Conversion of proinsulin to insulin: Involvement of a 31,500 molecular weight thiol protease

(prohormone processing/affinity labeling/cathepsins/islets of Langerhans/chloromethyl ketones)

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ABSTRACT A lysed crude secretory granule fraction from rat islets of Langerhans was shown to process endogenous proinsulin to insulin with a pH optimum of 5.0–6.0. The converting activity in the lysed fraction was not inhibited by serine protease inhibitors (diisopropyl fluorophosphate, soybean trypsin inhibitor, and aprotinin) or metalloprotease inhibitors (EDTA and *o*-phenanthroline) but was inhibited by some thiol protease reagents (*p*-chloromercuribenzenesulfonic acid, antipain, and leupeptin) but not by others (*N*-ethylmaleimide and iodoacetamide). *N*^α-*p*-Tosyl-L-lysyl chloromethyl ketone only mildly inhibited at higher concentrations, whereas L-alanyl-L-lysyl-L-arginyl chloromethyl ketone was a powerful inhibitor. L-Alanyl-L-lysyl-L-arginyl chloromethyl ketone was [¹²⁵I]iodotyrosylated and used as an affinity labeling agent for the converting activity. Because the crude granule preparation contained contaminating lysosomes the affinity labeling of the granule preparation proteins was compared with that in liver lysosomes purified from rats injected with Triton WR1339. In the crude granule fraction the affinity label bound in a cysteine-enhanced manner to a single 31,500 molecular weight protein, but in purified liver lysosomes the major affinity-labeled protein had a molecular weight of 25,000 and minor 31,500 and 35,000 molecular weight proteins were also labeled. Evidence suggests that these proteins are thiol proteases and that in islets the 31,500 molecular weight thiol protease is involved in the conversion of proinsulin to insulin.

Insulin is synthesized as a larger precursor, preproinsulin (1), which is rapidly cleaved to proinsulin (2). The conversion of proinsulin to insulin is then a relatively slow process, which is thought to commence in the Golgi apparatus and continue into the maturing secretory granule (3). The unequivocal identification of the converting activities present in these organelles has proven to be an elusive problem. Conversion is thought to be mediated by two enzymes: one with trypsin-like specificity and the other similar to carboxypeptidase B. Evidence for this is based on the known structure of the cleavage products and intermediate forms (4), the ability of these exocrine pancreatic enzymes to correctly process proinsulin *in vitro* (5), and the detection of similar activities in the appropriate subcellular fractions of rat islets (6). Previous studies have demonstrated that both activities have a slightly acidic pH optimum and that the trypsin-like activity is activated by thiols and inhibited by thiol reagents (6–8). This suggests that this enzyme may be related to the lysosomal cathepsins. Alternatively, serine proteases [kallikrein (9) and plasminogen activator (10)] have also been implicated in the conversion process (see ref. 11 for review).

In the experiments reported here, we have further characterized the inhibitor profile of the trypsin-like converting activity associated with a crude secretory granule preparation from rat islets, and on the basis of this information have at-

tempted to affinity label the enzyme. A preliminary account of some of this work has been reported (12).

