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A Cysteine Metalloproteinase from Mouse Liver Cytosol (41919)

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Abstract. A cysteine metalloproteinase that degrades ^{125}I-insulin B chain at neutral pH values was isolated from C3H mouse liver. The enzyme was partially purified from the 100,000*g* supernatant fraction by ammonium sulfate precipitation, DEAE-cellulose chromatography, and fast protein liquid chromatography. The molecular weight of the proteinase was estimated to be 190,000 by gel filtration on Sephadex G-200. Degradation of ^{125}I-insulin B chain by the proteinase was inhibited by *p*-hydroxymercuribenzoate (PHMB) and iodoacetate (cysteine proteinase inhibitors) and by ethylenediaminetetraacetic acid (EDTA) and 1,10-phenanthroline (metalloproteinase inhibitors). The proteinase also degraded ^{125}I-glucagon but did not hydrolyze ^{125}I-insulin, leucine-2-naphthylamide, or several large proteins. Equivalent levels of EDTA- and PHMB-inhibitable ^{125}I-insulin B chain-degrading activity were observed in the 100,000*g* supernatant fractions of brain, liver, lung, kidney, heart, and spleen from four mouse strains (C3H/HeN, CBA/J, ICR, and C57BL/6). High levels of ^{125}I-insulin B chain-degrading activity were found in the particulate fraction of kidneys and lungs from these four mouse strains; these activities were inhibited by EDTA but not by PHMB. The activity of the soluble liver cysteine metalloproteinase was not altered in C3H mice treated ip with metal chelators, bacterial endotoxin, phenobarbital, dexamethasone, or insulin. Starvation for 24 or 48 hr and alloxan-induced diabetes diminished total activity of this enzyme in liver by about 50 and 30%, respectively. This soluble polypeptide-degrading enzyme appears to be ubiquitous in mice and to be regulated by nutritional conditions.

Intracellular proteases serve a number of important physiological functions such as degradation of proteins to their constituent amino acids, posttranslational modification of proteins, removal of abnormal proteins, and processing of polypeptide hormones (1). The acidic proteases of lysosomes have been studied extensively, in part because of their purported role in degradation of intracellular proteins (2–4). There is a growing body of evidence that lysosomes are not the only important site of intracellular protein degradation, and increasing attention has been given to extralysosomal proteases (5–7) and in particular to neutral, soluble proteases (8–10).

Proteases may modulate the availability and biological action of polypeptide hormones (11); for example, Larner *et al.* (12) have proposed that binding of insulin to its receptor initiates a proteolytic event that produces a peptide mediator of insulin's biochemical actions. Proteases are also involved in degrading insulin and may be important in terminating its effects (13). Proteases that degrade insulin but not insulin B chain (14) and proteases that degrade insulin B chain but not insulin (10, 15) have been described but the degradative pathway for the intact insulin molecule remains unresolved (cf. (13)).

In this study, we examined extracts from mouse tissues for capability to degrade insulin B chain. Insulin B chain is a good general substrate for the detection of endoproteinase activity (6) and has been used to determine peptide bond specificity of proteinases (16). Furthermore, the peptide is easily iodinated thereby affording a reproducible and sensitive assay for proteolytic activity. Insulin B chain-degrading activity was found in the soluble fraction of all tissues examined. The soluble proteolytic activity in mouse liver was partially purified and characterized; it is a unique neutral cysteine metalloproteinase that readily degrades insulin B chain and glucagon but not intact insulin. Additionally, the activity of this proteinase was measured in mice treated with biological and pharmacological effectors that alter protein degradation (17–

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26); only starvation and alloxan-induced diabetes affected activity *in vivo*.

