TEM CHANGES IN SKELTAL MUSCLE CONNECTIN

GE, H. NAKAI and *K. TAKAHASHI
al Institute of Animal Industry, Tsukuba Norindanchi, Ibaraki 305, Japan
tment of Animal Science, Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido 060, Japan

ectin is a long, filamentous protein found in striated muscle, which links the thick filament to the Z line. rotein is also responsible for positioning the thick filament at the center of a sarcomere. There are two types nectin, α-connectin and β-connectin, and their molecular masses are estimated as 2,800 kDa and 2,100 kDa, tively. In this study we report the postmortem changes in connectin of chicken muscles.
ken leg muscles were stored at 5℃. At the appropriate postmortem time, myofibrils were prepared from the stored . Then myofibrillar proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS- to detect the changes in connectin. Furthermore, myofibrils prepared from freshly excised muscles were suspended Ca^{2+} solution containing 0.1 mM $CaCl_2$ and 30 μg of leupeptin/ml. This suspension was stirred gently at 5℃. Then Ca^{2+}-treated myofibrils were also applied to SDS-PAGE. In both stored muscles and Ca^{2+}-treated myofibrils, a kDa peptide appeared. Polyclonal antibodies against this 1,200 kDa peptide were obtained in a rabbit. Using blot analysis, the origin of this 1,200 kDa peptide was investigated.
amount of α-connectin decreased with storage time in chicken leg muscles. The other hand, the amount of β- tin increased, and the 1,200 kDa peptide appeared during storage. The densitometric analytic data showed that the kDa peptide was stoichiometrically produced from α-connectin. The same changes also occurred in Ca^{2+}-treated rils. Using immunoblot analysis, it was shown that the origin of the 1,200 kDa peptide was α-connectin. We ded that α-connectin was divided stoichiometrically into β-connectin and a 1,200 kDa peptide in postmortem n leg muscle, and this division was induced by 0.1 mM Ca^{2+}.

CTION

nectin (also called titin) is a elastic protein of striated muscles found by MARUYAMA et al (1976). It is thought his protein extends from Z-line to the vicinity of M-line as one giant molecule (MARUYAMA et al,1984a, FURST et al, 989). It exists as a elastic thin filament (MARUYAMA et al,1984a), and is responsible for positioning the thick nt at the center of sarcomere (HOROWITS et al,1989).
bilization of myofibrils by sodium dodecylsulfate (SDS) provides a connectin doublet in gel electrophoresis. The band is called α-connectin (titn I) and the lower one is called β-connectin (titin II), and their molecular are estimated as 2,800 kDa and 2,100 kDa respectively (MARUYAMA,1986). β-connectin is a product by limited lysis of α-connectin (MARUYAMA,1986). The shape of a purified β-connectin molecule is like a very thin string, hat length is estimated~0.6 ~ 1.2 μm (MARUYAMA et al,1984b, TRINICK et al,1984, WANG et al,1984, NAVE et al,1989). e major changes in postmortem myofibrils are a weakening of Z-line, a weakening of the interactions between myosin tin and a degradation of connectin (TAKAHASHI,1989). These changes are responsible for tenderization of meat. AHASHI et al (1979) found that in postmortem skeltal muscle, the amount of α-connectin decreases with increasing of storage in parallel with the decrease in elasticity of the muscles. So, they suggested that connectin was nsible for tenderization of meat.
this report, we study the postmortem changes in connectin contained in chicken leg muscles. We demonstrate that ly β-connectin but also a new 1,200 kDa peptide is stoichiometrically produced from α-connectin. And we show changes are induced by 0.1 mM Ca^{2+}. Furthermore, we show immunologically that the 1,200 kDa peptide is produced α-connectin, not β-connectin.