MATERIALS AND METHODS

Preparation of a Crude Secretory Granule Fraction from Rat Islets. Islets of Langerhans were isolated from 200- to 300-g adult male Sprague-Dawley rats as described (13), and in some experiments the islet proteins were labeled with [³H]leucine (L-[4,5-³H]leucine, 170 Ci/mmol, Amersham; 1 Ci = 3.7 × 10¹⁰ becquerels), by incubating the islets at 37°C for 30 min in 0.2 ml of Hanks' buffer supplemented with 33 mM glucose, amino acids according to Eagle's minimal essential medium (omitting leucine), and 200 μCi of [³H]leucine (2). The buffer was then removed and the islets were incubated at 37°C for 15 min in 0.2 ml of the same buffer (without [³H]leucine), containing 1 mM leucine. In all other experiments the islets were preincubated at 37°C for 45 min in 0.2 ml of this latter buffer. All procedures during the preparation of the crude granule fraction were then performed at 0–4°C. Approximately 3,000 islets (from eight rats) were homogenized in 0.5 ml of 0.25 M sucrose/0.1% bovine serum albumin in a motor-driven Teflon-on-glass Potter-Elvehjem homogenizer and the homogenate was centrifuged at 600 × *g*_{av} for 10 min onto a 2 M sucrose cushion (0.5 ml) in a 1.5-ml plastic Eppendorf tube, using a Sorvall SS-34 rotor fitted with an appropriate adaptor. The supernatant was collected, and the pellet was rehomogenized (0.5 ml) and recentrifuged (600 × *g*_{av} for 10 min). The supernatants were pooled and layered onto a sucrose step gradient composed of 2 ml of 2 M sucrose and 2 ml of 0.6 M sucrose, and the gradient was centrifuged at 15,000 × *g*_{av} for 15 min (Beckman SW 50.1 rotor). The crude granule fraction at the 2 M/0.6 M sucrose interface was carefully collected, the volume was adjusted to 5 ml with 0.25 M sucrose/0.1% bovine serum albumin, and the preparation was centrifuged at 15,000 × *g*_{av} for 20 min. The pellet was then suspended in an appropriate amount of incubation buffer.

Preparation of Liver Lysosomes. Liver lysosomes were prepared from adult male rats injected with Triton WR1339 as described (14).

Iodination of L-Alanyl-L-lysyl-L-arginyl Chloromethyl Ketone (Ala-Lys-ArgCH₂Cl). This peptide analog (15) was [¹²⁵I]iodotyrosylated on the free amino groups by the method of Assoian *et al.* (16). The iodinated inhibitor was separated from the reaction mixture by paper electrophoresis [30% (vol/vol) formic acid, 200 V for 4 hr], and extracted from the paper overnight at room temperature in 30% formic acid. The formic acid was evaporated and the *tert*-butoxycarbonyl group was removed from the tyrosine by treatment with 90% (vol/vol) formic

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Abbreviations: Ala-Lys-ArgCH₂Cl, L-alanyl-L-lysyl-L-arginyl chloromethyl ketone; [¹²⁵I]-Tyr-Ala-Lys-ArgCH₂Cl, [¹²⁵I]iodotyrosylated Ala-Lys-ArgCH₂Cl; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Tos-Lys-CH₂Cl, *N*^α-*p*-tosyl-L-lysyl chloromethyl ketone.

acid for 4 hr. The iodotyrosylated Ala-Lys-ArgCH₂Cl was then vacuum dried, suspended in 1 mM HCl, and stored at -20°C prior to use.

Electrophoresis. NaDodSO₄/polyacrylamide gel electrophoresis was performed on slab gels at 200 V (12.5% acrylamide, 0.25% bisacrylamide) (17). The gels were vacuum dried and exposed to x-ray film (XAR-5, Eastman Kodak) for autoradiography.

Assays. The method of measuring endogenous [³H]proinsulin conversion by a crude granule fraction (i.e., by separating substrate and product by gel filtration) has been described (18). The samples were made 3 M with respect to acetic acid, 1 mg of carrier bovine insulin was added, and the sample was applied to a Bio-Gel P-30 column (1 cm × 50 cm, Bio-Rad) equilibrated in 3 M acetic acid. The total ³H dpm eluting in the position of proinsulin and insulin was calculated, and the extent of conversion was determined by the increase in the percentage of the total ³H dpm in the insulin peak.

Insulin was measured by radioimmunoassay as previously described (19), with the modification that formalin-fixed *staphylococcus A* was used to precipitate the antibody-antigen complex. *N*-Acetylglucosaminidase was measured fluorometrically by using methylumbelliferyl *N*-acetylglucosaminide as substrate (20). Protein was measured by the method of Lowry *et al.* (21), using bovine serum albumin as a standard.

