Muskulare und ultrastrukturelle Anderungen im Rindermuskel hervorgerufen durch hohe Temperatur und niedrigen pH während der Inkubation

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Um festzustellen welche Änderungen in den Myofibrillproteïnen vorkommen wenn Muskel hoher Inkubationstemperatur ausgesetzt ist, wurden longissimus- Muskelproben von sechs USDA Good qualifizierten Rindern vor der Starre ausseziert und bei 37°C (HT) und 4°C inkubiert. Wegen des niedrigen pH Wertes in HT Muskeln wurden Proben von acht sternomandibularis Muskeln gleichfalls bei 37°C inkubiert und auf pH 7,0 und 5,4 gehalten. Nach 12 und 24 Studen Inkubation wurden kleine Mengen von jeder Muskelprobe zur Elektronenmikroskopie vorbereitet. Nach der Inkubation wurden von jeder Probe Myofibrille purifiziert und zur SDS Polyacrylamid-Elektrophorese vorbereitet. In ihrer Zellstruktur zeigten die HT Proben kompaktere Z-Linien, dünnere, fadenartige Myosinfilamente und völligen Strukturverlust in transversen Dünnschnitten wenn sie mit denen der C-Proben verglichen wurden. SDS Gels der HT-Proben zeigten weniger Myosin auf, Änderungen in M und C Proteïnen, mehr Substanz in der α-Aktininzone und in der 50,000 - 100,000 Dalton-Zone, weniger Troponin-T und mehr Substanz in der 28,000 - 30,000 Dalton-Zone. Proben die auf pH 5,4 gehalten worden waren, waren den Proben der HT-Gruppe ähnlich in Bezug auf ihre Zellstruktur, während Proben die auf pH 7,0 gehalten worden waren denen der C-Gruppe glichen. SDS Gels der Proben die auf pH 5,4 gehalten worden waren glichen denen der HT-Gruppe, die auf pH 7,0 gehalten worden waren denen der C-Gruppe. Um vermitteln zu können welche dieser Änderungen durch die einzelnen Behandlungen hervorgerufen worden waren, wurden Muskelproben mit Papaïn dehandelt. Pyrophosphatextrakte von Papaïn-behandelten Muskeln ergaben ähnliche Resultate mit SDS Gels als Pyrophosphatextrakte von HT-Proben und denen die auf pH 5,4 gehalten worden waren, eine Indikation dafür, dass einige dieser Änderungen in Muskelproteïnen die durch hohe Temperaturen (37°C) und niedere pH-Werte hervorgerufen werden, denen solcher hervorgerufen durch Papaïn-proteolyse ähnlich sind.

Molecular and ultrastructural alterations in bovine muscle caused by high temperature and low pH incubation

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To determine what changes in myofibril proteins occur during high temperature incubation of muscle, pre-rigor excised longissimus muscle samples from six USDA Good Grade carcasses were incubated at 37°C (HT) and at 4°C (C). Due to the low pH of HT muscles, samples from eight sternomandibularis muscles were also incubated at 37°C while being maintained at pH 7.0 and pH 5.4 After incubation for 12 and 24 hours, portions of each muscle sample were prepared for electron microscopy. Myofibrils were purified from each sample after incubation and prepared for SDS polyacrylamide gel electrophoresis. Ultrastructurally, HT samples had more dense Z lines, thinner more thread-like myosin filaments and a complete loss of structure in transverse section when compared to C samples. SDS Gels of HT samples show less myosin, alterations in M and C proteins, more material in the region of α actinin, more material in the 50,000 - 100,000 dalton region, less troponin-T and more material in the 28,000 - 30,000 dalton region. The samples held at pH 5.4 were ultrastructurally similar to samples from the HT treatment, whereas the samples held at pH 7.0 were similar to samples from the C treatment. SDS gel patterns of samples held at pH 5.4 were similar to those of the HT treatment, whereas samples held at pH 7.0 showed patterns similar to those of the C treatment. To determine what changes were actually being caused by these treatments, muscle samples were treated with papain. Pyrophosphate extracts of papain treated muscle gave a similar appearance on SDS gels to pyrophosphate extracts of HT samples and samples held at pH 5.4, indicating that some of the changes in muscle proteins caused by high temperatures (37°C) and low pH conditions are similar to those caused by papain proteolysis.

