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Quantitative Determination of the Myofibrillar Proteins and Connective Tissue Content in Selected Porcine Skeletal Muscles[1]

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The new analytical chromatographic methods developed to quantitate the unique amino acids that occur in proteins have been successfully applied for the determination of the myofibrillar and connective tissue content of both select porcine skeletal muscles and the intracellular and extracellular protein fractions. The proposed chemical approach is based on the direct determination of the myofibrillar myosin and actin contents of skeletal muscles from the amounts of protein bound N^{τ}-methylhistidine present. Collagen and collagen-like proteins can be calculated from the amounts of 5-hydroxylysine found and the elastin content from the amounts of desmosine or isodesmosine present. These quantitations are based on the total protein content of the selected porcine muscles determined by their detailed amino acid composition. Actin accounts for an estimated 10.2–11.5% of the total porcine muscle mass or about 21.1% of the total myofibrillar protein, while myosin ranged from 21.3 to 24.0% of the total muscle protein corresponding to 43.9% of the myofibrillar proteins (52.14% of protein). Total porcine muscle collagen ranged from 2.84 to 5.89% in select porcine muscles, while elastin accounts for an estimated 0.063–0.143%.

The possibility of using N^{τ}-methylhistidine [His(τ-Me)] as an index for determining the absolute mass of the myofibrillar proteins myosin and actin in skeletal muscles and composite meat products has stimulated considerable interest recently (Hibbert and Lawrie, 1972; Olsman and Slump, 1981; Ranken, 1984; Expert Work Group, FSIS, 1984; Benedict, 1987; Ashworth, 1987; McNeal, 1987). This quantitation is based on the following findings. Sequence studies have shown that actin contains 1 mol of His(τ-Me) at position 73 in its amino sequence (Elzinga et al., 1973; Vanderkerckhove and Weber, 1978, 1979) and that myosin isolated from adult fast-twitch white skeletal muscles contains 1 mol of His(τ-Me) at position 755 in each of the two heavy chains of this protein (Okamoto and Yount, 1975; Maita et al., 1987). Other studies have indicated that His(τ-Me) is absent from all other muscle and nonmuscle proteins (Huszar, 1984). In addition, Yates and Greaser (1983) have shown that the psoas skeletal muscle myosin accounts for 43% and actin for an estimated 22% of the myofibrillar protein mass (57.7%) of skeletal muscle. Moreover, the in situ molar ratio of actin to myosin in the myofibrils of skeletal muscle has been shown to be 6/1 (Murakami and Uchida, 1985).

Measurements of the levels of His(τ-Me) in several experimental animals (Asatoor and Armstrong, 1967; Johnson et al., 1967; Haverberg et al., 1975; Holbrook et al., 1979) and in bovine, ovine, and avian skeletal muscle tissues (Rangeley and Lawrie, 1976; Olsman and Slump, 1981; White and Lawrie, 1985; Jones et al., 1985, 1987), using a variety of chromatographic methods (Hancock and Harding, 1984; Ashworth, 1987), have yielded variable amounts of His(τ-Me) among the muscle tissues studied, ranging from 2.24 to 10.6 μmol of His(τ-Me)/g of tissue. Although some of this variation was attributed to the distribution of porcine muscle fiber types or the presence of variable amounts of balenine (Carnegie et al., 1982, 1984; Harris and Milne, 1981, 1987), a histidine dipeptide, β-

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alanyl-L-N^{τ}-methylhistine, which must be extracted from muscle tissues prior to acid hydrolysis, most of this variation appears to be due to incomplete separation of such small amounts of His(τ-Me) from other components present in such complex muscle tissue hydrolysate by the multicolumn systems employed (Hancock and Harding, 1984).

This paper describes the application of the new analytical chromatographic methods developed (Zarkadas et al., 1986, 1987b) to quantitate the unique methylated basic amino acids, including 5-hydroxylysine [Lys(5-OH)], desmosine [Des], isodesmosine [iDes], and related compounds in typical porcine skeletal muscle tissues, in purified actin, and the two major intracellular muscle protein and connective tissue fractions, prepared and quantitated by the methods of McCollester (1962) and Laurent et al. (1981). The aim was to determine whether the levels of these unique basic amino acids in the selected porcine skeletal muscle tissues could be used for the determination of their myofibrillar and connective tissue proteins from the amounts of protein-bound His(τ-Me) and Lys(5-OH) found in their acid hydrolysates, respectively. The elastin content in muscles can also be calculated from the amounts of Des or iDes found (Zarkadas, 1981; Zarkadas et al., 1986). These calculations are based on the total protein content of porcine muscle tissues and the isolated intracellular muscle protein and extracellular matrix fractions determined by their detailed amino acid compositions.

MATERIALS AND METHODS

Materials. Types DC-4A (Lot No. 750) and DC-5A (Lot No. 746) cation-exchange spherical resins, sized to 9.0 ± 0.5 and 6.0 ± 0.5 μm, respectively, were purchased from Dionex Chemical Co., Sunnyvale, CA. Type AA-10 resin, which is equivalent to Dionex Type DC-4A resin, and Type I standard amino acid calibration mixture were obtained from Beckman Instruments, Inc., Palo Alto, CA. The diastereoisomer mixture of 5-hydroxy-DL-lysine, N^6-methyl-L-lysine, N^6,N^6-dimethyl-L- and N^6,N^6,N^6-trimethyl-L-lysines bis(*p*-hydroxyazobenzenesulfonate) monohydrate, D-glucosamine monohydrochloride, D-galactosamine monohydrochloride, N^{τ}-methyl-L-histidine, and N^{τ}-methyl-L-histidine monohydrate were purchased from Calbiochem-Behring Corp., La Jolla, CA. DL-Ornithine was purchased from Schwarg/Mann, Orangeburg, NY. Norleucine and L-2-amino-3-guanidinopropionic acid was from Pierce Chemical Co., Rockford, IL, and 3-nitro-L-tyrosine from Aldrich Chemical Co., Milwaukee, WI. Because Des and iDes are not commercially available, these cross-linking amino acids were isolated by the preparative method described by Zarkadas (1979) using bovine *Ligamentum nuchae* elastin purchased from Sigma Chemical Co., St. Louis, MO. Sodium dodecyl sulfate (SDS) was also from Sigma. The highly purified citrate buffers (pH 3.28, 0.20 M; pH 4.25, 0.20 M; pH 6.40, 1.0 M), sample dilution buffer (pH 2.2, 0.20 M), and ninhydrin recommended for high-sensitivity standard single-microcolumn analysis were purchased as either concentrates or ready-to-use solutions from Beckman Instruments. The chemicals and reagents employed for determining the methylated basic amino acids were purchased from Pierce. Sephadex G-10, G-25, and G-200 (fine bead type) were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. AG-500-X8(D) analytical-grade mixed bed (20–50 mesh fully regenerated), acrylamide (>99.9% purity), *N*,*N*′-methylenebisacrylamide, TEMED (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine), ammonium persulfate, Coomassie Brilliant Blue G-250 and R-250, Bromophenol Blue, glycine, Bio-Lyte (pH 3–10), and sodium dodecyl sulfate (SDS) were purchased from Bio-Rad Laboratories, Richmond, CA. Ampholine, pH 3.5–10 (LKB, Broma, Sweden), was obtained from Fisher Scientific Canada, Montreal, Quebec. Electrophoresis calibration Kit H (Lot No. 9015) for molecular weight determination was obtained from Pharmacia. The 2-mercaptoethanol was obtained from Eastman Kodak Co., Rochester, NY. Nonidet P40 was supplied from BDH Chemicals Ltd., Poole, England. High-purity urea were purchased from Schwarz/Mann, which was further purified by passing through an AG 501-X8(D) mixed-bed column (5 × 20 cm). The distilled water used in the preparation of all buffers and reagents was purified as described previously (Zarkadas et al., 1987b) and stored in glass until use. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Sampling and Preparation of Muscle Tissues. The following three typical porcine skeletal muscles were selected for this investigation: semimembranosus, semitendinosus, and external sternomandibularis. These muscle tissues were excised as 10-cm-thick muscle sections from the left side of randomly selected commercial-grade porcine carcasses (approximately 63–105 kg) from female (sows) mature Yorkshire pigs supplied by North Packers Ltd., Montreal, Quebec, and Abattoir Regional Valleyfield, Inc., Valleyfield, Quebec. All muscle tissues (approximately 200 g each) were cleaned of adhering fat, cut into small cubes, ground, frozen (−175 °C), and lyophilized. The freeze-dried samples were then pulverized in a standard electrically driven end runner mill, passed through a No. 40 mesh sieve, and then stored at −20 °C in polypropylene bottles until use. Proximate and elemental analyses were carried out in each of these samples, and the results have been reported elsewhere (Zarkadas et al., 1987a).

Porcine skeletal muscle tissues are known to contain variable amounts of the histidine dipeptides carnosine, anserine, and balenine (Rangeley and Lawrie, 1977; Carnegie et al., 1982, 1984; Harris and Milne, 1987). To effectively remove all these histidine dipeptides from porcine tissue, the muscle samples were extracted with a mixture of 75% ethyl alcohol in 0.1 M HCl (Rangeley and Lawrie, 1976) as described previously (Karatzas and Zarkadas, 1988).

Extraction and Quantitation of Porcine Skeletal Muscle and Connective Tissue Protein Fractions (F1 and F2). The procedure employed for the extraction and preparation of the porcine intramuscular skeletal muscle protein (F1) and extracellular matrix connective tissue protein fractions (F2) combines the original method of McCollester (1962) and the procedures described by Laurent et al. (1981) and Light and Champion (1984) as follows. Approximately 200 g of porcine skeletal muscle samples (external sternomandibularis, semimembranosus, semitendinosus), which were excised fresh from mature female Yorkshore sows, were cut into small cubes and homogenized in 100 mL of 0.05 M $CaCl_2$ solution in a VirTis homogenizer (Model 45) for 10 s (speed set at 30/100) at 4 °C. The homogenate was centrifuged at 4000*g* (SS-34, Sorvall rotor; 6000 rpm) for 20 min at 4 °C; the supernatant was decanted through eight layers of cheesecloth to trap fat particles and was retained so that muscle proteins could be removed by precipitation with 5% trichloroacetic acid and quantitated by amino acid analysis. The pellet was resuspended in 70 mL of phosphate-buffered saline (PBS; 0.15 M NaCl/0.02 M sodium phosphate buffer, pH 7.4) at 20 °C and rehomogenized for 3 min. The homogenate was again centrifuged at 4000*g* for 20 min, and the supernatants were combined. The

pellet was resuspended in 70 mL of 2% sodium dodecyl sulfate (SDS) and rehomogenized for 3.0 min, and the supernatants were combined. The wash procedure with SDS was repeated a further four times as described for lung tissues by Laurent et al. (1981), and the separated muscle protein fractions (F1 and F2), after desalting, drying under vacuum, and powdering, were designated SDS-solubilized intracellular protein (F1) and SDS-insoluble extracellular matrix (F2) fractions as described previously (Zarkadas et al., 1988).