Materials and Methods. *Animals.* Mice, weighing 19–24 g, were obtained from Frederick Cancer Research Center, Frederick, Maryland (female C3H/HeN and C57BL/6 mice), Jackson Laboratories, Bar Harbor, Maine (male CBA/J mice), and Dominion Laboratories, Dublin, Virginia (male ICR mice). Mice were maintained on a 12-hr light:dark cycle and were allowed free access to Purina Lab Chow (Ralston Purina, St. Louis, Mo.) and water (except as noted).

Purification of the proteinase. Mice were sacrificed by cervical dislocation and their livers were immediately perfused with ice cold 0.9% NaCl. The livers were homogenized in 9 vol (w/v) of ice-cold 20 m*M* tris-(hydroxymethyl)aminomethane (Tris)/HCl buffer (pH 7.5) containing 350 m*M* sucrose and 150 m*M* KCl, and the homogenate was centrifuged at 700*g* for 10 min at 4°C. The resulting supernatant fluid was centrifuged at 100,000*g* for 60 min at 4°C. In some experiments, the 100,000*g* particulate fraction was suspended in 2 ml of the Tris/sucrose/KCl solution. Otherwise, this sediment was discarded and the 100,000*g* supernatant fraction was subjected to $(NH_4)_2SO_4$ fractionation. The majority of the proteinase activity was associated with the 40–60% $(NH_4)_2SO_4$ fraction. Residual salt was removed from this $(NH_4)_2SO_4$ fraction by gel filtration on a Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, N.J.) column (3 × 45 cm) equilibrated with 10 m*M* Tris–HCl, pH 8.5, at 4°C. The eluted protein peak was then applied to a diethylaminoethyl (DEAE)-cellulose (Whatman De-52, Whatman, Clifton, N.J.) column (2 × 9 cm) equilibrated with 10 m*M* Tris–HCl, pH 8.5, at 4°C. Fractions were eluted in a stepwise fashion with 0, 0.1, 0.3, and 1 *M* NaCl in buffer. Eluted fractions containing proteinase activity were pooled and salt was removed by gel filtration on Sephadex G-25. The eluted protein peak was subjected to fast protein liquid chromatography (FPLC) with a Pharmacia Fast Protein Liquid Chromatograph on a Mono Q (Pharmacia) anion exchange column (0.5 × 5.5 cm) that had been equilibrated with 20 m*M* Tris–HCl, pH 8.5, at 22°C. Protein was eluted from the column with a linear gradient of 0 to 500 m*M* KCl in buffer at a flow rate of 1 ml/min.

Assay of proteolytic activity. Proteolytic activity was assayed routinely using ^{125}I-insulin B chain as the substrate. The oxidized form of insulin B chain (Schwarz/Mann, Orangeburg, N.Y.) was radiolabeled with [^{125}I]NaI (Amersham Corp., Arlington Heights, Ill.) using the chloramine-T method, and degradation of the labeled substrate was assayed following a modification of the method of George and Kenny (27) as described previously (28). The incubation mixture contained 100 μl enzyme, 20 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) buffer, pH 7.6, 150 m*M* NaCl, and 10 μg/ml ^{125}I-insulin B chain in a total volume of 0.5 ml.

Protein assays. Protein was determined by the method of Lowry *et al.* (29) using bovine serum albumin as a standard. More purified samples (fractions from DEAE-cellulose and FPLC columns) were assayed for protein content by the method of Kalb and Bernlohr (30).

Assays for activity against other proteinase substrates. Unless otherwise stated, all assays were performed at 37°C in 20 m*M* Hepes buffer, pH 7.6, containing 150 m*M* NaCl. Degradation of ^{125}I-glucagon (preparation from crystalline glucagon) and ^{125}I-insulin (both polypeptides having been radiolabeled as described above for insulin B chain) was assayed in the same manner as ^{125}I-insulin B chain except that 10% (w/v) trichloroacetic acid was used to terminate the assay with ^{125}I-insulin. Azocasein degradation was determined as described by Beynon and Kay (31). Hydrolysis of hemoglobin was determined by measuring release of trichloroacetic acid-soluble Folin-positive material (29). Casein was coupled with a fluorescent label, 2-methoxy-2,4-diphenyl-3(2H)furanone (Polysciences, Inc., Warrington, Pa.) using the method of Weigele *et al.* (32) and degradation was assayed by the method of Weisner and Troll (33). Activity of fructose-1,6-bisphosphate aldolase was assayed as described previously (34). Degradation of azocasein, hemoglobin, casein, and aldolase was assayed for a period of up to 2 hr using 15 to 20 μg of enzyme and 1 to 10 mg of protein substrate in a final reaction volume of 1 ml. Leucine-

