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Postmortem Proteome Changes of Porcine Muscle Related to Tenderness

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Proteome analysis was used to investigate the relation between changes in postmortem proteome of porcine muscle and tenderness development. Muscle samples were taken at slaughter and 72 h postmortem, and the registered changes in the proteome were related to Warner−Bratzler shear force. One hundred and three protein spots were found to change significantly ($P < 0.01$) over time, and of these the 27 most pronounced changes were identified. Eleven out of the 27 changes were fragments of actin. Other identified myofibril proteins or fragments included myosin heavy chain, titin, myosin light chain I, myosin light II, CapZ, and cofilin. Correlation analysis revealed significant correlations between three of the identified actin fragments and the myosin heavy chain fragment to shear force. Moreover, myosin light chain II and triose phosphate isomerase I were also found to correlate significantly to shear force. The results clearly demonstrate that postmortem degradation of actin and myosin heavy chain is related to meat tenderness.

KEYWORDS: Proteome analysis, pork; porcine muscle; tenderization; two-dimensional gel electrophoresis; 2DE; matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: MALDI-TOF MS; peptide-mass mapping; protein identification; shear force; Warner-Bratzler; M. longissimus dorsi.

INTRODUCTION

It is well-established that meat tenderizes during storage; however, the underlying biochemical and physiochemical mechanisms during the tenderization processes are still a matter of dispute (*1*).

In general, postmortem degradation of muscle proteins is an important factor in the meat tenderization process (*2*, *3*), as postmortem degradation of several structural proteins including troponin T, nebulin, titin, vinculin, desmin, dystrophin, and troponin T has been demonstrated using one-dimensional SDS−PAGE and immuno-blotting (4). This is supported by the fact that titin, a giant muscle protein spanning from the Z-line to M-line region, and nebulin, which runs parallel with the thin filaments to the Z-line (5), both have been shown to degrade faster in tender meat than in tough meat (*6*, *7*). Moreover, electron microscopy studies of the myofibrils during tenderization have shown that the attachment of the sarcolemma to myofibrils and the junctions between myofibrils at the level of the Z-disk and M-line are disrupted postmortem (8), which suggests that proteolysis of the costamere proteins desmin, dystrophin, and vinculin, which attach the myofibrils to the sarcolemma, is taking place.

Strong correlations have been found between postmortem degradation of troponin T and shear force (*9*−*11*). However, whether the degradation of troponin T is an essential part of the tenderization process or just a marker for postmortem proteolytic activity is still unclear.

Despite the fact that postmortem degradation of several structural proteins has been studied in great detail, it is still far from established whether any of these are directly responsible for the tenderization of meat. This might partly be due to the low resolving power of one-dimensional SDS−PAGE, which has been the dominating technique used in the analysis of changes in the protein profile postmortem. Recently, we have shown that proteome analysis based on two-dimensional gel electrophoresis (2DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALD I-TOF MS) is far more informative than SDS−PAGE, and we have previously reported the findings of 18 proteins and peptides that change during the first 48 h postmortem in M. longissimus dorsi from pigs (*12*).

The aim of the present study was to relate specific postmortem protein changes using two-dimensional gel electrophoresis (2DE) in combination with MALDI-TOF MS to Warner−Bratzler shear force in pork, representing an objective measurement of degree of tenderization.

MATERIALS AND METHODS

Animals and Tissue Samples. The animals were treated and slaughtered according to Danish regulations on treatment of livestock. The 25 female pigs used in this study were offspring from Danish Landrace × Danish Yorkshire sows and Duroc boars. They were all

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reared at the experimental farm at the Research Centre Foulum, The Danish Institute of Agricultural Sciences (DIAS). The pigs were either fed a standard grower-finishing diet ($n = 13$) or a standard grower diet combined with a three-week strategic finishing ($n = 12$) according to ref 13. The pigs were slaughtered at the experimental slaughter plant at DIAS. The pigs were stunned by 85% CO_2 for three min, cleaned, and eviscerated within 30 min. One hour postmortem, the carcasses were placed in a cooling room at 4 °C.