Actin Purification. Porcine skeletal muscle G-actin was purified from acetone-extracted semimembranosus Yorkshire sows muscle tissues by the low-salt buffer extraction method of Spudich and Watt (1971), followed by three polymerization and depolymerization steps as recommended by Pardee and Spudich (1982). The porcine semimembranosus actin was further purified by gel permeation chromatography on a 2.6 × 95 cm Sephacryl S-200 column eluted with the depolymerization buffer of Pardee and Spudich (1982). The flow rate was maintained at 60 mL/h, the effluent was collected in 3.0-μL fractions, and the fractionation was monitored by absorbancy measurements at 280 nm. Protein concentration in each fraction was determined either by the method of Lowry et al. (1951) as modified by Peterson (1983) using bovine serum albumin as a standard or by the method described by Horstmann (1979). The fractions containing the G-actin from the Sephacryl S-200 column were concentrated by the polymerization method of Pardee and Spudich (1982). Prior to use, the concentrated G-actin solutions were centrifuged for 1 h at 100000*g* and the supernatants were passed through a Sephadex G-25 column (40 cm × 2.5 cm) preequilibrated with the same depolymerization buffer of Pardee and Spudich (1982) so that excess ATP and 2-mercaptoethanol and other impurities could be removed. To rule out any possible effects of aging of the samples, freshly prepared and chromatographically purified G-actin was used.

One- and Two-Dimensional Polyacrylamide Gel Electrophoresis (PAGE). One-dimensional SDS-PAGE was performed in a Bio-Rad Model 172A analytical–electrophoresis apparatus (Bio-Rad Laboratories) equipped to accommodate 12 gel tubes according to the procedure of Weber and Osborn (1975). The technique described by Laemmli (1970) using 10% acrylamide gel with a 3% stacking gel followed by two-dimensional SDS-PAGE according to O'Farrell (1975) was applied to further test the purity of porcine semimembranosus G-actin. The Ampholine or Bio-Lyte used for isoelectric focusing in the first dimension was pH 3.5–10.0. Second-dimension separation was on a 5–20% gradient gel with a 3% stacking gel.

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman Model 120C) or an updated and fully automated amino acid analyzer (equivalent to Beckman Model 121MB). The automated instrument was equipped with a Varian Vista 402 chromatographic data reduction system (Varian Instruments Group, Walnut Creek, CA) as described previously (Zarkadas et al., 1986, 1987b).

Porcine muscle tissue samples (0.1 g) were hydrolyzed in Pyrex test tubes (18 × 150 nm) under vacuum (below 10 μmHg) with 10 mL of triple-glass-distilled constant-boiling HCl (6.0 M) at 110 °C in duplicate for each of four times, 24, 48, 72, and 96 h, respectively, with the usual precautions described previously (Hunt, 1985; Nguyen et al., 1986). The data reported for serine, threonine, and tyrosine represent the average of values extrapolated to zero time of hydrolysis. The values for valine, isoleucine, leucine, and phenylalanine are average values from 48, 72, and 96 h of hydrolysis. All other values are reported as the average values from 24, 48, 72, and 96 h of hydrolysis (Zarkadas, 1979, 1981). 4-Hydroxyproline was determined separately from a concentrated hydrolysate (equivalent to 0.1 mg of protein/analysis) as described previously (Zarkadas et al., 1986), and recoveries were calculated relative to alanine.

Methionine and cyst(e)ine were determined separately (0.1 g) by the performic acid procedure of Moore (1963) as described previously (Nguyen et al., 1986). Recovery of cyst(e)ine as cysteic acid and methionine as the dioxide was calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids and relative to alanine and leucine present in the sample. Tryptophan in porcine muscle samples (0.1 g) was determined separately after alkaline hydrolysis (Hugli and Moore, 1972) by an improved chromatographic procedure (Zarkadas et al., 1986) using 3-nitrotyrosine as an internal standard.

The determinations of the methylated basic amino acids, the diastereoisomers of 5-hydroxylysine, and related compounds were carried out with concentrated 96-h hydrolysates (equivalent to 100–300 μg of protein/analysis) by the single-microcolumn 50 × 0.28 cm) system packed with Dionex DC-4A resin (Zarkadas et al., 1987b) so that peaks adequate for these components could be obtained.

Determination of Protein Mass in Skeletal Muscle. Recoveries of these unique amino acids were calculated on the protein content of each 96-h hydrolysate determined by the procedure described by Horstmann (1979). According to this method, a mean residue weight (WE, μg/nmol) is calculated for the amino acids constituting the proteins in skeletal muscle samples as

$$\mathrm{WE} = \sum_{i=1}^{20} (a_i b_i) \tag{1}$$

where a_i is the mole fraction of an amino acid i found in the analyzed aliquot and b_i is the molecular weight of amino acid residue i as described by Horstmann (1979). A conversion factor F (μg/nmol) is used for determining the protein mass in each hydrolysate sample analyzed, which is the apparent mean residue molecular weight increased in proportion to the missing tryptophan (a_{Trp}) and cys(e)ine (a_{Cys}) values from

$$F = \mathrm{WE}/[1 - (a_{\mathrm{Trp}} + a_{\mathrm{Cys}})] \tag{2}$$

The conversion factor F' (μg/mol) was also calculated according to eq 2, but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and Pro(4-OH). These factors (F and F') are constants characteristic for each protein or protein mixture. Thus, the amount of protein (P, μg) in each hydrolysate can then be calculated as

$$P = F \sum_{i=1}^{16} x_i \tag{3}$$

where x_i are the nanomoles of each amino acid i found in the analyzed aliquot. Protein determination has also been carried out by the standard Kjeldahl procedure, and the results have been reported (Zarkadas et al., 1987a).

(*a*) *Determination of Connective Tissue Proteins.* The amounts of the unusual protein-bound amino acids Lys(5-OH) and Des, which occur exclusively in vertebrate connective tissue proteins, i.e., collagens and elastin, have been used to determine the contents of these extracellular matrix proteins in three selected porcine skeletal muscles. In this chemical approach the distribution of elastic fibers

Table I. Average Values for 5-Hydroxysine and 4-Hydroxyproline Computed from the Known Distribution of Types I, III, and IV Collagens in Skeletal Muscles

collagen type standard	chain assoc	av no. res/1000 total[a] Pro(4-OH)	av no. res/1000 total[a] Lys(5-OH)	collagen type distribn,[b] % total muscle collagen	av no. res contrib by collagen type Pro(4-OH)	av no. res contrib by collagen type Lys(5-OH)
I	$[\alpha1(I)]_2\alpha2(I)$	98	10	61.5	60.3	6.2
III	$[\alpha1(III)]_3$	118	5	33.5	39.5	1.7
IV	$[\alpha1(IV)]_2\alpha2(IV)$	120	42	5.0	6.0	2.1
$\sum_{i=1}^{3} n_i'$					105.8	10.0

[a] Average values computed from the data of Miller and Cay (1982), Laurent et al. (1981), and Light (1985). (See also Table VIII.) [b] Data taken from Light and Champion (1984) and Light et al. (1985).

(elastin) could be calculated from the amounts of Des found in the acid hydrolysates of these tissues and the total collagen and collagen-like proteins (Porter and Reid, 1978; Anglister et al., 1976) from the amounts of the aLys(5-OH) diastereoisomers present. Amorphous elastin (Foster, 1982), which was used as standard for comparison, contains 3 residues of Des/1000 total amino acids and has a mean residue weight (WE) of 85.06 (Table I). The anhydrous $M_r(i)$ of Des is 454.54. Light and Champion (1984) and Light et al. (1985) have shown that types I and III collagens accounted for 61 and 35% of the recovered collagen in the epimysium, perimysium, and endomysium of skeletal muscle, while type IV collagen accounted for the remaining 5%. Thus, a mean for the diastereoisomers of Lys(5-OH) content of $n_i' = 10.0$ residues/1000 total amino acid residues in muscle collagen could be computed from the relative distribution of collagen types and their respective Lys(5-OH) contents presented in Table I. The average residue weight (WE) for collagen is 91.1, and each of the diastereomers of Lys(5-OH) has an anhydrous M_r of 145.18.

A method to calculate the amount of a specific protein in skeletal muscle is

$$P_j = C_i \cdot \frac{[1000]}{n_i'} \cdot \frac{\mathrm{WE}(P_j)}{M_r(i)} \quad (4)$$

where $\mathrm{WE}(P_j)$ is the weight equivalent of a specific muscle protein j determined from eq 1 as described by Horstmann (1959), n_i' is the number of amino acid residues (i)/1000 amino acid residues, and $M_r(i)$ is the anhydrous molecular weight of the unique amino acid i.

The following analytical conventions, derived from eq 4, can therefore be used for calculating the

$$\text{amt of collagen } (P_C) = \text{amt of Lys(5-OH)} \times 63.3 \quad (4a)$$

and

$$\text{amt of elastin } (P_E) = \text{amt of Des} \times 62.4 \quad (4b)$$

Thus, the total connective tissue content of porcine skeletal muscle (in grams per kilogram of total protein) could then be calculated from the sum of collagen (P_G) and elastin (P_E) found in these tissues.

An alternative method for calculating the amount of collagen and elastin involves substitution into eq 6 below the total amount of Pro(4-OH) found in both proteins (Berg, 1982; Etherington and Sims, 1981; Rucker, 1982) from the known Pro(4-OH) contents of collagen ($n_i' = 105.8$; see Table I) and elastin ($n_i = 22$) as described previously (Zarkadas et al., 1988). The anhydrous molecular weight of Pro(4-OH) is 113.12.

The relative distribution (P_C/P_E) of collagen and elastin per unit of tissue can therefore be calculated either from the Lys(5-OH) and Des contents (eq 4a and 4b) or from the amounts of Pro(4-OH) found with eq 4c

$$\text{amt of connective tissue } (P_C + P_E) = \text{amt of Pro(4-OH)} \times 8.03 \quad (4c)$$

This value is in close agreement with that reported by Etherington and Sims (1981).

(b) *Determination of the Amount of a Mixture of Two Proteins in Skeletal Muscle.* The amount of two proteins (P_1 and P_2) containing the same or different amounts of the same unique amino acid (C_1 and C_2) can also be determined from the total amount (C_T) of that amino acid as described previously (Zarkadas et al., 1988):

$$C_T = C_1 + C_2 \quad (5)$$

By substituting C_1 and C_2 by the amounts of each protein from eq 4, the following relationship can be derived:

$$\sum_{j=1}^{2}(P_1 + P_2) = \frac{P_1/P_2 + 1}{n_2'/\mathrm{WE}(P_2) + (P_1/P_2)n_2'/\mathrm{WE}(P_1)} \frac{1000C_T}{M_r(i)} \quad (6)$$

The amount of the two proteins (P_1 and P_2) is dependent on the relative composition of the two proteins in the sample (P_1/P_2), the number of residues of the unique amino acid (n_i') per 1000 amino acid residues, and the total amount of the unique amino acid (C_T). Equation 6 is analogous to eq 4 for one protein with a unique amino acid. As $P_1/P_2 \rightarrow 0$, eq 6 will simplify to eq 4.