RESULTS

Characterization of Subcellular Fractions. The purification of secretory granules in high yield from rat islets has proven difficult due to limitations in the amount of starting material that can be prepared conveniently (6, 22). In the work described here we have not attempted to purify a granule fraction free of lysosomal contaminants. During the preparation, about 75% of lysosomes and 70% of the secretory granules are lost through breakage and the final fraction consists of almost equal amounts of the homogenate lysosomal (25.8%) and secretory granule (31.0%) markers (Table 1). In the affinity labeling experiments the impure nature of this preparation has been circumvented by comparing the labeling with that in a highly purified liver lysosomal preparation from rats injected with Triton WR1339. This preparation had a specific activity for *N*-acetylglucosaminidase (relative to that in the homogenate) of 48.2.

Conversion of Endogenous [³H]Leucine-Labeled Proinsulin. The time course of conversion of endogenous proinsulin to insulin by the crude granule fraction (Fig. 1) shows that the reaction proceeds fairly slowly, with an increase in the ³H dpm in the insulin peak of 28% of the total ³H dpm (in the proinsulin and insulin peaks) after 16 hr of incubation at pH 6.0. The data suggest that the enzyme may be losing activity during the prolonged incubation, but the insulin formed is not further degraded. The pH profile of the conversion process (Fig. 2) demonstrates a broad pH optimum between pH 5.0 and 6.0, with the activity dropping off above pH 6.0. Consequently in the following experiments on the effect of inhibitors on the con-

FIG. 1. Time course of conversion of [³H]proinsulin to insulin in an islet intact crude secretory granule fraction. The crude secretory granule pellet was gently suspended in 0.05 M 2-(*N*-morpholino)ethanesulfonic acid (Mes), pH 6.0, containing 0.1% bovine serum albumin and incubated at 37°C. At the indicated times, aliquots were removed and the reaction was terminated by the addition of acetic acid to 3 M. The proinsulin and insulin were then separated by Bio-Gel P-30 chromatography (18).

verting activity, the incubations were performed at pH 6.0 for 6 hr.

A major problem in using an intact granule preparation is that a lack of effect of inhibitors may be due to the inability of the inhibitor to permeate the granule membrane (6). It was therefore necessary to determine whether a lysed granule preparation would convert endogenous proinsulin to insulin. Lysis by freezing and thawing had no effect on the ability of the granule fraction to convert, and substantial conversion was also observed after lysis by Triton X-100, deoxycholate, or sonication (Table 2).

When various inhibitors were then tested in the lysed granule preparation (Table 3), the results showed that the serine protease inhibitors diisopropyl fluorophosphate, soybean trypsin inhibitor, and aprotinin and the metalloprotease inhibitors EDTA and *o*-phenanthroline did not substantially inhibit. Chloroquine also did not inhibit, although it is doubtful whether it would be expected to inhibit conversion in a lysed granule prep-

FIG. 2. Effect of pH on the conversion of endogenous [³H]proinsulin to insulin in a lysed islet crude secretory granule fraction. The crude secretory granule pellet was suspended in 0.25 M sucrose and lysed by repeated (six times) freezing and thawing (see Table 2). Equal aliquots were then added to buffers (0.1 M final concentration) containing 0.1% bovine serum albumin, at the indicated pH values. The samples were incubated at 37°C for 6 hr and treated as described in the legend to Fig. 1. The buffers used were pH 4.0–5.0, sodium acetate; pH 5.5–6.5, Mes/NaOH; and pH 7.0–8.0, 2-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid (Tes)/NaOH.

Table 1. Distribution of secretory granule and lysosomal markers during the fractionation of rat islets

Cell fraction	Total insulin		Total <i>N</i> -acetylglucosaminidase	
	μg	%	nmol/min	%
600 × <i>g</i> supernatant	23.9 ± 1.9 (2)	100	31.0 ± 6.0 (3)	100
Crude granules	7.3 ± 2.1 (2)	30.5	8.0 ± 2.0 (3)	25.8

The results represent the mean ± SEM of the number of experiments indicated in parentheses. When there were two experiments, the mean and range are given.

