Physicochemical Properties and Partial Purification of Porcine Muscle Cathepsin

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Properties of porcine muscle cathepsin extracted with water and dilute salt solutions were studied. Its activity in hydrolyzing denatured hemoglobin was maximal at pH 4.0, and there were also optima at pH 8.0 and pH 10.0. The enzyme was readily inactivated at 65° C., and it had an activation energy of approximately 7.0 kcal. per mole. Its activity was stimulated by ferrous ions and by calcium chloride but not by cysteine, L-adrenaline, or iodoacetate. These properties are similar to those of spleen cathepsin D. The enzyme was partially purified by DEAE-cellulose chromatography and by fractionation with ammonium sulfate and zinc-ethanol. Degree of purity was monitored by polyacrylamide gel electrophoresis.

Much of the previous work with cathepsins has been on protein from spleen (*8*, *10*, *13*, *15*, *20*, *21*, *27*, *32*), lung (*6*, *7*), erythrocytes (*25*), endocrine glands (*1*, *33*), and brain (*2*); and considerable basic knowledge concerning the properties of these enzymes has been gained. Properties of cathepsin from muscle tissue, on the other hand, have been studied only sparingly and little information has accumulated (*4*, *5*, *16*, *17*, *19*, *30*, *31*) concerning proteases from this source.

The investigation of the physicochemical properties of muscle cathepsins is particularly significant to the meat researcher, since autolysis of muscle tissue has been implicated in meat tenderness changes during post-mortem aging. Aseptic autolysis has been studied by Zender and coworkers (*35*) and Sharp (*29*); both reported that autolysis plays a role in muscle protein breakdown post-mortem.

The purpose of this study was to investigate some of the fundamental properties of cathepsins associated with the water and dilute salt-soluble proteins of porcine muscle.

Materials and Methods

Preparation of Enzyme. Immediately following slaughter, muscle tissue was removed aseptically from the ham or loin of pork carcasses, cut into small sections, and frozen immediately with dry ice. The tissue was stored in glass jars at −17.8° C. prior to subsequent study; cathepsins were extracted by blending 2 parts of solvent with 1 part of tissue. Solvents used for extraction of the sarcoplasmic proteins included distilled water, 0.1*M* acetate buffer (pH 5.6), 0.1*M* phosphate buffer (pH 6.0), and 2% potassium chloride. The mixtures were blended for 10 minutes at 1.5° C. in a Servall Omni-mixer and centrifuged (500 × g, 30 minutes) and the centrifugate was dialyzed against deionized water for 24 hours at 1° C. Following dialysis, the supernatant was centrifuged (500 × g, 15 minutes) to remove flocculent material and the centrifugate used as the crude enzyme source.

Assay of Cathepsin Activity. A procedure similar to that of Anson (*3*), modified by Sliwinski and coworkers (*30*), was used to assay cathepsin activity.

Equal parts (10 ml.) of enzyme and substrate were incubated at 37° C. along with other flasks containing reagents necessary for determining exogenous activity of the substrate and endogenous activity of the enzyme. Duplicate 2-ml. samples of each were withdrawn from the reaction mixtures after a 5-minute equilibration period and following designated reaction intervals (usually 120 minutes) and were pipetted into centrifuge tubes containing 2 ml. of 10% trichloroacetic acid (TCA). Samples were cooled to 4° C. prior to centrifugation (500 × g, 15 minutes) and measurement of TCA-soluble substances at 274 mμ or alternatively at 655 mμ after reaction with the Folin-Ciocalteu color reagent (*23*). When L-adrenaline was used in the enzyme-substrate mixture, tyrosine was determined by the method of Udenfriend and Cooper (*34*).

Relative activity was expressed as the increase in absorbance in excess of exogenous activity of substrate and endogenous activity of the enzyme during the reaction period. Specific activity was expressed as the increase in absorbance in excess of endogenous and exogenous activity per milligram of protein per milliliter times 1000. Protein was determined by a biuret procedure (*14*).

Assays of crude porcine cathepsin and purified porcine cathepsin (50 to 60% ammonium sulfate precipitate of crude preparation) activities were made on the synthetic substrates, carbobenzoxy-L-glutamyl-L-tyrosine, benzoyl-L-argininamide, and glycyl-L-phenylalanine amide. Substrates (0.1*M*) were dissolved in 0.1*M* citrate buffer, pH 5.0. A mixture containing equal parts of enzyme and substrate was incubated at 37° C. Aliquots of 0.2 ml. were withdrawn at 0, 30, and 60 minutes, and rate of hydrolysis was determined by titration in acetone with alcoholic hydrochloric acid (*22*).

pH Optima Studies of Crude Porcine Cathepsin. Optimum pH was determined by buffering urea-denatured hemoglobin to pH 2.0, 3.0, 4.0, and 5.0 with 0.1*M* sodium acetate–hydrochloric acid buffer; to pH 6.0, 7.0, and 8.0 with 0.1*M* sodium phosphate buffer; and to pH 9.0 and 10.0 with 0.1*M* glycine–sodium hydroxide buffer. Equal quantities of crude porcine cathepsin and buffered hemoglobin were incubated for 2 hours at 37° C., and activities determined.

