TIGHTLY BOUND CALCIUM WITHIN I BAND: POSSIBLE FUNCTION IN TITIN AGGREGATION AND CALPAIN 1 BINDING TO TITIN

G. Coulis*, L. Aubry, C. Herrera-Mendez and A. Ouali

QuaPA, Muscle biochemistry group, INRA-THEIX, 63122 Saint Genès Champanelle,France.
Email: gcoulis@clermont.inra.fr

Keywords: titin, calpain 1, calcium, N-line regions, titin polymerization

Introduction

Meat tenderisation is based upon an enzymatic process implying several proteolytic systems (Sentandreu *et al.*, 2002). Amongst them, calpains have been and are still extensively investigated by meat scientists. Besides the implication of ubiquitous calpains in the meat tenderising process, some recent investigations provide contradictory findings about a possible contribution of muscle specific calpain 3 (Parr *et al.*, 1999; Ilian *et al.*, 2000; Geesink *et al.*, 2005). Within myofibrils, calpain 1 and calcium are colocalised at N1- and N2-line levels (Raynaud *et al.*, 2005; Vignon *et al.*, 1989). Calpain 3 has been also localised at the N1 and N2 levels of the sarcomere (unpublished data). Hence, the question arising is: what relationship between these different partners, i.e. calcium, calpains and titin may exist? The purpose of the present study was to provide partial answers to that question by investigating the identification of the calcium binding site at the N1 line region of titin and the consequences of calcium binding on titin itself and titin/calpain 1 interactions.

Materials and Methods

Recombinant fragment preparation:
Recombinant titin fragment spanning Z9 to I1 domains of titin and located in the N1-line region of the protein was expressed in *Escherichia coli* using the pET vectors.
Calcium titration of the recombinant Z9-I1 titin fragment:
Calcium titration of the recombinant fragment was carried out as described previously by Johnson and Tikhunova (2002). Increasing concentrations of calcium were added, from a 2M stock solution to the recombinant fragment and changes in the intrinsic tryptophan fluorescence recorded using a Perking Elmer LS50 spectrofluorometer (λexc 305 nm λ Em 358nm). The fluorescence data were fit to the one-site model of the non-linear Hill equation: y= ymax /1+10(n*(Kd-pCa))
Polymerization of the titin fragment in the presence of calcium:
After overnight treatment of the Z9-I1 recombinant fragment with Chelex-100 resin, the mixture was run on a Superose 12 HR 10/30 column. The monomeric form eluted at a Mr of about 53000 Da was collected and used for the calcium-induced polymerization study. The fragment was incubated at room temperature without calcium (control) or with 0.1 and 1µM calcium for 30 min. An aliquot of each mixture was then loaded on a Superose 12 10/30 column. Proteins were eluted at a flow rate of 0.3 ml/min and fractions of 0.3 ml were collected.
Titin/calpain 1 interaction
Binding assays were carried out with both bovine or porcine calpain 1 and Z9-I1 titin fragment by using solid phase ELISA whether in presence 4mM calcium or 4mM EGTA.

Results and Discussion

Calcium titration of the recombinant Z9-I1 titin fragment:

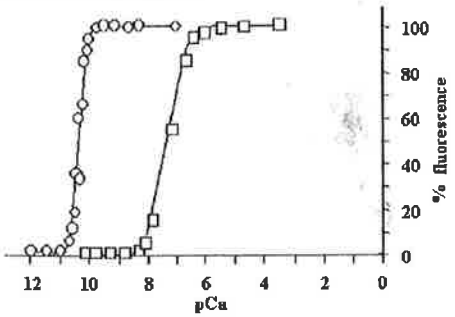

Figure 1: Calcium titration of Quin-2 (open squares) and of the Z9-I1 titin fragment (open circles).

As shown in Figure 1, increasing concentrations of calcium causes a sharp increase in the Trp fluorescence and half-maximal binding was found to occur at pCa 10,31±0.03 giving a Kd value of $4{,}92\times10^{-11}$ M.

The free calcium concentration was calibrated by titrating Quin-2 in similar conditions (λexc 330 nm λem 495nm). The Kd value of 61nM obtained for Quin-2 was identical to the value of 60nM reported by the supplier suggesting that the free calcium concentrations are accurate up to the nM range.

