Amino Acid Composition and N^{τ}-Methylhistidine Contents of Bovine and Porcine Cardiac Muscle Tissues†

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The actin and collagen contents of adult bovine and porcine cardiac muscle tissues have been determined from the amounts of N^{τ}-methylhistidine and 5-hydroxylysine found in their acid hydrolysates. Bovine and porcine cardiac α_c-actins from hearts of adult cows and sows contained 375 and 376 amino acids, respectively, and 1 mol of N^{τ}-methylhistidine/mol of protein, 42 000 Da, consistent with the values for all eukaryotic actins. Since the molar ratio of myosin to actin in cardiac muscles is known to be 1:4, the myosin content can be estimated from the amounts of α_c-actin present. α_c-Actin accounts for an estimated 9.7% of total cardiac muscle mass and myosin for about 27.6−28.0% of total protein, corresponding to 16.3 and 48.6% of the total myofibrillar proteins (57.6%). Total cardiac collagen ranged from 3.9% in porcine to 4.8% in bovine hearts. Cardiac muscles have a very good balance of EAA_9 ranging from 49.8 to 50.3%, compared to the FAO/WHO reference essential amino acid (EAA_9) pattern value of 33.9% for a 2−5-year-old child. The results indicate that assessment of protein quality of cardiac muscles can be based on their amino acid composition and myofibrillar and connective tissue protein contents.

Keywords: *Cardiac muscles; bovine; porcine; actin; myosin; collagen; connective tissue; assessment; protein quality; amino acids; composition*

INTRODUCTION

Beef and pork hearts are edible byproducts of the meat-packing industry. The major proportion of hearts are sold fresh or frozen, primarily for sausage manufacture or processed luncheon meat (Ockerman and Hansen, 1988). Beef hearts weigh approximately 1.4 kg and pork hearts approximately 225 g (Rust, 1988). The myofibrillar and sarcoplasmic protein fractions, including mitochondria (Anversa et al., 1978), account for about 85−95% of the total cardiac muscle proteins. Although numerous studies indicate that the amino acid composition of cardiac muscle tissues is nutritionally equivalent to lean skeletal muscle tissue (USDA, 1983, 1986), little is known about the relative amino acid composition of the myofibrillar and extracellular cardiac muscle proteins nor is much known about the levels and variation of connective tissue proteins of cardiac muscle and how they influence the protein quality (Hay, 1981; Wiens et al., 1984; Sanes, 1986). In a recent review, Prockop and Kivirikko (1995) indicated that in the collagen superfamily there are at least 19 collagen types and an additional 10 proteins that have collagen-like domains such as the subcomponent C1q of complement and the tail structure of acetylcholinesterase.

Quantitation of N^{τ}-methylhistidine [His(τ-Me)] has been proposed as an index for determining the total actin content of muscle tissues (Zarkadas et al., 1988c; Zarkadas, 1992). Studies have shown that actin contains 1 mol of His(τ-Me) (Elzinga et al., 1973; Vanderkerckhove and Weber, 1978, 1979) and myosin, isolated from adult fast-twitch white skeletal muscles, contains 1 mol of His(τ-Me) in each of its two heavy chains (MHC) (Maita et al., 1987, 1991; Nakayama et al., 1994). Other studies have indicated that His(τ-Me) is absent from cardiac myosin (Kuehl and Adelstein, 1970; Huszar, 1984). The *in situ* molar ratio of myosin to actin in the myofibrils of cardiac muscle has been shown to be 1:4 compared to 1:6 found in skeletal muscles (Murakami and Uchida, 1985).

Studies by Alsmeyer et al. (1974), Happich et al. (1975), Lee et al. (1978), and Pellett and Young (1984) have shown a significant positive correlation between rat protein efficiency ratio (PER) values and the essential amino acids of meat and poultry products and a negative correlation between the collagen content of meats. As a result, the U.S. Department of Agriculture's Food Safety and Inspection Services (Expert Work Group, 1984) and Pellett and Young (1984, 1990) have recommended the use of accurate protein and amino acid composition and connective tissue data of meat and poultry products as a simple and practical method for assessing their protein quality, for both regulatory and scientific purposes, as well as for consumer information and international trade.

The aims of the present study were (1) to provide statistically and experimentally sound amino acid data on cardiac muscle tissue to assess protein quality from their digestibility and FAO/WHO (1991) amino acid scoring pattern; (2) to show that the myofibrillar α_c-actin, collagen, and collagen-like proteins in cardiac muscle tissues can be determined from the amount of His(τ-Me) and 5-hydroxylysine [Lys(5-OH)] present, respectively; and (3) to determine the His(τ-Me) content and molecular weights of highly purified bovine and porcine cardiac α_c-actins.

MATERIALS AND METHODS

Materials. Types DC-6A (lot 3280), DC-4A (lot 750), and DC-5A (lot 746) cation-exchange spherical resins, sized to 11.0 ± 1.0, 9.0 ± 1.0, and 6.0 ± 0.5 μm, respectively, were purchased from Dionex Chemical Co., Sunnyvale, CA. The amino acid standards were obtained as follows: N^6-lysinoalanine [N^G-(DL-2-amino-2-carboxyethyl)-L-lysine] from Miles Ana-

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lytical Laboratories, Inc., Elkart, IN; the diastereoisomer mixture of 5-hydroxy-DL-lysine, N^6-methyl-L-lysine, N^6-dimethyl-L- and N^6-trimethyl-L-bis(*p*-hydroxyazobenzene-*p*′-sulfonate)·H_2O, N^τ-methyl-L-histidine, N^π-methyl-L-histidine hydrate, D-glucosamine monohydrochloride, D-galactosamine monohydrochloride, and 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; DL-ornithine(5-aminonorvaline) from Schwarz/Mann, Orangeburg, NY; norleucine and L-2-amino-3-guanidinopropionic acid from Pierce Chemical Co., Rockford, IL; and 3-nitro-L-tyrosine from Aldrich Chemical Co., Milwaukee, WI. The standard amino acid calibration mixture was purchased from Beckman Instruments, Inc., Palo Alto, CA. Sephadex G-25 and Sephacryl S-200 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Acrylamide (>99.9% purity), *N,N*′-methylenebis(acrylamide), ammonium persulfate, Coomassie Brilliant Blue R-250, Bromophenol Blue, glycine, Bio-lyte (pH 3−10), TEMED, and sodium dodecyl sulfate (SDS) were purchased from Bio-Rad Laboratories, Richmond, CA. Nonidet P40 was supplied from BDH Chemicals Ltd., Poole, England. High-purity urea was purchased from Schwarz/Mann. All reagents and buffers were made with high-purity laboratory water as described previously by Zarkadas et al. (1988c). All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Animals, Sampling, and Preparation of Cardiac Muscle Tissues.* Hearts from three mature (8 years old) Holstein-Friesian cows and three adult (4 years old) Yorkshire sows were obtained from a local abattoir and trimmed as described by Spooncer (1988). After trimming, the hearts were mechanically washed with cold water. Sections (1.0 cm thick) were taken from the right and left auricles and ventricles of the hearts. All cardiac muscle tissues (approximately 20.0 g each) were cut in small cubes, minced twice in a precooled (4 °C) meat grinder ($^3/_{16}$ then $^1/_8$ in. plates), frozen (−170 °C), and lyophilized. The freeze-dried samples were then pulverized in a standard electrically driven end runner mill (coffee mill; Moulinex Canada Ltd., Weston, ON) to pass through a 0.5 mm mesh sieve and stored at −20 °C in polypropylene bottles until used.