Muscle samples were taken from six carcasses immediately after exsanguination (time 0) and from all 25 carcasses 72 h postmortem. The samples were taken from the M. longissimus dorsi, at the position of the last rib. The samples were frozen immediately in liquid nitrogen and were kept at −80 °C until the time of protein extraction.

Warner−Bratzler Shear Force. The Warner−Bratzler shear force was measured in samples from M. longissimus dorsi taken 24 h after slaughter, vacuum-packed, and either frozen immediately (WB1) or aged for three day at 4 °C (WB4) before freezing, and stored at −20 °C until shear force was measured. All samples were thawed in a water bath at 4 °C for 4 h and cut in $5 \times 4.5 \times 8$ cm^3 samples across the muscle. The samples were subsequently heated in a water bath at 70 °C for 50 min (internal temperature of 69 °C). Water bath thawing and cutting to identical sizes prior to heating was done to obtain identical heating patterns in the samples. After heating, the samples were cooled in a water bath at 4 °C until the next day. The shear force was measured in $10 \times 10 \times 50$ mm^3 strips using a Stable Micro Systems Texture Analyzer HD100 (Godalming, U.K.) equipped with a Warner−Bratzler shear blade with a rectangular hole, 11 mm wide and 15 mm high, and a blade thickness of 1.2 mm (*14*). The maximum shear force for 8 strips per sample, sheared across the fiber direction, was recorded at a test speed of 50 mm/min according to the procedure described in ref 14.

Extraction of Muscle Proteins. Pieces (100 mg) of frozen muscle tissue were cut and weighed at −20 °C in order to minimize protein degradation. The frozen muscle tissues were homogenized in 1 mL of 8 M UREA, 2 M thiourea, 65 mM DTT, 2% CHAPS-O, and 1% carrier ampholytes (Ampholyte 3-10, Pharmacia, Uppsala, Sweden) using a handheld glass homogenizer. Crude extracts were transferred to Eppendorf tubes, vigorously shaken for 2 h, and centrifuged (30 min at $10\,000 \times g$) in order to remove unextracted cellular components, high molecular weight protein complexes, and unsoluble proteins. The protein content was determined with Bradford protein assay (Bio-Rad, Hercules, CA) and were found to be between 8 and 10 μg/μL.

Two-Dimensional Electrophoresis (2DE). The preparation and running of the 2DE analysis was made as previously described (*15*). The first dimension of protein separation was made in immobilized pH gradient strips (18 cm, IPG-strips), which span the pH range of 4−7, while 13% SDS−PAGE was used for second dimension separation. For preparative 2DE, 500 μg of protein was loaded onto every IPG strip, whereas for analytical gels 50 μg of protein was loaded. Preparative gels were silver-stained according to ref 16, and the analytical gels were silver-stained according to ref 15.

Image Analysis. The 2DE gels were scanned using a U-Max office scanner (UMAX Technologies, Freemont, CA) and analyzed using the Bioimage 2D analyzer software (Genomic Solutions, Ann Arbor, MI). First, all spot positions were recognized and relative integrated spot intensities in the individual gels were estimated as the percents of the total spot intensity. Subsequently, the 2DE images were matched by comparing the relative positions and the integrated intensities of the individual spots on each gel. For comparative image analysis, the images were grouped, after which the relative expressions of the individual spots were analyzed and compared within and between the image groups. Finally, the changes in spot patterns revealed by computer-based image analysis were individually inspected and confirmed.

In-Gel Digestion of Protein Spots. In-gel digestion was performed as described by ref 17. The excised gel plugs were washed in 50 mM NH_4CO_3, followed by 50% and 100% acetonitril, and dried by vacuum centrifugation. Fifteen microliters of trypsin (sequencing grade, Roche, Basel, Switzerland), dissolved in 50 mM NH_4CO_3 pH 7.8 (12 ng/μL), was added to the dry gel pieces and incubated for reswelling on ice for 1 h. The supernatant was removed, 30 μL of 50 mM NH_4CO_3 buffer pH 7.8 was added, and the digestion was incubated overnight at 37 °C.