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Les changements moléculairs et ultrastructurels dans le muscle bovine causés par la température haute et l'incubation à pH bas

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Afin de déterminer les changements dans les protéines myofibrilleuses pendant l'incubation du muscle à température haute, des échantillons des muscle longissimus de six boeufs USDA Good, excisés préalable de rigidité, furent incubés à 37°C (HT) et 4°C (C). A cause du pH bas du muscle HT, échantillons de huit muscles sternomandibularis furent incubes aussi à 37°C et maintenus à pH 7,0 et 5,4. Après l'incubation de 12 et 24 heurs, part de chaque échantillon des muscle fût preparée pour la microscopie électronique. De chaque échantillon myofibrilles furent purifiées après l'incubation et preparées pour l'électrophorèse SDS polyacrylamide. Dès qu'il concerne l'ultrastructure, les échantillons HT possèdent des lignes Z plus compactes, des filaments de myosine plus minces; il y avait une perte totale de la structure dans les coupes transverses si comparées aux échantillons C. Les gels SDS des échantillons HT montrent moins myosine, des changements dans les protéines M et C, plus de la matière dans la region de l'actinine α, plus de la matière dans la region 50,000 - 100,000 de Dalton moins de troponine T et plus de la matière dans la region 28,000 - 30,000 de Dalton. Les échantillons maintenus à pH 5,4 sont similairs aux échantillons HT en consideront l'ultrastructure, pendant que les échantillons maintenus à pH 7,0 sont similairs aux échantillons C. Les gels SDS des échantillons maintenus à pH 5,4 sont similairs à ceux du traitement HT, pendant que les échantillons maintenus à pH 7,0 montrent des figures similaires à ceux du traitement C. Afin de déterminer quel changements furent actuellement causés par ces traitements, des échantillons de muscle furent traités avec la papaine. Des extraits pyrophosphates du muscle traité avec la papaine donnait une image similaire à ceux d' extraits pyrophosphate d'échantillons HT et ceux maintenu a pH 5,4 sur les gels SDS, ce qu'indique que quelques changements dans les protéines des muscle evoqués par les températures hautes (37) et des conditions à pH bas sont similairs à ceux causés par la protéolyse papaine.

Молекулярные и ультраструктурные перемены в бычих мышцах причененные высокой температурой и низкой pH инкубацией.

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Чтобы определить какие изменения происходят в протеине миофибрилв течении высоко-температурной инкубации мышцей, двоякобие образцы акцизных longissimus мышцов, шести USDA одобренной хорошего качества тушей были инкубированные при температуре 37°C(HT) и 4°C(C). Из за низких pH мышцей HT, образцы восьми sternomandibularis мышцей были так же инкубированные при 37°C, все же соблюдая их при pH 7,0 и pH 5,4. После 12 и 24 часовей инкубации, часть каждого образного мышца были приготовленны для электронной микроскопии. Миофибрили были очищенны с каждого образца после инкубации и приготовленны к электрофорисису пользуясь гелими СДС полиакриламида. Ультраструктурно, образцы HT имели более плотные Z линии, теньше и более нитевидные миосинные нити и совершенная утрата структуры в поперечном срезе если сравнить с образцами C. Гелии СДС образцев HT показывают меньше миосина, перемены в M и C протеинах, больше материала в зоне асинина α, больше материала в 50,000 -100,000 в зоне далтона, меньше тропонина-T и больше материала в зоне 50,000-100,000 далтона. Образцы содержанны на уровне pH 5,4 были ультраструктурно похожи на образцы обработки HT, тогда как образцы на pH 7,0 были похожи на образцы обработки C. Форма образцев СДС при pH 5,4 были похожи на образцы обработки HT, тогда как образцы при pH 7,0 проявили формы похожи образцам обработки C. Чтобы определить какие изменения были действительно причененные этими обработками, образцы мышцей были обработанны папаином. Экстакты пирофосфатов обработанных мышцей папаином дали тоже-самые по виду гели СДС экстрактам пирофосфатй образцов HT и образцам при pH 5,4; показывая, что некоторые из изменений в протеинах мышцей причиненные высокой температуре (37°C) и низким состоянием pH, похожи тем причиненные папаином протеолисисом.