(c) *Determination of the Myofibrillar Proteins Myosin and Actin.* Since sequence studies (Elzinga et al., 1973; Elzinga and Collins, 1977; Vandekerckhove and Weber, 1979; Tong and Elzinga, 1983; Maita et al., 1987) have shown that 1 mol of actin (A) contains 1 mol of His(τ-Me) and that 1 mol of myosin (M) contains 2 mol of His(τ-Me), the quantitation of protein-bound His(τ-Me) can be used as an index for determining these two principal myofibrillar proteins in muscle tissues. Considerable care must be taken, however, to extract all soluble histidine dipeptides, especially balenine (Carnegie et al., 1982; Harris and Milne, 1987), prior to acid hydrolysis of muscle tissues. Thus, the total amount of His(τ-Me) in the selected porcine tissues determined by the present method (Zarkadas et al., 1987b) represents the sum of the distribution of His(τ-Me) in the myosin and actin present in each of the porcine skeletal muscle tissue investigated and can be calculated according to

$$C_T = C_A + C_M \quad (5)$$

where C_T is the total protein-bound His(τ-Me) in myosin and actin (in grams per kilogram of the total protein), C_A is the amount (g) of His(τ-Me) in actin, and C_M is the amount (g) of His(τ-Me) in myosin in 1 kg of total protein (Zarkadas et al., 1988).

Since the relative amounts of actin (M_r(A) 41 782; Elzinga et al., 1973) and myosin (M_r(M) 521 000; Yates and Greaser, 1983) per unit of tissue (see eq 4), expressed as moles per kilogram of total protein, are related to the distribution of His(τ-Me) in myosin and actin of skeletal

Table II. Amino Acid (AA) Composition (Grams of Amino Acid per Kilogram of Protein) of Selected Porcine Skeletal Muscles Excised from Mature Yorkshire Sows

	mean ± SEM[a]					
amino acid	external sternomandibularis	semitendinosus	semimembranosus	weighted	CV[a]	F^a
aspartic acid	91.06 ± 1.43	89.48 ± 0.47	90.88 ± 0.48	90.47	2.26	0.85[ns]
threonine	46.87 ± 0.77[f]	43.93 ± 0.32[g]	45.30 ± 0.33[f,g]	45.37	1.94	8.76*
serine	42.79 ± 0.77[f]	40.00 ± 0.63[g]	40.56 ± 0.46[g]	41.12	1.65	14.01*
glutamic acid	144.36 ± 2.11	143.93 ± 1.02	147.24 ± 0.72	145.18	2.46	1.26[ns]
proline	44.03 ± 1.12[f]	45.01 ± 0.63[f]	38.62 ± 0.85[g]	42.55	4.78	7.85*
glycine	52.62 ± 1.91[f]	51.40 ± 0.67[f]	41.83 ± 1.52[g]	48.62	6.65	9.53*
alanine	59.17 ± 0.58	59.28 ± 0.35	56.88 ± 0.42	58.44	1.69	4.07[ns]
cysteine	8.01 ± 0.25	8.99 ± 0.20	8.55 ± 0.25	8.52	5.74	2.94[ns]
valine	50.44 ± 0.68[f]	48.11 ± 0.55[g]	50.44 ± 0.42[f]	49.66	1.87	7.54*
methionine	36.64 ± 5.84	42.77 ± 0.85	42.35 ± 1.42	40.59	14.01	1.11[ns]
isoleucine	45.72 ± 0.64[f,g]	43.03 ± 0.67[g]	48.72 ± 1.72[f]	45.82	3.55	10.26*
leucine	78.26 ± 1.07	78.46 ± 0.16	79.45 ± 0.61	78.72	1.74	1.34[ns]
tyrosine	41.06 ± 0.93	37.96 ± 0.66	39.91 ± 0.38	39.64	4.48	2.50[ns]
phenylalanine	37.62 ± 2.53	40.07 ± 0.07	40.13 ± 0.19	39.27	6.82	0.92[ns]
histidine	35.02 ± 0.86[g]	33.61 ± 0.22[g]	41.28 ± 1.08[f]	36.64	5.05	15.54*
lysine	88.34 ± 0.94	87.06 ± 0.20	88.56 ± 0.49	87.99	1.52	1.95[ns]
arginine	68.78 ± 0.43[g]	69.96 ± 0.15[f]	67.90 ± 0.38[g]	68.88	0.68	8.98*
tryptophan	6.38 ± 0.10	7.43 ± 0.32	7.64 ± 0.17	7.15	8.69	3.68[ns]
4-hydroxyproline	13.73 ± 0.72[f]	15.53 ± 1.79[f]	5.12 ± 1.16[g]	11.46	24.86	11.36*
5-hydroxylysine	3.53 ± 0.41	3.28 ± 0.150	2.015 ± 0.028	2.941	12.15	10.33*
N^6-methyllysine	0.205 ± 0.085	0.167 ± 0.002	0.290 ± 0.003	0.221	31.48	1.64[ns]
N^6,N^6-dimethyllysine	0.040 ± 0.0	0.227 ± 0.018	0.141 ± 0.054	0.227	25.22	6.82[ns]
N^6,N^6,N^6-trimethyllysine		1.35 ± 0.16	0.589 ± 0.05	0.971	54.12	2.12[ns]
N^π-methylhistidine	1.665 ± 0.055	2.962 ± 0	2.408 ± 0.43	2.345	15.42	6.48[ns]
N^τ-methylhistidine	4.315 ± 0.175	6.987 ± 0.556	14.674 ± 0.50	8.659	29.56	8.83*
unknown peak 17	0.715 ± 0.105	0.323 ± 0	0.147 ± 0.037	0.393	23.20	20.75*
ammonia	12.62 ± 0.017	13.63 ± 0	14.19 ± 1.30	14.45	5.82	2.24[ns]
total AA N[b]	175.79	176.90	177.97			
total protein, g protein/kg dry wt	513.58 ± 9.54	613.85 ± 24.94	677.15 ± 15.31			
EAA index[c]	74.28	74.96	77.70			
protein score[c]	40.36	47.03	47.31			
WE,[d] μg/nmol	0.109411	0.109896	0.111470			
F,[d] μg/nmol	0.110767	0.111450	0.113028			
F',[e] μg/nmol	0.118294	0.119442	0.118977			

[a] Mean values and standard error of measurements (SEM) for 3 replicates and 48 determinations. Significance: F, values from analysis of variance; **, $P < 0.01$; *, $P < 0.05$. Key: ns, not significant; CV, coefficient of variation. (f, g) Means along a horizontal column with different superscripts are significantly different. [b] Calculated according to Heidelbaugh et al. (1975). [c] From Oser (1951) and Block and Mitchell (1946). [d] The WE and F constants calculated according to Horstmann (1979). [e] The conversion factor F' calculated according to eq 2 but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline.

muscle, the amounts of myosin (P_M) and actin (P_A) in porcine muscles (grams per kilogram of total protein) can thus be calculated from the total amount (C_T) of His(τ-Me) from eq 5 and the molar ratio of actin and myosin by the following equation:

$$\sum_{j=1}^{2}(P_A + P_M) = \left[\frac{A}{A+2M}\frac{41\,782}{151.2} + \frac{M}{A+2M}\frac{521\,000}{151.2}\right]C_T \quad (6)$$

Substituting the molar ratio of actin to myosin reported by Murakami and Uchida (1985) ($A/B = 6$) in eq 6, the sum of actin and myosin in porcine skeletal muscles can be made as

$$\sum_{j=1}^{2}(P_A + P_M) = [207 + 431]C_T \quad (6a)$$

or

$$\sum_{j=1}^{2}(P_A + P_M) = 638C_T \quad (6b)$$

Yates and Greaser (1983) have shown that the sum of actin and myosin in the myofibril accounts for 65% of the total myofibrillar protein by weight. Thus, the total myofibrillar protein in grams per kilogram of total protein in porcine skeletal muscle can also be calculated as

$$\text{amt of myofibrillar protein} = \frac{\sum_{j=1}^{2}(P_A + P_M)}{0.65}C_T = 981C_T \quad (7)$$

Statistical Analysis. Data processing and linear regression analysis of the results were carried out by a Fortran computer program developed for this purpose. Analysis of variance conducted on the amino acid data for a completely randomized block design (factorial) was carried out by the Statistical Analysis System (SAS, 1982) general linear model procedure.

RESULTS AND DISCUSSION

Amino Acid Composition of Selected Porcine Muscles. The overall amino acid compositions of the selected external sternomandibularis, semitendinosus, and semimembranosus porcine skeletal muscles excised from mature Yorkshire sows, usually being marketed for meat processing, and levels of statistical significance obtained from analysis of variance are summarized in Table II. The results expressed as grams of amino acid residues per kilogram of total protein show deviations of less than 100 ± 3.0% from the weighted mean between three animals within the same treatment. The main advantage of this

Table III. Recoveries of the Intracellular and Extracellular Matrix Protein Fractions (F1 and F2) Isolated from Three Selected Porcine Skeletal Muscles by the Combined Methods of McCollester (1962) and Laurent et al. (1981)

	mean ± SEM[a]			
	F1 intracellular SDS-sol muscle protein fraction		F2 extracellular SDS-insol protein fraction	
porcine skeletal muscle	g dry matter/ 10.0 g wet tissue	g protein/ 100 g protein[b]	g dry matter/ 10.0 g wet tissue	g protein/ 100 g protein[b]
external sternomandibularis	2.3517 ± 0.021	89.78 ± 0.80	0.1689 ± 0.02	10.22 ± 1.21
semitendinosus	2.2806 ± 0.009	94.55 ± 0.373	0.1573 ± 0.021	5.45 ± 0.073
semimembranosus	2.2632 ± 0.17	94.80 ± 0.007	0.1729 ± 0.007	5.20 ± 0.211

[a] Mean ± standard error of measurement (SEM) for three replicates. [b] Protein content was determined by amino acid analysis according to Horstmann (1979).

unit of expressing the composition of a protein mixture is that it reflects the relative amounts of the amino acids present (Tristram and Smith, 1963; Eastoe, 1967) since the influence of fat, ash, and moisture is eliminated. Protein determinations were carried out in each acid hydrolysate as described previously (Horstmann, 1979; Nguyen et al., 1986). This method is based upon knowledge of the amino acid composition of the protein or protein mixture and yields accurate estimates of the amount of protein present calculated by eq 1–3. The constants, weight equivalent (WE, μg/nmol) and conversion factors (F and F', μg), for each of the porcine muscles (Table II) or muscle protein fractions (Tables IV and V) investigated have been determined and can be used in all subsequent quantitations of these muscle tissues following standard procedures as described by Horstmann (1979), Peterson (1983), and Nguyen et al. (1986).

