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LIBERATION OF PHOSPHOLIPIDS FROM BOVINE SKELETAL MUSCLE Z-DISCS BY 0.1 mM CALCIUM IONS—THE WEAKENING MECHANISM OF Z-DISCS DURING POST-MORTEM AGEING OF MEAT—

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Background:

The weakening of skeletal muscle Z-discs contributes to tenderization of meat during post-mortem ageing. Z-Discs are composed of Z-filaments, whose key component is α-actinin, and an unstructured amorphous-matrix (Takahashi & Hattori, 1989). Amorphous-matrix materials fill up the space between individual Z-filaments, and cement neighbouring Z-filaments. The structure of Z-discs is non-enzymatically weakened by 0.1 mM calcium ions without release of α-actinin (Takahashi *et al.*, 1987). Recently, we have identified the main components of amorphous-matrix materials as lipids. The content of lipids in bovine *semitendinosus* muscle Z-discs is 1.8 g per 100 g of myofibrillar proteins. They are composed of phospholipids, triacylglycerols, cholesterol and free fatty acids; the proportions are 65.8, 23.2, 8.6 and 2.4%, respectively. The liberation of phospholipids by the binding of 0.1 mM calcium ions is the main cause for Z-disc weakening during post-mortem ageing of chicken (Shimada *et al.*, 1998). We report here the weakening mechanism of Z-discs during post-mortem ageing of beef.

Objectives:

We studied the interaction between 0.1 mM calcium ions and phospholipids in Z-discs using bovine myofibrils in order to confirm that the post-mortem weakening of Z-discs is caused by the liberation of phospholipids from Z-discs.

Materials and Methods:

Japanese Black steers aged 32-months old were stunned and slaughtered conventionally. *Semitendinosus* muscle was dissected from carcasses after about 6 h. The muscle was aged at 4℃. Myofibrils at resting length were prepared by the method of Tatsumi *et al.* (1993). The myofibrils were treated with a salt solution containing 1 μM calpastatin at 5℃ for 12 h with gentle stirring to inactivate calpains adsorbed at Z-discs. Myofibrils were then treated with a solution containing 0.1 M KCl, 0.1 mM $CaCl_2$, 1 μM calpastatin, 1 mM dithiothreitol, 1 mM NaN_3, and 10 mM Tris-maleate buffer, pH 7.0 at 5℃. To prepare I-Z-I brushes, myofibrils were extracted with 5 vol of a modified Hasselbach-Schneider solution containing 0.6 M KCl, 10 mM $Na_2P_2O_7$, 1 mM $MgCl_2$, 0.1 M potassium phosphate buffer, pH 6.4 for 30 min at 5℃ with gentle stirring, and centrifuged at 10,000 rpm for 10 min. This extraction was repeated. The precipitated I-Z-I brushes were extracted with methanol : chloroform : 0.1 M KCl = 2 : 1 : 0.8 (v/v) (Bligh & Dyer, 1959). The determination of remaining phospholipids in Z-discs was carried out by the method of Bartlett (1959). The amount of liberated phospholipids from Z-discs was calculated by subtracting the remaining amount from the original amount of phospholipids in Z-discs, and expressed as g per 100 g of myofibrillar proteins. The degree of the Z-disc weakening was measured by the method of Takahashi *et al.* (1967). Samples for SDS-PAGE were subjected to gel electrophoresis according to the method of Laemmli (1970) using linear 7.5–17% polyacrylamide and 1.75–3.3% (w/v) glycerol gradient gels. Myofibrillar proteins separated by SDS-PAGE were transferred electrophoretically onto nitrocellulose membranes for immunoblotting, and the binding of anti-α-actinin antibodies was detected by horseradish peroxidase reaction using goat anti-rabbit IgG. The binding of calcium ions to phospholipids was examined by ^{45}Ca-autoradiography.

Results and Discussions:

During post-mortem ageing of beef, the Z-disc weakening agreed precisely with the liberation of phospholipids, both changes reaching a maximum within 14 days post-mortem. The amount of α-actinin remained unchanged in these processes. Myofibrils

prepared from bovine *semitendinosus* muscle were uncontaminated with calpains, because the liberation of phospholipids and the Z-disc weakening were unaffected by calpastatin. After the myofibrils had been treated with a solution containing 0.1 mM $CaCl_2$, the Z-disc weakening correlated well with the liberation of phospholipids. Both reached a maximum within 21 days, each maximum value being 0.62, and 42% of the original amount in Z-discs. On the other hand, they were inhibited in the presence of 5 mM EGTA instead of 0.1 mM $CaCl_2$. We examined dependence of the Z-disc weakening and the liberation of phospholipids on calcium ion concentrations. The Z-disc weakening agreed well with the liberation of phospholipids, which increased above 10 μM and reached a maximum at 0.1 mM calcium ions. The calcium-specific liberation of phospholipids, which induced the weakening of Z-discs, was affected by pH and temperature. When myofibrils were treated with a solution containing 0.1 mM $CaCl_2$ at various pH values, the liberation of phospholipids was minimal at pH 6.5, as was the Z-disc weakening. When myofibrils were treated with 0.1 mM $CaCl_2$ at pH 7.0, the liberation of phospholipids increased almost linearly with increasing temperature and reached a maximum at 35℃ corresponding with that for the Z-disc weakening. The binding of calcium ions to lipids, which had been extracted from I-Z-I brushes, determined by ^{45}Ca-autoradiography. The lipids were radioactive, indicating that calcium ions bind to phospholipids and induce their liberation from Z-discs. These findings strongly support 'the calcium theory of meat tenderization.'

Conclusion:

The liberation of phospholipids by the binding of 0.1 mM calcium ions is the main cause for weakening of Z-discs during postmortem ageing of beef.

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