Thermal Activation and Inactivation. Energies of activation were computed from Arrhenius plots using activities obtained by incubating both crude and purified porcine cathepsins at 30°, 35°, 40°, and 45° C. Temperature coefficients (ratio of velocity at temperature $t + 10°$ to that at temperature $t°$) were determined on both enzyme preparations between 35° and 45° C.

The half life of crude porcine cathepsin to heating at 65° C. was determined by removing aliquots from the water-extracted cathepsin preparation at zero time and after 10, 20, 30, 40, 50, and 60 minutes. The samples were chilled in ice water for 5 minutes and assayed for cathepsin activity as outlined above.

Addition of Activators and Inhibitors. Urea-denatured hemoglobin substrate was prepared to contain the appropriate amounts of inorganic or organic activator or inhibitor. Additional control tubes containing substrate, ferrous ions, and buffer were incubated along with the usual experimental samples when ferrous ammonium sulfate was used as an activator because of the possibility of oxidation of ferrous ion to ferric ion which absorbs light in the ultraviolet.

Purification Studies. The proteins

in water extracts of porcine muscle were fractionated with ammonium sulfate at 10% saturation intervals from 0 to 100% saturation using the amounts of salt listed in a table published by Green and Hughes (*12*). Proteins precipitated within each 10% saturation interval were retained for analysis. In all cases the salt was added slowly with continuous stirring at 0° C., and after addition of ammonium sulfate the solutions were kept at 1° C. overnight and then centrifuged at 10,000 × g for 30 minutes. The precipitated proteins studied for cathepsin activity were dissolved in cold distilled water and dialyzed for 24 hours against cold distilled water prior to analysis.

In subsequent experiments, proteins were precipitated by adding 313 grams of solid ammonium sulfate to each liter of supernatant from water extracts of porcine muscle to make a 50% saturated solution. The precipitate was discarded and 66 grams of solid ammonium sulfate was added to each liter of supernatant to make a 60% saturated solution. The resulting precipitate was referred to as the 50 to 60% ammonium sulfate fraction. It was dissolved in distilled water and dialyzed as outlined above prior to assay. The zinc-ethanol method described by Snoke and Neurath (*31*) was also used to fractionate water-extracted porcine sarcoplasmic proteins.

DEAE-cellulose (Bio-Rad, Cellex D, exchange capacity of 0.56 meq. per gram) slurries were prepared and columns packed according to the method of Peterson and Sober (*26*). The packed columns were cooled by circulating ice water. Protein (100 mg.) precipitated by the addition of ammonium sulfate (50 to 60% fraction) to the water extract of porcine muscle was further purified by stepwise elution chromatography, using 300 ml. of carbon dioxide–free water followed by 900 ml. of carbon dioxide–saturated water (*24*). Ten-milliliter fractions were collected and protein content was measured at 280 mμ. Fractions containing protein were assayed for cathepsin activity with urea-denatured hemoglobin substrate (pH 4.0) and protein was determined by a biuret method (*14*).

Polyacrylamide (Cyanogum) Gel Electrophoresis (PAGE) of Bovine and Porcine Sarcoplasmic and Bovine Granular Proteins. Electrophoresis was carried out with an EC470 vertical gel electrophoresis apparatus supplied by the E-C Apparatus Corp. The gel was prepared by adding 10.5 grams of Cyanogum to 150 ml. of 0.08*M* tris (hydroxymethyl)aminomethane, 0.01*M* boric acid, and 0.02*M* sodium EDTA, ionic strength 0.08, pH 8.8. A catalyst (0.1 ml. of TMED) was added to the gel, followed by filtering, and then 0.1 gram of ammonium persulfate was added just prior to use. The gel was poured into the tray, where it polymerized. Buffer was poured into the reservoir and samples [50 μl. containing 8% (w./v.) sucrose] were pipetted into sample slots.

A field strength of 12.9 volts per cm. was applied across the gel for the first 30 minutes. Subsequently, the field strength was increased to 16.1 volts per cm. for the remaining 120 minutes of the run. The gel was chilled to 20° with circulating water during the 150-minute period. At the end of this time the gel was stained for 5 minutes with a dye solution composed of 500 ml. of methanol, 500 ml. of water, 100 ml. of acetic acid, and 2 grams of amido black 10B. The destaining of the gel background was accomplished by washing with a solution of water, 4 liters of methanol, and 1 liter of acetic acid.

Protein bands were measured and mobilities calculated by using a weighted mean field strength of 15.5 volts per cm. The following equation was used to calculate electrophoretic mobilities:

$$u = \frac{d}{Ft}$$

where u = electrophoretic mobility
d = distance over which protein migrates, cm.
t = time, seconds
F = field strength, volts per cm.