Procedure for Ethanol−HCl Extraction of Cardiac Muscle Tissues. To remove histidine dipeptides and soluble amino acids, lyopholized cardiac samples (10.0 g) were extracted three times with a mixture (200 mL) of 0.1 M HCl in 75% ethanol (v/v) (Rangeley and Lawrie, 1976).

Preparation of Acetone Powders. Fresh cardiac muscle tissues (approximately 20 g each) were cleaned of adhering fat and connective tissue, cut into small cubes, chilled on ice, washed free of blood with distilled water, and ground at 4 °C in a prechilled meat grinder. The mince was then quickly extracted for 10 min with 1.0 L of ice-cold 0.1 M KCl and 0.15 M potassium phosphate (pH 6.5) and filtered through four layers of cheesecloth following the method of Spudich and Watt (1971). The filtered muscle mince was then extracted with stirring for 10 min at 4 °C in 2.0 L of ice-cold water for 5 min each. The final five extractions were with 1.0 L of acetone for 10 min each at 20 °C. The filtered residue was air-dried in a fume hood to obtain dried acetone powder and was stored at −20 °C as described previously (Khalili and Zarkadas, 1988).

Purification of Cardiac Actins. Bovine and porcine cardiac G-actins were purified from acetone-extracted heart samples. Extraction was carried out according to the low-salt buffer method (Spudich and Watt, 1971), followed by three polymerization and depolymerization steps (Pardee and Spudich, 1982). Cardiac actins were further purified 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 mL fractions, and the fractionation was monitored at 280 nm. Protein concentration in each fraction was also determined either according to the method of Lowry et al. (1951) as modified by Peterson (1983) using bovine serum albumin as a standard or according to the method described by Horstmann (1979). The fractions containing the G-actin from the Sephacryl S-200 column were identified either by one-dimensional polyacrylamide gel electrophoresis and absorbency measurements at 280 nm or by amino acid analysis and were then concentrated according to 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 × 2.5 cm) preequilibrated with the same depolymerization buffer used by 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.

Polyacrylamide Gel Electrophoresis (PAGE). The molecular weight determination of purified bovine and porcine α_c-actins was also carried out by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE). Electrophoresis was conducted in a Bio-Rad Protean apparatus (Bio-Rad Laboratories, Richmond, CA) on 0.75 mm slab gels (140 × 120 mm) containing either 12% acrylamide or linear gradient slab gels (8−20% acrylamide). The high-molarity Tris buffers in the resolving gel (0.75 M) and in the running buffer (0.05 M) were as in the Fling and Gregerson (1986) modification of the method of Laemmli (1970).

The first-dimensional isoelectric focusing (IEF) of α_c-actins was carried out in 11.5 mm long rod gels with 2.0 mm diameter as described by Laemmli (1970) and O'Farrell (1975). IEF was performed for 16 h at 338 V for a total of 5400 V h at 16 °C. Electrode solutions were 0.01 M phosphoric acid at the anode (bottom reservoir) and 0.02 M sodium hydroxide at the cathode (upper reservoir). IEF gels were equilibrated for 2 h in 5 mL of 2.3% SDS sample buffer containing 5% β-mercaptoethanol, 10% glycerol, and 62.5 mM Tris-HCl buffer (pH 6.8).

The second-dimensional (2-D) SDS−PAGE was performed on linear gradient resolving slab gels (140 × 120 × 0.75 mm) using the Bio-Rad Protean apparatus as described by O'Farrell (1975). Acrylamide was poured in 8−20% linear gradients containing 0.1% SDS, 0.375 Tris-HCl (pH 8.8), 0.025% (v/v) TEMED, and ammonium persulfate. Electrophoresis was performed at 6−8 mA constant current overnight (16 h) until the dye front reached the bottom of the gel. One of the 2-D gels was stained with Coomassie Brilliant Blue R-250 (Bio-Rad) as described by Fling and Gregerson (1986), while the other 2-D gel was silver stained according to the modified procedure of Blum et al. (1987).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman Model 120C) or a fully automated amino acid analyzer (Beckman Model 121MB) as described previously (Zarkadas et al., 1986, 1987, 1990). The conventional instrument was equipped with a module control Autolab System AA (Beckman Methodology Bulletins AA-TB-001 to AA-TB-014) for computing the areas under peaks and determining the amino acid concentrations (Zarkadas, 1975). The automated instrument was interfaced with a Beckman Model 406 analog interface module, the system Gold (Beckman Instrument, Inc., Altex Division, San Ramon, CA) chromatographic data reduction system, and an IBM (AT series) compatible personal computer to enable quantitation of amino acids at the picomole level as described previously (Zarkadas et al., 1987).

Complete amino acid analyses were carried out on each of the three lyophilized beef and pork hearts following standard chromatographic procedures (Zarkadas et al., 1986, 1987). Each of the three dried heart samples (50.0 mg) was hydrolyzed in Pyrex test tubes (18 × 150 mm) under vacuum (below 10 mmHg) with 5.0 mL of triple-glass-distilled constant-boiling HCl (6.0 M; 20.5% v/v) at 110 °C in quadriplicate for each of four times, 24, 48, 72, and 96 h, respectively, following the precautions described previously (Zarkadas et al., 1987, 1988a). The clear filtrate and washings were combined, evaporated to dryness in a Rotary EvapoMix (Buchler Instruments, Fort Lee, NJ) at 45 °C, and brought to volume (usually to 5 mL) with 0.2 M sodium citrate buffer (pH 2.2).

The data reported for serine, threonine, and ammonia in Tables 1 and 2 represent the average values at each hydrolysis time extrapolated to zero time using linear regression. The values for valine, isoleucine, leucine, and phenylalanine are the average of 24 determinations obtained from 72 and 96 h of hydrolysis. All other values are reported as the average

Table 1. Comparison of the Amino Acid (AA) Composition, Protein and Nitrogen Contents (Grams of Amino Acid per Kilogram of Protein), and Essential Amino Acid Indices of Bovine and Porcine Cardiac Muscle Tissues

