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# Temperature- and pH-dependent effect of lactate on *in vitro* redox stability of red meat myoglobins ☆

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ABSTRACT

Our objective was to evaluate the influence of lactate on *in vitro* redox stability and thermostability of beef, horse, pork, and sheep myoglobins. Lactate (200 mM) had no effect ($P > 0.05$) on redox stability at physiological (pH 7.4, 37 °C) and meat (pH 5.6, 4 °C) conditions. However, lactate increased ($P < 0.05$) metmyoglobin formation at a condition simulating stressed live skeletal muscle (pH 6.5, 37 °C). The redox stability of myoglobins at stressed live skeletal muscle and meat conditions was species–specific ($P < 0.05$). Myoglobin thermostability at 71 °C was lower ($P < 0.05$) in the presence of lactate compared with controls and was influenced ($P < 0.05$) by species. The results of the present study indicate that the effects of lactate on myoglobin are temperature and pH dependent. The observed lack of influence of lactate on myoglobin redox stability at meat condition suggests that the color stability of lactate-enhanced fresh meat is not due to direct interactions between the ingredient and the heme protein.



## 1. Introduction

Color is a major quality trait influencing meat purchase decisions, and discoloration is generally considered a sign of spoilage leading to rejection (Faustman & Cassens, 1990). The formation of metmyoglobin results in meat discoloration, which is responsible for an estimated annual revenue loss of $1 billion to the United States beef industry (Smith, Belk, Sofos, Tatum, & Williams, 2000). Several processing strategies have been employed to minimize surface discoloration in fresh meats (Mancini & Hunt, 2005).

Lactate, widely used as a non-meat ingredient in enhanced fresh meats, exerts a color-stabilizing effect by minimizing surface discoloration and promoting formation of ferrous myoglobin redox forms (Kim et al., 2006; Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2004; Mancini, Suman, Konda, & Ramanathan, 2009; Suman et al., 2010). Myoglobin redox stability is species-specific and is influenced by the primary structure (Brown & Mebine, 1969; Gutzke & Trout, 2002). Previous studies (Giardina et al., 1996; Tamburrini, Romano, Giardina, & di Prisco, 1999) reported a species-specific influence of lactate on oxygen affinity of horse, sperm whale, and Emperor penguin myoglobins. Although lactate improved redox stability of horse myoglobin *in vitro* (Mancini & Ramanathan, 2008), the species-specific effect of lactate on redox stability of red meat myoglobins is yet to be investigated. Examining the effect of lactate on red meat myoglobins will help characterizing the molecular mechanisms through which lactate influences meat color stability. Furthermore, this approach will enable meat industry to engineer species-specific processing strategies to improve color stability.

Thermostability of myoglobin depends on redox state; deoxymyoglobin is the most resistant to heat denaturation followed by oxymyoglobin and metmyoglobin (Machlik, 1965; Sepe et al., 2005). Since lactate influences myoglobin redox state by promoting the formation of ferrous forms (Mancini & Ramanathan, 2008), it is logical to anticipate that lactate can influence thermostability. This hypothesis was further supported by previous research (Kim, Keeton, Hunt, & Savell, 2010), which documented that lactate-enhancement decreased cooking-induced myoglobin denaturation in beef steaks by maintaining the heme protein in a ferrous state. Nevertheless, the direct influence of lactate on the thermostability of red meat myoglobins in model systems has not been examined.

The objective of the present study was to examine the direct effect of lactate on redox and thermal stabilities in beef, horse, pork, and sheep myoglobins at physico-chemical conditions encountered in physiological skeletal muscle, stressed live skeletal muscle, and meat.

## 2. Materials and methods

### 2.1. Materials and chemicals

Sephacryl 200HR, ammonium sulfate, Tris–HCl, EDTA, horse heart myoglobin, sodium hydrosulfite, sodium citrate, sodium phosphate, and sodium lactate were procured from Sigma Chemical Co. (St. Louis,

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MO, USA), and PD-10 columns were obtained from GE Healthcare (Piscataway, NJ, USA). All chemicals were of reagent grade or greater purity.

### 2.2. Myoglobin isolation and purification

Beef, pork, and sheep myoglobins are not commercially available and therefore were isolated from cardiac muscles. Fresh hearts were obtained locally within 1 h of exsanguination, placed on ice, and transported to the laboratory. Myoglobin was purified via ammonium sulfate precipitation and gel-filtration chromatography (Faustman & Phillips, 2001). Briefly, cardiac muscle tissue was homogenized in buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0, 4 °C) and centrifuged at 5000 ×*g* for 10 min. The supernatant was brought to 70% (for beef and pork) or 50% (for sheep) ammonium sulfate saturation and centrifuged at 18000 ×*g* for 20 min. The resulting supernatant was saturated with ammonium sulfate (100%) and centrifuged at 20000 ×*g* for 1 h. The precipitate was re-suspended in homogenization buffer and dialyzed (3 × 40 volumes) against 10 mM Tris–HCl, 1 mM EDTA, pH 8.0, at 4 °C for 24 h. Myoglobin was separated from hemoglobin using a Sephacryl 200HR gel-filtration column (2.5 cm × 100 cm). The elution buffer contained 5 mM Tris–HCl, 1 mM EDTA, at pH 8.0 and 4 °C, and the flow rate was 60 mL/h.