Results and Discussion

Extraction with Inorganic Solvents. Results obtained from study of various extraction media are shown in Table I. These data indicate very little difference in the extraction of cathepsins with the various solvents employed. Acetate buffer–extracted protein contained slightly more catheptic activity as measured by absorbance at 274 mμ and by Folin's reagent. Phosphate buffer–extracted protein contained slightly greater activity as measured by Folin's reagent. Increased activity was not sufficient to warrant routine use of these buffers as extracting media. Subsequent experiments on porcine cathepsin activity were on water-extracted protein.

Assay of Cathepsin A, B, and C Activities. Considerable difficulty was encountered in detecting hydrolysis of synthetic peptides by crude porcine cathepsin. The synthetic substrates for spleen cathepsins A, B, and C (carbobenzoxy-L-glutamyl-L-tyrosine, benzoyl-L-argininamide, and glycyl-L-phenylalanine amide, respectively) were only slightly hydrolyzed after 1 hour at 37° C. in the presence of crude porcine muscle extracts. The per cent hydrolysis was 3 to 5% for the substrates of cathepsins A and B, and 12% for the substrate of cathepsin C. Cathepsin prepared by precipitating protein with ammonium sulfate (50 to 60% saturated) did not catalyze hydrolysis of the synthetic substrates, even though this purified cathepsin catalyzed the hydrolysis of hemoglobin. The activity measured was apparently different from that of cathepsins A, B, and C. Several workers have studied muscle cathepsins that did not hydrolyze synthetic substrates for proteases (*1, 5, 33*). Press, Porter, and Cebra (*27*) isolated and studied properties of a protease from bovine spleen and found that it accounted for two thirds of the total cathepsin activity and existed in ten forms, none of which catalyzed hydrolysis of synthetic substrates for cathepsin A, B, or C. This enzyme did, however, catalyze hydrolysis of hemoglobin and was referred to as cathepsin D. Lapresle and Webb (*21*) later described a proteolytic enzyme in bone marrow which did not catalyze hydrolysis of the substrates for cathepsin A, B, or C and was also different from cathepsin D. They named this proteolytic enzyme cathepsin E. The activity of porcine cathepsin in the presence of hemoglobin in most respects is similar to that of bovine spleen cathepsin D.

Table I. Crude Porcine Cathepsin Activity in Extracts Obtained by Using Various Solvents

Extracting Solvent	Specific Activity[a] 665 mμ[b]	274 mμ[c]
Distilled water	25.2	14.6
0.1*M* acetate buffer (pH 5.6)	25.2	16.5
0.1*M* sodium acetate	19.4	12.2
0.1*M* citrate buffer (pH 5.6)	21.6	10.5
0.1*M* phosphate buffer (pH 6.0)	27.6	14.5
2% potassium chloride	20.5	9.9

[a] Increase in absorbance (2-hour reaction time) per mg. of protein per ml. × 1000.
[b] Change in absorbance at 665 mμ following reaction with Folin's reagent.
[c] Change in absorbance at 274 mμ.

Data in Figure 1 show that the reactivity of the original extract (13.0 mg. per ml. of protein) was not linear with time, and it appeared that insufficient substrate was present to saturate the enzyme or that an inhibitor was present. The activities of samples diluted 1-to-1, 1-to-3, 1-to-7, and 1-to-15 were proportional after 2 hours' reaction and their activities were linear during the reaction period. There may be some type of inhibition of the reaction at the higher enzyme concentration, but the kinetics of the reaction do not indicate inhibition. Finkenstaedt (*9*) studied an inhibitor of cathepsin B in the soluble fraction of rat liver extracts, and Sliwinski, Doty, and Landman (*30*) found cathepsin inhibitors in extracts of beef muscle.

pH Optima. The effect of hydrogen ion concentrations between pH 2.0 and 10.0 on porcine cathepsin activity is shown in Figure 2. Three pH optima for the enzyme-hemoglobin reaction were found. Activity peaks occurred at pH 4.0, 8.0, and 10.0, but the activities of all samples below pH 5.0 were greater than those of samples above pH 6.0 and maximal activity occurred in media buffered at pH 4.0. Subsequent analyses used to measure hydrolysis of hemoglobin were carried out at pH 4.0.