ALS AND METHOD

paration of myofibrils. Chicken leg muscle (M. adductor) was used. Myofibrils were prepared from fresh or muscle according to the method of PERRY et al (1956).
cium-treatment of myofibrils. Ca^{2+}-treatment of myofibrils was carried out according to the method of TAKAHASHI (1992). Fresh myofibrils were prepared from the whole muscle of a chicken leg. These prepared myofibrils were ed with a solution containing 0.1 M KCl, 0.1 mM $CaCl_2$, 30 μg of leupeptin / ml, 1 mM dithiothreitol, 1 mM NaN_3 and Tris-maleate buffer, pH 7.0, at 5℃ with gentle stirring.
-polyacrylamide gel electrophoresis (SDS-PAGE). The myofibrils were dissolved in a solution containing 1% SDS, mercaptoethanol, 5 mM EDTA, 5mM Tris-HCl, pH 8.0, and 10% glycerol. Then samples were heated in boiled water for

2 min., and clarified by centrifugation at 10,000 g for 5 min. The proteins were separated by SDS-PAGE using 2-12% gradient of polyacrylamide in the buffer system according to Fairbanks et al (1971) or 2% gel of polyacrylamide containig 0.5% agarose according to Takahashi et al (1992).

Antiserum. In both the stored muscle and the $Ca2^{+-}$treated myofibrils, a peptide of 1,200 kDa appeared. Antiserum against this peptide was obtained by the following procedure: this peptide was cut out from slab gels and approximately 0.3 mg of peptide in dispersed gels was injected in a rabbit 3 times at 7 days intervals.

Immunoblot. The proteins separated by SDS-PAGE were electrophoretically transfered onto a nitrocellulose sheet (TOWBIN et al,1979). This sheet was treated with the antiserum against the 1,200 kDa peptide, and antibody binding was detected by horseradish peroxidase reaction using anti rabbit IgG. (Bio-rad,CA)

Amino acids composition analysis. β-connectin and the 1,200 kDa peptide separeted by SDS-PAGE were electrophoretically transfered onto a polyvinylidene difluoride (PVDF) membrane (Millipore Corp.). The amino acid composition of the transfered proteins was analyzed by the method of Nagasawa et al (1989).

RESULTS AND DISCUSSION

Figure 1 shows the electrophoretic pattern of myofibrillar protein from chicken M. adductor stored at 5℃. At 0 day postmortem, only α-connectin exists with no β-connectin. With increasing postmortem time, the band of α-connectin becomes weaker, while the band of β-connectin becomes stronger. Furthermore, a new 1,200 kDa peptide appears during storage. The molecular weight of this peptide is higher than nebulin which has large molecular weight next to connectin. The densitometric analysis sugggests that these changes continue until about 4 days postmortem, and β-connectin and the 1,200 kDa peptide are produced stoichiometrically from α-connectin (Fig. 2). The same changes are observed in chicken M. semimembranosus and M. tensor fasciae latae (data not shown). These data suggest that α-connectin degrades to β-connectin and the 1,200 kDa peptide until about 4 days postmortem in chicken leg muscles.

Using chicken breast muscles, samples at 0 day postmortem include both α- and β-connectins. During storage, α-connectin decreases and β-connectin increases, but the 1,200 kDa peptide doesn't appear. It is thought that this difference between breast and leg muscles is caused by differences in the structures of connectin between the two muscles. It has been demonstrated that there are differences in molecular weights of connnectins among species of animals (LOCKER et al,1986, HU et al,1986). Furthermore, FURST et al(1988) reported that their monoclonal antibody T12 reacted with both α- and β-connectin of skeltal muscles, but didn't react with β-connectin of cardiac muscles. Their data suggest that there are differences in the structure of connectin in the two types of muscles. Thus, it is thought that differences in the structure of connectin is responsible for the differrences in postmortem changes in connectin.

Figure 3 shows changes in myofibrillar proteins of chicken leg muscles during Ca^{2+}-tratment. Basically, the changes in connectin are the same as that occured in stored muscles. But the band of the 1,200 kDa peptide is stronger and sharper than in stored muscles. The densitometric analysis (Fig. 4) shows that the changes in connectin continue for about 24 hours. This data also suggests that β-connectin and the 1,200 kDa peptide are produced stoichiometrically from α-connectin.