A comparison of the amino acid profiles of the porcine external sternomandibularis, semitendinosus, and semimembranosus muscles investigated shows that although many of the individual amino acid values appeared to be very similar; muscle to muscle variations in amino acid content from the weighed mean were significant with respect to 12 amino acids among these muscles. The variation noted for threonine, serine, proline, glycine, and valine among the three muscles evaluated was statistically significant ($P < 0.05$), with the external sternomandibularis muscle being consistently higher in these amino acids than either of the other two muscles. Mean proline values ranged from 48.13 g/kg of protein in sow semitendinosus to 41.6 g/kg of protein in semimembranosus for sows. The mean value obtained for arginine in the semitendinosus muscle was significantly higher ($P < 0.05$) compared to that found in the other two muscles. The weighted mean values obtained for the amino acid composition of the three muscles investigated are consistently higher compared to those obtained for the semimembranosus muscle. In all the tissues studied, Pro(4-OH) and Lys(5-OH) varied significantly between porcine muscles, with the semimembranosus muscle containing the lowest mean Pro(4-OH) and Lys(5-OH) values.

The variation noted in the His(τ-Me) content among the three untreated porcine muscles evaluated (Table II) was significant ($P < 0.05$), with the semimembranosus muscle being much higher in total His(τ-Me), 14.67 g/kg of protein, compared to that found in the semitendinosus (6.99 g/kg of protein) and external sternomandibularis (4.3 g/kg of protein) muscles. Skeletal muscles are known to contain variable amounts of soluble histidine dipeptides including balenine (Carnegie et al., 1982; Harris and Milne, 1987), β-alanyl-L-N^{τ}-methylhistidine. These dipeptides must be extracted from muscle tissues prior to acid hydrolysis. One major limitation of the muscle tissue extraction procedures (Rangeley and Lawrie, 1976; Bligh and Dyer, 1959; Happich et al., 1984; Carnegie et al., 1984), however, is that often high incidences of unemptied muscle cell segments are encountered. This emptying phenomenon, although not fully understood (McCollester, 1962), has been related to irreversible contraction of muscle cells during homogenization (McCollester and Semente, 1964). Thus, conventional methods of cellular disruption are unable to break down contracted muscle cell cytoskeletal membranes, i.e., endoplasmic reticulum, Z-bands, etc., to produce a satisfactory dissolution and separation of the intracellular soluble structures from the extracellular matrix insoluble proteins.

Unique Basic Amino Acid Content of Porcine Intracellular and Extracellular Protein Fractions (F1 and F2). To quantitatively establish the occurrence and variation of protein-bound His(τ-Me) in porcine skeletal muscles, the combined procedures of McCollester (1962) and Laurent et al. (1981) have been used to effectively separate the intracellular SDS-soluble muscle protein fraction (F1) from the extracellular SDS-insoluble protein fraction (F2). Each of these fractions (F1 and F2) was subjected to detailed amino acid analysis and protein determination by the use of the chromatographic methods (Zarkadas et al., 1986, 1987b) developed to quantitate the unique amino acids that occur in those proteins. The yields obtained for F1 and F2 are summarized in Table III. The total yield obtained for the intracellular muscle protein fraction (F1) in the porcine external sternomandibularis muscle averaged 89.8% compared to 96.6% and 96.8% found in the semitendinosus and semimembranosus muscles, respectively. The external sternomandibularis muscle contained a higher concentration of extracellular matrix proteins (10.2%) compared to that found in the semitendinosus (5.45%) and semimembranosus (5.2%). Since recoveries for each of the fractions have been calculated on total protein determined from their resepective amino acid composition, as presented in Tables IV and V, the average protein yields reported in Table III represent accurate estimates of the amount of protein present. These results are comparable to those reported by Bendall (1967) and Dransfield (1977) for bovine muscles.

Typical chromatographic separations obtained, when samples of the intracellular and extracellular porcine muscle protein fractions (F1 and F2) were analyzed on a 50 × 0.28 cm column of Dionex DC-4A resin with two 0.35 M sodium citrate buffers, pH 5.701 and 4.501 (buffer changed at 215.7 min), at two column temperature (28 °C changed to 73 °C at 294.7 min), are illustrated in Figure 1. As may be seen in Figure 1A, the analysis of an intracellular muscle (external sternomandibularis) protein fraction (F1) 96-h hydrolysate (100-μL equivalent to 200 μg of protein/analysis) by the present method (Zarkadas et al., 1987b) revealed the complete separation, at pH 5.700, of both methylated lysines along with four as yet unidentified ninhydrin-positive peaks, designated 2, 7, 8, and 10. The Arabic number assigned to each unknown

Table IV. Comparison of the Amino Acid Composition (Grams of Amino Acid per Kilogram of Protein) of SDS-Solubilized Intracellular Muscle Protein Fractions (F1) Isolated from Three Typical Porcine Skeletal Muscles of Mature Yorkshire Sows

	porcine skeletal msucle SDS-sol muscle protein fraction (F1)								
	external sternomandibularis ($N = 4$)		semitendinosus ($N = 4$)		semimembranosus ($N = 4$)		wt mean ($N = 12$)	signif level (between muscles)[b]	
	mean ± SEM[a]	CV	mean ± SEM[a]	CV	mean ± SEM[a]	CV		CV	F
aspartic acid	96.16 ± 0.31[f]	0.63	98.33 ± 0.22[f]	0.44	96.63 ± 0.60[g]	1.22	97.71	0.83	5.47*
threonine	48.48 ± 0.38[f]	1.58	47.04 ± 0.41[f]	1.74	48.59 ± 0.36[g]	1.50	48.04	1.67	6.07*
serine	42.75 ± 0.76[f]	3.58	36.04 ± 0.27[f]	1.47	41.82 ± 0.70[g]	3.37	40.20	3.08	35.92**
glutamic acid	163.38 ± 0.49	0.60	162.82 ± 0.40	0.50	164.07 ± 0.64	0.79	163.42	0.64	2.63[ns]
proline	37.95 ± 1.19	6.27	36.94 ± 2.90	2.95	37.13 ± 0.44	2.33	37.34	4.27	0.60[ns]
glycine	30.18 ± 0.11[g]	0.69	31.04 ± 0.08[f]	0.53	29.54 ± 0.11[i]	0.72	30.25	0.65	52.66**
alanine	50.71 ± 0.15[f]	0.60	51.14 ± 0.20[f]	0.77	49.99 ± 0.28[g]	1.12	50.61	0.85	6.48**
cysteine	8.33 ± 0.07[g]	1.55	10.15 ± 0.14[f]	2.75	8.14 ± 0.01[g]	0.22	8.87	2.00	150.88**
valine	55.44 ± 0.11	0.39	56.07 ± 0.33	1.19	56.66 ± 0.47	1.68	56.06	1.21	2.69[ns]
methionine	28.83 ± 0.23	1.54	28.83 ± 0.09	0.59	28.64 ± 0.23	1.57	28.80	1.32	0.59[ns]
isoleucine	53.35 ± 0.23	0.86	54.47 ± 0.32	1.17	54.16 ± 0.36	0.37	53.99	1.15	2.20[ns]
leucine	88.11 ± 0.18	0.41	87.99 ± 0.36	0.83	88.01 ± 0.27	0.60	88.04	0.64	0.88[ns]
tyrosine	40.68 ± 0.39[g]	1.94	44.13 ± 1.51[f]	6.82	40.22 ± 0.65[g]	3.22	41.68	4.66	4.52*
phenylalanine	44.00 ± 0.40	1.81	44.61 ± 0.60	2.67	44.62 ± 0.84	3.80	44.41	2.88	0.18[ns]
histidine	27.66 ± 0.20[g]	1.43	29.08 ± 0.36[f]	2.53	27.88 ± 0.36[g]	2.62	28.21	2.27	4.85*
lysine	95.00 ± 1.67[f]	1.39	91.85 ± 0.67[g]	1.46	96.46 ± 0.41[f]	0.86	94.44	1.26	17.74**
arginine	68.94 ± 0.33[g]	0.97	68.77 ± 0.54[g]	1.58	70.59 ± 0.26[f]	0.72	69.43	1.14	6.63*
tryptophan	16.98 ± 0.03[g]	0.29	19.38 ± 0.09[f]	0.91	15.52 ± 0.03[i]	0.32	17.29	0.63	232.28**
ornithine	0.31 ± 0.003[f]	2.09	0.264 ± 0.005[g]	3.51	0.266 ± 0.01[g]	9.41	0.284	5.70	8.68**
N^6-methyllysine	0.10 ± 0.001[g]	3.95	0.095 ± 0.001[g]	2.53	0.109 ± 0.002[f]	3.73	0.101	3.51	15.36**
N^6,N^6,N^6-trimethyllysine	0.41 ± 0.01[g]	5.26	0.350 ± 0.007[i]	3.97	0.453 ± 0.01[f]	5.41	0.405	5.07	25.63**
N^τ-methylhistidine	0.592 ± 0.009[f]	3.16	0.589 ± 0.01[f]	3.47	0.521 ± 0.005[g]	2.07	0.568	3.02	22.16**
ammonia	15.30 ± 0.94[g]	12.26	10.58 ± 1.03[i]	19.50	25.01 ± 1.78[f]	14.24	16.96	15.39	31.77**
total AA N[c]	172.53		168.63		180.82				
WE,[d] μg/nmol	0.11262		0.11295		0.11278				
F,[d] μg/nmol	0.120256		0.120944		0.120170				
F',[e] μg/nmol	0.120508		0.121176		0.120428				

[a] Mean values and standard error of measurements (SEM) for four replicates. [b] Significance ($N = 12$): **, $P < 0.01$; *, $P < 0.05$. Key: ns, not significant; CV, coefficient of variation. (f, g, i) Means along a horizontal column with different superscripts are significantly different. [c] Calculated according to Heidelbaugh et al. (1975). [d] Calculated according to Horstmann (1979) and Nguyen et al. (1986), with eq 1 and 2. [e] The conversion factor F' was calculated according to eq 2 but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline.