Desalting and Concentration. Custom-made chromatographic columns were used for desalting and concentration of the peptide mixture prior to mass spectrometric analysis (*18*). A column consisting of 100−300 nL of Poros reverse phase R2 or Oligo R3 material (20−30 μm bead size, PerSeptive Biosystems, Wellesley, MA) was packed in a constricted GeLoader tip (Eppendorf, Hamburg, Germany). A 10-mL syringe was used to force liquid through the column. Twenty microliters of the tryptic protein digests were loaded onto the column and washed with 20 μL of 0.1% TFA. For analyses by MALDI MS the peptides were eluted with 0.5 μL of matrix solution (15−20 g/L of α-cyano-4-hydroxycinnamic acid in 70% acetonitrile) directly onto the MALDI target in very small droplets.

Peptide-Mass Mapping by MALDI-TOF MS. A Bruker REFLEX model MALDI-TOF mass spectrometer (Bruker-Franzen Analytik GmbH, Bremen, Germany) equipped with the scout source and variable detector bias gating was used in positive-ion reflector mode for mass analysis of peptide mixtures (peptide-mass mapping). The ion acceleration voltage was 20 kV. Calibration was preformed using the trypsin peaks at m/z 842.11 and 2211.1 resulting from autodigestion of trypsin. The average deviation in mass accuracy was 50 ppm.

Protein Identification. Protein identification by peptide-mass mapping was performed using the database search program ProFound (http://prowl.rockefeller.edu/cgi-bin/ProFound), which searches the NCBI (National Center for Biotechnology Information) protein sequence database. The peptide-mass maps and protein identifications were evaluated as described in ref 17.

Statistics. The data were analyzed using the SAS package, version 8.1 (SAS Institute, Carry, NC). Proc Corr was used for calculating Pearson linear correlation coefficients (r) between Warner−Bratzler shear force measured in meat aged for 1 or 4 days, and spot intensity was measured at 72 h postmortem. Proc T-test was used to test for significance in relation to time changes. The null hypothesis of the t-test was that the mean of two groups was equal.

RESULTS

Postmortem Changes. Identification of the postmortem proteome changes was based on a comparison of muscle samples taken immediately from six animals after slaughter and 72 h postslaughter. The muscle proteins were separated by 2DE, silver stained, and matched as previously described in ref 15. A total of 345 individual spots were matched and compared, of which 103 spots were found to have changed significantly ($P < 0.01$) during the first 72 h postslaughter. The most notable changes were selected for protein identification by peptide-mass mapping by MALDI-TOF MS. Twenty-seven altered spots were identified, and **Figure 1** shows a representative 2DE display where the migration patterns and the changes in spot intensities are illustrated for the 27 identified spots. **Table 1** summarizes the estimated molecular weights, isoelectric points, estimated minimum sequence coverage, matched peptides, and resultant SWISS-PROT entries, which matched the peptide-mass mapping based database search of each of the individual spots.

Eleven of the identified spots were found to be fragments of actin (**Figure 2**), ranging from 44 to 16 kDa in molecular weight and from pH 4.52 to 5.55 in their isoelectric points. The proportion between sequence coverage and molecular weight of the individual actin fragments, as seen in **Table 1**, shows that all of these spots are indeed fragments of actin, hence clearly showing that actin is degraded postmortem. Other spots were likewise identified to originate from myofibrillar proteins, e.g., myosin heavy chain, myosin light chain I, myosin light chain II, titin, CapZ, and cofilin 2, as outlined in **Figure 2**. Of these, titin and myosin heavy chain had a very low sequence coverage (see **Table 1**), as they are fragments of proteins with high

