DEGRADATION OF PORCINE MUSCLE PROTEINS BY BLOOD LYSOSOMAL PROTEINASES

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The molecular weights of porcine leukocyte lysosomal proteinases were determined by (SDS)-polyacryalamide gel electrophoresis and these enzymes used to degrade actomyosin, actin, myosin, tropomyosin and troponin.

Degradation of various myofibrillar proteins was demonstrated by viscosity measurements, electrophoresis, NPN-analyses and Sephadex gel filtration.

Functional properties of troponin, tropomyosin and actomyosin were changed by treating with blood lysosomal proteinases at pH 7.0 for 12 hours.

La Dégradation des protéines des muscles du porc par les protéinases des lysosomes du sang

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Les poids moléculaires des protéinases des lysosomes des leucocytes du porc ont été déterminés par électrophorèses sur gel (SDS) - polyacrylamide et à l'aide de ces enzymes on a dégradé de l'actomyosine, de l'actine, de la myosine, de la tropomyosine et de la troponine.

La dégradation des diverses protéines des myofibrilles a été démontrée par des mesures de viscosité, électrophorèse, analyses NPN et gel-filtration Sephadex.

Les propriétés fonctionnelles de la troponine, tropomyosine et actomyosine ont été changées par le traitement utilisant les protéinases des lysosomes du sang pendant 12 heures à pH 7.0.

Degradation Muskularen Proteins Von Schweinen mit Lysosomalen Blutproteinasen

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Die molekulargewichte der lysosomalen leukozyten-proteinasen von schweinen wurden mit (SDS) - polyacrylal-amid-gel-elektrophorese bestimmt, und diese enzyme wurden benutzt um actomyosin, actin, myosin, tropomyosin und troponin zu bestimmen.

Die degradation verschiedener myofilbrillarer proteine wurden mit viskositätsmessungen, elektrophorese, (NPN) - analyse und Sephadex-gel-filtration bestimmt.

Die funktionalen eigenschaften von troponin, tropomyosin, und actomyosin wurden durch 12 stunden lange behandlung mit blut-lysosomalen proteinasen mit einem pH von 7,0 bestimmt.

ДЕГРАДАЦИЯ СВИНЫХ МЫШЕЧНЫХ ПРОТЕИНОВ ЛИСОСОМАТИЧЕСКИМИ ПРОТЕИНАЗАМИ КРОВИ

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Молекулярный вес лисосоматичкеских протеиназ свиных лейкоцитов был определен при помощи электрофореза полиакриламидного геля /SDS / и эти энзимы использовались для деградации актомиозина, актина, миозина, тромиозина и тропонина.

Была продемонстрирована деградация различных миофибриллярных протеинов при помощи измерений вязкости, электрофореза, анализов NPN и фильтрации Сефадексового геля.

Путем обработки лисосоматическими протеиназами крови при pH 7.0 в течение 12 часов изменились функциональные свойства тропонина, тропомиозина и актомиозина.

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SUMMARY

Differential sedimentation procedures were used to isolate lysosomes from porcine leukocytes and their constituent proteinases studied.

The leukocyte lysosomes in various sedimentation fractions appeared under the electron microscope as dense bodies ranging in size from 0.13 to 0.34 μ.

SDS-polyacrylamide gel electrophoresis, ultracentrifugation and Sephadex chromatography were used to determine molecular weights of degradation products following reaction of lysosomal enzymes with actomyosin, actin, tropomyosin and troponin. Polypeptides of 10-12,000 M.W. and a smaller fraction (M.W.=4,600) accumulated during activity of these enzymes at 37°C.

Changes in structure of the various muscle proteins during hydrolysis by leukocyte lysosomal proteinases were also demonstrated by specific viscosity measurements and accumulation of TCA-soluble NPN.

Hydrolysis of troponin by lysosomal enzymes also influenced its interaction with tropomyosin to form viscous solutions.

Hydrolysis of actomyosin also influenced its emulsifying and gelling properties.

INTRODUCTION

Proteolysis of myofibrillar proteins and its relationship to post-mortem tenderization have received considerable emphasis. However, there is still confusion and controversy in the extent and role of proteolysis in post-mortem tenderization. The confusion may be attributed, partially to the methods of analysis and difficulty of interpreting results observed during study of this complex system.

Recent studies by Canonico and Bird (1970) indicate that there are at least two sources of lysosomes in normal muscle, one from macrophages and the other from muscle cells. Lysosomal enzymes in blood leukocytes are possibly responsible for post-mortem proteolysis of muscle proteins because some blood remains in numerous blood vessels which traverse muscle tissue. The extent and role of proteolysis in post-mortem muscle has been reviewed by Parrish (1971) and the morphology of porcine leukocytes lysosomes and properties of their constituent hydrolases by Bailey et al. (1971).

The objectives of this study were to demonstrate that leukocyte lysosomal enzymes can catalyze hydrolysis of myofibrillar proteins; to characterize breakdown products from myofibrillar proteins following proteolysis by leukocyte lysosomal enzymes; and to test functional properties of myofibrillar proteins treated with leukocyte lysosomal proteolytic enzymes.