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Introduction

Numerous articles have been published which show the relationship of postmortem temperature decline and muscle tenderness (Locker and Hagyard, 1963; Marsh and Leet, 1966a, 1966b; Marsh et al., 1968; McCrae et al., 1971; Busch et al., 1967; Parrish et al., 1969; Smith et al., 1971; Bouton et al., 1973; Bouton et al., 1974; Harris, 1975; Fields et al., 1976; Smith et al., 1976). Most of these articles attribute increases in tenderness during elevated postmortem temperatures to a reduction in cold shortening. However, studies by Locker and Daines (1976) and Dutson et al. (1978) have shown that marked increases in tenderness of muscles can be produced by increasing the pre-rigor temperature, even though no differences in muscle shortening were found. This indicates that there are factors, other than a reduction in cold shortening, which are causing tenderness increases of high temperature pre-rigor conditioned carcasses.

Moeller et al. (1976, 1977) have demonstrated that the reduced pH and elevated temperatures of high temperature pre-rigor conditioned muscle causes a rupture of the lysosomal membrane and a release of lysosomal enzymes into an environment which is conducive to action of these enzymes on tissue components.

The objectives of the study reported herein were to determine what effect high temperature pre-rigor conditioning and its associated increase in lysosomal enzyme activity might have on muscle proteins and muscle ultrastructure.

Materials and Methods

Incubation at 37°C (HT) and 4°C (C) - Sections of the longissimus muscle were excised one hour postmortem from the area of the first to sixth lumbar vertebrae of six USDA Good Grade carcasses. Each muscle was trimmed of fat and connective tissue, halved and placed in covered glass dishes. One half of each muscle was placed at 37°C for 24 hours (HT) and the other half was placed in a 4°C coldroom for the same period of time (C). Samples were removed from each muscle in the HT and C treatments at 1, 7, 12 and 24 hours postmortem for pH determinations by homogenizing in 0.005 M sodium iodoacetate. Additional samples were also removed at 1 and 12 hours postmortem, a portion of each sample was prepared for electron microscopy as outlined by Dutson (1974) and 40 nm sections were stained with uranyl acetate and bismuth subnitrate (Riva, 1974). The remainder of each sample was frozen and stored at -20°C until myofibrillar fractions were prepared according to the procedures of Goll et al. (1974).

Incubation at pH 5.4 and pH 7.0 - Both sternomandibularis muscles were dissected from each of four USDA Good Grade cattle at 30 minutes postmortem, ground and mixed with an equal volume (W/V) of 0.1 M KCl, 0.01 M PO_4, pH 7.0, 50 µg/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml neomycin. The mixture was divided equally into three treatments; control held at 4°C for 12 hrs and pH not adjusted, pH 5.4 treatment held at 37°C for 12 hrs and pH continually adjusted to maintain a pH of 5.4, and pH 7.0 treatment, held at 37°C for 12 hrs and pH continually adjusted to maintain a pH of 7.0. Myofibrillar fractions were prepared from each treatment by the procedure of Rome (1972). Portions of each myofibrillar fraction were centrifuged at 10,000 xg for 10 min. and processed as a pellet for electron microscopy according to the methods described previously.

SDS Gel Electrophoresis - Myofibrillar fractions from the temperature and pH incubations were denatured by boiling in SDS and mercaptoethanol and electrophoresed, stained and destained by the procedure of Weber and Osborne (1969). Destained gels were scanned on a Photovolt densitometer to quantitate the relative amount of each myofibrillar protein.

Results

The rate of pH decline of muscles given the HT treatment was significantly ($P<.05$) greater than control muscles, with the pH at 7 hrs postmortem being 5.65 for the HT samples and 6.40 for the C samples.

The mean values for myofibrillar proteins on SDS gels of the C and HT treatments are found in table 1. There was a significant ($P<.05$) decrease in the amount of protein found in the region of the gels for myosin heavy chains and a significant ($P<.05$) increase in the amount of protein found in the region of α-actinin, the 50,000-100,000 dalton region the 28,000-32,000 dalton region and the myosin light chain 1 region, of the HT samples when compared to controls. Although no significant ($P>.05$) differences were shown for densitometer tracings in the region of M-proteins and C-proteins, there was an observable alteration in the banding configuration in these areas.