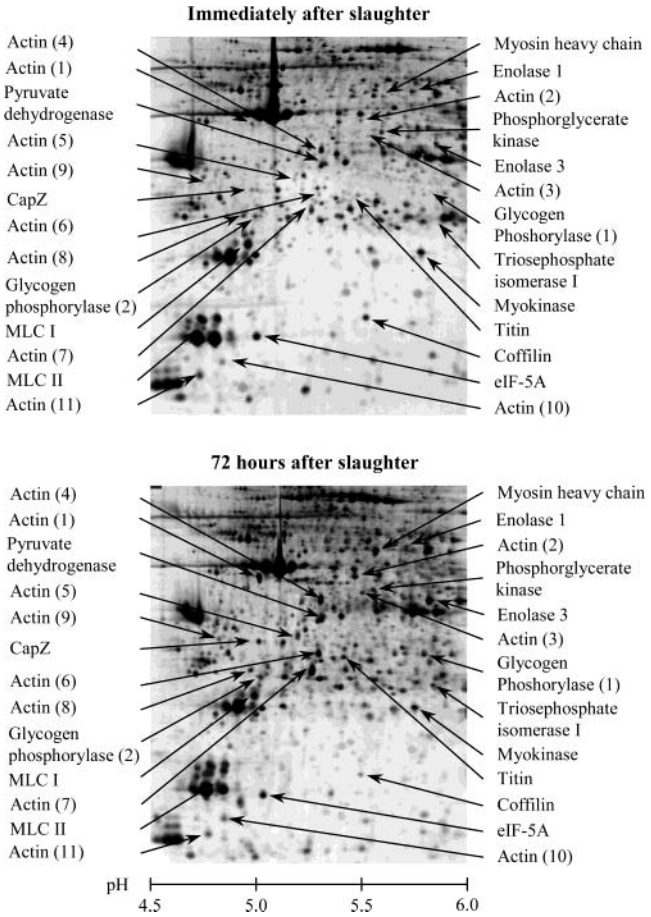

Figure 1. 2DE gels of pig longissimus dorsi immediately after slaughter and 72 h after slaughter. Arrows show the identified protein postmortem changes. MLC, myosin light chain.

molecular weight, and the sequence coverage is calculated relative to the full-length protein. In contrast to all other

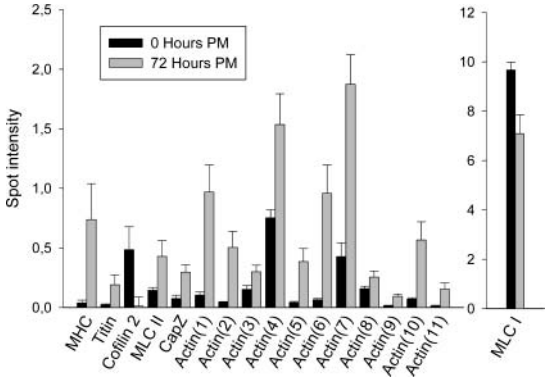

Figure 2. The spot intensity of myofibrillar postmortem proteins changes at slaughter and 72 h after slaughter. MHC, myosin heavy chain; MLC, myosin light chain. All changes are significant ($P < 0.01$).

identified proteins, the spot intensities of cofilin 2 and myosin light chain I were found to decrease during postmortem storage, as illustrated in **Figure 2**. Comparison of the molecular weights of the 2DE separated spots with the calculated molecular weights of the full-length sequences (see **Table 1**) shows that these two spots are full-length proteins rather than proteolytic fragments of cofilin 2 or myosin light chain I. The spot intensities of CapZ and myosin light chain II increase during postmortem storage. The observed molecular weights of CapZ and myosin light chain II are so close to the expected molecular weights of the full-length proteins that it is not possible to determine whether the increases are due to postmortem modification, protein expression, or a slight degradation of the full-length proteins.

Finally, eight sarcoplasmic proteins were found to change postmortem, namely, enolase 1, enolase 3, phoshoglycerate kinase, pyruvate dehydrogenase, glycogen phosphorylase, triosephosphate isomerase I, myokinase, and eukaryotic translation initiation factor 5A (eIF-5A) (**Figure 3**). Two fragments of glycogen phoshorylase, 27 and 30 kDa, respectively, were identified. Comparison of the observed and expected molecular

