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COMPARISON OF μ-CALPAIN ACTIVITY IN SKELETAL MUSCLE OF BROILER AND LAYER

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Abstract

It is well known that calpain [EC 3.4.22.17] plays an important role in the degradation of myofibrillar protein. The amount of calpain present in atrophying muscle tissue (where myofibril degradation is proceeding rapidly) is several-fold greater than that isolated from normal muscle tissue. The muscle protein degradation rates of layers are higher than those of broiler chickens, and exhibited downward trends with aging of chickens. It is likely that differences among genetic stocks in muscle protein degradation rate are related to protease activity in muscle. The m-calpain, has now been purified from a number of sources including rabbit, bovine chicken hamster and human skeletal muscle. A second calpain that requires only 1 to 70 μ M Ca^{2+} for half-maximal activity (μ -calpain) has been purified from all these same tissues with the exception of human and chicken skeletal muscle. Recent data reported that chicken breast muscle has three calpain, two requiring millimolar Ca^{2+} (m-calpain and high m-calpain) and one requiring micromolar Ca^{2+} (μ -calpain). The results showed that by using fluorescence assay, the calpain activities of broilers were lower than those of layers. μ -calpain activity in skeletal muscle was vary among the breeds that have different rates of muscle production.

Keywords: μ -calpain, Skeletal muscle, Broiler and layer

Introduction

The millimolar Ca^{2+} -requiring proteinase, m-calpain, has now been purified from a number of sources including rabbit, bovine, chicken, hamster, and human skeletal muscle (Ishiura *et al.*, 1978; Suzuki *et al.*, 1981; Dayton, 1982; Inomata *et al.*, 1984; Kawashima *et al.*, 1984; Penny *et al.*, 1985; Johnson and Guindon-Hammer, 1987). A second calpain that also has 80 and 28 Kda subunits but that requires only 1 to 70 μM Ca^{2+} for half-maximal activity (μ calpain) has been purified from all these same tissues with the exception of human and chicken skeletal muscle (Ishiura *et al.*, 1978; Kawashima *et al.*, 1984; Emori *et al.*, 1986).

Both μ- and m-calpain activities, however, are found in almost all mammalian cells (Takano *et al.*, 1988), and the isolation of a m-calpain from fish skeletal muscle (Taneda *et al.*, 1983) and a Ca^{2+} -dependend proteolytic activity from Drosophila

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(Pinter and Friedrich, 1988) indicate that μ- and m-calpains occur in non mammalian as well as mammalian tissue. Wolfe *et al.* (1989) reported that chicken breast muscle has three calpain, two requiring millimolar Ca^{2+} (m-calpain and high m-calpain) and one requiring micromolar Ca^{2+} (μ-calpain).

Materials and Methods

Birds and Management

The broilers and layers (1-d-old) from a commercial hatchery were individually housed in a continuously lighted room in wire-floored cages and allowed access to food (containing 21.5% CP and 2,950 Kcal ME) and water *ad libitum*. All chicks were reared until 3 weeks of age.

Preparation of μ-calpain Activity from Skeletal Muscle

Breast muscles were obtained from chicken within 2 to 5 minutes after exsanguinations at the Poultry Farm. The excised muscles were packed in ice and were transported immediately to the laboratory. Protein purification begins within 60 to 90 min after death. The calpain from skeletal muscle was isolated essentially with the method described by Wolfe *et al.* (1989). Briefly, the excised muscles were trimmed free from external fat and connective tissue and passed through a pre-chilled tissue grinder. The ground tissue was homogenized in 2.5 vol. (v/w) of buffer A (50 mM Tris-acetate, 5 mM 8.5) in a Ultra Turax TP 10N tissue grinder by using three bursts of 30 s each separated by 30-s cooling periods. The homogenate was centrifuged at 17.600 x g (max.) for 15 min. The supernatant (pH 7.3-7.5) was filtered through glass wool and was loaded directly onto a DEAE-Sephacel column. This column was eluted with a linear 0 to 500 mM KCl gradient.

Procedures of enzyme assay

The Calpain activity was assayed either by using casein as a substrate and measuring absorbency of a 2.5% trichloroacetic acid supernatant at 280 nm as described previously (Fagan *et al.*, 1983) or more frequently, by using fluorescent-casein as a substrate and measuring fluorescence of a 2.5% trichloroacetic acid supernatant (Twinning, 1994). Use of the fluorescence assay, which is 10-20 times more sensitive than the absorbency assay, to measure calpain activity has not been described before, so some details are given here (Wolfe *et al.*, 1989). 50 μl of stock solution containing 6.25 mg fluorescent isothiocyanate casein per ml, 250 mM Tris-acetate (pH 7.5), 250 mM KCl, and 12.5 mM MCE was added to a 1.5 ml micro centrifuge tube followed by 5 to 50 μl of the calpain solution to be assayed. Water was added to bring the volume to 100 μl, the tube was incubated at 25°C for 5 min., and the reaction was initiated by adding 25 μl of the appropriate concentration of $CaCl_2$. After 30 min., the reaction was stopped by adding 125 μl of 5% (w/v)