	mean ± SEM[a]		signif levels	
AA	bovine	porcine	CV	F[a]
aspartic acid	94.72 ± 0.27	93.07 ± 0.05	0.95	31.04*
threonine	44.55 ± 0.46	46.49 ± 1.07	3.27	3.11[ns]
serine	40.50 ± 0.28	42.94 ± 0.56	3.65	16.63[ns]
glutamic acid	150.26 ± 0.54	151.30 ± 0.68	0.67	2.31[ns]
proline	40.77 ± 0.03	40.28 ± 1.47	2.98	0.09[ns]
glycine	46.63 ± 0.25	45.11 ± 0.73	2.26	3.59[ns]
alanine	55.27 ± 0.32	56.63 ± 0.11	1.49	17.23[ns]
cysteine	10.95 ± 1.14	11.38 ± 0.51	9.25	0.14[ns]
valine	53.23 ± 0.41	56.00 ± 1.55	3.83	3.30[ns]
methionine	27.78 ± 0.03	28.39 ± 0.32	1.63	4.50[ns]
isoleucine	47.50 ± 0.08	45.25 ± 0.89	3.12	6.15[ns]
leucine	91.40 ± 0.56	90.60 ± 0.94	1.04	0.35[ns]
tyrosine	36.40 ± 0.41	40.17 ± 1.83	6.98	4.34[ns]
phenylalanine	44.56 ± 0.08	44.49 ± 0.89	1.61	0.00[ns]
lysine	86.58 ± 0.14	87.99 ± 0.27	0.74	11.92[ns]
histidine	30.24 ± 0.01	27.50 ± 0.05	5.38	2962.47**
arginine	71.65 ± 0.74	69.80 ± 0.49	1.74	4.02[ns]
tryptophan	18.10 ± 0.45	18.24 ± 1.42	6.58	0.01[ns]
4-hydroxyproline	7.93 ± 0.35	6.30 ± 0.04	13.74	21.67[ns]
ammonia	18.60 ± 1.46	13.53 ± 2.63	23.79	2.85[ns]
5-hydroxylysine	0.7487 ± 0.0542	0.7892 ± 0.0279	4.57	2.68[ns]
N^{τ}-methylhistidine	0.3364 ± 0.0180	0.3294 ± 0.0210	4.65	0.29[ns]
N^{π}-methylhistidine	0.00	0.00		
total N	181.10	176.52		
total EAA, mg/g of N	2587.3	2701.7		
total protein, g/kg of dry matter	612.07	644.96		
EAA index[b]	79.6	81.5		
chemical score[c]	78.1	78.9		
WE,[d] μg/nmol	0.110591	0.110589		
CF,[d] μg/nmol	0.118272	0.117989		

[a] A mean values and standard error of measurements (SEM) for 3 replicates and 24 determinations. Significance: *F* test, values from analysis of variance, **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [b] Essential amino acid index (EAA) calculated according to the method of Oser (1951). [c] Chemical score calculated according to the method of Block and Mitchell (1946). [d] WE and CF constants calculated according to the method of Horstmann (1979).

values of 48 determinations obtained from 24, 48, 72, and 96 h of hydrolysis.

Methionine and cyst(e)ine were determined on separate 50.0 mg samples by the performic acid procedure (Moore, 1963). Norleucine was added in the hydrolysates as an internal standard, and recoveries of cyst(e)ine as cysteic acid and methionine as methionine *S*,*S*′-dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids and relative to alanine, valine, leucine, and isoleucine present in the sample (Zarkadas et al., 1988a−c).

Tryptophan was determined separately in 50.0 mg samples after alkaline hydrolysis (Hugli and Moore, 1972) according to the procedure of Zarkadas et al. (1986) using 3-nitrotyrosine [Tyr(NO_2)] as an internal standard.

Determination of the Pro(4-OH) content of beef and pork hearts was carried out separately from a concentrated hydrolysate (equivalent to 50.0 μg of protein per analysis) using a single column (21 × 0.6 cm) packed with Dionex DC-6A resin (Zarkadas et al., 1986). Recoveries of Pro(4-OH) were calculated relative to alanine, isoleucine, and leucine. The Pro(4-OH) data represent the average values of 24 determinations.

The determination of the methylated basic amino acids, the diastereoisomers of Lys(5-OH), and related compounds were carried out with concentrated 96 h hydrolysates (equivalent to 100−250 μg of protein per analysis) by use of analytical chromatographic methods developed to quantitate these unique amino acids (Zarkadas et al., 1986, 1987) so that peaks adequate for these components (100−250 pmol) could be obtained. Recoveries of these unique amino acids were calculated on the basis of total protein found in each 96 h hydrolysate determined according to the procedure described by Horstmann (1979).

Protein Determination. Protein content in each of the hydrolysates was calculated as discussed by Horstmann (1979). The mean residue weight (WE, in micrograms per nanomole of amino acid) was determined with the 18 standard amino acid residues plus Pro(4-OH) using the expression

$$\mathrm{WE} = \sum_{i=1}^{19}(a_i b_i) \tag{1}$$

where a_i is the mole fraction of a specific amino acid *i* found in the analyzed aliquot and b_i is the molecular weight of the amino acid residue *i*. A conversion factor CF (in micrograms per nanomole of amino acid) was used for determining the protein mass in each hydrolysate sample analyzed in the absence of tryptophan, methionine, and cyst(e)ine.

$$\mathrm{CF} = \sum_{i=1}^{15}(a_i b_i)/[1 - (a_{\mathrm{Trp}} + a_{\mathrm{Cys}} + a_{\mathrm{Met}}] \tag{2}$$

The protein content *P* (in micrograms) of each hydrolysate was calculated by multiplying CF by the total nanomoles (χ_i) of amino acids found (Horstmann, 1979; Peterson, 1983) as follows:

$$P = (\mathrm{CF})\sum_{i=1}^{15}\chi_i \tag{3}$$

Determination of Connective Tissue Proteins in the Bovine and Porcine Hearts. The amount of a specific protein *j* in muscles has been calculated (Zarkadas et al., 1988a−c) as follows:

$$P_{j=1} = C_i \frac{[1000]}{n_i}\frac{\mathrm{WE}_{pj}}{M_{\mathrm{r}}(i)} \tag{4a}$$

Table 2. Comparison of the Amino Acid (AA) Composition and Protein and Nitrogen Contents (Grams of Amino Acid per Kilogram of Protein) of Bovine and Porcine Cardiac Muscle Tissues Extracted with 0.1 M HC1 in 75% Ethanol Prior to Hydrolysis

AA	mean ± SEM[a]		signif levels	
	bovine	porcine	CV	F[a]
aspartic acid	96.50 ± 0.15	94.84 ± 0.69	1.37	1.44[ns]
threonine	44.33 ± 0.48	46.92 ± 1.09	5.30	3.58[ns]
serine	42.16 ± 1.98	42.69 ± 0.50	7.89	0.17[ns]
glutamic acid	144.79 ± 0.24	146.06 ± 1.59	1.90	0.62[ns]
proline	42.67 ± 0.78	42.83 ± 0.95	4.70	0.04[ns]
glycine	45.77 ± 1.37	45.36 ± 0.47	5.17	0.10[ns]
alanine	53.95 ± 0.38	54.94 ± 0.48	2.17	1.90[ns]
cysteine	55.25 ± 0.53	56.18 ± 0.79	3.00	3.34[ns]
valine	10.36 ± 0.41	10.93 ± 0.27	8.01	0.89[ns]
methionine	28.48 ± 0.17	28.84 ± 0.47	2.94	0.46[ns]
isoleucine	49.28 ± 0.55	46.85 ± 0.67	3.79	3.85[ns]
leucine	92.01 ± 0.72	90.56 ± 0.44	1.61	1.46[ns]
tyrosine	37.12 ± 0.34	40.93 ± 0.59	6.03	38.60**
phenylalanine	46.97 ± 0.39	44.33 ± 0.84	4.30	7.29[ns]
lysine	84.66 ± 0.59	84.23 ± 0.98	2.20	0.00[ns]
histidine	28.31 ± 0.22	26.77 ± 0.27	3.38	8.21*
arginine	73.46 ± 0.46	70.98 ± 0.52	2.20	8.18*
tryptophan	15.54 ± 1.02	16.65 ± 0.26	11.74	0.52[ns]
4-hydroxyproline	6.99 ± 0.17	6.82 ± 0.51	13.06	0.16[ns]
ammonia	15.97 ± 2.23	11.66 ± 0.88	32.95	22.09**
5-hydroxylysine	1.0315 ± 0.0713	1.1408 ± 0.0518	14.16	0.58[ns]
N^{τ}-methylhistidine	0.3519 ± 0.0115	0.3494 ± 0.0246	11.44	0.02[ns]
N^{π}-methylhistidine	0.00	0.00		
total N	178.60	175.61		
total protein, g/kg dry matter	763.87	819.35		
WE,[b] μg/nmol	0.110577	0.110520		
CF,[b] μg/nmol	0.118218	0.118241		

[a] Mean values and standard error of measurements (SEM) for 3 replicates and 24 determinations. Significance: *F* test, values from analysis of variance, **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [b] WE and CF constants calculated according to the method of Horstmann (1979).

where WE_{Pj} is the mean residue weight of a specific connective tissue protein *j*, determined from eq 1, n_i is the number of residues of the amino acid *i* per 1000 amino acid residues, $M_{r(i)}$ is the anhydrous molecular weight of the unique amino acid *i*, and C_i is the mean concentration in grams per kilogram of total protein of a unique protein-bound amino acid *i* found in the analyzed acid hydrolysate of the heart.

