A MECHANISM FOR THE INVOLVEMENT OF NADH-DRIVEN METMYOGLOBIN REDUCING ACTIVITY IN MEDIATING LIPID OXIDATION IN MUSCLE POST-MORTEM

A. E. D. Bekhit*[1], M. M. Farouk[2], L. Cassidy[2], and R. Hurst[3]
[1]*Agriculture and Life Sciences Division, Lincoln University, Canterbury, New Zealand*
[2]*AgResearch Limited, Ruakura MIRINZ Centre, Hamilton, New Zealand.*
[3] *Health & Food Group, The Horticulture and Food Research Institute of New Zealand Ltd., Hamilton, New Zealand.*

Key Words: NADH, metmyoglobin, reducing activity, lipid oxidation

Introduction

The reduction of metmyoglobin is an important physiological phenomenon in live animals and is also important in maintaining a fresh colour in meat during post-mortem storage depending on the availability of cofactors. Metmyoglobin reduction was identified in skeletal muscle through the microsomal NADH-dependent electron transfer involving NADH-cytochrome b_5 reductase and cytochrome b_5 (Arihara et al., 1989; 1997). However, other enzymatic and non-enzymatic systems may be involved (Bekhit and Faustman, 2005). In an earlier study (Bekhit et al., 2005), myofibrillar myoglobin reducing activity (MMRA) and total myoglobin reducing activity (TMRA) – that is the sum of reducing activities present in the sarcoplasmic and myofibrillar fractions of the muscle – were found to be correlated to lipid oxidation as measured by thiobarbituric acid reactive substances (TBARS) [$r = 0.407$ ($P < 0.001$) and $r = 0.367$ ($P < 0.001$) for MMRA and TMRA, respectively]. However, in the presence of zinc ions (an antioxidant), oxidative processes were delayed. The current study was conducted to evaluate the mechanisms involved in meat lipid oxidation through two different free radical detecting systems.

Materials and Methods

Meat: Pre-rigor lamb longissimus muscles were obtained from a commercial slaughter house and stored in a chiller at 4°C for 3 days. Thin meat cores (30- 40 mg and 0.5 cm in diameter) were prepared using a filleting knife and a cork borer and used in all the analysis. The pro-oxidant activity of meat was measured using 2`,7`-dichlorofluorescin diacetate (DCFH-DA) based on the method described by Driver et al. (2000) with modifications. Meat cores were placed into 96-well plates and 150µl of MES-TRIS buffer (pH 5.5) was added. To each well, DCFH-DA was added to final concentration of 4mM. The plates were incubated at room temperature in dark for 15 min to allow the DCFH-DA to be incorporated into membrane-bound vesicles and the diacetate group to be cleaved by resident esterases. De-ionized H_2O and NADH (1mM final concentration) were added to each well to a final total assay volume of 250µl. To examine the system, we used 2 antioxidants (carnosine and resveratrol, 20-80 µM final concentration range) that have been shown to have pro-oxidant and antioxidants activities, respectively, in meat of normal (5.50- <5.8 range) pH (Bekhit et al., 2003). The pro-oxidant mediated oxidation of the DCFH to the highly fluorescent product DCF was measured over time using POLARstar galaxy fluorescence plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with excitation at 485 nm and emission at 520 nm. Data points were taken every minute for 200 minutes and the fluorescence values expressed as fluorescence units after adjustment to 200 mg sample weight. Pro-oxidation activity (specifically superoxide anion production) was also measured using a modified cytochrome c reduction assay (Barbacanne et al. 2000). Meat cores were transferred to cuvettes containing 735µl of phosphate buffered saline, and to each cuvette cytochrome c was added to give a final concentration of 12.5µM. The reduction rate of cytochrome c was then determined by measuring the change in absorbance between 550nm and 590nm on a Jenway 6405 UV/VIS spectrophotometer (Jenway Ltd, Essex, UK).

Results and Discussion

The superoxide anion production, as indicated by the reduction of cytochrome c, from muscle cores was increased three fold in the presence of NADH relative to its absence The pro-oxidant mediated oxidation of the DCFH was increased 22 fold in the presence, compared with in the absence, of NADH (Figure 1). Because NADH is required for the reduction of metmyoglobin in meat, the production of reactive oxygen species (ROS) as a by-product of the reaction should be expected. The ROS produced will lead to increased lipid oxidation. This confirms a hypothetical mechanism for the production of ROS earlier suggested by Bekhit (2004) as a result of increased metmyoglobin reducing activity in meat (Fig 2). The observed oxidation in the muscles (Figure 1) were found after 3 days of post-mortem and was promoted by carnosine (acts as pro-oxidant at pH 5.5) and inhibited by resveratrol (a potent antioxidant) in dose dependent fashion (Figure 3). Our results explain the increased lipid peroxidation in frozen beef patties supplemented with partially purified cytochrome b_5 reductase and cytochrome b_5 from beef liver in the presence of NADH (Mikkelsen and Skibsted, 1992). Earlier immunochemical studies on the pathway of electron flow in NADH-dependent microsomal lipid peroxidation by

Hirokata *et al.* (1978) has shown that the presence of Fe^{3+} ions can support NADH lipid peroxidation of liver microsomes. They pointed out that the electrons from NADH were supplied to the lipid peroxidation reaction via NADH-cytochrome b_5 and cytochrome b_5 because antibodies for these proteins inhibited NADH-dependent lipid peroxidation.