TAKAHASHI et al (1992) demonstrated that degradation of connectins doesn't occur in a Ca^{2+}-treatment solution containing 5 mM EDTA. So, they concluded that these changes are non-enzymatically induced reactions caused by 0.1 mM Ca^{2+}. The other hand, MARUYAMA et al (1986) thought that these changes are induced by an unkown $Ca2^{+-}$dependent protease. From our data revealed here, we think that these changes in connectin are induced by 0.1 mM Ca^{2+}.

Figure 5 shows results of immunoblot analysis. The antiserum against the 1,200 kDa peptide reacts with 1,200 kDa peptide and α-connectin. But it doesn't react with β-connectin. This data reveals that the origin of the 1,200 kDa peptide is α-connectin. Thus, it is confirmed immunologically that the 1,200 kDa peptide is produced from α-connectin and not β-connectin. This data also suggests that there are differences in structures between β-connectin and the 1,200 kDa peptide.

There are differences in amino acid composition between β-connectin and the 1,200 kDa peptide, especially Ser, Gly and Arg (Table 1). This data also suggests that there are differences in structures between β-connectin and the 1,200 kDa peptide.

These data suggest that α-connectin divides into β-connectin and the 1,200 kDa peptide at a certain point in the molecule. It is thought that this division occures at a point 0.31 μm from Z-line (R. TANABE and K. TAKAHASHI, manuscript in preparation). The 1,200 kDa peptide is composed of the Z-line side part of α-connectin molecule.

Thus, it is thought that postmortem changes in connectin as revealed in this study is related to the tenderization of meat.

CONCLUSION

postmortem chicken leg muscles, α-connectin devides stoichiometrically into β-connectin and a 1,200 kDa peptide, his division is induced by 0.1 mM Ca^{2+}.

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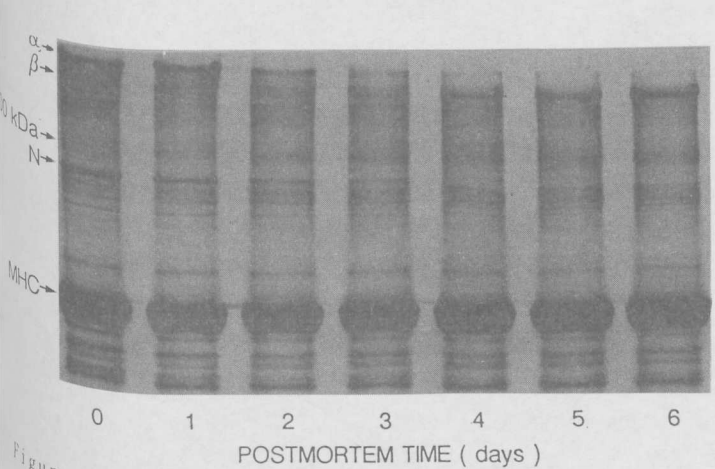

Figure 1. Postmortem changes in connectin of chicken M. adductor stored at 5℃. Electrophoresis was carried out using a gradient gel of 2-12% polyacrylamide. α = α-connectin; β = β-connectin; 1,200 kDa= a peptide of 1,200 kDa; N= nebulin; MHC= myosin heavy chain.

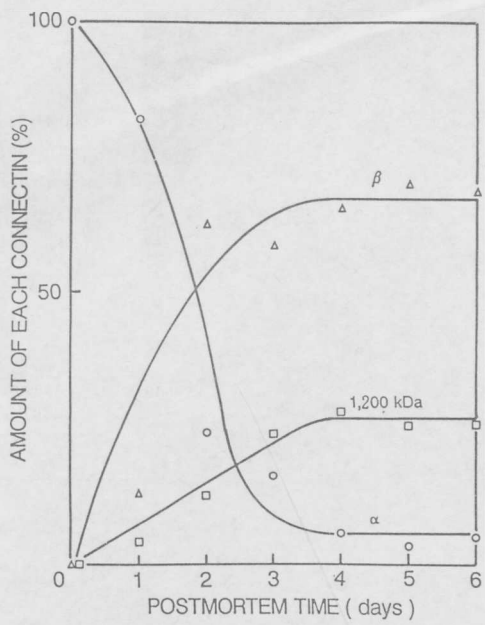

Figure 2. Postmortem changes in the amount of connectin. The amount of connectin was calculated with a densitogram from the electrophoretic pattern shown in Fig. 1. The amount of each type of connectin was expressed as percentage of the total amount of connectin. ○= α-connectin; △= β-connectin; □= the 1,200 kDa peptide.