Table 1. Identification of Postmortem Changes in Pig Longissimus Dorsi

identified fragments	MW[a] (kDa)	pI[b]	sequence coverage[c] (%)	match peptides[d]	SWISS-PROT[e]	calculated MW[f] (kDa)
actin (1)	43	4.76	60	17	P02568	42
actin (2)	44	5.46	51	13	P02568	42
actin (3)	41	5.55	54	15	P02568	42
actin (4)	40	5.30	56	12	P02568	42
actin (5)	34	5.18	42	10	P02568	42
actin (6)	32	5.29	38	12	P02568	42
actin (7)	28	5.25	30	12	P02568	42
actin (8)	28	4.87	26	6	P02568	42
actin (9)	34	4.52	20	6	P02568	42
actin (10)	17	4.87	16	5	P02568	42
actin (11)	16	4.78	11	4	P02568	42
myosin heavy chain	49	5.51	6	9	Q9TV62	223
CapZ	32	4.88	38	9	P47756	32
titin	30	5.42	4	7	O97791	4200
myosin light chain i	25	4.95	36	8	P05976	21
cofilin 2	19	5.52	31	5	Q9Y281	19
myosin light chain ii	18	4.74	49	7	P04466	19
enolase 1	50	5.71	16	5	P06733	47
phosphoglycerate kinase	41	5.52	24	6	P09411	44
enolase 3	39	5.87	29	12	P21550	47
pyruvate dehydrogenase	36	5.30	30	8	P26269	40
glycogen phosphorylase (1)	30	5.85	38	13	P00489	97
glycogen phosphorylase (2)	27	4.96	26	7	P00489	97
triosephosphate isomerase i	25	5.90	20	5	P17751	27
myokinase	23	5.69	56	10	P00571	22
eIF-5A	18	4.99	47	8	P10160	17

[a] Molecular weight of spot. [b] Isoelectric point of spot. [c] The minimum coverage of the matched peptides in relation to the full-length sequence. [d] The number of matched peptides in the database search. [e] Primary accession number in the SWISS-PROT database. [f] Calculated molecular weight of full-length protein.

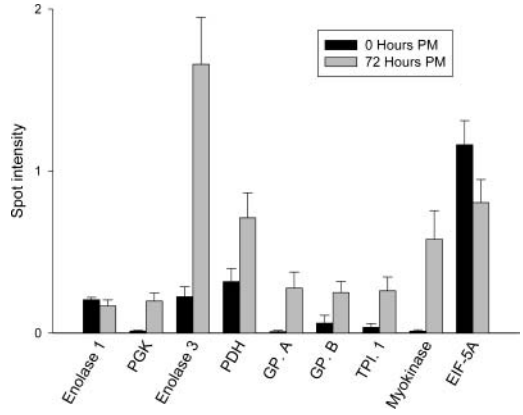

Figure 3. The spot intensity of sarcoplasmic postmortem proteins changes at slaughter and 72 h after slaughter. PGK, phosphoglycerate kinase; PDH, pyruvate dehydrogenase; TPI. 1, triosephosphate isomerase I. All changes are significant ($P < 0.01$).

weight of eIF-5A indicates that we observe postmortem degradation of the full-length protein. Regarding the molecular weight data (**Table 1** and **Figure 1**) of enolase 1, enolase 3, phosphoglycerate kinase, pyruvate dehydrogenase, and myokinase, the differences between the observed and the expected molecular weight of the full-length proteins are so small that it is not possible to determine whether the postmortem changes are due to modifications such as phosphorylation, protein expression, or minor fragmentations.

Correlation between Postmortem Changes and Shear Force Values. Warner−Bratzler shear force measurements were made on pork samples taken from the same 25 carcasses used to analyze the 72-h postmortem proteome patterns, at day 1 (WB1) and day 4 (WB4) after slaughter. Shear force data were correlated to the spot intensity measured at 72 h postmortem of the 27 spots that were found to change postmortem. In **Tables 2** and **3** are given calculated Pearson correlations between the individual spot intensity at 72 h postmortem and shear force values.

Significant correlations were found with several actin fragments ($P < 0.05$). The highest correlation coefficients (−0.54 and −0.55) were found for actin(6) to WB1 and WB4, respectively. The correlation coefficients for actin(2) and actin(7) to WB1 were −0.51 and −0.46 and to WB4 −0.47 and −0.44, respectively. Nine out of the 11 identified actin fragments had a negative correlation coefficient to WB1 and WB4, whereas actin(8) and actin(9) showed positive correlations. The fragment of myosin heavy chain showed negative correlations (−0.54 and −0.49) to WB1 as well as WB4 ($P < 0.05$), and the myosin light chain II fragment was positively correlated (0.59 and 0.49) to both WB1 and WB4 ($P < 0.05$).