These pH optima are in the range of those reported by many workers for the activity of proteases from muscle of

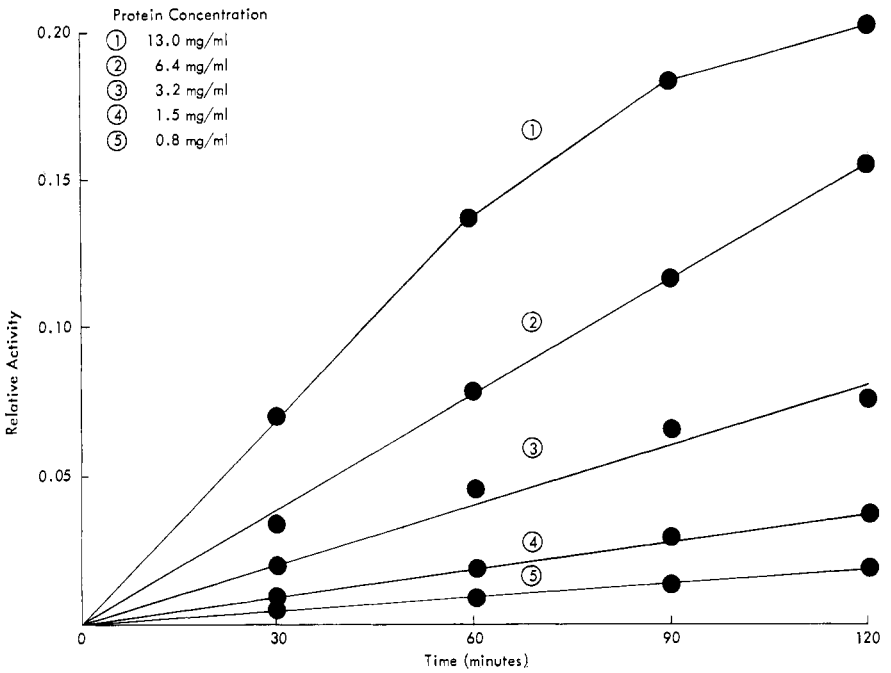

Figure 1. Effect of enzyme concentration on porcine cathepsin activity with hemoglobin substrate, pH 4.0

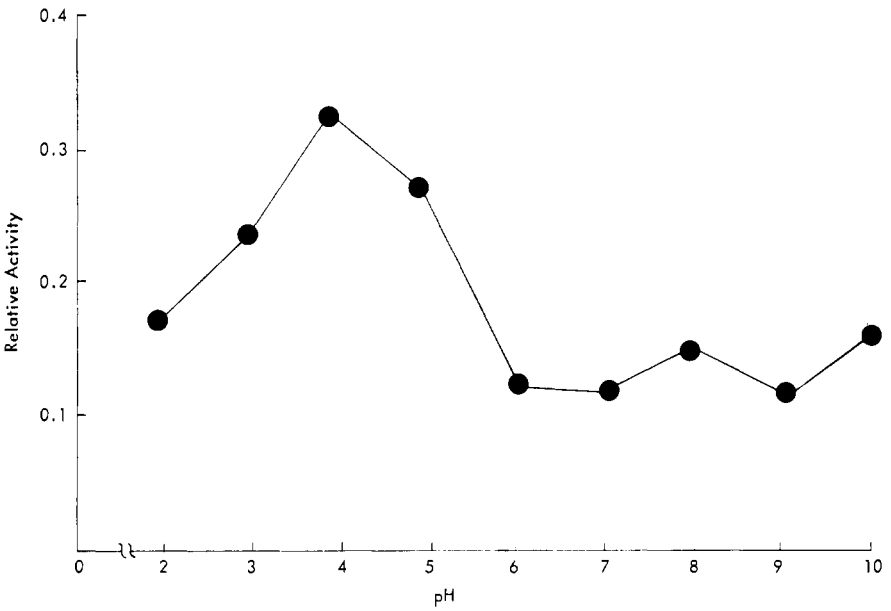

Figure 2. pH optima of porcine muscle cathepsin assayed at 37° C.

various species (*4*, *16*, *31*, *32*), and are also similar to those reported for protease activity of organ and glandular tissue (*1*, *6*, *34*). Cathepsin D from bovine spleen was reported (*27*) to have a pH optimum at 4.2 when albumin was used as substrate and cathepsin E from rabbit has a pH optimum at 2.5 (*21*).

Thermal Activation and Inactivation. Studies of the effects of heating on the activity of porcine cathepsins involved the examination of the relative activity at 30°, 35°, 40°, 45°, and 50° C. Activities (Figure 3) increased progressively until the temperature reached 45° C. and then decreased at 50° C. The optimum temperature for porcine cathepsin activity under the conditions employed would appear to be approximately 45° C.

Activation energy for both crude and purified porcine cathepsins was computed from Arrhenius plots (Figure 4). Values for the crude and purified pork cathepsins were 6.93 and 7.23 kcal. per mole, respectively. These data indicate that the crude and fractionated cathepsins are similarly affected by heating and that heating has less effect on placing the molecules in a reactive state than it does with other proteases (*18*). The temperature coefficients between 35° and 45° C. of the reactions were 1.39 and 1.45 for the crude and purified enzymes, respectively.

Heating at 65° C. reduced the activity of the crude enzymes rather rapidly. Figure 5 shows that the half life of the enzyme at 65° C. was approximately 6 minutes. The activity measured was not related to cathepsin C from beef spleen, which is active after heating at 65° C. for 40 minutes (*32*). Cathepsins D and E are readily inactivated at 60°C. (*20*, *27*). The cathepsin in crude porcine muscle extract was completely inactivated after heating at 100° C. for 2 minutes.