Table 2. Effect of membrane disruption on the conversion of endogenous proinsulin to insulin by an islet crude secretory granule fraction

Condition	Conversion, %
Intact granules	100
Freezing and thawing	
Three times	100.8 ± 3.4 (5)
Six times	99.7 ± 2.5 (3)
0.1% Triton X-100	60.2 ± 14.3 (4)
0.1% Deoxycholate	67.2 ± 4.1 (2)
Sonication	70.3 (1)

The crude secretory granule pellet was suspended in 0.05 M Mes, pH 6.0/0.1% bovine serum albumin/0.25 M sucrose, divided into six aliquots, and subjected to the above treatments. The granules were then incubated at 37°C for 6 hr and the proinsulin was separated from insulin by Bio-Gel P-30 chromatography. The results represent the mean ± SEM of the number of experiments indicated in parentheses or the mean and range for two experiments.

aration because it is thought to act as a weak base accumulating in the acid interior of vesicles (23) and inhibit proteolysis by raising the internal pH of the vesicle. Antipain and leupeptin were mild inhibitors, with antipain being stronger than leupeptin. *p*-Chloromercuribenzenesulfonic acid was a potent reversible inhibitor, whereas other thiol-directed reagents (*N*-ethylmaleimide and iodoacetamide) did not inhibit significantly. *N*^α-*p*-Tosyl-L-lysyl chloromethyl ketone (Tos-LysCH₂Cl) was a mild inhibitor at higher concentrations, whereas Ala-Lys-ArgCH₂Cl was a powerful inhibitor (Fig. 3).

Affinity Labeling with [¹²⁵I]iodotyrosylated Ala-Lys-Arg-CH₂Cl. From the inhibitor profile, Ala-Lys-ArgCH₂Cl was chosen as a potential affinity label because it inhibited strongly by alkylation (irreversible) and had free amino groups that could be [¹²⁵I]iodotyrosylated. In preliminary experiments the

Table 3. Effect of protease inhibitors on the conversion of endogenous proinsulin to insulin by an islet crude secretory granule fraction

Inhibitor	Conc., mM	Inhibition, %
Diisopropyl fluorophosphate	1	16.5 ± 3.7 (5)
Tos-LysCH ₂ Cl	1	10.9 ± 5.2 (5)
Ala-Lys-ArgCH ₂ Cl	1	50.6 ± 5.5 (7)
Soybean trypsin inhibitor	0.05 (1 mg/ml)	0 ± 1.3 (2)
Aprotinin	0.150 (1 mg/ml)	0 ± 5.3 (2)
Leupeptin	1	21.4 ± 5.0 (4)
Antipain	1	40.2 ± 8.9 (3)
<i>p</i> -ClHgPhSO ₃ H	0.1	79.0 ± 10.0 (2)
<i>p</i> -ClHgPhSO ₃ H + 1 mM cysteine	0.1	10.1 ± 1.6 (3)
<i>p</i> -ClHgPhSO ₃ H	1	84.5 ± 6.9 (4)
MalNEt	1	9.4 ± 4.8 (3)
MalNEt	10	7.9 (1)
Iodoacetamide	1	4.6 ± 3.6 (3)
α-Phenanthroline	1	6.1 (1)
EDTA	1	14.2 (1)
Chloroquine	0.1	-3.8 (1)
Chloroquine	1	-0.5 (1)
Chloroquine	10	-3.4 (1)

The crude granule pellet was suspended in 0.05 M Mes, pH 6.0/0.1% bovine serum albumin and divided into equal fractions. The inhibitors were then added to give the indicated final concentration. After lysis by repeated (six times) freezing and thawing the samples were incubated at 37°C for 6 hr. Proinsulin was separated from insulin by Bio-Gel P-30 chromatography. The results represent the mean ± SEM for the number of experiments indicated in parentheses (mean and range for two experiments). Tos-LysCH₂Cl, *N*^α-*p*-tosyl-L-lysyl chloromethyl ketone; *p*-ClHgPhSO₃H, *p*-chloromercuribenzenesulfonic acid; MalNEt, *N*-ethylmaleimide.