Figure 1. Pro-oxidant activities in fresh lamb (3 days postmortem) as detected using 2`,7`-dichlorofluorescin diacetate (DCFH-DA) assay (A) and cytochrome c reduction assay (B).

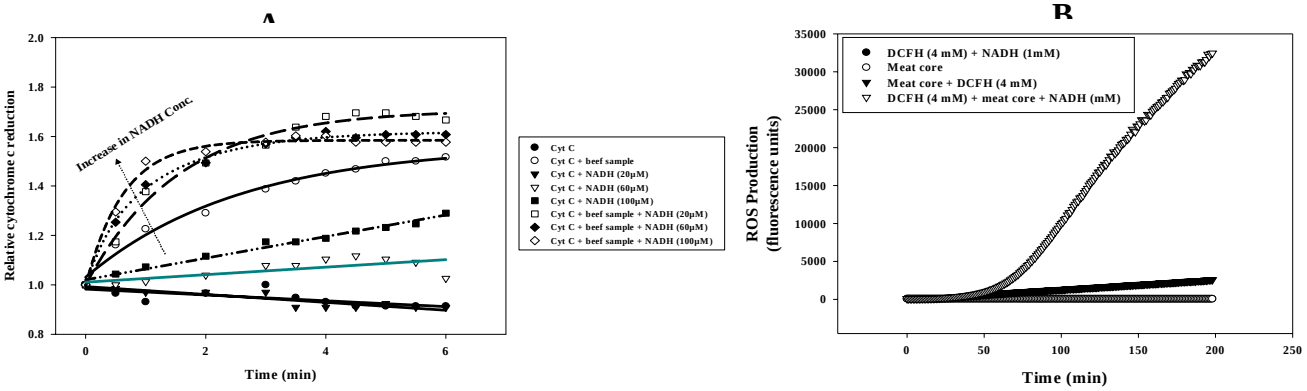


Figure 2. Effect of Zn^{++} induction on the oxidative pathway of meat as proposed by Bekhit (2004). Cycle B represent the reduction pathway of MetMb as proposed by Kuma (1976) and the production of superoxide as proposed by Hansen *et al* (1996). Cycle A represents the oxidative processes in meat due to free radicals.

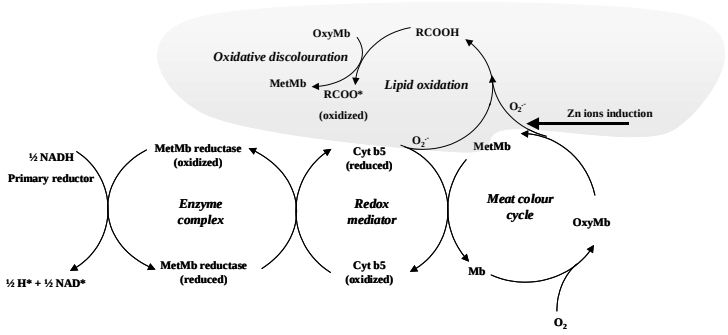
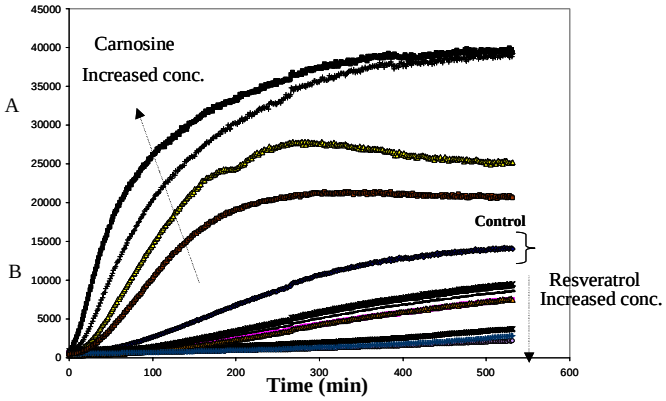


Figure 3. Increase (carnosine) and decrease (resveratrol) in DCFH oxidation compared with control (DCFH + meat core+NADH as in material and method section) in dose–dependent fashion reaction.



Conclusions

The above results confirm the production of ROS as a result of the activation of metmyoglobin reducing activity in meat during postmortem. These ROS can lead to increased oxidative processes and will probably mask any positive outcome of metmyoglobin reduction. Increasing the muscle antioxidant capacity seems to be crucial to maintain good colour stability by inhibiting/delaying the oxidation through this pathway.

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