Effect of Activators and Inhibitors on Porcine Cathepsin Activity. Data concerning the effects of two concentrations of various metal ions on the activity of purified porcine cathepsin are presented in Table II. Results indicate that 0.001*M* calcium chloride as well as 0.001*M* and 0.01*M* ferrous ammonium sulfate stimulated activity. Cathepsin activity was diminished slightly by calcium chloride, magnesium sulfate, and zinc acetate at 0.01*M*. Threefold increase in catheptic activity of rabbit muscle has been obtained with 0.02*M* ferrous ions (*32*), and with rat muscle cathepsin using 0.001*M* ferrous ions (*17*). Kozalka and Miller (*17*) also found that the divalent cations, barium, calcium, magnesium, manganese, and zinc, at 0.01*M* concentrations, inhibited proteolytic activity of rat muscle cathepsin.

The use of ferrous ammonium sulfate at concentrations of 1×10^{-2} to $5 \times 10^{-2}M$ resulted in increased catheptic activity (Table III). Maximum activ-

Table II. Effect of Two Concentrations of Metal Ions on Relative Activity of Purified Porcine Muscle Cathepsin[a]

Inorganic Salt	Relative Activity[b] 0.01 M[c]	0.001 M[c]
Control	0.070	0.079
Calcium chloride	0.065	0.100
Magnesium sulfate	0.054	0.083
Zinc acetate	0.056	0.072
Ferrous ammonium sulfate	0.178	0.111

[a] Ammonium sulfate fraction 50 to 60% of porcine muscle water extract.
[b] Change in absorbance at 274 mμ.
[c] Salt concentration.

Table III. Effect of Ferrous Ion Concentration on Relative Activity of Crude Porcine Muscle Cathepsin

Ferrous Ion Concn., Mole per Liter	Relative Activity[a]
0.00	0.121
0.01	0.152
0.02	0.195
0.03	0.226
0.04	0.271
0.05	0.251

[a] Change in absorbance at 274 mμ.