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EXPERIMENTAL PROCEDURES

Isolation of Porcine Leukocyte Lysosomes.
Blood was collected aseptically from hogs during exsanguination in sterilized 1 liter beakers containing 15 ml 5% EDTA in 1% NaCl. The leukocytes were then separated by the procedure of Fraenkel-Conrat et al. (1966) as modified by Venugopal (1970). Suspensions of leukocytes in 0.25M sucrose were sonicated (0°C) for 3 min in a Biosonic sonicator and further fragmented with an Elvejhem homogenizer. The sonicated suspension was centrifuged 500xg, 20 min to remove cellular debris and intact cells. The supernatant was centrifuged 10,000xg, 30 min, 0°C to sediment lysosomes.

Electron Microscopy of Leukocyte Sediments.
The method of Miller et al. (1966) for studying eosinophil granules was adopted with slight modification to study porcine leukocyte lysosomes. In some cases, mounted sections were subjected to electron shadow micrography instead of staining by depositing a thin layer (7nm) of chromium in vacuo at an angle of 21° on the preparation.

Preparation of Lysosomal Enzymes.
The lysosomal suspension (10,000xg fraction) was dialyzed against phosphate buffer (0.05M, pH 7.0 or pH 3.0), 24 hr (4°C), the dialysate centrifuged (0°C) 20,000xg for 1 hr and the clean supernatant used as enzyme source.

Preparation of Actomyosin.
Actomyosin was prepared by the method of Ebashi and Ebashi (1964).

Preparation of F-Actin.
F-actin was prepared by the method of Tsao and Bailey (1953), the actin polymerized with 0.1M KCl and the F-actin collected by centrifugation at 100,000xg for 3 hr.

Preparation of Troponin.
The procedure described by Greaser and Gergely (1971) was used for troponin preparation.

Preparation of Tropomyosin.
Tropomyosin was prepared from the pH 4.6, 1M KCl precipitate remaining after troponin preparation by the method of Greaser and Gergely (1971).

Viscosity Measurements.
Viscosity was measured in an Ostwald viscometer at 20°C.

Non-Protein Nitrogen (NPN).
NPN was measured as the absorbance at 274 nm following precipitation of proteins with 5% trichloroacetic acid (TCA).

RESULTS AND DISCUSSION

Electron Micrograph of Leukocyte Sediments.
Figure 1 is an electron micrograph containing dense bodies ranging in diameter from 0.15 to 0.18 μ partly masked by cellular debris. This fraction was sedimented at 3,500xg and contains dense bodies identified as lysosomes. Structures of similar morphology and size were visualized by electron microscopy of intact porcine leukocytes and other fractions separated by differential centrifugation.

Results of previous work with enzymes in the various sedimentation fractions (Venugopal, 1970) revealed that the constituent hydrolases, including proteinases, were confined within a lipoprotein sac which was lysed by hypo-osmotic media. This enzyme latency was thought to be due to the presence of dense bodies such as those shown in Figure 1.

SDS-Polyacrylamide Gel Electrophoresis of Actomyosin Reacted With Porcine Leukocyte Lysosomal Proteinases.
SDS-Polyacrylamide gel electrophoresis of actomyosin following reaction with proteinases for various time intervals from 10 min to 48 hr at 37°C resulted in 31 different bands. Relative mobilities and apparent molecular weights of the major degradation products are presented in Table 1. Most products appeared after 10 min incubation, although some required 48 hr. Small molecular weight peptides (10,000-12,000) increased continuously during the reaction.

Electrophoresis of actomyosin and enzyme samples incubated separately revealed no changes in these proteins during the reaction under conditions employed.

A polypeptide of molecular weight 4,600 soluble in 5% TCA also increased in quantity during the reaction. The molecular weight of this component was determined by ultracentrifugation and Sephedex gel filtration.

Change in Specific Viscosity of Actomyosin During Reaction With Leukocyte Lysosomal Proteinases.
Specific viscosity was measured at pH 6.7 and 8.5 during reaction with lysosomal proteinases at 37°C. The decrease in viscosity, particularly at pH 6.7 (Figure 2), indicated reduction in molecular weight of actomyosin. Changes in specific viscosity at pH 8.5 with time were less dramatic. The viscosity decreased more rapidly in the presence of 2 mM iodoacetamide at pH 7.0 which apparently inhibited transamidation reactions. After 24 hr incubation, there was a two-fold decrease in the final specific viscosity of a reaction mixture containing 2 mM iodoacetamide compared with a reaction mixture without inhibitor.

Besides the reduction in specific viscosity, reaction mixtures containing the inhibitor produced 4 times more NPN than noninhibited mixtures during 24 hr incubation at 37°C, pH 7.0.

Changes in NPN During Reaction of Actomyosin With Porcine Leukocyte Lysosomal Proteinases.
Other reaction mixtures used in viscosity measures were also analyzed for NPN. The 5% TCA-soluble NPN was inappreciable in samples incubated without enzymes but increased rapidly in actomyosin samples treated with enzymes at pH 6.7, 37°C. NPN production was considerably less (60%) in samples incubated at pH 8.5.