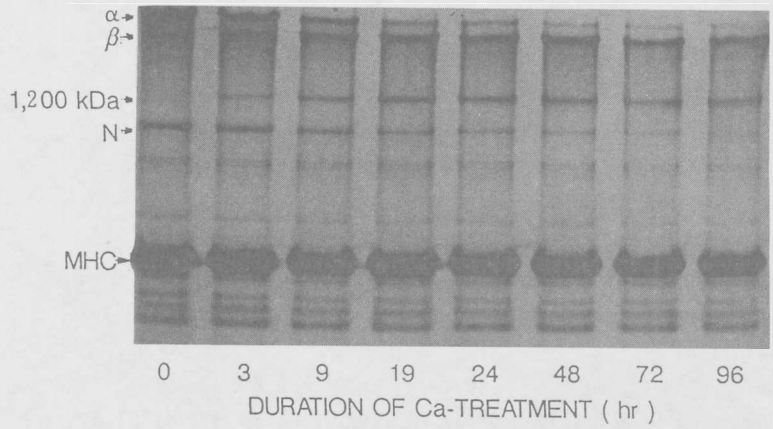

Figure 3. Changes in connectin during Ca^{2+}-treatment of chicken leg myofibrils. Electrophoresis was carried out by using a gradient gel of 2-12% polyacrylamide. Abbreviations are the same as used in Fig. 1.

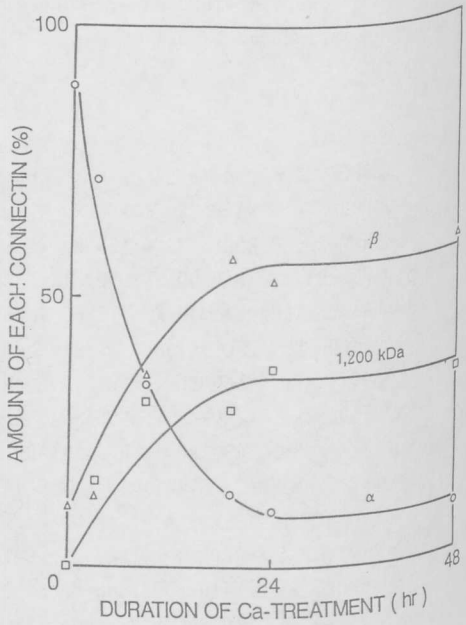

Figure 4. Changes in the amount of connectin during Ca^{2+}-treatment of myofibrils. The amount of connectin was calculated with a densitogram from the electrophoretic pattern shown in Fig. 3. The calculation procedures and abbreviations are the same as used in Fig. 2.

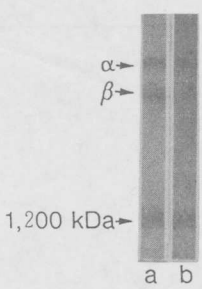

Figure 5. Immunoblot analysis using antiserum against the 1,200 kDa peptide. Ca^{2+}-treated myofibrils were analyzed with SDS-PAGE system using a slab gel of 2% polyacrylamide. The immunoblot was carried out by using antiserum against the 1,200 kDa peptide diluted 500 times. a= amidoblack 10B-stained myofibrils; b= immunoblot analysis.

Table 1. Amino acid composition of the 1,200 kDa peptide.

	1,200 kDa	β-connectin
Asp	107	116
Thr	85	82
Ser	77	68
Glu	149	136
Pro	49	62
Gly	62	80
Ala	66	65
Cys/2	4	4
Val	88	85
Met	12	10
Ile	53	53
Leu	64	60
Tyr	27	28
Phe	30	24
Lys	75	73
His	15	11
Arg	36	44

Number of residues per 1,000 residues.