Finally, postmortem changes in identified sarcoplasmic proteins were also correlated to shear force values (**Table 3**). Triose phosphate isomerase I was the only identified sarcoplasmic protein that was significantly correlated to both WB1 and WB4 with a correlation coefficient of −0.46 and −0.64, respectively.

DISCUSSION

The aim of the present work was to study whether specific postmortem proteome variation in porcine M. longissimus dorsi is related to the shear force values of pork.

The results clearly show that both actin and myosin heavy chain are degraded postmortem. The fact that actin and myosin heavy chain are the major structural proteins accounting for 20% and 45%, respectively, of the total myofibrillar proteins (*19*) implies that any postmortem degradation of these cannot be excluded to weaken the myofibrillar lattice and hereby also to influence the texture of meat. Our results are in contrast to most other reports, which have claimed that actin is not degraded postmortem (*5*, *7*, *20*, *21*) and that myosin heavy chain is only degraded when the muscle is kept at an elevated temperature (*4*, *22*). These statements have all been based on analysis of postmortem degradation using one-dimensional SDS−PAGE. The higher resolution obtained using 2DE proteome analysis in the present studies stresses that both actin and myosin heavy

Table 2. Pearson Correlations Coefficent for Postmortem Myofibrilar Protein Changes and Shear Force Measurements[a]

	actin(1)	actin(2)	actin(3)	actin(4)	actin(5)	actin(6)	actin(7)	actin(8)	actin(9)
WB1	–0.30	–0.51	–0.11	–0.26	–0.34	–0.54	–0.46	0.01	0.04
p-value	0.1520	0.0088	0.6052	0.2055	0.0922	0.0052	0.0204	0.9931	0.8627
WB4	–0.27	–0.47	–0.15	–0.31	–0.39	–0.55	–0.44	0.10	–0.06
p-value	0.2150	0.0244	0.4825	0.1548	0.0663	0.0064	0.0362	0.6648	0.7918

	actin(10)	actin(11)	MHC	MLC I	MLC II	titin	CapZ	cofilin 2
WB1	–0.23	–0.38	–0.54	0.03	0.59	–0.02	0.21	–0.28
p-value	0.2619	0.0636	0.0054	0.8996	0.0018	0.09438	0.3153	0.1757
WB4	–0.26	–0.18	–0.49	–0.02	0.49	0.02	0.32	–0.21
p-value	0.2302	0.4092	0.0170	0.9388	0.0182	0.9423	0.1391	0.3412

[a] Pearson correlation coefficients and *p*-values for the correlations between the spot intensity at 72 h after slaughter and WB1 and WB4 of postmortem myofibrilar protein changes. MHC, myosin heavy chain; MLC, myosin light chain.

Table 3. Pearson Correlation Coefficents for Postmortem Sarcoplasmic Protein Changes and Shear Force Measurements[a]

	enolase 1	enolase 3	PDH	GP(2)	GP(1)	TPI	myokinase	PGK	eIF-5A
WB1	0.28	–0.32	–0.40	–0.09	0.06	–0.46	0.03	–0.04	0.25
p-value	0.1755	0.1141	0.0501	0.6706	0.7623	0.0201	0.8980	0.8387	0.2338
WB4	0.33	–0.20	–0.33	–0.12	0.12	–0.64	0.10	–0.08	0.29
p-value	0.1232	0.3496	0.1275	0.5895	0.5900	0.0011	0.6507	0.7286	0.1755

[a] Pearson correlation coefficients and *p*-values for the correlation between the spot intensity at 72 h after slaughter and WB1 and WB4 of post mortem sarcoplasmic protein changes. PDH, pyruvate dehydrogenase; GP, glycogen phosphorylase; PGK, phosphoglycerate kinase.

chain fragments are formed during the tenderization process, as we also found in a previous study (*12*).