FIG. 3. Effect of Tos-LysCH₂Cl (■) and Ala-Lys-ArgCH₂Cl (●) on the conversion of endogenous [³H]proinsulin to insulin in a lysed islet crude secretory granule fraction. The crude secretory granule pellet was suspended in 0.05 M Mes, pH 6.0, containing 0.1% bovine serum albumin and divided into equal fractions. The inhibitors were then added to give the indicated final concentrations. After lysis by repeated (six times) freezing and thawing, incubation was at 37°C for 6 hr. The samples were treated as described in the legend to Fig. 1.

[¹²⁵I]iodotyrosylated inhibitor was shown by NaDodSO₄/polyacrylamide gel electrophoresis to bind to trypsin and papain but not to similar amounts (50 μg) of a variety of other proteins (data not shown).

The time course of binding of [¹²⁵I]-Tyr-Ala-Lys-ArgCH₂Cl to trichloroacetic acid-precipitable proteins in the crude granule preparation was very similar to that in liver lysosomes (Fig. 4). The binding was fairly rapid, was complete within 30–90 min, was displaced by Ala-Lys-ArgCH₂Cl (1 mM) and Tos-LysCH₂Cl (1 mM), and was enhanced by 1 mM cysteine.

When NaDodSO₄/polyacrylamide gel electrophoresis was performed on trichloroacetic acid-precipitated crude granule and lysosomal samples that had been incubated with [¹²⁵I]-Tyr-Ala-Lys-ArgCH₂Cl, several proteins were found to be affinity labeled (Fig. 5). In the crude granule preparation a protein of molecular weight 31,500 was labeled, whereas in the liver lysosomes the major affinity-labeled protein had a molecular weight of 25,000 and two other labeled proteins had molecular weights of 31,500 and 35,000. The labeling of all of these proteins was enhanced by 1 mM cysteine. In the crude granule samples another protein of molecular weight 65,000 was labeled, but this was also present in the buffer control (lane 5) and probably represented binding to the carrier bovine serum albumin (0.5 mg). No carrier bovine serum albumin was used during precipitation of the liver lysosomal samples. The facts that these three proteins bind [¹²⁵I]-Tyr-Ala-Lys-ArgCH₂Cl in a cysteine-enhanced manner and also bind to an organomercurial affinity column (data not shown) strongly suggest that they are thiol proteases. These results further suggest that the 31,500 molecular weight protein is the major thiol protease of the crude islet granule preparation, whereas the purified liver lysosome preparation contains a major thiol protease of molecular weight 25,000, which is probably cathepsin B (24), as well as lesser amounts of a 35,000 molecular weight thiol protease [possibly the tyrosine aminotransferase converting enzyme, cathepsin T (25)] and the 31,500 molecular weight thiol protease.

It should be noted that although the 31,500 molecular weight protein was the only affinity-labeled protein in the crude islet

FIG. 4. Binding of ^{125}I -Tyr-Ala-Lys-ArgCH₂Cl to trichloroacetic acid-precipitable proteins from islet crude secretory granules and purified liver lysosomes. Crude secretory granules (A) or purified liver lysosomes (B) were suspended in 0.05 M Mes, pH 6.0, and divided into equal fractions. Additions were made as indicated, and the samples were lysed by repeated (six times) freezing and thawing before preincubation at 37°C for 30 min. ^{125}I -Tyr-Ala-Lys-ArgCH₂Cl (50,000 cpm) was added, and the samples were further incubated at 37°C for the indicated times, whereupon aliquots were removed and the reaction was terminated by adding 1 mg of bovine serum albumin and trichloroacetic acid to 5%. After 30 min at 4°C the insoluble material was pelleted by centrifugation. The supernatant was aspirated and the pellet was washed once with 5% trichloroacetic acid before measurement of radioactivity by gamma spectroscopy. An incubated buffer control was subtracted from each value. Each point represents the binding of ^{125}I -Tyr-Ala-Lys-ArgCH₂Cl to 10 μg of lysosomal protein. Because carrier bovine serum albumin (0.1%) was used throughout the preparation of the islet crude granule fraction, it was not possible to determine the exact amount of islet protein in these samples. Δ , No additions; $\square$, 1 mM cysteine; $\bullet$, 1 mM Tos-LysCH₂Cl; and $\diamond$, 1 mM Ala-Lys-ArgCH₂Cl.