2-naphthylamide was assayed according to Barrett (2) with omission of cysteine and EDTA.

Effect of starvation, alloxan-induced diabetes, and various pharmacological agents on proteolytic activity. The supernatant fraction of livers from treated C3H mice was assayed for proteolytic activity against ^{125}I-insulin B chain in the presence and absence of 10 m*M* ethylenediaminetetraacetic acid (EDTA) or 1 m*M* *p*-hydroxymercuribenzoate (PHMB). The inhibitors were preincubated with the supernatant fraction for 1 hr at 37°C after which the assay proceeded as usual except that the concentration of inhibitor was maintained for the duration of the assay. The effect of chelators on proteinase activity was evaluated by injecting mice with EDTA (100 mg/kg) or ethylene glycol bis(β-aminoethyl ether) *N,N'*-tetraacetic acid (EGTA, 100 mg/kg) and sacrificing 1 hr later. Unless otherwise stated, all injections were given intraperitoneally (10 ml/kg), control mice received saline, and four mice were used for each treatment. Endotoxin (*Escherichia coli* 026:B6 lipopolysaccharide, isolated by the Westphal procedure, Difco Laboratories, Detroit, Mich.) was administered in doses of 2 and 24 mg/kg and, for each dose, groups of mice were sacrificed at 4 and 18 hr. Phenobarbital was administered on four consecutive days: 60 mg/kg on Days 1 and 2 and 80 mg/kg on Days 3 and 4. Mice were sacrificed 1 and 24 hr after the last dose. A single dexamethasone treatment of 3 mg/kg was given and mice were sacrificed 1 and 4 hr later. Insulin was administered in a single dose (1 mg/kg) and mice were sacrificed at 1, 4, 14, and 24 hr. Starved mice were deprived of food for 12, 18, 24, and 48 hr. Control mice were given food *ad libitum*. Both starved and fed mice were sacrificed at approximately 9:00 AM. To induce diabetes, mice were fasted for 18 hr and were then injected with alloxan (150 mg/kg; Baker Chemical Co., Phillipsburg, N.J.). A starved control was fasted for 18 hr and received saline. Both groups were then given food *ad libitum*. An additional group of control mice received saline and was allowed free access to food throughout the experiment. Alloxan-injected mice exhibited glycosuria (as determined by Clinistix reagent strips, Ames Division, Miles Laboratories, Inc., Elkhart, Ind.). Diabetic mice were sacrificed 1, 3, and 5 days after alloxan was administered. Controls were sacrificed 1 day after saline. Statistical analysis of the data was performed by analysis of variance and Dunnett's *t* test (36).

Materials. Unless otherwise stated, all chemicals were obtained from Sigma Chemical Company (St. Louis, Mo.).

Results. *Isolation and characterization of the partially purified proteinase.* The results of a typical purification (using ^{125}I-insulin B chain as substrate) are shown in Table I. All of the proteolytic activity applied to DEAE-cellulose and FPLC columns was adsorbed on the columns and the proteinase was recovered as a single peak during elution with 300 m*M* NaCl and 300 m*M* KCl, respectively. Fractions from the FPLC column were pooled and used for studies of substrate specificity, pH optimum, and susceptibility to inhibitors. Pooled fractions from the DEAE-cellulose column were also applied to a Sephadex G-200 column (3 × 45 cm) and the protease was recovered in a single peak with an elution volume corresponding to a molecular weight of 185,000 to 195,000.