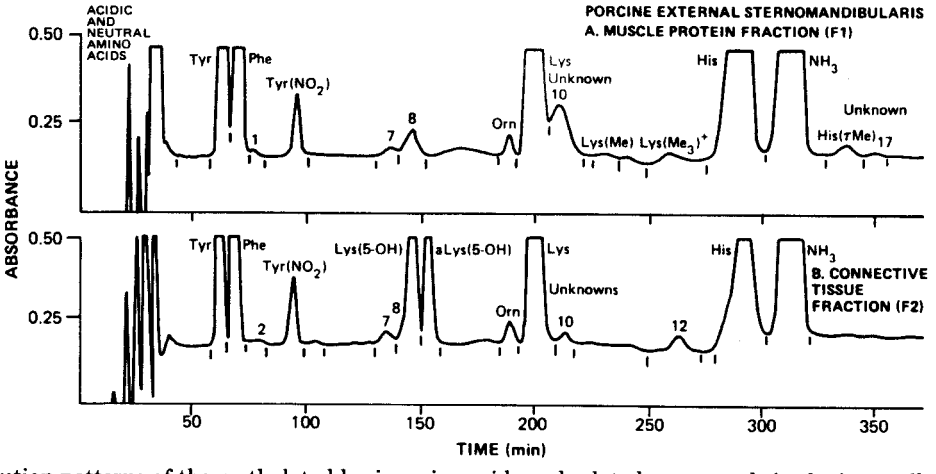

Figure 1. Typical elution patterns of the methylated basic amino acids and related compounds in the intracellular and extracellular protein fractions from the external sternomandibularis porcine skeletal muscle on an 0.28 × 50 cm analytical microcolumn of Dionex DC-4A resin. The curves show absorbance at 570 nm. (A) Separations of all methylated lysines and histidines in the intracellular muscle protein fraction (F1). (B) Analysis of a 96-h hydrolysate of the extracellular matrix protein fraction (F2). Key: Tyr(NO_2), 3-nitrotyrosine; Lys(5-OH), 5-hydroxylysine; aLys(5-OH), *allo*-5-hydroxylysine; Lys(Me), N^6-methyllysine; Lys(Me_3^+), N^6,N^6,N^6-trimethyllysine; His(τ-Me), N^τ-methylhistidine.

peak in consecutive order indicates its relative elution time from the microcolumn, and the results calculated relative to the internal standard [Tyr(NO_2)] are presented in Table VI. Since the positions of the methylated histidines in the chromatogram are very sensitive to pH, it was found that, at pH 4.501 and 71 °C, it has been possible to accomplish the complete separation of His(τ-Me) from an unknown ninhydrin-positive peak, designated 17 (Table VI). This unknown compound (17) has also been found in variable amounts in other proteins and tissues.

Analysis of an acid hydrolysate (96 h) of the extracellular matrix protein fraction (F2) from the external sternomandibularis porcine muscle (Figure 1B) revealed the complete separation of the diastereoisomers of Lys(5-OH) along with five as yet unidentified stable components, designated 2, 7, 8, 10, and 11 (Table VI). Although pre-

Table V. Amino Acid Composition (Grams of Amino Acid per Kilogram of Protein) of SDS-Insoluble Muscle Connective Tissue Fractions (F2) Isolated from Porcine Skeletal Muscles of Mature Yorkshire Sows

	porcine skeletal muscle SDS-insol connective tissue fractions (F2)							signif levels (between muscles)[b]	
	external sternomandibularis		semitendinosus		semimembranosus		wt mean		
	mean ± SEM[a]	CV	mean ± SEM[a]	CV	mean ± SEM[a]	CV	(N = 12)	CV	F
aspartic acid	63.45 ± 0.52[g]	1.65	65.44 ± 1.04[f]	3.19	67.43 ± 0.49[f]	1.42	65.44	2.27	17.83**
threonine	20.24 ± 0.35[i]	3.44	21.34 ± 0.26[g]	2.48	25.97 ± 0.94[f]	7.27	22.52	5.32	27.60**
serine	39.18 ± 0.89[g]	4.53	40.68 ± 0.33	1.65	46.36 ± 1.85[f]	7.99	42.07	5.68	11.59**
glutamic acid	109.23 ± 0.57[g]	1.05	111.37 ± 2.46[f]	4.25	115.53 ± 0.96[f]	1.67	112.04	2.75	10.04**
proline	119.59 ± 1.18[f]	1.97	107.87 ± 2.28[g]	4.25	99.23 ± 0.93[i]	1.88	108.90	2.94	30.69**
glycine	189.27 ± 0.63[f]	0.67	183.95 ± 4.14[f]	4.51	167.17 ± 0.71[g]	0.85	180.13	2.79	21.03**
alanine	79.87 ± 1.38[f,g]	3.46	77.66 ± 1.47[f]	3.79	74.24 ± 0.50[g]	1.36	77.26	3.12	5.48**
cysteine	3.30 ± 0.12[i]	7.56	4.43 ± 0.11[f]	5.13	3.87 ± 0.04[g]	2.14	3.87	5.16	44.11**
valine	29.16 ± 0.54[g]	3.75	29.34 ± 0.80[g]	5.49	32.67 ± 0.36[f]	2.25	30.39	3.99	12.30**
methionine	9.16 ± 0.45[g]	9.99	10.74 ± 0.47[f]	8.90	10.17 ± 0.73[f,g]	15.01	9.97	11.58	3.29*
isoleucine	16.60 ± 0.27[i]	3.28	18.44 ± 0.42[g]	4.51	21.99 ± 0.14[f]	1.31	19.01	3.18	91.73**
leucine	34.37 ± 0.42[i]	2.48	36.23 ± 0.71[g]	3.92	40.94 ± 0.31[f]	1.58	37.18	2.79	50.82**
tyrosine	12.79 ± 0.95[g]	14.83	12.45 ± 0.81[g]	13.06	16.55 ± 1.00[f]	12.14	13.93	13.25	5.97*
phenylalanine	23.65 ± 0.55[g]	4.69	23.71 ± 0.38[g]	3.23	26.05 ± 0.37[f]	2.85	24.47	3.61	11.44**
histidine	7.73 ± 0.14[i]	3.71	8.72 ± 0.31[g]	7.08	10.83 ± 0.34[f]	6.32	9.09	6.17	34.37**
lysine	37.94 ± 0.62[i]	3.29	39.43 ± 0.86[g]	4.18	43.88 ± 0.60[f]	2.73	40.42	3.43	24.42**
arginine	82.98 ± 1.20	2.89	79.69 ± 1.46	3.67	80.74 ± 0.61	1.51	81.14	2.84	0.28[ns]
4-hydroxyproline	111.50 ± 1.87[g]	3.37	118.12 ± 2.19[f]	3.70	107.10 ± 1.68[g]	3.14	112.24	3.44	16.23**
5-hydroxylysine	9.11 ± 0.05[g]	1.04	9.81 ± 0.06[f]	1.16	8.64 ± 0.07[i]	1.60	9.19	1.27	101.01**
isodesmosine	0.224 ± 0.003[g]	2.75	0.291 ± 0.00[f]	0.58	0.185 ± 0.001[i]	2.04	0.234	1.84	620.28**
desmosine	0.225 ± 0.002[g]	2.00	0.291 ± 0.002[f]	1.83	0.194 ± 0.001[i]	1.80	0.237	1.90	486.86**
ornithine	0.362 ± 0.004[g]	2.25	0.458 ± 0.004[f,g]	1.91	0.588 ± 0.098[f]	32.91	0.472	24.14	4.45*
ammonia	24.61 ± 7.53	61.65	32.86 ± 6.77	41.25	53.00 ± 19.93	75.19	36.76	70.21	1.29[ns]
total AA N[c]	199.28		203.55		219.07				
WE,[d] μg/nmol	0.093110		0.93726		0.095323				
F,[d] μg/nmol	0.093389		0.094105		0.095665				
F',[e] μg/nmol	0.117781		0.118034		0.117862				

[a] Mean values and standard error of measurements (SEM) for four replicates. [b] Significance: **, $P < 0.01$; *, $P < 0.05$. Key: ns, not significant; CV, coefficient of variation. (f, g, i) Means along a horizontal column with different superscripts are significantly different. [c] Calculated according to Heidelbaugh et al. (1975). [d] Calculated according to Horstmann (1979) and Nguyen et al. (1986), by eq 1 and 2. [e] The conversion factor F' calculated according to eq 2 but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline.

Table VI. Elution Times and Contents (Nanomoles per Milligram of Protein) of Unknown Ninhydrin-Positive Compounds Separated from Selected Porcine Skeletal Muscle Protein and Connective Tissue Fractions

		porcine skeletal muscles							signif levels (between muscles)[b]	
unknown compd	elution time, min	external sternomandibularis		semitendinosus		semimembranosus		wt mean		
		mean ± SEM[a]	CV	mean ± SEM[a]	CV	mean ± SEM[a]	CV		CV	F
		SDS-Solubilized Muscle Protein Fraction F1								
2	(77)	1.907 ± 0.39[c]	40.87	0.838 ± 0.126[d]	29.98	0.983 ± 0.33[d]	6.82	1.243	38.18	5.99*
7	(137)	0.783 ± 0.21[c]	53.14	0.139 ± 0.007[d]	9.61	0.367 ± 0.025[d]	13.58	0.430	56.26	7.26*
8	(146)	5.538 ± 0.54[c]	19.48	1.968 ± 0.075[d]	7.67	2.958 ± 0.255[d]	17.24	3.488	19.91	28.19**
10	(211)	17.900 ± 0.66[e]	7.36	23.953 ± 0.35[d]	2.93	26.310 ± 0.578[c]	4.40	22.72	4.79	63.34**
17	(350)	0.747 ± 0.016[d]	4.22	0.914 ± 0.08[d]	18.75	1.283 ± 0.125[c]	19.52	0.98	17.95	9.69**
		SDS-Insoluble Muscle Connective Tissue Fraction F2								
2	(77)	0.536 ± 0.053[d]	19.92	1.143 ± 0.058[c]	10.19	0.640 ± 0.031[d]	2.31	0.77	12.67	43.88**
7	(137)	2.239 ± 0.098[c]	8.72	0.821 ± 0.244[d]	59.31	2.684 ± 0.032[c]	2.31	1.92	15.93	40.70**
10	(211)	3.802 ± 0.326[c,d]	17.16	2.995 ± 0.038[d]	2.19	4.810 ± 0.814[c]	33.84	3.87	26.18	3.22[ns]
12	(267)	6.199 ± 0.258[c]	8.35	3.824 ± 0.246[d]	12.85	5.071 ± 0.577[c,d]	22.75	5.02	15.58	9.06**

[a] Mean values and standard error of measurements (SEM) for 3 replicates and 48 determinations. [b] Significance (N = 12): **, $P < 0.01$; *, $P < 0.05$. Key: ns, not significant; CV, coefficient of variation. (c–e) Means along a horizontal column with different superscripts are significantly different.

vious work from this laboratory showed that the determination of Lys(5-OH) can be made from the sum of the values obtained from its diastereoisomers after epimerization in 6 M HCl at 110 °C for 96 h (Zarkadas, 1975), as shown in Figure 1B unknown peak 8 partially coelutes with the Lys(5-OH) diastereoisomer, even after epimerization, thus interfering with its quantitation. For this reason, only the aLys(5-OH) diastereoisomer after epimerization (96 h) is now being routinely used in the quantitation of this important amino acid found in collagen and collagen-like proteins (Reid, 1982; Anglister et al., 1976), as well as in the extracellular matrix and muscle tissues.