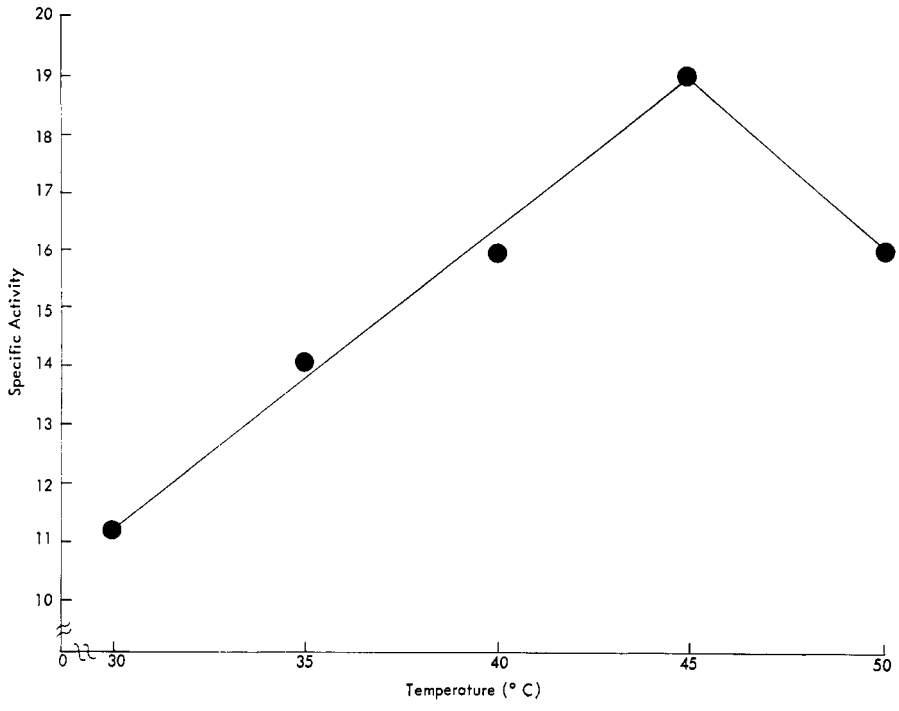

Figure 3. Optimum temperature for activity of porcine cathepsin

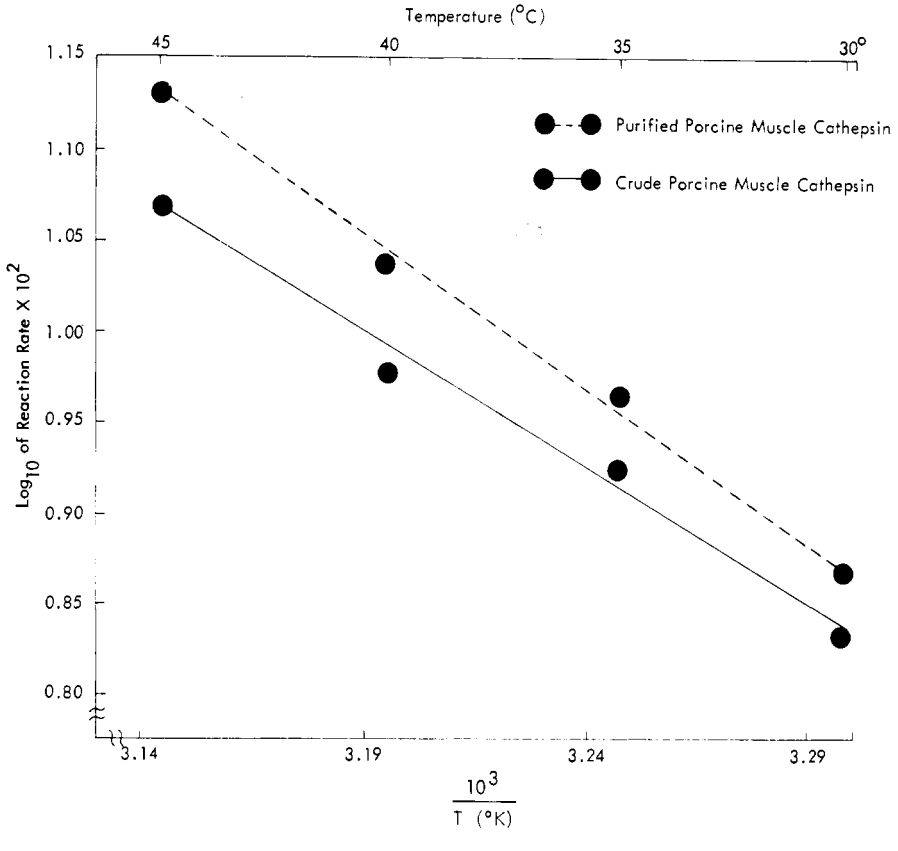

Figure 4. Arrhenius plots of crude and purified porcine muscle activities between 30° and 45° C.

ity occurred when the salt was used at a concentration of $4.0 \times 10^{-2} M$.

Iodoacetic acid ($1 mM$) and cysteine $0.01 M$ had no effect on the activity of crude or purified (50 to 60% ammonium sulfate fraction) cathepsin. The ineffectiveness of iodoacetic acid on activity and the inability of cysteine to potentiate activity indicate that sulfhydryl groups are not necessary for the function of porcine muscle cathepsin. L-Adrenalin in concentrations from 11 to 55 μM also did not affect activity of porcine cathepsin. This was somewhat surprising, since Radouco-Thomas (*28*) showed that ante-mortem injections of adrenalin suppressed the proteolytic activity of muscle, which resulted in more desirable post-mortem muscle characteristics. Conversely, Gordon and Zak (*11*) found that the addition of physiological doses of L-adrenalin enhanced cathepsin activity of a myosin preparation from rabbit muscle.

Purification Studies. Results of ammonium sulfate fractionation are given in Table IV. A $2^1/_2$-fold purification was obtained by precipitating protein from the aqueous extract of pork with ammonium sulfate (50 to 60% saturation).

Results obtained using zinc-ethanol as a protein precipitant are shown in Table V. The initial specific activity of the crude enzyme preparation was 18.8. The activity was increased to 26.9 by heating the crude preparation at 37° C. for 10 minutes followed by centrifugation (500 × g, 15 minutes) to remove insoluble protein. Addition of TCA and pH adjustment to 4.0 produced a precipitate which when dissolved in water had a specific activity (35.1) almost twice as great as that of the crude extract. The greatest increase in activity was obtained with protein precipitated as the zinc salt in 19% ethanol (fraction A). This re-

Table IV. Ammonium Sulfate Fractionation of Porcine Cathepsin[a]

Ammonium Sulfate, %	Specific Activity[b]	Purification
0–10	None	...
10–20	4.7	...
20–30	5.7	...
30–40	11.8	...
40–50	16.0	...
50–60	47.6	2.53
60–70	20.3	1.08
70–80	17.4	...
80–90	19.9	1.06
90–100	7.0	...

[a] Water extract of porcine muscle cathepsin; specific activity 18.8.
[b] Change in absorbance at 274 mμ per mg. of protein per ml. × 1000.

Table V. Effect of Heating at 37° C. and Precipitation with Sodium Trichloroacetate, Zinc - Ethanol, and Alkali on Porcine Cathepsin Activity[a]

Treatment	Specific Activity[b]	Purification
Crude extract heated at 37° C. for 10 minutes	26.9	1.43
Heated extract after trichloroacetate treatment at pH 4.0	35.1	1.87
Zinc - ethanol precipitation at pH 5.8 (fraction A)	183.7	9.77
Alkaline precipitation at pH 7.5 (fraction B)	96.0	5.11

[a] Change in absorbance at 274 mμ per mg. of protein per ml. × 1000.
[b] Water extract of porcine muscle cathepsin; specific activity 18.8.