TABLE I. PARTIAL PURIFICATION OF A CYSTEINE METALLOPROTEINASE FROM C3H MOUSE LIVER

	Proteinase activity (units)[a]	Protein (mg)	Sp act (units/mg protein)	Purification (fold)	Yield (%)
Homogenate	60,500	3008	20	1	100
100,000*g* supernatant	32,490	884	37	1.8	53
Ammonium sulfate precipitate (40–60%)	13,242	264	50	2.5	21
DEAE-cellulose	9,945	126	79	3.9	16
FPLC	847	1.3	641	32.0	1

[a] Nanograms ^{125}I-insulin B chain degraded/min.

The partially purified enzyme degraded ^{125}I-insulin B chain optimally between pH 7.4 and 7.8 (data not shown). The susceptibility of the ^{125}I-insulin B chain-degrading activity to a number of inhibitors (preincubated with the proteinase for 1 hr prior to assay) was evaluated using both crude (100,000*g* supernatant) and partially purified (FPLC fractions) preparations (Table II). The most potent inhibitors were the cysteine proteinase inhibitors, PHMB and iodoacetate, and the metalloproteinase inhibitors, EDTA and 1,10-phenanthroline. Protease activity was not inhibited by diisopropyl phosphofluoridate or pepstatin, which are serine and aspartate proteinase inhibitors, respectively, nor by bestatin, an aminopeptidase inhibitor (2). A small degree of inhibition was observed with leupeptin, an inhibitor of certain cysteine and serine proteinases (2).

Studies of the substrate specificity of the proteinase revealed that it did not degrade the intact insulin molecule, leucine-2-naphthylamide (a substrate for N-terminal exopeptidases), or several large proteins (azocasein, casein, aldolase, and hemoglobin) under the conditions described under Methods, but glucagon, a polypeptide hormone similar to insulin B chain in molecular weight, was degraded at approximately one-third the rate of insulin B chain degradation (data not shown). Both glucagon and insulin B chain degradation were inhibited by EDTA and PHMB corroborating that both substrates were hydrolyzed by the same enzyme.

Tissue and strain distribution of insulin B chain-degrading activity. Cytosolic (100,000*g* supernatant fraction) and particulate (100,000*g* sedimented fraction) preparations of six tissues from each of four strains of mice were assayed for proteolytic activity using ^{125}I-insulin B chain as substrate and susceptibility to both EDTA and PHMB inhibition as criteria for the proteinase described above. On the basis of these criteria, the proteinase was found in the cytosol of all six tissues and in all four strains of mice (Table III). By contrast, the profile of proteolytic activity in the particulate preparations (Table IV) showed a marked divergence from the uniform pattern seen in the cytosolic preparations. Proteolytic activity in particulate fractions of brain, liver, spleen, and heart were similar to each other and to corresponding tissues of other strains. However, the activities in particulate fractions from the kidney and lung were 10- to 20-fold and 5- to 10-fold higher, respectively, than the activity in the other four tissues. In addition, the proteolytic activities in most of the particulate preparations were metal dependent but showed variable susceptibility to the cysteine inhibitor.

TABLE II. EFFECT OF POTENTIAL INHIBITORS ON PROTEINASE ACTIVITY OF FPLC FRACTIONS

Inhibitor	Concentration	Inhibition (%)
Diisopropyl phosphofluoridate	1 m*M*	0
p-Hydroxymercuribenzoate	1 m*M*	100
Iodoacetate	5 m*M*	82
EDTA	10 m*M*	100
1,10-phenanthroline	1 m*M*	89
Leupeptin	10 μ*M*	18
Pepstatin	1 μg/ml	0
Bestatin	10 μg/ml	8