A summary of the amino acid composition of the SDS-solubulized intracellular muscle protein fractions (F1) isolated from three typical porcine skeletal muscles of mature Yorkshire sows, and levels of statistical significance obtained from analysis of variance, is presented in Table IV. Although the amino acid profiles (grams per kilogram of protein) of the intracellular protein fractions from external sternomandibularis, semitendinosus, and semimembranosus porcine muscles are very similar, characteristic differences were noted in their contents of aspartic acid, threonine, serine, glycine, alanine, cyst(e)ine, tyrosine, histidine, lysine, arginine, and tryptophan. The variation

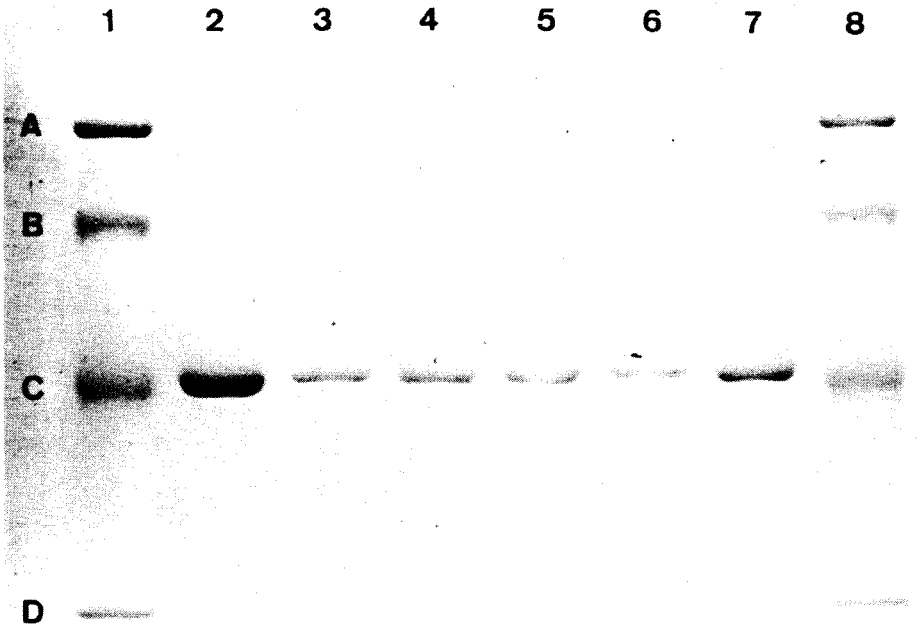

Figure 2. Comparison of one-dimensional SDS-PAGE on 10% polyacrylamide slab gel electrophoretic separations of G-actin monomers purified from selected muscle tissues as outlined in the purification scheme in Materials and Methods. The G-actin monomers were reduced with dithiothreitol, denatured with SDS sample buffer at 100 °C for 5 min, and subjected to electrophoresis (Weber and Osborn, 1975). The gels were then stained for protein with Coomassie Brilliant Blue C-250 and destained (Weber and Osborn, 1975). Lanes: 1, standards [A, phosphorylase (92 500 Da); B, bovine serum albumin (67 000); C, ovalbumin (40 000); D, carbonic anhydrase (30 000)]; 2, chicken breast actin; 3, chicken leg muscle actin; 4, porcine semimembranosus actin; 5, porcine cardiac muscle actin; 6, bovine semimembranosus actin; 7, bovine cardiac muscle actin; 8, standards.

noted for serine, glycine, alanine, cyst(e)ine, lysine, and tryptophan among the three muscles evaluated was highly significant ($P < 0.01$), with the semitendinosus muscle being consistently higher in total glycine, alanine, cyst(e)ine, and tryptophan than either of the other two muscles. The external sternomandibularis and semimembranosus muscles contained the highest levels of lysine but had the lowest mean values of histidine. Variations, however, due to 36 determinations between three replicates within the same muscle were small, indicating that analytical errors were also small relative to biological variability observed between muscles. Thus, the data presented in Table IV defined variability in amino acid content of the intracellular muscle protein fractions (F1) among selected muscles.

A comparison of the amino acid data presented in Tables II and IV indicate that practically all of the His(π-Me), 17.8–36.1% of total histidine, and about 93% of the total His(τ-Me) found in porcine skeletal muscles were present as free or as soluble histidine-bound dipeptides, which on acid hydrolysis yield β-alanine, histidine, and His(π-Me). These results are in accord with those reported by Harris and Milne (1981, 1987) and Carnegie et al. (1982) for porcine muscles. These authors have shown that in young animals balenine constitutes more than 90% of the total nonprotein-bound His(τ-Me), reaching 99.8% of the total His(τ-Me) in older animals. Although the physiological function of anserine and balenine in skeletal muscle tissues has not been established, both β-alanine and carnosine are now being considered as neurotransmitters (Griffith, 1986).

The values obtained for protein-bound His(τ-Me) in the intracellular muscle protein fractions (F1) of the selected porcine muscles (Table IV) show high reproducibility and low coefficients of variation, and within the precision of the chromatographic method (100 ± 2.5%), recoveries were found to be quantitative. These results show that while the protein-bound His(τ-Me) contents of the external sternomandibularis (0.592 g/kg of protein) and semitendinosus (0.589 g/kg of protein) muscles appeared to be very similar, there was a small but statistically significant variation ($P < 0.01$) in the protein-bound His(τ-Me) content (0.521 g/kg of protein) of the semimembranosus muscle. While most of this variation may be attributed to the presence of variable amounts of fast (type IIA), slow (type I), or mixed fast/slow (types IIA and IIB) classes of myosin heavy chains, the factors responsible for the small differences in His(τ-Me) contents among porcine skeletal muscles are less clear. The distribution of heavy myosin chain isoforms among different muscle fiber types with fast, mixed fast/slow, and slow properties has been shown to vary in a muscle-specific manner (Gauthier and Lowey, 1979; Beecher et al., 1968; Beerman et al., 1978; Suzuki and Cassens, 1980; Kiessling et al., 1982; Kiessling and Hanson, 1983; Lowey, 1986a,b; Mahdavi et al., 1986; Staron and Pette, 1987a,b).

N^{τ}-Methylhistidine Content of Porcine Semimembranosus Actin. To establish whether this small variation (11.9%) in protein-bound His(τ-Me) among porcine muscles could be attributed to myosin heavy chain or actin isoforms, the His(τ-Me) content of purified G-actin from sow's semimembranosus muscles was analyzed by the present method (Zarkadas et al., 1987b). The purification of actin was carried out by the procedure of Spudich and Watt (1971), followed by the polymerization and depolymerization steps recommended by Pardee and Spudich (1982), as described in Materials and Methods. The purified G-actin eluted from the Sephacryl G-200 column (2.6 × 95 cm) with the depolymerization buffer of Pardee and Spudich (1982) was subjected to one- and two-dimensional SDS–polyacrylamide gel electrophoresis (Weber and Osborn, 1975; Laemmli, 1970; O'Farrell, 1975), and a single band was observed after staining the gels for protein (Figure 2)8, demonstrating that the G-actin obtained by this procedure is essentially homogeneous (Pollard and Cooper, 1986). As may be seen in Figure 2, similar single bands were observed with actins purified from bovine

Table VII. Amino Acid Composition of Porcine G-Actin from the Semimembranosus Muscle

amino acid	mean, nmol/1000 μg protein (c_i + SEM)[a]	mole fraction[a] (a_i)	res no.[b] (n_i ± SE)	$k_{best}n_i$[b]	nearest integer ratio[b] (I_i)	
aspartic acid	899.36 ± 9.7	0.0944	35.43 ± 0.38	35.20	35	34[c]
threonine	699.33 ± 14.1	0.0702	26.37 ± 0.55	26.20	26	27
serine	541.13 ± 15.7	0.0568	21.32 ± 0.62	21.18	21	22
glutamic acid	1062.09 ± 11.3	0.1114	41.84 ± 0.44	41.57	42	39
proline	484.82 ± 11.1	0.0509	19.10 ± 0.44	18.97	19	19
glycine	750.77 ± 9.8	0.0788	29.58 ± 0.38	29.38	29	28
alanine	745.51 ± 8.4	0.0782	29.37 ± 0.33	29.18	29	29
cysteine[c]	119.41 ± 1.0	0.0125	4.70 ± 0.39	4.67	5	5
valine	563.27 ± 12.5	0.0561	22.19 ± 0.49	22.04	22	21
methionine	382.12 ± 1.0	0.0401	15.05 ± 0.04	14.95	15	16
isoleucine	734.89 ± 11.8	0.0771	28.95 ± 0.46	28.76	29	30
leucine	639.60 ± 11.2	0.0671	25.20 ± 0.44	25.03	25	26
tyrosine	409.07 ± 6.0	0.0429	16.11 ± 0.24	16.01	16	16
phenylalanine	267.68 ± 6.3	0.0281	10.55 ± 0.25	10.47	10	12
lysine	457.24 ± 23.7	0.0480	18.01 ± 0.93	17.89	18	19
histidine	198.23 ± 10.9	0.0208	7.81 ± 0.43	7.76	8	8
arginine	462.31 ± 9.9	0.0464	18.21 ± 0.39	18.09	18	18
tryptophan[c]	119.41 ± 1.0	0.0125	4.70 ± 0.04	4.67	5	5
N^{τ}-methylhistidine	24.52 ± 0.6	0.0026	0.97 ± 0.02	0.96	1	1
total	9560.76	1.0000	375.46		373	375[c]
WE,[a] μg/nmol	0.11125					
F, μg/nmol	0.11441					
MW					41 648	41 872[c]

[a] The protein concentration, mole fraction (a_i), weight equivalent (WE), and conversion factor (F) for G-actin from porcine semimembranosus skeletal muscle were computed according to Horstmann (1979). [b] Determination of the residue number (n_i ± SE), the goodness of fit (f) to integral amino acid residue numbers (I_i) as a function of the scaling factor (k_{best}), equivalent to a molecular weight of 41 648 for G actin, was carried out by the computer-assisted procedures of Black and Hogness (1969) and Hoy et al. (1974). [c] Data taken from Vandekerckhova and Weber (1978).

