INHIBITION OF POSTMORTEM TENDERIZATION BY ZINC

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Introduction

It is generally accepted that proteolysis of myofibrillar proteins is the major reason for meat tenderization during postmortem aging of carcasses at refrigerated temperatures (Penny, 1980; Goll et al., 1983; Koohmaraie, 1989). However, Takahashi et al. (1987b) reported that the postmortem weakening of Z-disks is non-enzymatically induced by the raised Ca^{2+} concentration.

Whiting and Richards (1978) reported that injecting prerigor muscle with zinc increased shear force of the meat after aging and cooking. The zinc concentration of muscle, in combination with 3 other ions, has also been positively correlated with sensory tenderness and shear force (Vavak et al., 1976).

Previous work conducted in this laboratory has indicated that Ca^{2+} probably exerts its effect through activation of Ca^{2+}-dependent proteases (Koohmaraie et al., 1988a,b). Since Zn^{2+} inhibits the activity of Ca^{2+}-dependent proteases (Guroff, 1964), the objective of these experiments was to examine the effect of zinc on postmortem tenderization.

Material and Methods

A total of twelve lambs (8 to 12 months old) were slaughtered on the same day. Control lambs were slaughtered and processed according to the normal procedures. $ZnCl_2$-infused lambs were slaughtered and infused with 50 mM $ZnCl_2$ (at a volume equal to 10% of the live weight) according to the procedures described by Koohmaraie et al. (1989). Twenty-four hours after slaughter, the entire loins from control and $ZnCl_2$-infused lambs were removed, divided into two sections and assigned to day 1 or day 14 postmortem for the following determinations: shear force, zinc content, Myofibrillar Fragmentation Index (MFI), CDP-I, CDP-II, CDP inhibitor, Cathepsin B, Cathepsins B+L, and SDS-PAGE of isolated myofibrils at day 1 postmortem. The same measurements (with the exception of zinc content) were made after 14 days of storage at 2-4°C.

Shear forces, MFI, and zinc content were determined according to the procedures described by AMSA (1978), Culler et al. (1978) and Nakamura (1973a,b), respectively. Myofibrils were isolated after 1 and 14 days of postmortem storage, according to the procedure described by Olson et al. (1976) and analyzed by SDS-PAGE (Laemmli, 1970) using 7.5 to 15% gradient gels (acrylamide: bisacrylamide, 75:1). Data were analyzed by least-squares procedures (SAS, 1985).

Results and Discussion

Results indicated that $ZnCl_2$ infusion of carcasses blocked postmortem changes in these carcasses (Table 1). This conclusion is based on the results that between 1 and 14 days of postmortem storage, no change occurred in the shear force or MFI values of the samples obtained from longissimus muscle of animals infused with $ZnCl_2$. To examine the effect of $ZnCl_2$ infusion on the classes of proteases thought to be involved in the postmortem tenderization process, the activities of CDP-I, CDP-II, CDP inhibitor and Cathepsins B and B+L were measured after 1 and 14 days of postmortem storage (Table 1). Results indicated that $ZnCl_2$ infusion had no effect on the lysosomal enzymes and CDP-II activities. The differences in CDP-I activities between control and $ZnCl_2$-infused animals were significant at 1 but not 14 days of postmortem storage. However, $ZnCl_2$ infusion prevented

the loss in the activity of CDP inhibitor which was observed in Control samples. It has been demonstrated that under normal postmortem conditions (i.e., storage at 2°C for up to 14 days) CDP-II is remarkably stable, while there is a gradual decline in activity of CDP-I, and CDP inhibitor loses its activity rapidly (Vidalenc et al., 1983; Ducastaing et al., 1985; Koohmaraie et al., 1987). We have suggested that autolysis is the reason for loss of CDP-I activity during postmortem storage (Koohmaraie et al., 1987). However, another possible explanation is that the reason for loss of CDP-I activity is its hydrolysis by another protease. The results of the experiments reported presently support the latter explanation, since Zn^{2+} inhibits CDP activity (Guroff, 1964) and its activity continued to decline in $ZnCl_2$-infused samples between 1 and 14 days of storage (Table 1). Further experimentation will be needed to determine the mechanism of loss of CDP-I activity during postmortem storage. Although the reason for loss of CDP inhibitor activity in control samples during postmortem storage is not known, it has been demonstrated that both CDP-I and CDP-II are capable of hydrolyzing CDP inhibitor (Goll et al., 1985). Because Zn^{2+} inhibits the activities of both CDP-I and CDP-II (Koohmaraie, unpublished results) and because $ZnCl_2$ infusion prevented the loss in the activity of CDP inhibitor, we propose that CDP-I and/or CDP-II hydrolysis of the inhibitor is the reason for loss of inhibitor activity during postmortem storage in control carcasses. Further experiments must be conducted to test the accuracy of this speculation.

In summary, the results of this experiment indicate that $ZnCl_2$ infusion completely blocks postmortem changes, thereby preventing meat tenderization. Based on these and previous studies (Koohmaraie et al., 1986, 1988a,b, 1989) it is concluded that Ca^{2+}-dependent proteases are probably responsible for proteolysis of myofibrillar proteins during postmortem storage. It is possible that protease(s) other than Ca^{2+}-dependent proteases could be involved. It would appear, however, that their activity must be stimulated by Ca^{2+}, inhibited by Zn^{2+} and be endogenous to skeletal muscle cells.

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TABLE 1. LEAST SQUARE VALUES FOR SHEAR FORCE, Zn CONTENT, MYOFIBRILLAR FRAGMENTATION INDEX AND PROTEASE ACTIVITY OF LONGISSIMUS MUSCLE FROM CONTROL AND $ZnCl_2$-INFUSED CARCASSES AT TWO DIFFERENT AGING PERIODS

	Control		$ZnCl_2$-infused		
	d_1	d_{14}	d_1	d_{14}	SE
Zn (μg/g)	8.3		62.5		
Shear Force (kg)	11.5	5.7	11.2	10.1	.4
MFI	43.6	75.9	39.0	39.6	2.2
CDP-I[a]	76.9	17.2	115.1	22.8	4.5
CDP-II[b]	115.2	109.3	126.0	92.9	6.8
CDP inhibitor	125.5	10.0	142.2	187.5	16.7
Cathepsin B[c]	85.2	105.8	93.9	97.6	7.6
Cathepsin B+L[d]	77.6	90.7	92.6	84.1	7.3

[a]Low-Calcium-requiring Ca^{2+}-dependent protease (Caseinolytic activity).

[b]High-Calcium-requiring Ca^{2+}-dependent protease (Caseinolytic activity).

[c]Activity against Z-Arg-Arg-NHMec.

[d]Activity against Z-phe-Arg-NHMec.