Effect of biological and pharmacological manipulations on proteolytic activity in mouse liver cytosol. Table V summarizes the results of studies on the proteolytic activity in liver cytosol from C3H mice that were subjected to a variety of treatments. Neither administration of EGTA nor EDTA had any effect on proteolytic activity; only the data for EDTA are shown. Likewise, no effect of endotoxin was observed either 4 or 18 hr after doses of 2 or 24 mg/kg; the data shown are for samples prepared 18 hr after a dose of 2 mg/kg. Similarly, all phenobarbital, dexamethasone, and insulin treatments resulted in protease activities that were no different from controls; values shown in Table V are for samples prepared 24 hr after administration of pentobarbital, 1 hr after dexamethasone, and 1 hr after insulin. Food deprivation, however, altered proteinase activity in the liver and the effects appeared to be somewhat dependent on the length of starvation. A small decrease (20–30%) in specific activity, total units of activity, and total protein in the cytosol was observed after 12 or 18 hr of starvation. In another experiment, these effects were somewhat more pronounced by 24 hr, but an additional 24 hr

TABLE III. DISTRIBUTION OF EDTA- AND PHMB-INHIBITABLE INSULIN B CHAIN-DEGRADING ACTIVITY IN THE 100,000*g* SOLUBLE FRACTION OF TISSUES FROM FOUR MOUSE STRAINS[a]

Tissue	Enzyme activity[b]			
	CBA/J	C57BL/6	ICR	C3H/HeN
Kidney	30.0 ± 2.2	31.5 ± 1.1	39.6 ± 1.6	32.2 ± 0.2
Brain	23.2 ± 1.1	28.0 ± 1.4	30.1 ± 1.0	24.4 ± 0.9
Lung	28.4 ± 2.4	23.1 ± 2.1	30.8 ± 1.5	30.8 ± 1.8
Liver	21.5 ± 1.3	16.8 ± 0.6	32.1 ± 0.8	30.6 ± 3.7
Spleen	23.2 ± 1.5	24.8 ± 2.3	57.2 ± 3.0	36.2 ± 1.4
Heart	15.5 ± 1.3	19.1 ± 1.3	19.4 ± 1.0	24.2 ± 3.4

[a] ^{125}I-insulin B chain-degrading activity in all tissues was inhibited 95–100% by 10 m*M* EDTA and by 1 m*M* PHMB.

[b] Enzyme activity expressed as ng ^{125}I-insulin B chain degraded/min/mg protein. Values given as means ± SE for groups of four mice.

of food deprivation produced no further change. Alloxan-induced diabetic mice also showed a diminution of total protein and proteolytic activity in the cytosol (compared to the fed control) but no change in specific activity.

Discussion. The protease from mouse liver described herein is a large (185,000 to 195,000), soluble, cysteine metalloproteinase (abbreviated here as SOCEM) that degrades small proteins such as oxidized insulin B chain and glucagon. Classification of SOCEM as an endo- rather than exopeptidase is supported by the observation that SOCEM does not degrade leucine-2-naphthylamide and that its proteolytic activity against insulin B chain is not inhibited by bestatin. Furthermore, degradation of ^{125}I-insulin B chain is not likely to be catalyzed by exopeptidases but rather implicates endoproteinase activity (6). The proteinase is being purified further so that the enzyme can be characterized with regard to the degradation of other peptides, identification of peptide products, peptide bond specificity, and kinetic properties.