Using this approach, the content of collagen and collagen-like proteins in cardiac muscle tissue can be calculated from the amounts of the aLys(5-OH) diastereoisomer found in the acid hydrolysates. For the diastereoisomers of Lys(5-OH) the mean content of 10.0 residues/1000 total amino acid residues in muscle collagen was calculated from the relative distribution of collagen types and their respective Lys(5-OH) contents as described previously (Light et al., 1985; Zarkadas et al., 1988b,c). The average residue weight (WE) for collagen is 91.1, and each of the diastereoisomers of Lys(5-OH) has an anhydrous M_r of 144.18.

From eq 4a, collagen (grams per kilogram of total protein) can be calculated (Zarkadas et al., 1988b,c) as follows:

$$\text{collagen } [P_c;\text{g/kg}] = \text{Lys(5-OH) (g/kg)} \times 63.2 \quad (4b)$$

To calculate the amount of total connective tissue proteins in the bovine and porcine hearts (in grams per kilogram of total protein), Pro(4-OH) and amino acid composition of purified collagen and elastin (Light, 1985, 1987; Miller and Gay, 1987; Zarkadas et al., 1988a−c; Timpl, 1989; van der Rest and Garrone, 1991) were used. It involves substituting into eq 4a the total amount of Pro(4-OH) found in both proteins (Berg, 1982; Etherington and Sims, 1981), the known Pro(4-OH) contents of collagen ($n_i = 105.8$) and elastin ($n_i = 22$), the relative distributions of collagen (P_C) and elastin (P_E) per unit of tissue, and the anhydrous molecular weight [$M_{r(i)} = 113.12$] of Pro(4-OH). The following analytical convention described previously (Zarkadas et al., 1988c) can also be used for computing total connective tissue proteins, i.e., collagen, collagen-like proteins, and elastin, in grams per kilogram of total protein, of cardiac muscle tissues:

$$\text{connective tissue } (P_{CT};\text{g/kg}) = \text{Pro(4-OH) (g/kg)} \times 8.03 \quad (4c)$$

Determination of the Myofibrillar Proteins. Since 1 mol of actin (A) contains 1 mol of His(τ-Me), and His(τ-Me) is absent from cardiac myosin and all other cardiac muscle and non-muscle proteins (Elzinga et al., 1973; Vandekerchkhove and Weber, 1979; Maita et al., 1987), protein-bound His(τ-Me) can be used as an index for determining actin in cardiac muscle tissues. Care must be taken, however, to extract all soluble histidine dipeptides (Carnegie et al., 1984; Harris and Milne, 1987; Kohen et al., 1988) prior to acid hydrolysis.

The amount of α_c-actin (P_A) in cardiac muscles can be calculated from the total concentration, C_T (in grams per kilogram of total protein), of His(τ-Me) present in the acid hydrolysates by the following equations:

$$P_A = \frac{M_{r(A)}}{n_i + M_{r(i)}} C_T \quad (5a)$$

or

$$P_A = \frac{41782}{1 + 151.2} C_T \quad (5b)$$

P_A is the amount of actin in cardiac muscles (grams per kilogram of total protein), $M_{r(A)}$ is the molecular mass of α_c-actin (41 782 Da), $n_i = 1$ is the number of unique amino acid residues *i* per mole of α_c-actin, and $M_{r(i)}$ is the anydrous molecular mass of His(τ-Me), which is 151.2 Da.

Therefore, the following equation can be used to calculate α_c-actin as grams per kilogram of total protein:

$$\text{amt of } P_A \text{ (g/kg)} = \text{His}(\tau\text{-Me}) \text{ (g/kg)} \times 276 \quad (5c)$$

The myosin content (grams per kilogram of total protein) in bovine and porcine cardiac muscle tissues can be calculated relative to α_c-actin as follows:

$$P_M = P_A \frac{M_{r(M)}}{M_{r(A)}} \frac{1}{4} \quad (6a)$$

P_M and P_A are the concentrations of cardiac myosin and α_c-actin (grams per kilogram of total protein), respectively, and $M_{r(M)}$ and $M_{r(A)}$ are the respective molecular weights of cardiac myosin and α_c-actin.

Substituting the molar ratio of cardiac α_c-actin to myosin reported by Murakami and Uchida (1985) (A/M = 4:1) and the molecular mass of myosin, $M_{r(M)}$ = 480 000 Da (Kawamoto and Adelstein, 1991; Babij et al., 1991; Kelley et al., 1992, 1993), and α_c-actin (41 782 Da; Elzinga et al., 1973) in eq 6, the total amount of myosin in cardiac muscle can then be calculated as

$$P_M = P_A \frac{480000}{41782} \frac{1}{4} = 2.872 P_A \quad (6b)$$

Yates and Greaser (1983) have shown that the sum of actin and myosin in myofibrils accounts for 65% of the total myofibrillar protein by weight. Therefore, the total myofibrillar protein in grams per kilogram of total protein in bovine and porcine cardiac muscle tissues is

$$\text{myofibrillar protein (g/kg)} = \frac{\sum_{j=1}^{2}(P_A + P_M)}{0.65} \quad (6c)$$

Determination of the Nearest Integer Ratios of Amino Acid Residues in Bovine and Porcine α_c-Actins. The computer-assisted methods of Black and Hogness (1969) and Hoy et al. (1974) were used to determine both the nearest integer ratios (i.e., a_i mole ratio) of amino acid residues and a minimum molecular weight for bovine and porcine α_c-actins. As the values of n_i (residue number) are seldom integral numbers, the n_i values obtained for α_c-actin from cardiac muscles were multiplied by a series of values for the k scaling factor. This factor ranged from 0.90 to 1.15 in steps of 0.0001, with 20 000 points available for plotting in the search for k_{best} = 1.0277 and 0.99990 for bovine and porcine cardiac α_c-actins, respectively. A good fit was indicated by the values for f = 0.29932 and 0.90822 for bovine and porcine α_c-actins, respectively.

Statistical Analysis. Data processing and statistical 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 according to the Statistical Analysis System (SAS, 1991) by the general linear model procedure.

RESULTS AND DISCUSSION

An accurate determination of all amino acids, including the methylated basic amino acids, Lys(5-OH), and Pro(4-OH) in cardiac muscle tissues, was carried out to evaluate the overall protein quality of bovine and porcine cardiac muscle tissues.