Calcium induced polymerization of the Z9-I1 titin fragment

Calcium induced the spontaneous polymerization of the recombinant fragment at the expense of the monomer which disappears almost totally at a higher calcium concentration. These findings suggest a calcium-dependant association of titin strands to form the end-filaments package at least in the N1-line region.

Calpain/titin interaction

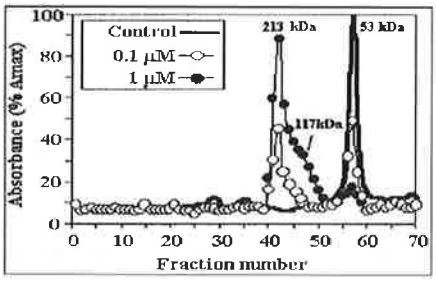

Figure 2: Elution profile of the recombinant fragment from a Superose 12 HR column after incubation for 30 min at room temperature, without calcium (inset and thick line) or with either 0.1 µM free calcium (open circles) or 1 µM free calcium (close circles). Fractions of 0.3 ml were collected.

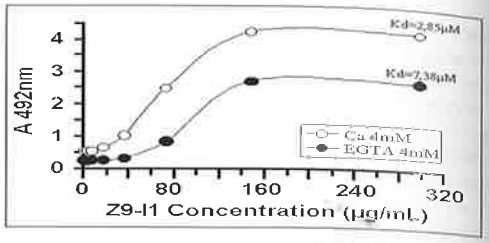

Figure 3: ELISA binding assays carried out with porcine calpain 1and Z9-I1 titin fragment whether in presence of 4mM calcium or 4mM EGTA.

Calcium increases the amount of titin fragment bound to coated calpain however with a comparable affinity in the presence (Kd=2,85µM) or absence(Kd=7,38µM) of calcium. Theses constants affinities are weaker than those observed with a 150kDa titin fragment corresponding to Z8-I5 domains (Raynaud *et al.*, 2005). Thus, calpain 1 probably interacts strongly in a calcium-dependent manner with a region including I2-I5 domains.

Conclusions

This study provides possible answers to the physiological significance of tightly bound calcium in skeletal muscle fibres. A calcium-dependent aggregation of titin molecules would allow a better coordination of the stretching and shortening of each titin strand and would ensure a better force transmission between adjacent sarcomeres as well. The calcium induced-aggregation could have beneficial effect upon titin-calpain interactions increasing the amount of calpain bound to titin.

References

Geesink, G. H., Taylor, R. G and Koohmaraie, M. (2005). Calpain 3/p94 is not involved in postmortem proteolysis *J. Anim. Sci.* 83 (7):1646-52.

Ilian, M.A., Morton, J.D., Kent, M.P., Le Couteur, C.E., Hickford, J, Cowley R and Bickerstaffe, R (2000) Intermuscular variation in tenderness: association with the ubiquitous and muscle-specific calpains. *J. Anim. Sci.* 79, 122-132.

Johnson, J.D. and Tikhunova, S.B. (2002) In: Calcium-Binding protein protocols, Vol. 2: Methods and techniques pp.89-102, Humana Press Inc., Totowa, NJ.

Parr, T., Sensky, P.L, Scothern, G.P., Bardsley, R.G., Buttery, P.J., Wood, J.D. and Warkup, C. (1999). Relationship between skeletal muscle-specific calpain and tenderness of conditioned porcine longissimus muscle. *J. Anim. Sci.* 77, 661-668.

Raynaud, F., Fernandez, E., Coulis, G., Aubry, L., Vignon X., Bleimling, N., Gautel, M., Benyamin, Y. and Ouali, A. (2005). Calpain 1-titin interactions concentrate calpain 1 in the Z-band edges and in the N2-line region within the skeletal myofibril. *FEBS J.* 272(10):2578-90.

Sentandreu, M.A., Coulis, G. and Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in food science and technology* 13, 400-421

Vignon, X., Beaulaton, J. and Ouali, A. (1989). Ultrastructural localization of calcium in post-mortem bovine muscle: a cytochemical and X-ray microanalytical study. *J. Histochem* 21(7):403-11