The characteristics of SOCEM reported here differentiate it from previously described extralysosomal hepatic proteinases. SOCEM is larger than tryase, a serine proteinase isolated from a rat liver membrane fraction, that has a molecular weight of 28,000 to 32,000 and readily hydrolyzes azocasein,

TABLE IV. DISTRIBUTION OF EDTA- AND PHMB-INHIBITABLE INSULIN B CHAIN-DEGRADING ACTIVITY IN THE 100,000*g* PARTICULATE FRACTION OF TISSUES FROM THREE MOUSE STRAINS[a]

Tissue	CBA/J		C57BL/6		C3H/HeN	
	Sp act[b]	%I[c] E P	Sp act	%I E P	Sp act	%I E P
Kidney	168.9 ± 16.2	95, 15	513.6 ± 76.3	99, 10	347.0 ± 16.0	98, 12
Brain	3.9 ± 0.6	96, 89	11.1 ± 1.0	100, 92	11.3 ± 0.3	96, 95
Lung	115.2 ± 2.0	98, 38	57.9 ± 8.7	100, 45	67.2 ± 8.4	95, 28
Liver	14.0 ± 0.3	78, 88	10.2 ± 0.3	82, 94	13.7 ± 0.4	88, 92
Spleen	18.8 ± 4.3	95, 69	24.0 ± 2.4	96, 56	23.7 ± 0.3	76, 58
Heart	11.9 ± 1.9	93, 85	8.2 ± 0.3	100, 88	9.4 ± 0.6	94, 76

[a] Data are given for only three mouse strains. Proteolytic activity in the fourth strain, ICR mice, was essentially the same as in C3H/HeN mice.

[b] Specific activity (Sp act) expressed as ng ^{125}I-insulin B chain degraded/min/mg protein. Values given as means ± SE for groups of four mice.

[c] Percentage inhibition (%I) of ^{125}I-insulin B chain-degrading activity in the presence of 10 m*M* EDTA (E) or 1 m*M* PHMB (P).

TABLE V. EFFECT OF BIOLOGICAL AND PHARMACOLOGICAL TREATMENTS ON INSULIN B CHAIN-DEGRADING ACTIVITY IN THE SOLUBLE FRACTION OF C3H MOUSE LIVER

Treatment[a]	Total activity[b]	Sp act[b]
EDTA	1991 ± 63 (103)	25.2 ± 0.9 (100)
Endotoxin	1560 ± 178 (94)	19.2 ± 0.9 (95)
Phenobarbital	2734 ± 121 (114)	23.9 ± 0.8 (98)
Dexamethasone	1936 ± 255 (108)	21.7 ± 1.8 (108)
Insulin	2725 ± 288 (100)	32.4 ± 3.1 (102)
Starvation		
12 hr	1966 ± 91 (81)	25.4 ± 1.6 (84)
18 hr	1644 ± 80 (68)[c]	24.2 ± 0.7 (80)[c]
Starvation		
24 hr	815 ± 63 (45)[d]	15.4 ± 2.0 (62)[c]
48 hr	933 ± 81 (52)[d]	16.6 ± 1.4 (67)
Starved control	995 ± 85 (80)	11.8 ± 1.0 (94)
Alloxan		
1 day	805 ± 37 (80)[e]	12.0 ± 0.6 (96)
3 day	712 ± 77 (55)[e]	10.8 ± 1.1 (86)
5 day	957 ± 103 (77)[e]	13.5 ± 0.8 (108)

[a] Means ± SE of nontreated controls from eight separate experiments: total activity, 2167 ± 102; sp act, 27.0 ± 1.1 See text for details of treatments.

[b] Units: same as Table I. Values given as means ± SE for groups of four mice. Each treatment is a separate experiment and numbers in parentheses indicate percentage of control value for that experiment. In all instances, proteolytic activity was inhibited 95–100% by 10 m*M* EDTA and by 1 m*M* PHMB.

[c] Significantly different from the corresponding fed control at $P < 0.05$.

[d] Significantly different from the corresponding fed control at $P < 0.01$.

[e] Significantly different from untreated controls at $P < 0.01$ (Days 1 and 3) and $P < 0.05$ (Day 5) but not significantly different when compared to starved controls.

a large protein which is not degraded by SOCEM (7). DeMartino (37) has isolated two proteinases from rat liver cytosol which are totally dependent on calcium for their activity whereas SOCEM does not require calcium (data not shown). The ATP-dependent soluble proteinase from rat liver which degrades large proteins (9) differs from SOCEM in substrate specificity as well as molecular size (550,000 vs 190,000).