The results of these analyses, and the levels of statistical significance obtained from analysis of variance between bovine and porcine cardiac muscle tissues and α_c-actins, are summarized in Tables 1−3. The data are expressed as grams of amino acids per kilogram of protein to allow comparisons to be made between the present results and those given in food compositional tables. This also enables the calculation of percentage recovery of the amino acids by simple summation (Tristram and Smith, 1963; Eastoe, 1967). On a dry weight basis the actual protein contents of bovine and porcine cardiac muscle tissues did not differ significantly and ranged from 61.2−64.5 to 76.4−81.9% in untreated and extracted cardiac muscle tissues, respectively.

Tables 1 and 2 compare the total amino acid nitrogen contents of bovine and porcine muscle tissues calculated from their amino acid nitrogen contents as described by Heidelbaugh et al. (1975). The amino acid profiles of lyophilized bovine and porcine hearts before and after extraction, as presented in Tables 1 and 2, indicate that many of the individual amino acid values are very similar. The values obtained for the amino acid contents of both bovine vs porcine cardiac samples showed high reproducibility. Certain characteristic features of the overall composition of bovine and porcine cardiac muscle may be noted (Tables 1 and 2). Glutamic acid accounted for approximately 15% of the amino acid content and aspartic acid for approximately 10%. Lysine, arginine, and histidine combined accounted for 17−18% compared to the acidic amino acids, which accounted for almost 25% of all residues. Valine, leucine, and isoleucine comprised about 18−20% of the hearts of both species.

Certain differences, however, were noted. Significant differences existed in the arginine ($P < 0.05$) and tyrosine ($P < 0.01$) contents between bovine and porcine cardiac muscle tissues (Table 2) which did not occurred in the unextracted samples (Table 1). The histidine content was significantly different ($P < 0.01$) in the unextracted samples (Table 1) and significantly different ($P < 0.05$) in the extracted samples (Table 2) with porcine compared to bovine tissue. These results are in accord with those reported by Carnegie et al. (1984), Harris and Milne (1987), and Kohen et al. (1988) for bovine and porcine muscle tissues. Skeletal muscles contain variable amounts of soluble histidine dipeptides including carnosine (β-alanyl-L-histidine), anserine (β-alanyl-L-N^{π}-methylhistidine), and balenine (β-alanyl-L-N^{τ}-methylhistidine), which upon acid hydrolysis yield β-alanine, histidine, His(π-Me), and His(τ-Me) (Carnegie et al., 1984; Harris and Milne, 1987; Kohen et al., 1988). The results in Tables 1 and 2 show that approximately 2.0−6.0% of the total histidine has been extracted at ambient temperatures by the 0.1 M HCl in 75% ethyl alcohol solvent. Thus, the differences noted in histidine content between extracted and untreated cardiac muscle tissues suggest that cardiac muscles must contain about 2−6% free histidine and probably most of it as the soluble dipeptide carnosine.

The chromatographic separations shown in Figure 1 are typical of those obtained when the 96 h hydrolysis products (100 μL equivalent to 250 μg of protein per analysis) of extracted and untreated bovine cardiac muscle tissue were analyzed. It should also be noted in parts B and C of Figure 1 that, unlike skeletal muscles, His(π-Me) was absent from the bovine cardiac muscle tissue 96 h hydrolysates, suggesting the absence of anserine in heart muscles.

The protein-bound His(τ-Me) and Lys(5-OH) contents of bovine and porcine cardiac muscles before and after extraction are presented in Tables 1 and 2 and represent the average values of sextuplet determinations. The values for His(τ-Me) in cardiac muscles, 0.349 and 0.352 g/kg of protein in adult porcine and bovine cardiac muscles, respectively, are considerably lower than the mean His(τ-Me) value of 0.548 g/kg of protein reported previously for skeletal muscle tissues (Zarkadas et al., 1988a,b). The low levels of protein-bound His(τ-Me) found agree with observations that His(τ-Me) is a constituent of both myosin and actin in fast-twitch rabbit skeletal muscles and is absent in myosins from cardiac muscles (Kuehl and Adelstein, 1970; Elzinga et al., 1973; Huszar, 1984). Recent studies by Maita et al. (1991) and Nakayama et al. (1994) suggest the

Table 3. Comparison of the Amino Acid (AA) Composition (Means, c_i SEM), Frequencies (a_i), Residue Number (n_i), Corrected Residue Numbers ($K_{best}n_i$), and Nearest Integer Ratios (I_i) of Bovine and Porcine Cardiac Actins

	means, nmol/1000 μg of protein $c_i \pm$ SEM		mole fractions a_i		$K_{best}n_i$ [k_{best} = 1.0277 (k_{best} = 0.99990, f = 0.29932) f = 0.90822]		no. of residues to nearest integer I_i		
AA	bovine	porcine	bovine	porcine	bovine	porcine	bovine	porcine	lit.[a]
aspartic acid	864.43 ± 3.73	876.64 ± 6.24	0.0904	0.0913	34.796	34.342	35	34	34
threonine	644.42 ± 2.56	657.46 ± 2.30	0.0674	0.0687	29.940	25.756	26	25	26
serine	600.81 ± 11.41	487.40 ± 15.00	0.0628	0.0509	24.185	29.094	24	19	23
glutamic acid	1018.77 ± 3.38	1035.82 ± 7.33	0.1065	0.1082	41.009	40.577	41	41	39
proline	477.67 ± 7.38	486.38 ± 5.60	0.0499	0.0508	19.228	29.054	19	19	19
glycine	725.73 ± 11.11	775.80 ± 2.99	0.0759	0.0810	29.213	30.391	29	30	28
alanine	731.01 ± 3.21	747.31 ± 4.72	0.0764	0.0781	29.426	29.275	29	29	29
cysteine	118.47 ± 1.00	119.41 ± 1.00	0.0125	0.0125	4.807	4.678	5	5	5
valine	546.43 ± 5.96	543.20 ± 3.28	0.0571	0.0571	21.996	21.280	22	21	21
methionine	405.90 ± 1.00	382.07 ± 1.00	0.0424	0.0399	16.339	14.967	16	15	16
isoleucine	717.50 ± 3.78	719.81 ± 5.75	0.0750	0.0752	28.882	28.198	29	28	30
leucine	689.38 ± 4.47	716.92 ±10.40	0.0721	0.0749	27.750	28.084	28	28	26
tyrosine	423.95 ± 2.84	414.30 ± 3.01	0.0443	0.0433	17.066	16.230	17	16	16
phenylalanine	267.55 ± 4.71	274.46 ± 2.97	0.0280	0.0287	10.770	10.752	11	11	12
lysine	499.06 ± 3.64	502.70 ± 6.70	0.0522	0.0525	20.089	19.693	20	20	19
histidine	249.68 ± 6.75	261.82 ± 6.48	0.0261	0.0274	10.051	10.257	10	10	8
arginine	440.63 ± 3.72	451.71 ± 2.84	0.0461	0.0472	17.695	18	18	18	18
tryptophan	119.41 ± 1.00	119.41 ± 1.00	0.0125	0.0125	4.807	4.679	5	5	5
N^{τ}-methylhistidine	22.56 ± 0.57	29.43 ± 0.17	0.0024	0.0035	0.908	1.1037	1	1	nd
total							375	376	375
WE,[b] μg/nmol							0.11466	0.11414	
CF,[b] μg/nmol							0.11152	0.11101	
MW[c] (AAA)							43.092	42.092	41.858[a]
MW (SDS−PAGE)							43.000	43.000	
pI (IEF)							5.43	5.43	5.40[d]

[a] Obtained from Vandekerckhove and Weber (1978, 1979). [b] Calculated by Horstmann (1979). [c] Calculated according to the procedure of Hoy et al. (1974). [d] Obtained from Garels and Gibson (1976).

presence of His(τ-Me) at position 754 in the amino acid sequence of both skeletal and ventricular cardiac myosins. Further studies will be needed to clarify this point.