Table VIII. Comparison of the Amino Acid (AA) Composition (Number of Amino Acid Residues/1000 Total Amino Acid Residues) of Extracellular Matrix Protein Fractions (F2) from Selected Porcine Skeletal Muscles with Mammalian Collagens and Avian Elastin

	porcine muscle[a] intramuscular connective tissue fraction			collagen std[b,e]			avian elastin std[c] (insol)	bovine semitendinosus elastin[d]
amino acid	external sternomandibularis	semi-tendinosus	semi-membranosus	type I	type III	type IV		
aspartic acid	51.33	53.29	55.84	43	42	46	2	7.4
threonine	18.64	19.70	24.49	18	13	29	3	8.2
serine	39.18	43.77	50.74	33	39	35	5	8.3
glutamic acid	78.78	80.85	85.30	71	71	74	12	17.1
proline	114.68	104.12	97.41	120	107	81	128	110.9
glycine	308.63	301.94	279.07	335	350	331	352	325.9
alanine	104.59	102.37	99.53	111	96	36	176	223.1
cysteine	2.98	4.03	3.58			1	1	
valine	27.40	27.75	31.42	26	14	31	175	145.8
methionine	6.50	7.67	7.27	6	8	15		
isoleucine	13.65	15.27	18.52	9	13	34	19	26.5
leucine	28.27	30.0	34.47	23	22	53	47	61.4
tyrosine	7.30	7.15	9.67	2	3	6	12	8.4
phenylalanine	14.96	15.10	16.87	12	8	30	23	29.8
histidine	5.25	5.96	7.52	6	6	6	1	0.6
lysine	27.56	28.83	32.63	23	30	6	4	5.3
arginine	49.46	47.82	49.27	50	46	29	5	5.7
3-hydroxyproline	nd	nd	nd	1		1		
4-hydroxyproline	91.79	97.89	90.27	98	118	120	22	12.4
5-hydroxylysine	5.88	6.40	5.71	10	5	42		
desmosine	0.0459	0.0600	0.0388				3	1.2
isodesmodine	0.0461	0.0600	0.0407				3	2.1
Pro(4-OH)/(Pro + Pro(4-OH))	0.44	0.48	0.48	0.45	0.48	0.52	0.15	
Lys(5-OH)/(Lys + Lys(5-OH))	0.18	0.17	0.15	0.27	0.17	0.15		

[a] Means of 4 replicates and 64 determinations, present study. [b] Average values recalculated from the data of Miller and Gay (1982), Light and Champion (1984), and Light (1985). [c] Data for amorphous elastin taken from Foster (1982). [d] Data for bovine semitendinosus muscle elastin taken from Bendall (1967). [e] The average WE values for muscle collagen and connective tissue calculated according to Horstmann (1979): $\sum_{i=1}^{3}$(WE muscle collagen) = [(0.615 × 91.14) + (0.335 × 90.90) + (0.05 × 95.94)] = 91.10 g/mol (see also Table I); $\sum_{i=1}^{4}$(WE muscle connective tissue) = [(0.0473 × 85.06) + (0.9527 × 91.10)] = 90.014 g/mol (see also Table I).

semimembranosus muscle and bovine and porcine cardiac muscles as well as from chicken breasts or leg muscles. The molecular weights of G-actin purified from porcine and bovine semimembranosus skeletal muscles were determined as 41 648 and 41 279, respectively. These results are in accord with those reported by Vandekerckhove and Weber (1978; 1979).

The mean values and standard errors of the amino acid

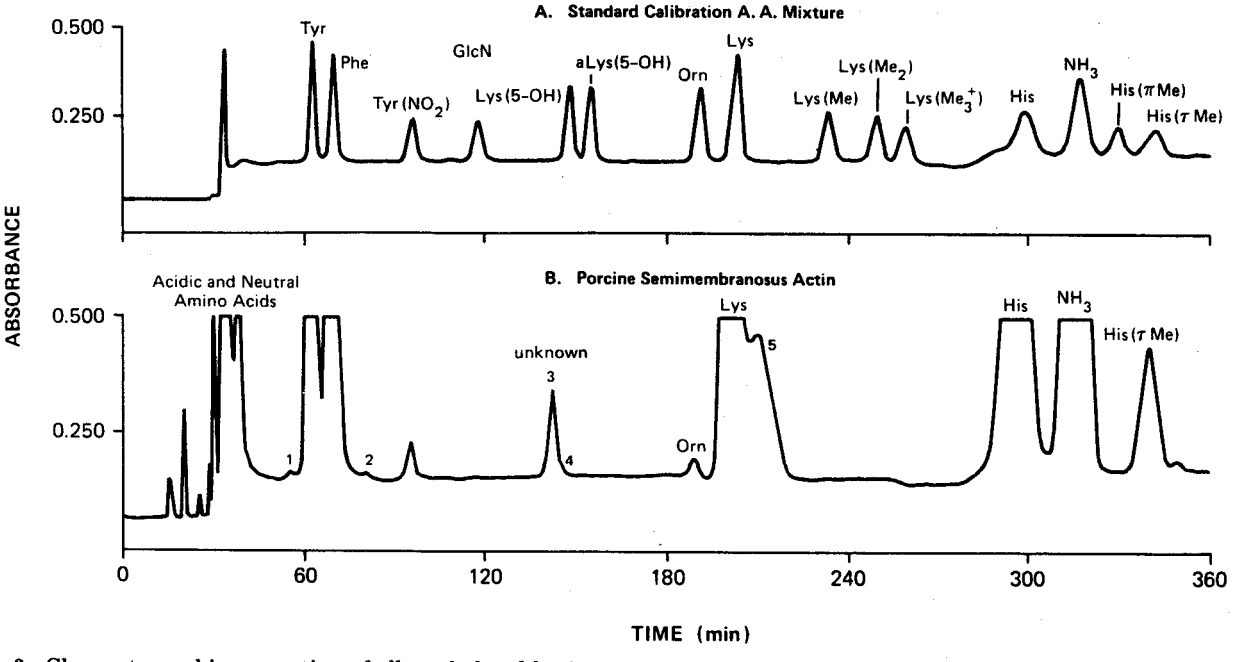

Figure 3. Chromatographic separation of all methylated basic amino acids and related compounds of actin from the porcine semimembranosus skeletal muscle. (A) Separation of a synthetic amino acid calibration mixture. (B) Typical separation of a 96-h hydrolysate of porcine semimembranosus actin. The curves show absorbance at 570 nm. Key: Tyr(NO_2), 3-nitrotyrosine; GlcN, glucosamine; Lys(5-OH), 5-hydroxylysine; aLys(5-OH), *allo*-5-hydroxylysine; Lys(Me), N^6-methyllysine; Lys(Me_2), N^6,N^6-dimethyllysine; Lys(Me_3^+), N^6,N^6,N^6-trimethyllysine; His(π-Me), N^τ-methylhistidine.

composition of G-actin purified from porcine semimembranosus are presented in Table VII. The data are expressed as nanomoles/1000 μg of protein and represent the average values of triplicate determinations obtained from duplicate 24-, 48-, 72- and 96-h hydrolysates. The values for only two amino acids, cysteine and tryptophan, are taken from Vandekerckhove and Weber (1978). The protein concentration of individual hydrolysate samples was determined by the procedure described by Horstmann (1979). The average weight equivalent (WE = 0.111 25 μg/nmol) and conversion factor (F = 0.114 41 μg/nmol) obtained are also listed in Table VII.

The large number of determinations used to obtain the data listed in Table VII were necessary to ensure low coefficients of variation (2%) for any given amino acid residue so that the computer-assisted method of Hoy et al. (1974) could be applied for determining both the nearest integer ratios (i.e., frequencies) of amino acid residues and a minimum molecular weight for this protein. The results obtained (n_i + SE) by this method are given in Table VII. Since the values of n_i are seldom integral numbers, the n_i values obtained for G-actin from the porcine semimembranosus muscle were multiplied by a series of values for the k scaling factor ranging from 0.91 to 1.1 in steps of 0.0001 with 20 000 points available for plotting in the search for k_{best} = 0.9934. A good fit was indicated by a value for f = 0.4905. The nearest integers (I_i) to the corrected residue number ($k_{best}n_i$) are those listed in column six of Table VII as minimum residue numbers and compared with those reported by Vandekerckhove and Weber (1978). This method yielded a total of 373 amino acid residues/mol of porcine G-actin and a molecular weight of 41 648 consistent with the values 41 871 and 375 amino acid residues reported by Vandekerckhove and Weber (1978). The results are also consistent with the currently favored concept that eukaryotic actins are highly conserved in evolution (Pollard and Cooper, 1986).

Porcine G-actin from the semimembranosus muscle contained 0.97 mol of His(τ-Me)/mol of protein (Table VII). Typical chromatographic separations obtained, when a synthetic mixture of all methylated basic amino acids and porcine actin from the semimembranosus muscle were analyzed on the 0.28 × 50 cm column of Dionex DC-4A resin by the method described previously (Zarkadas et al., 1987b), are illustrated in Figure 3. As may be seen in Figure 3B, the analysis of a 96-h hydrolysate of porcine actin (100 μL equivalent to 150 μg protein per analysis) revealed the complete separation, at pH 4.501, of His(τ-Me) following the ammonia peak, along with five as yet unidentified ninhydrin positive peaks, designated 1–5. The two major unknown compounds, 3 and 5, present in relatively large amounts in porcine actin have not been previously reported, and further investigations will be necessary for their isolation and characterization. These results are in close agreement with those reported by Elzinga et al. (1973), Vandekerckhove and Weber (1978, 1979), and Vandekerckhove et al. (1986), and for purposes of comparison, their data are included in Table VII. From these results it can be concluded that the lower protein-bound His(τ-Me) content of the porcine semimembranosus muscle could be attributed to differences in the distribution of myosin heavy-chain isoforms in this skeletal muscle tissue. Staron and Pette (1987a,b) have shown that a maximum of 54–60 myosin isoforms can coexist in just two rabbit skeletal muscles (soleus and tibalis anterior). From this experimental evidence it became apparent that the composition of muscle tissues is much more complex than anticipated earlier, and further studies are required.

The recent sequence studies of Maita et al. (1987), for example, have indicated that the His(τ-Me) is located at position 755 in the heavy-polypeptide chains of myosin rather than near the active site of this protein, at position 69, as originally reported by Elzinga and Collins (1977) and Huszar (1984). From amino acid and sequence data, spectral measurements, and radioactive labeling, Okamoto and Yount (1985) have shown that the location of the unusual amino acid N^6,N^6,N^6-trimethyllysine next to Trp-130 suggest that the positively charge [Lys(Me_3)$^+$] residue may provide part of the binding site for the triphosphate portion of ATP.

Table IX. Myofibrillar and Connective Tissue Protein Contents of Selected Porcine Skeletal Muscles from Mature Yorkshire Sows

	skeletal muscle protein fraction[a]	protein, g/kg total proteins			% of total muscle proteins				
		external sternomandibularis	semitendinosus	semimembranosus	external sternomandibularis	semitendinosus	semimembranosus	psoas major (rabbit) Yates and Greaser (1983)	psoas major (rabbit) Hanson and Hyxley (1957)
	F1, Intracellular: SDS-Soluble								
i	myofibrillar[b]	580.75 ± 8.83	577.81 ± 9.81	511.10 ± 4.91	52.14	54.63	48.45	57.71	62.00
	actin	122.54 ± 1.86	121.92 ± 2.07	107.85 ± 1.04	11.00	11.53	10.22	12.69	12.00
	mysoin	255.15 ± 3.88	253.86 ± 4.31	224.55 ± 2.16	22.91	24.00	21.29	24.82	34.00
	actomyosin	377.70 ± 5.74	375.78 ± 6.38	332.40 ± 3.19	33.91	35.53	31.51	37.52	46.00
ii	other sol proteins	419.25	422.19	488.90	37.64	39.92	46.35		34.00
	F2, Extracellular Matrix: SDS-Insoluble								
iii	connective tissue[c]	895.35 ± 15.02	948.50 ± 17.59	860.00 ± 13.49	9.15	5.17	4.47		
iv	collagen[d]	576.66 ± 3.17	620.97 ± 3.80	546.91 ± 4.43	5.89	3.38	2.84		
v	elastin[e]	14.04 ± 0.12	18.16 ± 0.12	12.11 ± 0.06	0.143	0.099	0.063		
vi	total iv + v	590.70	639.13	559.02	6.033	3.480	2.903		

[a] Separated by the combined procedures of McCollester (1962) and Laurent et al. (1981). [b] Calculated from eq 6a, 6b, 8, and 8a–c. [c] Calculated from Pro(4-OH) data (Table V) by eq 5c. [d] Calculated from Lys(5-OH) data (Table V) by eq 5a with the data taken from Miller and Gay (1982) and Light et al. (1985). [e] Calculated from eq 5b with data taken from Foster (1982).