Endoproteinases are typically characterized according to their substrate specificity and susceptibility to proteinase inhibitors. SOCEM is clearly different from the rat liver insulin-specific protease: (a) during purification the rat liver enzyme is assayed in the presence of EDTA at a concentration that would inhibit the mouse enzyme (8, 38), (b) the rat enzyme degrades insulin but not insulin B chain (14) whereas the mouse enzyme degrades insulin B chain but not insulin, and (c) the rat enzyme has a molecular size of 71,000 to 80,000 (38) whereas the mouse enzyme is approximately 190,000. Proteinases from human skeletal muscle (39), human erythrocytes (40), and rat skeletal muscle (41) are also capable of degrading insulin while SOCEM does not degrade insulin under comparable conditions. These insulin-degrading enzymes have either inhibitor profiles differing from SOCEM or their susceptibility to inhibitors has not been described.

The soluble mouse liver enzyme characterized herein may be related to a soluble proteinase which was isolated from red cells and degrades small polypeptides including insulin but which is much larger in size (mol wt 550,000; (10)). Iodoacetic acid, a cysteine proteinase inhibitor, inhibits the mouse enzyme but not the red cell enzyme. Furthermore, there are indications that SOCEM is a metalloenzyme whereas the red cell enzyme is metal activated; SOCEM loses no activity upon dialysis while the red cell enzyme loses 100% activity under similar dialysis conditions.

The distribution of SOCEM among different mouse strains was examined because polymorphism in one mouse proteinase has been described in the strains used (42). Meprin (metalloendopeptidase from renal tissue) is an extralysosomal membrane-bound proteinase whose activity is deficient in CBA and C3H mouse strains (42). The present studies, however, showed that SOCEM is distributed ubiquitously in the cytosol of all tissues and mouse strains examined. Insulin B chain degradation by the particulate fraction of mouse kidney homogenates was inhibited by EDTA but not PHMB indicating that, unlike the soluble enzymes, the membrane-associated enzymes are metallo- but not cysteine proteinases. The metalloproteinase activity of the particulate fraction of kidneys may be attributed in part to meprin (5) or endopeptidase-24.11 (43). Meprin activity may account for the observation that

insulin B chain-degrading activity was greatest in kidneys from ICR and C57BL/6 mice, meprin sufficient strains, and lowest in kidneys from CBA/J mice, a meprin-deficient strain (42). Most of the insulin B chain-degrading activity of particulate fractions of all tissues was inhibited by EDTA, demonstrating an unexpected correlation between membrane-associated neutral proteases and metal requirements.

Alterations in protein degradation have previously been reported in response to insulin (19, 22), dexamethasone (26), diabetes (17, 18, 20), and starvation (18, 23–25). Erdos and Wohler (21) have demonstrated that chelating agents (inhibitors of metalloproteinases) inhibited the *in vivo* proteolytic inactivation of bradykinin and kallidin. The liver microsomal P-450 system, which is inducible by phenobarbital, has been shown to initiate proteolytic inactivation of some enzymes (44). In the present study, previous experience or published reports guided the selection of doses and times of sacrifice for metal chelators (45), insulin (47), dexamethasone (48), phenobarbital (46), bacterial endotoxin (45), and alloxan (20). Under these conditions, metal chelators, insulin, dexamethasone, phenobarbital, and bacterial endotoxin had no effect on the liver soluble protease.

The finding that SOCEM activity is substantially decreased in livers of diabetic and starved mice indicates that this activity is regulated by the hormonal or dietary state of mice. Other hepatic proteinases are not affected or only slightly decreased in diabetic mice (17, 20). The decreased SOCEM activity may be related to the observed decrease in hepatic protein degradation (20) or to the decreased concentration of insulin available to the liver in diabetes and starvation.

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