***N*$^{\tau}$-Methylhistidine Content of Cardiac Actins.** In an attempt to understand the molecular basis for the difference (35%) observed between levels of His(τ-Me) in cardiac and skeletal muscles and to establish whether this difference was due to myosin isoforms or actin isotypes, highly purified cardiac muscle α_c-actins were prepared. A single band was observed on one-dimentional PAGE−SDS at MW 43 000 and on two-dimensional pH-IEF PAGE−SDS electrophoresis, demonstrating that both cardiac α_c-actins prepared from bovine and porcine hearts were essentially homogeneous. The molecular masses of α_c-actins from both cardiac muscles were determined as 43 000 Da by one-dimensional SDS−PAGE electrophoresis as described by Fling and Gregrerson (1986). These results are in accord with those reported for cardiac and skeletal muscle actins by Vandekerckhove and Weber (1978, 1979), Lowey (1986), and Vandekerckhove et al. (1986).

The amino acid composition with standard error values found for highly purified G-actin from bovine and porcine cardiac muscles is given in Table 3. The data are expressed as nanomoles per 1000 μg of protein (columns 2 and 3) as described previously (Hoy et al., 1974; Zarkadas et al., 1988b,c). This method yielded totals of 375 and 376 amino acid residues per mole of bovine and porcine cardiac α_c-actins, respectively. Molecular masses calculated from the amino acid compositions of bovine and porcine cardiac α_c-actins were 43 092 and 42 092 Da, respectively. These results are consistent with the values 41 871 Da and 375 amino acids residues reported by Vandekerckhove and Weber (1978) for bovine skeletal and cardiac muscle α_c-actins. The similarity of the results between bovine and porcine α_c-actins is consistent with the currently favored concept that eukaryotic actins are highly conserved in evolution (Pollard and Cooper, 1986) and that actins are the most abundant protein in many animal cells (Pollard et al., 1994).

Bovine and porcine cardiac α_c-actins contained 0.908 and 1.104 mol of His(τ-Me)/mol of protein, respectively. Typical chromatographic separations obtained are illustrated in Figure 2. The analysis of 96 h hydrolysates of bovine and porcine cardiac α_c-actins (100 μL equivalent to 150 μg of protein per analysis) reveals the separation, at pH 4.501, of His(τ-Me) following the ammonia peak, along with three as yet unidentified ninhydrin positive peaks, designated 2−5. These are in close agreement with those reported previously for skeletal muscle actin (Khalili and Zarkadas, 1988; Zarkadas et al., 1988b,c) and for bovine cardiac muscle α_c-actin by Vandekerckhove and Weber (1978, 1979) and Vandekerchkhove et al. (1986), and for purposes of comparison their data are included in Table 3. These results are also in accord with those reported on the primary structure of rabbit skeletal G-actin by Elzinga et al. (1973). The low levels of protein-bound His(τ-Me) can be attributed to the absence of His(τ-Me) in cardiac muscle proteins other than α_c-actin.

Other studies have indicated that, like skeletal muscle myosins (Maita et al., 1991), the ventricular cardiac myosin contains 1 mol of His(τ-Me) at position 754 in its amino acid sequence (Nakayama et al., 1994).

Determination of Myofibrillar and Connective Tissue Proteins in Cardiac Muscle. The myosin, actin, collagen, and total connective tissue protein contents of the adult bovine and porcine cardiac muscles are presented in Table 4. The quantities of these proteins were calculated on the basis of the amounts of His(τ-Me) for myofibrillar α_c-actin and Lys(5-OH) collagen found in their acid hydrolysates, respectively. Myosin was then determined from the molar ratio of