granule preparation, in an islet sonicate several proteins (61,000, 49,000, 40,000, 38,000, and 31,500) were affinity labeled with ^{125}I -Tyr-Ala-Lys-ArgCH₂Cl (data not shown). It is not known in which cellular compartment these proteins are located or whether they are all proteases, bearing in mind that Tos-LysCH₂Cl has been shown to affinity label the catalytic subunit of cAMP-dependent protein kinase (26).

DISCUSSION

The results reported in this paper suggest that a 31,500 molecular weight thiol protease may be involved in the conversion of proinsulin to insulin. This conclusion is based on the following lines of evidence: (i) The pH and inhibitor profiles of the conversion of endogenous [^3H]leucine-labeled proinsulin by the crude granule fraction from rat islets indicate the participation of a thiol protease having an acidic pH optimum in this process. (ii) Affinity labeling of the protease with an ^{125}I -labeled active site-directed reagent followed by NaDodSO₄/polyacrylamide

FIG. 5. NaDodSO₄/polyacrylamide gel electrophoresis of ^{125}I -Tyr-Ala-Lys-ArgCH₂Cl affinity-labeled proteins from islet crude secretory granules and purified liver lysosomes. Islet crude secretory granules (lanes 1–5) and purified liver lysosomes (lanes 6–10) were suspended in 0.05 M Mes, pH 6.0, preincubated for 30 min at 37°C, and treated with ^{125}I -Tyr-Ala-Lys-ArgCH₂Cl (50,000 cpm) for 90 min at 37°C. The trichloroacetic acid-precipitable proteins were then separated by NaDodSO₄/polyacrylamide gel electrophoresis, and the dried gels were exposed to film for 72 hr (lanes 1–5) and 24 hr (lanes 6–10), respectively. Preincubation conditions: lanes 1 and 6, no addition; lanes 2 and 7, 1 mM cysteine; lanes 3 and 8, 1 mM Ala-Lys-ArgCH₂Cl; lanes 4 and 9, 1 mM Tos-LysCH₂Cl; and lanes 5 and 10, buffer control. For the lysosome samples, 46 μg of protein was applied to each lane. Molecular weight protein standards were bovine serum albumin (65,000), ovalbumin (43,000), soybean trypsin inhibitor (21,500), and myoglobin (17,300).

gel electrophoresis shows that the putative converting enzyme has a molecular weight of $31,500 \pm 10\%$ and is the predominant affinity-labeled component of the crude granule preparation. It also appears to be present along with 25,000 and 35,000 molecular weight thiol proteases in purified liver lysosomes. (iii) Reagents that inhibited conversion of endogenous proinsulin to insulin by the crude islet granule fraction (Table 3) were shown to compete with ^{125}I -Tyr-Ala-Lys-ArgCH₂Cl for binding to the 31,500 molecular weight protein when preincubated with the granule preparation (data not shown).

The efficacy of the methods used to lyse the secretory granules (Table 2) proved difficult to assess. In some experiments we measured the amount of liberated (nonpelletable) insulin. These experiments were necessarily performed at pH 6.0, however, which is close to the isoelectric point of insulin. The results, which showed no increase in free (soluble) insulin, were therefore inconclusive in determining whether membrane breakage and insulin release had occurred because much of the released insulin may have pelleted as a precipitate along with the granule-enclosed insulin. It is also of interest that although a freeze-thawed granule fraction processed endogenous proinsulin it did not process exogenous ^{125}I -labeled bovine proinsulin, ^{125}I -labeled porcine proinsulin, or [^3H]leucine-labeled rat proinsulin (data not shown). This observation requires further investigation, but a possible explanation may be that lysis and subsequent resealing of granule membranes may be occurring.

