An enzyme recently purified from rat liver (Gohda, E., and Pitot, H. C. (1980) J. Biol. Chem., in press), which catalyzes the conversion of multiple forms of tyrosine aminotransferase, has been further characterized. The purified enzyme, termed a convertase, hydrolyzed oxidized ribonuclease A and acid-denatured hemoglobin in addition to Form I of tyrosine aminotransferase and azocasein. The enzyme exhibited little activity toward albumin, native ribonuclease A, cytochrome c, and oxidized insulin B chain. During the early stages of digestion of oxidized ribonuclease A with the purified convertase, two major cleavage products were detected. Oxidized ribonuclease A inhibited in a dose-dependent manner the conversion of Form I of tyrosine aminotransferase to Forms II and III. Hydrolysis by the convertase of several synthetic substrates for exopeptidases was not detectable. During the purification of the convertase from rat liver, the activities of cathepsins B, H, and L were eliminated from the convertase fractions, and the final purification of the convertase showed no detectable activity of these cathepsins. Cathepsin D activity, measured as acid hemoglobin-hydrolyzing activity, was still detectable in the purified convertase fraction, but its yield and purification were much less than that of the convertase (about 2% of the convertase). The purified convertase was activated by sulfhydryl compounds and EDTA, and inhibited by sulfhydryl-reactive reagents. The convertase was also sensitive to leupeptin, antipain, and N-acetyl-L-lysine chloromethyl ketone, but insensitive to phenylmethylsulfonyl fluoride and pepstatin. These characteristics of the purified convertase are consistent with the interpretation that the enzyme is a new thiol endopeptidase in rat liver lysosomes. We propose the name, cathepsin T, for this new protease.

Multiple forms of rat liver tyrosine aminotransferase (L-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5) have been reported (1-7). Recently, several laboratories including our own have demonstrated the presence of a lysosomal factor in rat liver catalyzing the conversion of the multiple forms of the aminotransferase (8-12). Hargrove et al. (7) have purified the three forms, designated Forms I, II, and III, of tyrosine aminotransferase from rat liver and showed that Forms I, II, and III were composed of two subunits of molecular weights 53,000 and 53,000, 53,000 and 49,000, and 49,000 and 49,000, respectively; this suggests that the conversion of the enzyme is a proteolytic process.

EXPERIMENTAL PROCEDURES

Chemicals—2-Methoxy-2,4-diphenyl-3(2H)-furanone was a gift from Dr. W. E. Scott, Hoffmann-La Roche, Inc., Nutley, NJ. The following substances were obtained from the sources indicated: ribonuclease A (bovine pancreas), oxidized ribonuclease A, cytochrome c (horse heart), crystalline bovine serum albumin, bovine pancreatic trypsin inhibitor, bacitracin, hippuryl-L-arginine, hippuryl-L-phenylalanine, glycyl-L-phenylalanine-β-naphthylamide, L-tyrosine-β-naphthylamide, L-tyrosine-β-naphthylamide, L-lysine-β-naphthylamide, L-leucine-β-naphthylamide, L-leucinamide, L-arginine-β-naphthylamide, N-benzoyl-L-tyrosine ethyl ester, BANA, 1 n-glucose 6-phosphate, bovine hemoglobin (type II), p-hydroxymercuribenzoate, iodoacetate, iodoacetamide, N-ethylmaleimide, 4,4′-dipyrididyl disulfide, PMSF, TLCK, pepstatin A, leupeptin, antipain, soybean trypsin inhibitor, Fast Garnet GBC salt, β-naphthylamide, Mops, and Mes were all obtained from Sigma Chemical Co.; yeast glucose-6-phosphate dehydrogenase and dibuthreitol were from Calbiochem; SDS, acrylamide, and other electrophoretic reagents were from Bio-Rad. Other compounds were obtained from commercial sources and were of the highest grade available.

Animals—Albino male Holtzman rats (Madison, WI), weighing 200-250 g, were used in all experiments.

Separation of Multiple Forms of Tyrosine Aminotransferase by Hydroxyapatite Chromatography—Hydroxyapatite chromatography of multiple forms of tyrosine aminotransferase by use of a stepwise elution procedure was performed as described previously (13) at 4°C, except that 0.5 M potassium phosphate buffer, pH 6.9, was used instead of 0.32 M buffer for the elution of Form III of the enzyme.

Purification and Assay of Tyrosine Aminotransferase—Purified and partially purified Form I of tyrosine aminotransferase was prepared from rat liver as described previously (13). The specific activities of the preparations were 720 and 8.82 units/mg of protein, respectively. Tyrosine aminotransferase activity was assayed by the modification of Diamondstone’s method (14) described by Iwasaki and Pitot (3). One unit of activity is defined as that amount of enzyme

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In a previous report (13), we have purified the converting factor (termed "convertase") to homogeneity from rat liver. The purified convertase was a monomeric protein with a molecular weight of 33,500 to 35,000 and had a neutral optimum pH. The purified convertase catalyzed the conversion of Form I of tyrosine aminotransferase to Form II, and subsequently to Form III in vitro with concomitant production of 48,000-Mr subunits from 52,000-Mr subunits of the aminotransferase. Those values are almost the same as the molecular weights of subunits of purified tyrosine aminotransferase forms reported by Hargrove et al. (7). The purified convertase also showed a potent azocaseinolytic activity. These results indicate that the convertase is a proteinase. In this report, we demonstrate that the convertase is a new thiol endopeptidase in rat liver lysosomes.
that forms 1 \text{mMol} \text{of p}-\text{hydroxybenzylpyruvate}/\text{min at 37°C. A value of 19.900} \text{cm}^{-1} \text{ was used for the molar extinction coefficient of p}-\text{hydroxybenzylpyruvate} (14).