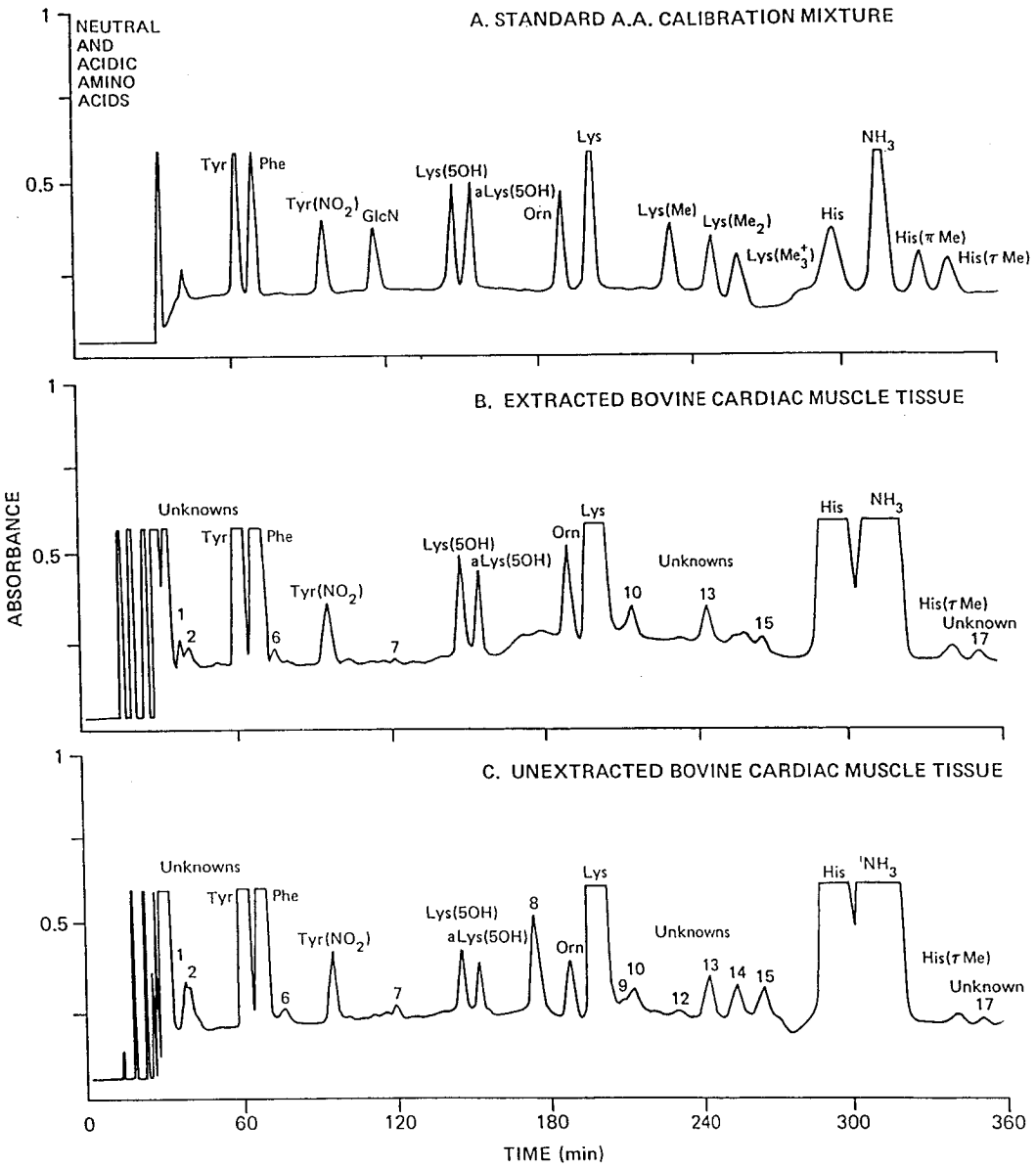

Figure 1. Typical elution patterns of the methylated basic amino acids and related compounds of bovine and porcine cardiac muscle tissues. The curves denote absorbance at 570 nm. (A) Separation of a synthetic mixture of 15 unique basic amino acids on an analytical 50 × 0.28 cm microcolumn of Dionex DC-4A resin; (B) analysis of an extracted bovine cardiac muscle tissue sample (96 h); (C) elution profile of an untreated bovine cardiac muscle tissue 96 h hydrolysate sample. Abbreviations: Tyr(NO_2), 3-nitrotyrosine; GlcN, glucosamine; Lys(5-OH), 5-hydroxylysine; aLys(5-OH), *allo*-5-hydroxylysine; Orn, ornithine; Lys(Me), N^6-methyllysine; Lys(Me_2), N^6-dimethyllysine; Lys(Me_3^+), N^6-trimethyllysine; His(π-Me), N^{π}-methylhistidine; His(τ-Me), N^{τ}-methylhistidine.

myosin to actin in the myofibrils of cardiac muscle (1:4) (Murakami and Uchida, 1984). Total connective tissue protein was calculated from the amounts of Pro-(4-OH) (Berg, 1982).

The myofibrillar proteins of bovine and porcine hearts (Table 4) appeared to be very similar. In cardiac muscles, α_c-actin accounts for an estimated 9.64−9.71% of the total muscle proteins or about 16.3% of the myofibrillar proteins, and myosin accounts for another 27.7−28.0% of the total muscle mass corresponding to about 48.6% of the myofibrillar proteins. These results are in accord with those reported by Murakami and Uchida (1984) and in close agreement with those reported previously for skeletal muscles (Yates and Greaser, 1983; Zarkadas et al., 1988b,c; Khalili and Zarkadas, 1988).

The total connective tissue protein and weighted mean collagen contents of beef and pork hearts before extraction are also presented in Table 4. The amount of collagen in bovine hearts before extraction averages 4.73% and in the porcine cardiac muscle averages 4.98% of the total muscle proteins. Table 4 shows that the mean values for total connective tissue proteins before extraction ranged from 6.37 to 5.06% in the porcine and bovine cardiac muscle tissues, respectively. These results are in reasonably good agreement with the values reported by Rust (1988) for beef (4.32%) and pork (4.35%) hearts and 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. The difference noted in the collagen contents between the extracted bovine (6.58%) and porcine (7.21%) cardiac tissues, while at present is without explanation, may be attributed to the extaction of considerable amounts of non-collagen-containing cardiac muscle proteins (Tables 1 and 2) according to the method of Rangeley and Lawrie (1976).

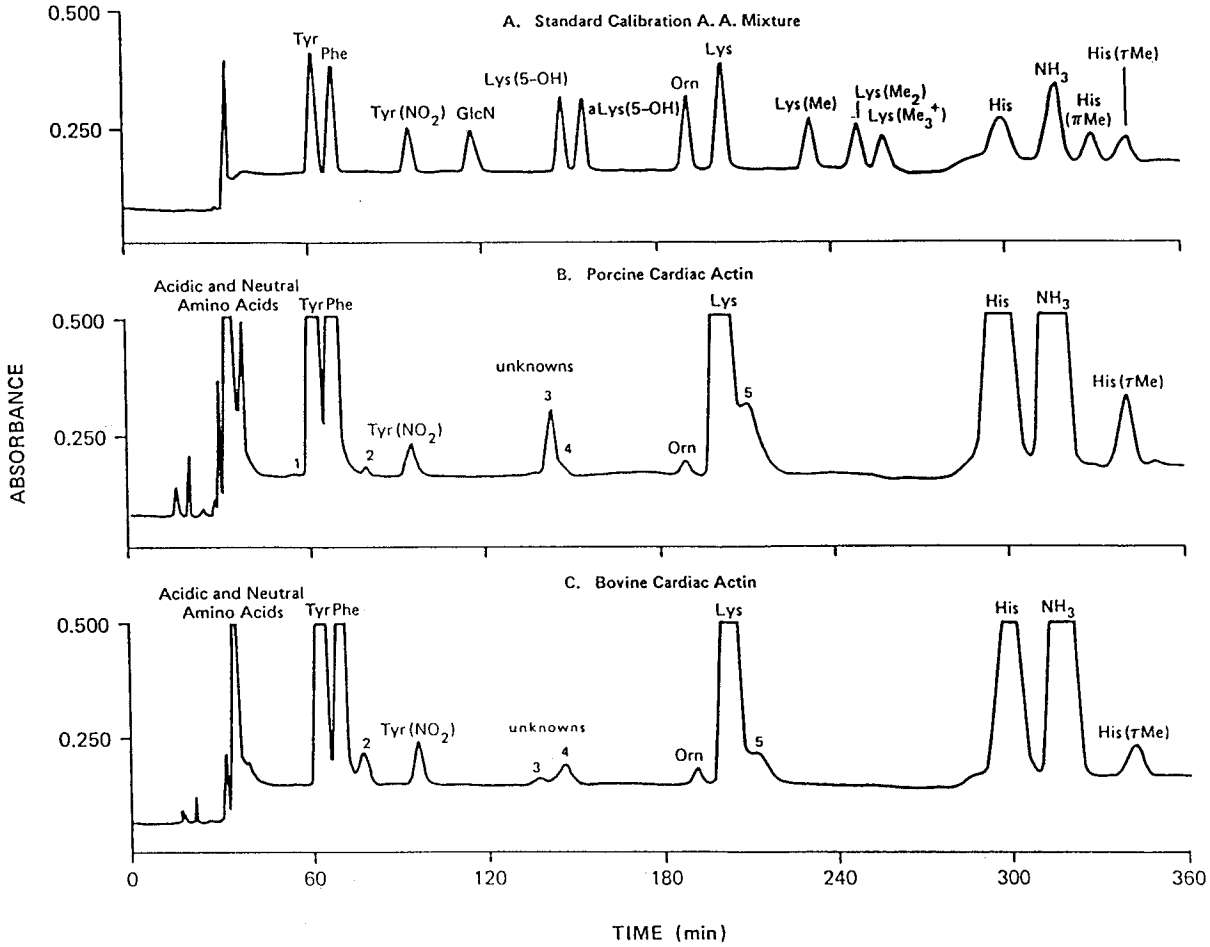

Figure 2. Chromatographic separation of methylated lysines and histidines, the diastereoisomers of 5-hydroxylysine, and related compounds on an analytical 50 × 0.28 cm microcolumn of Dionex DC-4A resin. The curves denote absorbance at 570 nm. (A) Separation of a synthetic mixture of 15 unique basic amino acids; (B) elution profile of a 96 h hydrolysate of highly purified porcine cardiac α_c-actin; (C) analysis of highly purified bovine cardiac ac-actin. Abbreviations: Tyr(NO_2), 3-nitrotyrosine; GlcN, glucosamine, Lys(5-OH), 5-hydroxylysine; aLys(5-OH), *allo*-5-hydroxylysine, Orn, ornithine; Lys(Me), N^6-methyllysine; Lys(Me_2), N^6-dimethyllysine; Lys(Me_3^+), N^6-trimethyllysine; His(π-Me), N^π-methylhistidine; His(τ-ME), N^τ-methylhistidine.