The inhibitor profile (Table 3) is also interesting in that the thiol reagents *N*-ethylmaleimide and iodoacetamide did not inhibit conversion, whereas *p*-chloromercuribenzenesulfonic acid was a powerful reversible inhibitor. Enzyme inhibition with *N*-ethylmaleimide is generally slow and requires substantial preincubation times, whereas iodoacetamide might be expected to

inhibit more powerfully at alkaline pH (27). In fact, this difference between *N*-ethylmaleimide and *p*-chloromercuribenzenesulfonic acid sensitivity has been used to affinity label the active thiols in complex V of the mitochondrial oxidative phosphorylation system (28).

Although the enzyme content of islet lysosomes may not be similar to that of liver lysosomes, it is interesting that no detectable affinity labeling of cathepsin B was observed in the islet crude granule fraction even after lengthy (10-day) exposure. It may be that the 31,500 molecular weight protease is related to cathepsin B, possibly representing a (membrane-bound) form (of cathepsin B) containing an additional sequence of approximately 6,000 molecular weight, which may alter its substrate specificity or serve to anchor it to membranes through hydrophobic sequences. In the purified liver lysosomes the 25,000 molecular weight form predominates. [The possible role of the detergent (Triton WR1339) used in the preparation of the lysosomes in generating the observed forms will require further study.] Although similarities in the inhibitor profile of conversion of endogenous proinsulin by the crude granule fraction and of labeling the 31,500 molecular weight band implicate this component as the processing enzyme, it is unclear whether it is present solely in the secretory granules. It seems possible that it may also occur in appreciable amounts in islet lysosomes, because it seems also to be present in hepatic lysosomes.

Many other polypeptide hormones and neuropeptides undergo conversion from larger precursors at sites marked usually by pairs of dibasic amino acids (11, 29), an example being the pro-corticotropin/ β -endorphin precursor, which is processed to several bioactive peptides (30). A thiol protease is thought to be involved in this processing in the rat pituitary intermediate lobe (31). Its activity was inhibited by leupeptin, *p*-chloromercuribenzoate, and pepstatin A, but not by serine or metalloprotease inhibitors. A thiol protease has also been implicated in the conversion of proinsulin, proglucagon, and prosomatostatin in anglerfish islets (32, 33). This acidic protease, which may be bound to the secretory granule membrane, has an inhibitor profile qualitatively similar to that reported here. It has also been proposed that the conversion of proalbumin to albumin in rat liver may involve a thiol protease, possibly cathepsin B (34). However, evidence against a general role for cathepsin B in propolypeptide processing comes from the observation that when purified cathepsin B was tested on proparathyroid hormone the enzyme did not remove the terminal hexapeptide but instead cleaved in the central part of the molecule (35). It is interesting to speculate that the 31,500 molecular weight thiol protease in the liver lysosomal preparation may also be involved in the conversion of proalbumin to albumin. On the basis of the above evidence, it may also be appropriate to propose a general role for this protease in propolypeptide processing, but definitive proof of this must await further characterization of the activity in various tissues.

Several thiol proteases with acidic pH optima have been described. Most of these are of lysosomal origin and include cathepsin B (*M*_r 25,000), cathepsin H (*M*_r 28,000) (24), cathepsin L (*M*_r 23,000) (36, 37), and cathepsin T (*M*_r 33,000–35,000) (25). In this paper we have described another unique acid thiol protease with a molecular weight of 31,500 in liver and islets that may play a general role in propolypeptide processing. We tentatively propose that it should be called cathepsin P. However, the possible relationship of cathepsin P to cathepsin B will require further investigation.

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