Antioxidant Capacity of Calcium Lactate on m-Calpain Activity *In Vitro*

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Summary and Implications

To investigate the antioxidant capacity of calcium lactate on m-calpain activity, porcine m-calpain was pre-incubated with various combinations of hydrogen peroxide (H_2O_2), calcium chloride ($CaCl_2$), and/or different concentrations of calcium lactate (CaLac). The m-calpain was activated by CaLac, and the extent of m-calpain oxidation by H_2O_2 was decreased with increasing CaLac concentrations. These results suggest that CaLac addition to early postmortem muscle may lead a significant improvement of meat tenderness by providing an activation of endogenous calpain enzymes and protection against protein oxidation.

Introduction

Calpains play significant role in postmortem proteolysis of cytoskeletal proteins and thus affect tenderization of meat. Oxidation of postmortem muscle negatively affects proteolytic activity of calpains, and subsequently decreases tenderness of meat. CaLac has free radical scavenging and antioxidant effects. Hence, it can be hypothesized that CaLac may both activate calpain and retard oxidative inhibition of calpain under oxidative conditions. The objective of this study was to determine the effects of CaLac on calpain activity under oxidizing conditions in vitro.

Materials and Methods

Porcine skeletal muscle m-calpain (66 U/mg) was incubated with 40mM Tris-HCl, at 23°C for 5 min with various combination of 100 μM H_2O_2, 5 mM $CaCl_2$, and/or 5 mM CaLac generating 8 treatments; 1) control, 2) H_2O_2, 3) $CaCl_2$, 4) CaLac, 5) H_2O_2 + $CaCl_2$, 6) H_2O_2 + CaLac, 7) $CaCl_2$ + H_2O_2, and 8) CaLac + H_2O_2. The m-calpain activity was measured in a standard casein assay under both reducing and non-reducing conditions (with and without 0.2% mercaptoethanol) in triplicate. In order to determine the effects of lactate concentration on calpain activity, m-calpain (66 U/mg) was incubated at the same conditions as above but with 50 μM H_2O_2 and with 5 mM $CaCl_2$, or CaLac (5, 10, 15, and 20 mM).

Results and Discussion

Under both reducing and non-reducing conditions, CaLac activated m-calpain. Pre-exposure of calpain with H_2O_2 + CaLac treatment significantly lowered calpain activity compared to the H_2O_2 + $CaCl_2$ under the reducing condition. These results suggest that CaLac scavenges oxidants, which allows more calpain activity and thus more autolysis during pre-incubation, and consequently it results in lower calpain activity ($P < 0.05$). Increasing CaLac concentration decreased ($P < 0.05$) the activity of m-calpain pre-incubated with H_2O_2 under the reducing condition (Fig. 1). Taken together these data suggest that the antioxidant capacity of CaLac may be concentration dependent, because the extent of m-calpain oxidation by H_2O_2 was decreased with increasing lactate levels. Thus, it can be concluded that CaLac is able to activate m-calpain, and may provide antioxidant capacity against m-calpain oxidation.

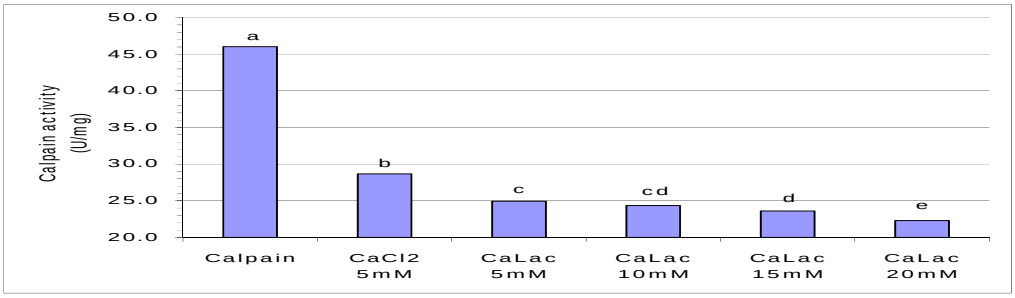

Figure 1. Effect of calcium lactate on m-calpain activity under oxidizing condition. Porcine skeletal muscle m-calpain was pre-incubated with different concentrations of CaLac. Activity was measured in a reducing buffer. The lower residual calpain activity indicates the more activity during pre-incubation. Means with a different letters (a-e) are different ($p < 0.05$). The standard error of the mean was 0.33.