The protein-free supernatants from 5% TCA extracts also contained nucleotides and free amino acids. Glutamic acid, aspartic acid and alanine were the most abundant amino acids released from actomyosin during proteolysis.

Similar changes in NPN were also obtained with F-actin in the presence of leukocyte lysosomal enzymes. The pH optimum of porcine leukocyte lysosomal proteinase with F-actin as substrate was 7.0 when the reaction was carried out at 37°C for 8 hr.

Influence of Native and Enzyme-Treated Troponin on Specific Viscosity of Tropomyosin.
Figure 3 contains results of viscosity measurements of tropomyosin containing various concentrations of lysosomal proteinase, treated and untreated troponin, and troponin treated with papain.

The rate of viscosity increase was slower for tropomyosin to which various amounts of troponin treated with leukocyte enzymes was added, indicating that troponin was degraded and its functional properties destroyed by the proteinases. From these results, it would be anticipated that thin filaments containing proteinase-degraded troponin would be less rigid than those of intact fibers.

Other protein functional qualities influenced by porcine leukocyte lysosomal enzyme treatments, pH 7.0, 37°C for 12 hr were emulsifying and gelling capacities of actomyosin. The emulsifying capacity of actomyosin treated with lysosomal enzymes was greater than actomyosin without treatment even though specific viscosity of the former was considerably greater. Actomyosin treated with lysosomal enzymes did not gel following removal of KCl while control actomyosin formed a firm gel.

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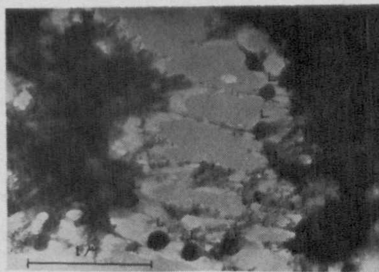

Figure 1. Electron micrograph of lysosomes from 3,500xg sediment of sonicated porcine leukocytes (magnification 54,000). Objects marked L were identified as lysosomes.

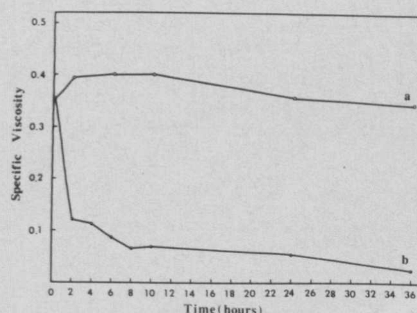

Figure 2. Change in specific viscosity of actomyosin during reaction with leukocyte lysosomal proteinases at pH 6.7, 37°C. The reaction mizture was composed of 10:1 (v/v) 2.7/mg/ml actomyosin and 2.7 m/ml enzyme. a. actomyosin; b. actomyosin + enzyme.

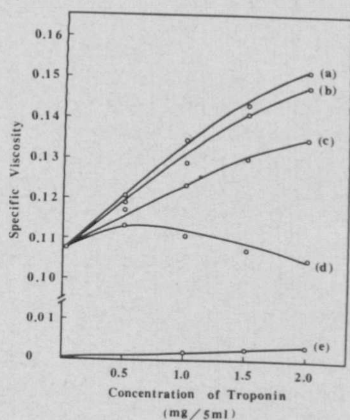

Figure 3. Influence of native and enzyme-treated troponin on the specific viscosity of tropomyosin. Two ml of various concentrations (0-2.0 mg 15 ml) of troponin were added to 4 ml of tropomyosin (4.2 mg/ml).
a. Fresh troponin + tropomyosin
b. Troponin at 37°C, pH 7.0 for 12 hr + tropomyosin
c. Troponin incubated with proteinase 37°C, pH 7.0 for 12 hr + tropomyosin
d. Troponin incubated with papain 37°C, pH 7.0 for 12 hr + tropomyosin
e. Troponin

TABLE I

RELATIVE MOBILITIES AND MOLECULAR WEIGHTS OF MAJOR DEGRADATION PRODUCTS PRODUCED FROM ACTOMYOSIN BY LEUKOCYTE LYSOSOMAL PROTEINASES[a]

Band No	Relative mobility	Molecular weight[b]
1	0.031	107,000
2	0.054	101,000
3	0.116	89,000
4	0.140	85,000
5	0.194	75,000
6	0.209	73,000
7	0.240	68,000
8	0.287	62,000
9	0.357	51,000
10	0.380	50,000
11	0.403	48,000
12	0.442	44,000
13	0.450	43,000
14	0.465	42,000
15	0.504	38,000
16	0.527	37,000
17	0.543	35,000
18	0.558	34,000
19	0.574	33,000
20	0.589	32,000
21	0.612	30,000
22	0.659	28,000
23	0.667	27,000
24	0.744	23,000
25	0.760	22,000
26	0.806	20,000
27	0.868	17,000
28	0.923	16,000
29	0.961	14,000
30	1.031	12,000
31	1.109	10,000

a. Mobility relative to cytochrome C. SDS-Polyacrylamide gel electrophoresis.
b. Calculated from: Log M.W.=-0.938x + 5.058: where x-relative mobility. Constants determined experimentally.