The established significant correlation between three of the actin fragments and the fragments of myosin heavy chain to shear force values (**Table 2**) clearly emphasizes that postmortem degradation of these two major myofibrillar components contributes to the tenderization process of the pork, even though only a minor part of actin and myosin heavy chain is degraded postmortem, as was evaluated from the amount of actin and myosin heavy chain fragments detectable on the gels (**Figure 1**). However, it is reasonable to believe that even a minor degradation of actin will weaken the myofibrillar lattice and thereby influence the texture of the meat, even though it could be expected that the degree depends on where in the thin filaments actin is degraded. Especially, even a minor degree of actin degradation within the I-band near the Z-disk could be expected to have a major influence on meat texture, considering the suggested structural changes of importance for tenderness (*8*). We have previously described the postmortem accumulation of a myosin heavy chain fragment being part of the globular myosin head domain (*12*). Likewise, actin degradation, cleavage within the myosin head domain, even to a minor degree, must be expected to weaken the myofibrillar lattice, as such a cleavage leads to disruption of the actin/myosin interaction.

This paper is the first to report postmortem degradation of myosin light chains I and II. The found positive correlations between myosin light chain II and WB1 and WB4 are surprising, as it is expected that the correlation coefficient is negative if the postmortem changes of myosin light chain II are an outcome of postmortem proteolysis. However, it could be that this myosin light chain II fragment is an intermediate product that is further degraded. Further studies are needed to reveal how postmortem changes of myosin light chain II influence myofibrillar lattice structure in a way that affects the texture of meat. One suggestion could be that the interaction between myosin and actin is influenced in a way that weakens the myofibrillar lattice.

Considering that both CapZ and cofilin 2 have important roles in the control of actin polymerization (*23*) it is interesting to see that both these proteins change postmortem. CapZ is located in the Z-disk and binds to the barbed end of actin filaments, where it prevents the addition or loss of actin monomers (*24*). Moreover, CapZ plays a significant role in the structure of the Z-disk, where together with α-actinin it has been proposed to make up an anchoring complex for thin filaments in the Z-disk (*19*, *25*). The registered postmortem change of CapZ is probably due to accumulation of the full-length protein rather than a result of protein degradation, as the molecular weight of the observed protein spot is identical to the expected molecular weight of the full-length protein (see **Table 1**). The observed postmortem accumulation is likely to be due to modification or protein expression. In contrast to CapZ, Cofilin 2, which is an actin-depolymerizing factor, is directly involved in the regulation of the polymerization of actin at the ends of the filaments (*26*). Even though postmortem change in neither CapZ nor cofilin 2 was significantly related to tenderness in the present study, further studies of these proteins in the postmortem period are needed to reveal their potential influence on changes in myofibrillar lattice postmortem considering their critical roles in these structures, which are known to be of importance for the tenderness of meat.

Finally, eight sarcoplasmic proteins were found to change postmortem. Although changes of sarcoplasmic proteins are not considered to play an important role in the tenderization process of meat, we observe that postmortem variation of triose phosphate isomerase I was highly correlated to both WB1 and WB2, −0.46 and −0.64, respectively. Even if triose phosphate isomerase I has no direct effect on meat texture, the observed postmortem accumulation may reflect increased proteolysis and might be a potential marker for tenderness development. The difference between the molecular weight of the spot and the theoretical molecular weight of the full-length protein is only 2 kDa, and the postmortem accumulation could be due to either a minor degradation, a modification, or a protein expression. However, the observation that the level of triose phosphate isomerase I correlates with shear force may also indicate that postmortem glycolysis plays an important role in postmortem tenderization of meat. Consequently, further studies of the postmortem changes of triose phosphate isomerase I are needed to acquire a better understanding of the relation between these changes and tenderness development.

CONCLUSION

For the first time we report on the potential of postmortem proteome analysis in the elucidation of the postmortem tenderization process in relation to tenderness in pork measured by Warner−Bratzler shear force values. We found significant correlations between the postmortem degradation of actin and myosin heavy chain to tenderness, which clearly indicates that the postmortem degradation of actin and myosin heavy chain influences meat texture. Moreover, myosin light chain II and triosephosphate isomerase I were also found to be significantly correlated to tenderness of pork. However, the present study cannot elucidate the reason these changes should effect meat texture.

ABBREVIATION USED

MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; 2DE, two-dimensional gel electrophoresis; WB, Warner−Bratzler shear force; SDS−PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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