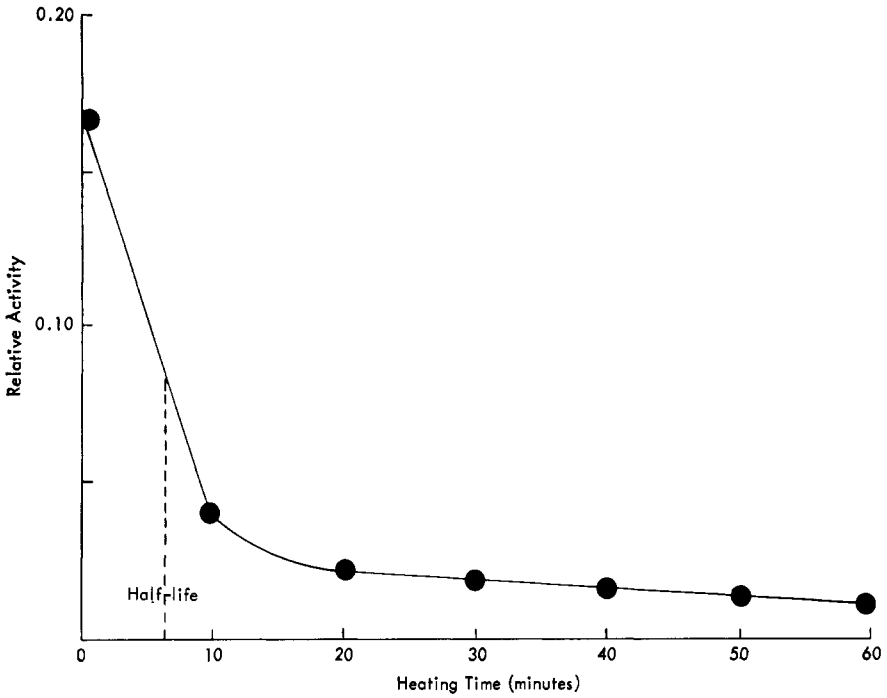

Figure 5. Effect of heating at 65°C. on porcine muscle cathepsin activity

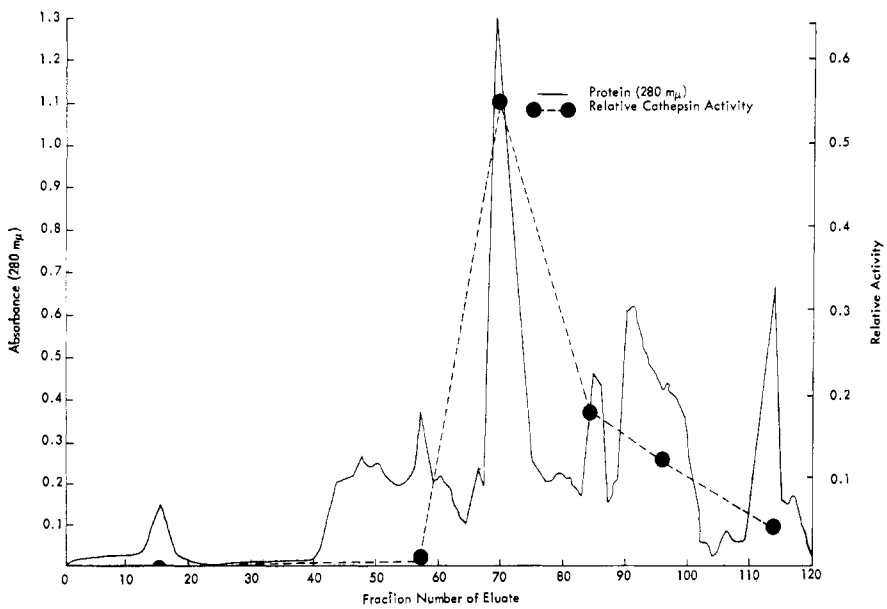

Figure 6. DEAE-cellulose chromatogram of proteins from 50 to 60% ammonium sulfate fraction of porcine muscle extract

sulted in a tenfold increase over the crude and more than a $3^1/_2$-fold increase in specific activity over the 50 to 60% ammonium sulfate fraction. More than a fivefold increase in specific activity was obtained with protein precipitated by 1*M* alkali at pH 7.5 (fraction B).

Snoke and Neurath (*37*) obtained a 1100-fold purification of rabbit muscle (cathepsin) in fraction B. A similar fractionation procedure used to purify proteases of chicken breast muscle resulted in a 163-fold purification in fraction A (*4*).