The mean values for myofibrillar proteins on SDS gels of the control, the 5.4 and the 7.0 pH incubation samples are found in table 2. There was significantly less ($P<.05$) protein in the myosin heavy chain region, the Troponin T - α tropomyosin region and the myosin light chain 2 region of the pH 5.4 treatment when compared to the pH 7.0 treatment. In addition there was significantly more ($P<.05$) protein in the M and C protein region, the α-actinin region, the 50,000-100,000 dalton region the β-tropomyosin region and the 28,000-32,000 dalton region of the pH 5.4 treatment in comparison to the pH 7.0 treatment. In general the control had protein values intermediate between the pH 5.4 and 7.0 treatments, but in most cases not significantly different ($P>.05$) than the pH 7.0 treatment.

Electron micrographs showing longitudinal sections of muscle tissue from the C and HT treatments are shown in figures 1 and 2, respectively; transverse sections of these same treatments are shown in figures 3 and 4, respectively. In comparing figures 1 and 2, more dense Z-lines can be observed for the HT treatment and heavier appearing thick filaments can be observed for the C treatment. In transverse section the thick myofilaments from the C treatment are clear and distinct, whereas the thick myofilaments from the HT treatment are difficult to distinguish. Electron micrographs of myofibrils from samples incubated at pH 5.4 had an appearance very similar to those of the C treatment (figure 1).

Table 1. Mean values for the percentage of myofibrillar proteins on SDS polyacrylamide gels prepared from the C and HT longissimus muscle samples incubated for 12 hours.

Region[a]	Percentage of myofibrillar protein	
	4°C	37°C
Myosin Heavy Chain	29.5[b]	24.8[c]
M-protein	8.3[b]	9.0[b]
C-protein	3.0[b]	3.2[b]
α-actinin	3.6[b]	6.6[c]
50,000-100,000 Dalton region	1.6[b]	3.0[c]
Actin	27.8[b]	26.6[b]
Troponin-T+Tropomyosin	17.3[b]	16.0[b]
28,000-32,000 Dalton region	1.1[b]	1.8[c]
MLC A-1	2.4[b]	3.2[c]
Troponin-I	2.3[b]	2.4[b]
Troponin-C	3.4[b]	3.5[b]

[a]Region identification was based on relative molecular weights.

[b,c]Mean values in the same row bearing a common superscript letter are not (P>.05) significantly different.

Table 2. Mean values for the percentage of myofibrillar proteins on SDS polyacrylamide gels from control, pH 5.4 and pH 7.0 incubations of sternomandibularis muscle samples.

Region[a]	pH 5.4	Control	pH 7.0
Myosin Heavy Chain	32.6[b]	39.4[c]	41.7[d]
M and C-proteins	18.5[b]	15.0[c]	14.0[c]
105-135K daltons	3.0[b]	2.9[b]	3.2[b]
α-actinin	8.1[b]	7.6[bc]	6.8[c]
50-100K daltons	6.6[b]	4.9[bc]	4.3[c]
Actin	22.2[b]	21.5[b]	21.8[b]
Troponin-T and α-tropomyosin	2.2[b]	3.0[c]	2.6[c]
β-tropomyosin	3.5[b]	2.5[c]	2.4[c]
28-32K daltons	1.2[b]	0.5[c]	0.8[d]
Myosin Light Chain-1	0.8[b]	0.9[b]	0.8[b]
Troponin-I	0.4[b]	0.5[b]	0.5[b]
Troponin-C	0.4[b]	0.3[b]	0.3[b]
Myosin Light Chain-2	0.8+[b]	1.0[c]	0.9[bc]

[a]Region identification was based on relative molecular weights.

[b,c,d]Mean values in the same row bearing a common superscript letter are not (P>.01) significantly different.