trichloroacetic acid that had been chilled to 2°C. The tubes were placed in an ice slush for 5 min., and then centrifuged at 2°C for 5 min. at 2700 x g (Ave). 200 μl of the supernatant was added to 800 μl 0.3 M sodium phosphate (pH 8.5), and fluorescence was read on a Hitachi F-2000 fluorescence spectrophotometer with an excitation of 287 nm and an emission of 518 nm. Specific activity expressed as fluorescence unit per ml was calculated as follows: fluorescence of calpain plus Ca^{2+} tube – fluorescence of same calpain plus 5 mM EDTA tube X 2/mg of calpain sample added to the assay.

Result and Discussion

The putative μ-calpain activity from the phenyl-Sepharose column was subjected to successive calpain assay with fluorescence spectrophotometer (Table 1). The crude-, m- and μ- calpain in total protein, total activity and specific activity differed significantly ($P<0.01$) between broiler and layer birds.

Table 1. Total protein, specific and total activity at each step in separation of crude-, m-, and μ- calpain from chicken skeletal muscle[1]

	Total protein (mg)	Total activity[2] (fluorescence units x 10^{-3})	Specific activity[2] (fluorescence units/mg protein x 10^{-3})
Broiler:			
Crude calpain	30.184	1230[a]	40.8[a]
m-calpain	14.724	662[a]	45.0[a]
μ-calpain	3.001	64[a]	21.4[a]
Layer:			
Crude calpain	30.678	1533[b]	50.0[b]
m-calpain	14.965	825[b]	55.1[b]
μ-calpain	3.075	90[b]	29.4[b]

[1] Figures are for a preparation that began with 80 g chicken breast muscle, fresh weight.
[2] Arbitrary fluorescence units obtained using fluorescence-casein as a substrate with 5.0 mM Ca^{2+} as described in Material and Method.
[a, b] Row values with different superscript are statistically significant ($P<0.01$).

To compare the accuracy of μ-calpain measurement with previous results (Johari *et al.*, 1993) in skeletal muscle of broiler and layer chicken, the comparison of μ-calpain in broiler and layer with different method of measurement are given in Table 2. The comparison rates of broiler and layer (B/L) were 0.75 and 0.80 for previous method, and 0.80 and 0.82 for these methods, respectively. The results show that measuring of these methods is more accurate than previous method when the number of accuracy is one.

Table 2. The comparison of broiler and layer in μ-calpain activity with different units of measurement

	Broiler (B)	Layer (L)	B/L
Units/g muscle (Johari *et al.*, 1993)	0.779 ± 0.10^a	1.042 ± 0.21^b	0.75
Units/mg muscle (Johari *et al.*, 1993)	0.353 ± 0.01^a	0.440 ± 0.07^b	0.80
Fluorescence units	662 ± 27^a	825 ± 37^b	0.80
Fluorescence units/mg protein	45 ± 1.4^a	55.1 ± 1.9^b	0.82

[1] Mean ± S.D

[a, b] Row values with different superscript are statistically significant ($P<0.01$)

No proteolytic activity resembling the third calpain (μ-calpain, high μ-calpain and μ-calpain) described in report of Wolfe *et al.* (1989) has been reported before. Recently, there is no report about μ-calpain activity in skeletal muscle of poultry, especially in chicken. So, author has a little bit difficulty to discuss about it.

The result of crude-, m- and μ-calpain in specific activity (fluorescence units per mg protein x 10^{-3}) of broiler and layer chickens are similar to the report of Wolfe *et al.* (1989) that using breast muscle of mature laying hen.

Crude-, m-, and μ-calpain in total protein, total activity and specific activity of broiler are lower compare than those of layer. These differences may be related to differences in muscle protein degradation and growth. Maeda *et al.* (1990) reported that meat type stock with a high growth rate was relatively high in K_d (the fractional rates of synthesis of muscle protein) and low in K_s (the fractional rates of degradation of muscle protein).

From the result, the fluorescence assays of μ-calpain have 7% and 2.5% more accurate than absorbance assay. Twinning (1984) and Wolfe *et al.* (1989) reported that using the fluorescence assay, which is 10-20 times more sensitive than the absorbance assay, to measure calpain activity.

Conclusion

By using fluorescence assay, the crude-, m- and μ-calpain activities of broilers were lower compare than those of layers. These results suggest that μ-calpain activity in skeletal muscle vary between breeds that have different rates of muscle production.

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