Cellulose Ion Exchange Chromatography. DEAE-cellulose chromatography separation of the 50 to 60% ammonium sulfate preparation by the stepwise elution method of Mitz and Yanari (*24*) is shown in Figure 6. The only protein peak obtained with carbon dioxide–free water was a breakthrough peak at fraction 15. The addition of carbon dioxide–saturated water eluted several protein peaks. However, most activity was obtained from fraction 70, which had a specific activity of 366.4. This was approximately an 8-fold purification of the 50 to 60% ammonium sulfate fraction and a 20-fold purification of the original extract. An 18-fold increase in specific activity of bovine muscle cathepsins was obtained by Sliwinski, Doty, and Landman (*30*), who used a similar chromatographic procedure.

Combined CM- and DEAE-cellulose ion exchange chromatography utilized for the purification of bovine spleen cathepsin D by Press, Porter, and Cebra (*27*) was not as effective as the DEAE-cellulose ion exhange method discussed above for the purification of procine cathepsin as measured by hemoglobin hydrolysis. Over-all purification obtained through use of the former method with porcine muscle cathepsin was approximately 16-fold.

Polyacrylamide Gel Electrophoresis. Data concerning the electrophoretic migration of proteins from porcine muscle are presented in Table VI. Protein of water extracts of porcine semitendinosus muscle were separated into 11 distinct bands under the con-

Table VI. Electrophoretic Mobilities of Porcine Sarcoplasmic Proteins

	PAGE[a] Bands and Mobilities, Sq. Cm. Volt^{-1} Sec.$^{-1}$ × 10^5										
Preparation	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Crude extract	0.39	0.98	1.43	1.56	1.76	2.02	2.48	2.80	3.06	3.58	4.80
$(NH_4)_2SO_4$ precipitate (50 to 60% cut)	0.52	1.17	1.43	...	1.62	...	2.48	...	...	3.58	5.08
Zinc-ethanol precipitate (fraction A)	...	...	...	...	...	...	...	...	...	3.58	5.00

[a] Polyacrylamide gel electrophoresis.

Gel contained tri(trihydroxymethyl)aminomethane-borate buffer pH 8.8; ionic strength 0.08; protein load approximately 250 μg.; field strength was 12.9 volts cm.$^{-1}$ for 30 minutes and subsequently 16.1 volts cm.$^{-1}$ for 2 hours.

ditions employed. The fastest migrating band had an electrophoretic mobility of 4.80 sq. cm. volt^{-1} sec.$^{-1}$ $\times$ 10^{-5}. Fractionation with ammonium sulfate (50 to 60% cut) removed bands 4, 6, 8, and 9. Further fractionation of the sample with zinc-ethanol (fraction A) removed all proteins except those migrating as bands 10 and 11. Protein in band 11 was extremely low in concentration compared to that in band 10, but was present in all fractions having cathepsin activity. Attempts to remove protein from the gel to determine which of the two bands contained the active enzyme were unsuccessful.

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Received for review July 6, 1965. Accepted January 12, 1966. Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 2957.

SWEET POTATO AMYLASE

New Characteristic Alpha-Amylase in Sweet Potatoes

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A new amylolytic enzyme has been demonstrated in raw sweet potato juice. Its dextrinizing activity places it with the α-amylases. Among its unusual characteristics were high optimum activity temperature, heat stability, and low activity at ordinary temperatures. The optimum temperature was 70° to 75° C. The enzyme showed maximum resistance to heat inactivation at pH 6.0, while a much wider range of pH (3.6 to 8.1) was tolerated at 30° to 45° C. At these temperatures, however, the activity was low. Freshly harvested sweet potatoes contain relatively small amounts of this enzyme, which increases about sixfold after 9 months' storage. Unlike the sweet potato β-amylase, this enzyme is distributed almost uniformly throughout the inner tissues of the root and is more soluble in water than in sweet potato juice.

THE occurrence of an active diastase in sweet potatoes was clearly shown in 1920 by Gore (*7*), who demonstrated that slow cooking of sweet potatoes through a range of 60° C. to the boiling point gave a very high conversion of starch into soluble carbohydrates. Giri (*6*) reported that sweet potato amylases were similar to malt β-amylase. Balls, Thompson, and Walden (*3*) crystallized sweet potato β-amylase and observed that the amylolytic activity was almost entirely due to β-amylase. Since that time many workers have concentrated or isolated β-amylase from sweet potato juice. Giri (*5*) and Ikemiya (*9*) have reported that sweet potatoes show some α-amylase activity.

This paper reports a series of experiments designed to show the nature of, and the cause for, observed changes in processing characteristics of sweet potatoes during storage. These experiments demonstrate that freshly pressed juice contains dextrinizing (α-amylase) activity which is readily apparent only at higher temperatures than are normally considered optimum for α-amylase activity. Some properties of the enzyme are presented: optimum pH and temperature, effect of the interaction of pH and temperature on the activity, and destruction of the enzyme. Preliminary data on the concentration of the enzyme, the yields from different extraction treatments, distribution in the roots, and increase in enzyme concentration in the juice during storage are also included.

Materials and Methods

Samples of several shipments of Goldrush variety sweet potatoes from the 1963