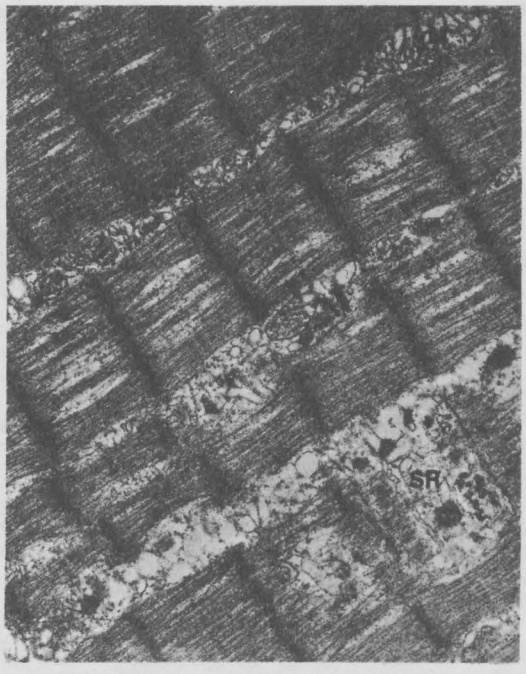

Figure 1. Electron micrograph of a longitudinal section of muscle tissue from the C treatment. Z = Z lines, M = mitochondria and SR = sarcoplasmic reticulum. X 17,500

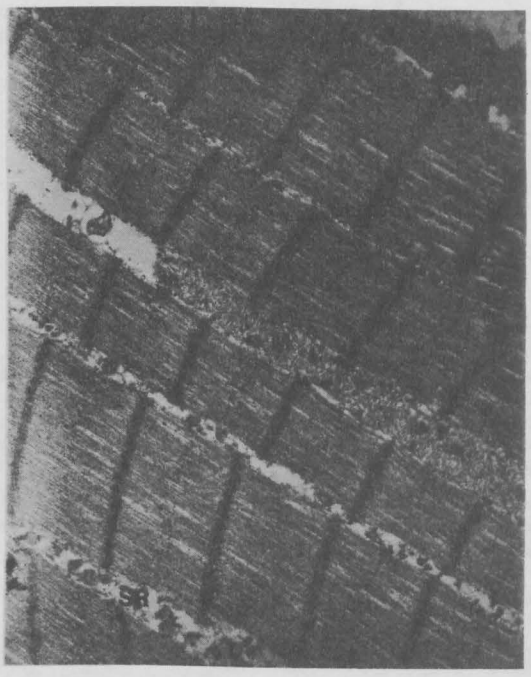

Figure 2. Electron micrograph of a longitudinal section of muscle tissue from the HT treatment. Z = Z lines, M = mitochondria and SR = sarcoplasmic reticulum. X 17,500

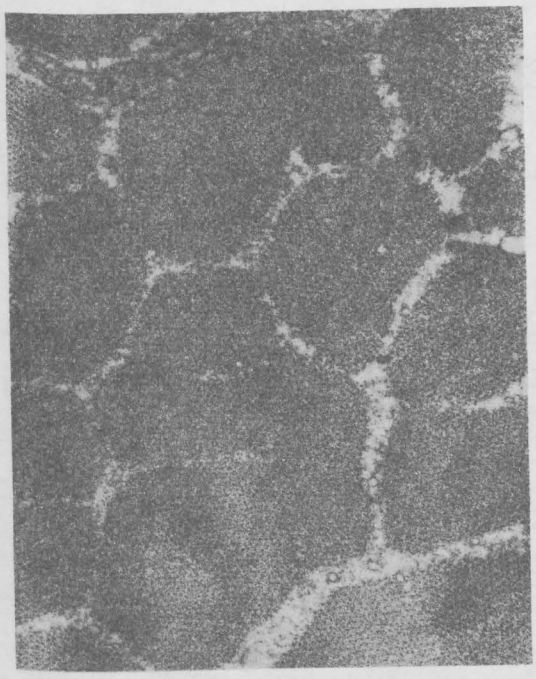

Figure 3. Electron micrograph of a transverse section of muscle tissue from the C treatment. M = mitochondria. X 26,800