Table 4. Myofibrillar and Connective Tissue Protein Contents (Grams per Kilogram of Total Muscle Protein) of Mature Cardiac and Skeletal Muscle Tissues

			skeletal muscle of total muscle protein		
				psoas major (rabbit)	
	cardiac muscle tissues (extracted)[a]		bovine diaphragm	Yates and Greaser	Hanson and Huxley
muscle protein	bovine	porcine	(Zarkadas et al., 1988)	(1983)	(1957)
i. myofibrillar[b]					
actin	97.12	96.43	110.4	126.9	120.0
myosin	278.93	276.95	229.4	248.2	340.0
total	578.54	574.43	522.6	577.1	620.0
ii. other proteins	364.45	370.68	430.0		340.0
iii. connective tissue[c]	63.68	50.59	259.0		
collagen[d]	47.32	49.88	262.0		

[a] Extracted according to the method of Rangeley and Lawrie (1976). [b] Calculated from His(τ-Me) data (Table 2) using eqs 5b, 5c, 6b and 6c. These calculations were based on the molar ratio of myosin to actin of 1:4 reported by Murakami and Uchida (1985). [c] Calculated from Pro(4-OH) data (Table 1) using eq 4c. [d] Calculated from Lys(5-OH) data (Table 2) using eq 4b.

In addition to myofibrillar and connective tissue proteins (such as collagen), cardiac muscles contain a large quantity of other intracellular proteins, which on the average represent 36.5−37.1% of the total muscle proteins (Table 4). Hanson and Huxley (1957) have shown that the average quantity of soluble protein (sarcoplasmic) washed out of the glycerol-extracted skeletal muscle after it has been broken up into fibrils is 28−32% of total protein.

Evaluation of Protein Quality of Cardiac Muscles. Amino acid composition data for the evaluation of protein quality of foods and diets has been widely used since the amino acid composition of egg was introduced as a standard by Block and Mitchell (1946). Mean values for total EAA were 2587 mg/g of nitrogen (N) in the cardiac muscle of mature cows and 2702 mg/g of N found in the porcine cardiac muscle excised from mature sows (Table 5). Similar results were obtained from the EAA indices and chemical scores (Oser, 1951; FAO/WHO, 1965).

A more accurate assessment of meat and poultry protein quality uses their amino acid composition and

Table 5. Essential Amino Acid (EAA) Scores of Bovine and Porcine Cardiac Muscle Tissues and Other Animal Proteins and EAA Requirements of Preschool Child

	(EAA)				
	EAA[a] requirements of	cardiac muscle tissue		other animal products	
(EAA)	preschool child (2−5 years old)	bovine	porcine	egg[a]	beef[b]
	Milligrams of Amino Acid per Gram of Total Protein[c]				
histidine	19	31	28	22	34
isoleucine	28	48	46	54	48
leucine	66	93	92	86	81
lysine	58	88	89	70	89
methionine + cyst(e)ine	25	39	40	57	40
phenylalanine + tyrosine	63	82	86	93	80
threonine	34	45	47	47	46
tryptophan	11	18	18	17	12
valine	35	54	57	66	50
mg of EAA/g of N[d]		2578	2702		
EAA_9[c]	33.9	49.8	50.3		
EAA_{10} including arginine[e]		57.2	57.4		
EAA index[f]		79.6	81.5		
chemical score[f]		78.1	78.9		
protein efficiency ratio (PER_{10})[e]		3.46	3.47		
	Percent Protein Digestibility in Man[a]				
		95	95	97	98
	Percent Amino Acid Score[c]				
		100	100	100	100
	Protein Digestibility Corrected Amino Acid Score[c]				
		95	95	97	98

[a] Data from FAO/WHO/UNU (1985) and FAO/WHO (1991). [b] Data taken from Bodwell (1987). [c] Calculation of protein ratings was carried out by comparison of the amino acid composition of bovine and porcine cardiac muscle tissues with that of the reference pattern established by FAO/WHO/UNU (1985) for a preschool child (2−5 years old). EAA_9: histidine, isoleucine, leucine, lysine, methionine plus cysteine, phenylalanine plus tyrosine, threonine, tryptophan, and valine. [d] Computed from reference protein standards (FAO/WHO, 1965). [e] Calculated according to the method of Lee et al. (1978). EAA_{10}: EAA_9 plus arginine. PER_{10} were calculated from eq 7 [$PER = 0.06320(EAA_{10}) - 0.1539$]. [f] Calculated according to the methods of Block and Mitchell (1946) and Oser (1951).

connective tissue protein content to calculate PER (Expert Work Group, 1984; Lee et al., 1978; Pellett and Young, 1984). Lee et al. (1978) identified the 10 EAA (EAA_{10}) as being threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine, and tryptophan. The mean value for EAA_{10} averaged 57.3% (Table 5), and the calculated mean PER_{10} value for cardiac muscles was 3.46 (Table 5), compared to 3.2 reported for skeletal muscle (FAO/WHO, 1990), both of which are considerably higher than the reported average rat bioassay PER values of 2.4−3.1 for various meat and poultry products reported by others (Happich et al., 1975).

The Joint FAO/WHO Expert Consultation Group (FAO/WHO/UNU, 1985; FAO/WHO, 1991) has recommended that, in conjuction with *in-vivo* protein digestibility data (Carpenter, 1984), the reference amino acid patterns for the 2−5-year-old child be used as the reference pattern (Table 5) in the evaluation of foods for all persons except infants. The nine essential amino acids included all of the above (EAA_{10}) except arginine (FAO/WHO/UNU, 1985). The two sulfur-containing (methionine plus cystine) and the two aromatic (phenylalanine plus tyrosine) amino acids are usually considered together. Cardiac muscles contained all of the EAA_9, ranging from 49.8 to 50.3%, which is considerably higher than the 33.9% reference value given by FAO/WHO (1991) required for the 2−5-year-old child. Cardiac muscle has a true protein digestibility of about 85% (Happich et al., 1975; Carpenter, 1984). The mean EAA_9 value for corrected amino acid scores for cardiac muscles was 85%. The mean value for amino acid score, corrected for digestibility, was 85%, indicating that cardiac muscles, which are low in connective tissue proteins, are of high protein quality (Anderson, 1988).

The results of the present study have provided statistically and experimentally sound amino acid data on bovine and porcine cardiac muscle tissues and have shown that the protein quality of cardiac muscles can be assessed from their FAO/WHO (1991) amino acid scoring pattern. The study has also shown that the myofibrillar α_c-actin, collagen, and collagen-like proteins in cardiac muscle can be determined from the amounts of His(τ-Me) and Lys(5-OH) present, respectively, and has reported, for the first time, the His(τ-Me) content and molecular weights of highly purified bovine and porcine cardiac α_c-actins.

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