peroxiredoxin-2 and Prdx6 were identified in multiple spots, but the nature of the modification of those Prdx was not reported. Understanding the differences between these various spots on 2D SDS-PAGE gels will be essential because, currently, limited conclusions can be drawn from the data. Identifying the modifications between the different spots of Prdx will further the understanding of the mechanism of the heat stress response. By understanding the molecular responses to stress, livestock can be genetically selected to respond better to oxidative stress, and livestock management practices can be modified to improve meat quality.

One model for studying the efficiency of livestock production has been to genetically select for residual feed intake (RFI). Residual feed intake is a measure of feed efficiency based on the difference between an individual animal's expected and actual feed intake. While RFI is not a direct measure of meat quality, differences in skeletal muscle growth of less efficient livestock can be explained by altered metabolic activity and energy production efficiency. The loss of electrons because of a less efficient energy production system could contribute to physiological stress because more electrons would be available to react to form ROS. It has been shown that the production of H_2O_2 in the mitochondria of red and white porcine *semitendinosus* muscle from low-RFI (more efficient) pigs was less than the high-RFI (less efficient) pigs (Grubbs et al., 2013). In a subsequent RFI generation, pigs exposed to a respiratory pathogen challenge were less efficient than non–disease-challenged (control) pigs (Helm et al., 2018). The infected pigs tended ($P = 0.088$) to have greater production of H_2O_2 in mitochondria isolated from *longissimus* muscle than control pigs. Based on RFI and infection status, the greater production of H_2O_2 in skeletal muscle of less efficient pigs may explain part of the discrepancy in growth efficiency. More energy would be needed to reduce oxidized antioxidant proteins and repair oxidatively damaged tissues caused by greater H_2O_2 production in less efficient livestock.

Peroxiredoxins exist in multiple forms that can be distinguished using SDS-PAGE and immunoblotting techniques. The profile of Prdx in skeletal muscle is not well characterized. A lack of understanding of the biochemical changes related to Prdx in skeletal muscle hinders the ability to draw clear connections between oxidation, oxidative stress, skeletal muscle growth, and meat quality. In order to classify these changes in Prdx, Patterson et al. (2021) prepared low ionic strength protein extracts in the presence and absence of a reducing agent from skeletal muscle from a subset of RFI and pathogen challenge pigs (Helm et al., 2018). The samples prepared in the absence of a reducing agent would maintain inherent disulfide bonds and other oligomeric structures of proteins, including Prdx. Patterson et al. (2021) focused primarily on Prdx2 because it has been consistently identified and associated with meat quality and physiological stress. The forms of Prdx2 that were quantified included the total reduced monomer, hyperoxidized monomer, non-reduced Prdx2, and decamer. The total reduced monomer and the non-reduced decamer were greater in the LL of pigs from high versus low RFI. In the absence of a reducing agent, Prdx2 was observed in 2 distinct bands based on migration on an SDS-PAGE gel. The faster-migrating band was greater in the LL of pigs from high versus low RFI. The hyperoxidized Prdx2 (as a proportion of total Prdx2 in a lane) was greater in the LL of pigs from low versus high RFI (Patterson et al., 2021). These results highlight the variety of oxidation states of Prdx that exist in skeletal muscle. The oxidation state of Prdx2 influences the formation of larger oligomeric structures like the decamer. A greater proportion of reduced or hyperoxidized monomers has contributed to decamer formation (Rhee and Kil, 2017). In a separate study of RFI lines, Vincent et al. (2015) reported a greater abundance of Prdx6 in LD from pigs classified as high versus low RFI with 2D SDS-PAGE. The less efficient pigs may require a greater total amount of Prdx to mitigate the increased level of H_2O_2 and other oxidants, whereas the energy-producing systems in more efficient pigs may have less ROS production.

Importance of context

Primarily the monomers of Prdx1, Prdx2, Prdx3, and Prdx6 have been identified, quantified, and associated with meat quality measurements. However, there are often multiple spots of Prdx identified in 2D gels, which could be due to PTM. Up to 3 spots of Prdx6 have been quantified with immunoblots, and at least 2 spots of Prdx1, Prdx2, and Prdx3 have also been identified. There are a variety of modifications that Prdx can undergo. The specific modifications that result in different spots due to isoelectric point focusing are unknown but have been hypothesized to result from hyperoxidation of Prdx (Jia et al., 2009) or other known PTM (Rhee and Woo, 2020). Because multiple spots of Prdx can be identified on a 2D gel, and the different modifications between the spots are unknown, the

context in which results are interpreted is not fully understood.

In muscle and meat tissue, Prdx exist as a homodimer, decamer, or other oligomeric structures that form inter- and intramolecular disulfide bonds. These quaternary and tertiary structures are almost always disrupted during protein quantification due to the inclusion of detergents and reducing agents. In disrupting these protein structures, the context in which results are interpreted is altered. Patterson et al. (2021) extracted proteins from pork skeletal muscle in a denaturing, non-reducing buffer and evaluated the decamer and dimeric structures of Prdx2. In quantifying these structures under non-reducing conditions, a better understanding of the oxidation state of proteins can be enumerated.

The application of 2D gels and LC-MS methods to study meat quality has been beneficial in advancing the knowledge of mechanisms of meat quality development. These methods detect proteins and modifications of proteins associated with meat quality. There are limitations to consider with both methods. The use of 2D gels allows for many protein spots to be resolved; however, the identity of many proteins or specific PTM of proteins may not be identified. Primarily only protein spots that are differentially abundant between comparisons are identified, whereas those not significantly different are commonly not identified, reported, or discussed. With most LC-MS experiments, samples are often reduced and digested with trypsin. These proteases generate peptides from intact parent proteins and degraded protein fragments, all of which contribute to the reported protein abundance. The consideration of degradation products' contribution to overall protein abundance must be recognized. Regardless of the methods used, it is imperative to put research results and observations in context and not overstate the observations of research experiments.

In postmortem muscle, aerobic metabolism is limited, and metabolism ceases due to decreased pH, oxygen availability, accumulation of waste products, lower temperature, and energy or substrate availability. Because of these occurrences, NADPH is limiting, and thus the role of Prdx as antioxidants in later postmortem products may not be relevant. However, while NADPH is present in the muscle tissue, Prdx and thioredoxin reducing systems would still be available. It is unclear when NADPH depletion occurs in postmortem skeletal muscle, but differences in NADPH production and consumption in skeletal muscle may ultimately impact the overall reducing capacity of Prdx and other antioxidant systems. In later postmortem skeletal muscle, it is unclear what role Prdx may play in mitigating oxidative damage since thioredoxin reducing systems may not be active, and Prdx would primarily exist as disulfide bound, hyperoxidized, or in aggregated dimer forms. Antioxidant activity of Prdx is likely more relevant early postmortem, when NADPH would still be present at some levels. The ability of Prdx in early postmortem skeletal muscle to maintain a reducing environment may ultimately impact meat quality development later postmortem. Studies that emphasize a greater understanding of the context of Prdx form, abundance, and activity under postmortem skeletal muscle conditions will provide greater insight into their role in skeletal muscle and meat quality.

Conclusions

Oxidation of proteins in postmortem muscle is possible when the cellular environment reflects a response to oxidative stress. Protein oxidation is generally detrimental to protein function and fresh meat color, water-holding capacity, and tenderness. Peroxiredoxins are indicators of oxidative events in perimortem and postmortem muscle. Variations in abundance, form, and modifications of Prdx proteins are associated with fresh meat quality traits. Continued investigations should focus on the complexity of these antioxidant proteins and their functions to establish a clear connection between their response to oxidative stress and ultimate meat quality.

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