Determination of the Actin and Myosin Components of the Selected Porcine Skeletal Muscles. From the His(τ-Me) contents of the SDS-solubilized intracellular muscle protein fractions (F1) isolated from three typical skeletal muscles of mature Yorkshire sows (Table IV), it has been possible to calculate the myosin, actin, actomyosin, and myofibrillar proteins present with eq 8–8c, and the results are summarized in Table IX. The data expressed as grams of protein per kilogram of total protein show deviations of less than 100 ± 3.0% from the average values.

In porcine skeletal muscles actin accounts for an estimated 11.00–11.53% of the total muscle protein or about 21.1% of the myofibrillar proteins, and myosin accounts for another 21.3–24.0% of the total muscle mass corresponding to about 43.9% of the myofibrillar proteins (weighted mean 51.74%; range 48.45–54.63%). These values are in close agreement with those reported previously for the myofibrillar protein contents of the costal region of the bovine diaphragm and all-beef hamburger which were 52.26% and 52.31% of the total bovine muscle proteins, respectively (Zarkadas et al., 1988; Karatzas and Zarkadas, 1988). These results are also in accord with those reported by Yates and Greaser (1983) who have used different methods and different muscle tissue for these determinations. These authors have shown that the rabbit psoas muscle contains 57.71% myofibrillar protein of the total muscle mass and that the myofibrils contain 22% actin and 43% myosin by weight. Hanson and Huxley (1957) found by quantitative extraction that the myofibril would contain 21% of actin and 43% of myosin weight, compared to the myosin value reported by Szent-Gyorgi et al. (1955) which accounts for 38% of the myofibrillar proteins.

Although the relative amounts of actin and myosin per unit of tissue among the porcine muscles investigated remains constant, the small differences noted in myofibrillar protein content of semimembranosus from the average figure of 53.3% reported in Table IX may reflect variations in the levels of fast, slow, or mixed fast/slow classes of myosin heavy-chain isoforms present in this skeletal muscle. Recent evidence has indicated that all three adult forms of myosin in skeletal muscle, corresponding to two adult fast myosin heavy chains (fast oxidative IIA and fast glycolytic IIB) and one slow myosin heavy chain (slow oxidative, type I), are tissue-specific and developmentally regulated, with more than one myosin heavy-chain gene expressed in each muscle and developmental stage (Whalen, 1985; Lowey, 1986; Gauthier, 1986; Mahdavi et al., 1986). It has also been shown that the slow (type I) myosin heavy chain is identical with the cardiac β-myosin heavy chain, since both isoforms are encoded by the same gene in slow-twitch skeletal muscles (soleus) of the mouse (Mahdavi et al., 1986). These findings indicate that the small differences noted in His(τ-Me) content among the porcine skeletal muscles evaluated (Table VI) may reflect the amounts of slow-twitch or cardiac β-myosin heavy isoforms present in the semimembranosus muscle. This explanation is consistent with earlier studies by Huszar (1984) who has shown that His(τ-Me) is absent from the myosin heavy chains of slow and cardiac muscles and in fast muscles of unborn and newborn animals. As mentioned earlier, further structural studies of myosin isoforms are required to clarify the early reports on the His(τ-Me) contents of myosin from different muscles.

Determination of the Connective Tissue Proteins. The extracellular matrix protein fraction (F2) of the porcine skeletal muscles, which is high in collagen and elastin content, was isolated and quantitated (Table III) by the combined methods of McCollester (1962) and Laurent et al. (1981). Tables III, V, VI, and VIII show that the external sternomandibularis muscle contained the highest levels (10.2%) of extracellular matrix proteins, compared to the other two muscles, which ranged from 5.20 to 5.45 g of protein/100 g of total muscle protein. The contents of Des, iDes, Lys(5-OH), and Pro(4-OH) per unit of the extracellular matrix protein (grams per kilogram of total protein), determined by the sensitive methods described previously (Zarkadas et al., 1986, 1987b), were used as markers for assessing the levels of the connective tissue proteins, i.e., collagen and elastin, in skeletal muscles. In this approach the content of collagen and collagen-like proteins (Porter and Reid, 1978; Reid, 1982; Anglister et al., 1976) can be calculated by multiplying the amounts of aLys(5-OH) found in the acid hydrolysates of fraction F2 by the conversion factor 63.3 (eq 5a) and the elastin content from the amounts of Des present (Gunja-Smith, 1985; Zarkadas et al., 1986, 1987b) by eq 5b. The total connective tissue protein content of the extracellular matrix has also been calculated from the amounts of Pro(4-OH) found in tissue hydrolysates (Berg, 1982) by eq 5c.

Table IX shows that the mean values for total collagen ranged from 2.84% in sow semimembranosus, 3.38% in semitendinosus, to 5.89% in the external sternomanidibularis muscle for sows. These results are in reasonably good agreement with those reported by Bendall (1967), Dransfield (1977), and Light et al. (1985) for the distribution of collagen (average 4.35%; range 1.22–15.1%) in

34 bovine skeletal muscles investigated. These results also demonstrate that the content of collagen is higher in slow-twitch postural muscles than that of porcine locomotory muscles, which contain primarily fast-twitch fiber types. This suggestion is supported by recent studies of Kovanen (1980, 1984) and others (Garcia-Bunnel and Garcie-Bunnel, 1967) who have shown that differences in muscle collagen content appear to be related to the level of individual muscle fibers or even at the level of perimysium and endomysium within a given fiber type.

The highest level of elastin (0.143%) was found in the external sternomandibularis muscle, with the semitendinosus being much lower in total elastin (0.099%) compared to 0.063% found in the semimembranosus (Table IX). This datum was based on the Des value (3 residues/1000 total residues) reported by Foster (1982) for purified amorphous elastin. Bendall (1967) has shown that the semitendinosus bovine muscle, which contained exceptionally high levels of elastin (1.82%), had rather low levels of Des and iDes. If his results, as summarized in Table VIII, are used to quantitate elastin in the extracellular matrix of porcine muscles, the amounts of elastin would be about 40% higher. Rowe (1986) has provided evidence to show that both high (semitentinosus) and low (longissimus dorsi) elastin content bovine muscles appear to have the same anatomical organization in the two structural forms of elastin present in the epimysium and perimysium and that both elastin fibers are aligned with the long axis of the muscle fibers.

The content of total connective tissue proteins in the semimembranosus, semitendinosus, and external sternomandibularis porcine muscles, estimated from the sums of collagen and elastin, were respectively 2.0, 3.48, and 4.03% (Table IX). The mean values for the transcellular matrix proteins that remained ranged from 1.97% in semitendinosus, with the external sternomandibularis being considerably higher in total transcellular proteins (4.19%), compared to 2.30% found in the semimembranosus. Loewy et al. (1983) reported that the transcellular matrix fiber ghost proteins in skeletal muscles account for 1% of the original muscle proteins. These authors have shown that the transcellular matrix proteins are rich in glycine and alanine but very low in basic and acidic amino acids, they are covalently cross-linked with ϵ-(γ-glutamine)lysine, and they have little or no Pro(4-OH). These results suggest that, in addition to collagen and elastin, the extracellular matrix from porcine muscles contains various other proteins. Blumenfeld et al. (1983) indicated that the extracellular matrix in most tissues consists of collagen, elastin, microfibrillar proteins, proteoglycans (Carrino and Caplan, 1982, 1986), and glycoproteins such as fibronectin or laminin, all of which are involved in maintaining proper alignment of the muscle fibers and in a variety of morphogenetic and developmental processes (Wiens et al., 1984). At each stage of development and maturation the extracellular matrix consists of an array of components whose specified compositions, amounts, and relative proportions are characteristic of the individual tissues. Little is known about how these macromolecules are assembled to form the matrix.

On the basis of known distributions of Pro(4-OH) in elastin and the various collagen isotypes given in Table I and VIII, the contents of total connective tissue in porcine muscles could be calculated from the amounts of Pro(4-OH). The results listed in Table IX indicate that the porcine muscles contained higher amounts of Pro(4-OH) than other vertebrate connective tissues and that this unique to collagen and elastin amino acid shows larger variation in level in porcine muscle than either Des or Lys(5-OH).

Other Muscle-Soluble Proteins. From the foregoing results, it became apparent that, in addition to myofibrillar and connective tissue proteins of the extracellular matrix (Table IX), a large quantity of SDS-soluble proteins is found in the intracellular muscle protein fraction of porcine muscles which ranged from 37.64 to 46.4% of the total muscle proteins. These results are considerably higher than those found (28–34%) by Hanson and Huxley (1957) as soluble proteins (sacroplasmic) washed out of the glycerol-extracted muscle after it has been broken up into fibrils, but they are in accord with the 41.6% figure quoted by Szent-Gyorgi et al. (1955) using two different extraction procedures. These results suggest that the intracellular muscle protein fraction includes sarcoplasmic proteins, organelles, Z-band, and other membrane proteins, etc., which are soluble in 2% sodium dodecyl sulfate by the combined procedures of McCollester (1962) and Laurent et al. (1981).

The results presented show the variation that exists in amino acid content among selected porcine muscles from mature Yorkshire sows that are being marketed today for meat processing. From these results, it is evident that the methods of analysis developed for determining all methylated basic amino acids, Lys(5-OH), the stable elastin cross-links Des and iDes, and related compounds in proteins and muscle tissues, can be easily applied for the determination of the myofibrillar and connective tissue protein contents of skeletal muscle tissues from porcine and the other major meat-yielding species. From these results it can also be concluded that a potentially useful means for evaluating the protein quality of different muscles or meats might be based on knowledge of their amino acid composition, as recommended by the Expert Work Group (FSIS, 1984) and Young and Pellett (1984).

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Registry No. His(τ-Me), 332-80-9; Lys(5-OH), 1190-94-9; Pro(4-OH), 51-35-4; Lys(Me_2), 2259-86-1; Lys(Me_3), 23284-33-5; desmosine, 11003-57-9; isodesmosine, 991-01-5.

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