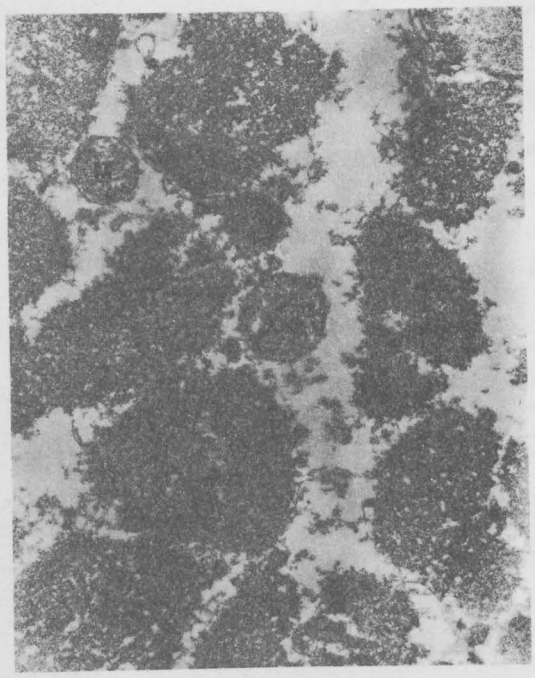

Figure 4. Electron micrograph of a transverse section of muscle tissue from the HT treatment. M = mitochondria. X 26,800

In order to gain some insight into what proteolytic changes might be taking place in the myofibrillar proteins under high temperature low pH conditions, samples of ground muscle tissue were incubated with papain and extracted with pyrophosphate according to the methods of Cooke (1972). Pyrophosphate extracts of papain treated muscle were compared to pyrophosphate extracts of C and HT muscle and pyrophosphate extracts of muscle that had been given the pH 5.4 and 7.0 treatments using SDS gel electrophoresis. Analysis of gels showed similar banding patterns for muscle treated with papain and muscles from the HT and pH 5.4 treatments, with a definite band being present in the 95,000 dalton region, probably corresponding to heavy meromyosin S-1. This same band was not evident on gels of the pyrophosphate extracts from muscles of the C and pH 7.0 treatments, indicating that these treatments are not producing the free heavy meromyosin S-1 subunit.

Discussion

When comparing the SDS gel data of purified myofibrils from the various treatments, myofibrils from those treatments having a combination of low pH and high temperature (the HT and pH 5.4 treatments) had a reduction in amount of myosin heavy chains and increases in amounts of material at lower molecular weights when compared to the other treatments (C and pH 7.0). The indicates that there was probably some proteolysis of myosin under high temperature, low pH conditions. Another indication that proteolysis was occuring during high temperature, low pH conditions is the modified appearance of the thick filaments in both transverse and longitudinal sections of the HT and pH 5.4 treatments. Also in support of myosin proteolysis is the similarity between papain treatment and treatment at 37°C and pH 5.4 when pyrophosphate extracts are compared using SDS gel electrophoresis. This similarity also indicates that proteolysis under high temperature, low pH conditions may be destroying the rigor bond by removing the heavy meromyosin S-1 subunit from the myosin molecule, leaving it attached to the thin filament. Proteolysis of the nature is likely promoted by lysosomal enzymes which have been shown to be released under high temperature, low pH conditions (Moeller et al., 1976; 1977) and could be responsible for the tenderness increases in muscles held at higher temperatures (Parrish et al., 1969; Smith et al., 1971; Fields et al., 1976; Smith et al., 1976; Dutson et al., 1978).

There was also a tendency for myofibrils from those treatments that had a combination of low pH and high temperature, to have less troponin-T and more protein in the 28,000-30,000 dalton region of SDS gels. The 30,000 dalton subunit has been shown by MacBride and Parrish (1977) and Olsen et al. (1977) to be associated with muscle tenderness. However, these authors attribute this subunit to be a result of the action of CAF (calcium activated factor) on troponin-T and thus attribute tenderness improvement to the action of this enzyme (CAF). The results of the present study show that the amount of protein in the region of 30,000 daltons on SDS gels is increased by treatments that would promote little or no CAF activity due to the low pH involved (Dayton et al., 1976). Also, electron micrographs of muscle which had a low pH early postmortem and more protein at 28,000-30,000 daltons on SDS gels (HT and pH 5.4 treatments), show that very little CAF activity was present because of the more dense Z lines of the low pH, high temperature muscle. Conversely, those muscles which had a higher postmortem pH and less material in the 28,000-30,000 dalton region, show marked Z line degradation, an indication that CAF activity was present (Dayton et al., 1976). Samejima and Wolfe (1976) also reported a 30,000 dalton subunit to be present on SDS gels of chicken muscle that had been incubated at pH 5.4 but this subunit was not present on SDS gels of chicken muscle incubated at pH 7.0, a pH of optimal activity for CAF.

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