### 2.3. Oxymyoglobin preparation

Oxymyoglobin was prepared by hydrosulfite-mediated reduction (Brown & Mebine, 1969). The residual dithionite was removed by passing through PD-10 desalting columns, and the pH of the myoglobin solution was adjusted to pH 5.6 or 6.5 with 50 mM sodium citrate buffer, or to pH 7.4 with 50 mM sodium phosphate buffer. The absorbance spectra (from 650 to 500 nm) were recorded using UV-2401PC spectrophotometer (Shimadzu Inc., Columbia, MD, USA), and the purity of oxymyoglobin (100%) was ensured using wavelength maxima at 503, 557, and 582 nm, representative for metmyoglobin, deoxymyoglobin, and oxymyoglobin, respectively (Tang, Faustman, & Hoagland, 2004).

### 2.4. Myoglobin redox stability in the presence of sodium lactate

Beef (0.15 mM), horse (0.15 mM), sheep (0.15 mM), and pork (0.075 mM) oxymyoglobins were incubated with sodium lactate (200 mM) at pH 5.6 and 4 °C (typical meat storage condition) in 50 mM sodium citrate buffer, at pH 6.5 and 37 °C (stressed live skeletal muscle condition; Gollnick, Bertocci, Kelso, Witt, & Hodgson, 1990) in 50 mM sodium citrate buffer, and at pH 7.4 and 37 °C (physiological skeletal muscle condition) in 50 mM sodium phosphate buffer. The interactions between lactate and myoglobin are relevant in live skeletal muscles at physiological (pH 7.4 and 37 °C) as well as stressed (pH 6.5 and 37 °C) conditions, and therefore, these conditions were also investigated. The concentration of myoglobin reflected previously reported concentrations for each species, whereas that of lactate corresponded to levels used in enhanced beef (Mancini et al., 2009). Controls consisted of oxymyoglobin plus a volume of deionized water equivalent to that used to deliver the lactate. Samples were scanned spectrophotometrically (UV-2401PC, Shimadzu Inc., Columbia, MD, USA) from 650 to 500 nm at specific incubation times, and metmyoglobin formation was calculated (Tang et al., 2004).

### 2.5. Myoglobin thermostability in the presence of sodium lactate

The thermostability of beef, pork, sheep, and horse oxymyoglobins in the presence of sodium lactate (200 mM) was assessed at meat conditions by determining the percentage myoglobin denaturation (PMD) at 71 °C, the recommended internal cooking temperature for meat (USDA, 1997). Oxymyoglobins were prepared as described earlier, and the pH was adjusted to 5.6 by passing through PD-10 desalting columns equilibrated with 50 mM sodium citrate buffer. Oxymyoglobin samples were incubated with sodium lactate (200 mM) at pH 5.6 and 4 °C for 48 h to simulate post-enhancement refrigerated storage of meat. Controls consisted of oxymyoglobin plus a volume of deionized water equivalent to that used to deliver the lactate. After 48 h incubation, the absorbance spectra were recorded, and the myoglobin samples were incubated at 71 °C in a water bath for 20 min. Samples were withdrawn at specific intervals (5, 10, 15, and 20 min) and cooled immediately by immersing in slushed ice to prevent post-incubation temperature rise. After centrifugation at 16,000 ×*g* for 2 min, myoglobin concentration was determined by measuring the absorbance at 525 nm (Tang et al., 2004) using a UV-2401PC spectrophotometer (Shimadzu Inc., Columbia, MD, USA). PMD was calculated as:

PMD = 100 × [(pre-incubation myoglobin concentration − post-incubation myoglobin concentration) / pre-incubation myoglobin concentration]

### 2.6. Statistical analysis

The experimental designs for redox and thermostability experiments were completely randomized designs with repeated measures, and each experiment was replicated three times ($n = 3$). It is well established that pH affects myoglobin oxidation; therefore, data for each pH were analyzed separately. Type-3 tests of fixed effects for species, lactate, changes in oxymyoglobin redox state during incubation, and their interactions were evaluated using the Mixed procedure of SAS (SAS, 2011). The repeated option in mixed was used to assess covariance–variance structure resulting from repeated measurements on the same sample during incubation. Least square means were generated for significant *F*-tests ($P < 0.05$), and differences among means were detected at the 5% level using the least significant difference (LSD) test.

## 3. Results

### 3.1. Myoglobin redox stability in the presence of lactate

Metmyoglobin formation in beef, horse, pork, and sheep oxymyoglobins at pH 7.4, 37 °C (physiological skeletal muscle condition) in the presence of lactate is presented in Fig. 1. Lactate had no effect ($P > 0.05$) on metmyoglobin formation at physiological skeletal muscle condition. The percentage metmyoglobin increased ($P < 0.05$) over

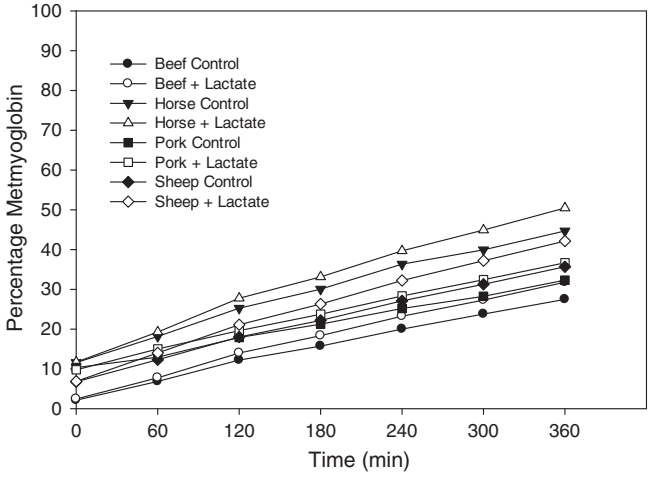


**Fig. 1.** Metmyoglobin formation in beef, horse, pork, and sheep myoglobins incubated with sodium lactate (200 mM) at pH 7.4 and 37 °C. Standard error for species × treatment × time = 5.1.