**Purification and Assay of the Convertase**—The convertase was purified from rat liver as described previously. Where the convertase free of sialylated-reducing compounds was required, the purified convertase was dialyzed overnight against 50 mM potassium phosphate buffer, pH 6.5, containing 12.5\% glycerol and 0.1 M KCl (Buffer A). The convertase activity was determined by measuring the formation of Form I from Form I of tyrosine aminotransferase according to the method described (13). The units of the convertase were determined by measuring the activity of the purified convertase in 50 mM potassium phosphate buffer, pH 6.5, containing 12.5\% glycerol and 0.1 M KCl (Buffer A) The convertase activity was determined by measuring the formation of Form I from Form I of tyrosine aminotransferase according to the method described (13). For the assay of the convertase activity during purification, partially purified Form I of tyrosine aminotransferase (11.7 mg, 11.2 units) was used. One unit of the activity of the convertase activity is defined as that amount of enzyme forming 1 unit of Form III from partially purified Form I of tyrosine aminotransferase/min at 37°C. The activity of the purified convertase was also assayed by use of purified Form I of tyrosine aminotransferase (3.75 \text{pg}, 2.73 units). In both systems, no conversion of Form I of tyrosine aminotransferase to other forms was observed during incubation in the absence of a convertase preparation. All assays were controlled by heating the reaction mixture for 10 min at 60°C immediately after adding a convertase preparation and albumin solution.

**Digestion of Proteins and Peptides by the Purified Convertase**—Bovine serum albumin and acid-denatured hemoglobin (15) were incubated at 1\% (w/v) concentration with the purified convertase in 50 mM Mes buffer (pH 6.5) for bovine serum albumin and pH 5.8 for acid-denatured hemoglobin containing 2 mM dithiothreitol for 10-30 min at 37°C. After termination of the reaction with trichloroacetic acid at a final concentration of 5\% and filtration of the mixture, the clear filtrate was used for the determination of released peptides by the method of Lowry et al. (16) with slight modifications (17). Other enzymes and peptides (ribonuclease A, oxidized ribonuclease A, cytochrome c, and oxidized insulin B chain) were incubated at 0.04\% (w/v) concentration with the purified convertase in 50 mM Mops buffer, pH 7.0, containing 2 mM dithiothreitol for 30-60 min at 0°C. After incubation, aliquots were heated in 20 mM sodium phosphate buffer, pH 7.0, containing 0.2\% (w/v) SDS and 0.05\% (v/v) β-mercaptoethanol for 10 min at 65°C. MDPF conjugation of peptides and SDS-polycrylamide gel electrophoresis of fluorescent-labeled peptides were performed according to the method described by Chen-Kiang et al. (18). The migration of MDPF-conjugated peptide bands was visualized with a UV lamp and photographed. Horse heart cytochrome c (M\text{r} = 12,300), bovine pancreatic trypsin inhibitor (M\text{r} = 6,160), and bacitracin (M\text{r} = 1,400) were used as molecular weight markers.

**Hydrolysis of Synthetic Substrates of Small Molecular Weight**—The purified convertase (1-10 μg) was incubated with various synthetic substrates in 1 ml of 50 mM Mops buffer, pH 6.5, containing 1-2 mM dithiothreitol at 25°C. Hydrolysis of glycyl-L-phenylalanine-β-naphthylamide, L-tryosine-L-tyrosine-β-naphthylamide, L-arginine-β-naphthylamide, and L-arginine-β-naphthylamide at 0.5 mM concentration was measured according to the method described by Barrett (19). Activity with 50 μM of L-leucine was determined at 238 nm (20). Hydrolysis of 1 mM of hippuryl-L-arginine and hippuryl-L-phenylalanine was measured at 234 nm (21, 22). Activity with 0.5 mM of N-benzoyl-L-tyrosine ethyl ester was determined at 256 nm (23).

**Assay of Carphinsin—**Cathpsin D activity was measured at pH 3.5 and 37°C with bovine hemoglobin as substrate by the method of Barrett (17). One unit of enzyme activity is defined as that amount of enzyme releasing 1 μg of tyrosine/min. Cathepsin L activity was assayed with yeast glucose-6-phosphate dehydrogenase as substrate following the procedure of Towatari et al. (24). The reaction mixture contained 0.1 M potassium phosphate buffer, pH 6.0, 5 mM β-mercaptoethanol, 2.75 units of yeast glucose-6-phosphate dehydrogenase (0.4 μg), and a proteinase preparation in a final volume of 200 μl. One unit of enzyme activity is defined as that amount of proteinase inactivating 50\% of the substrate enzyme in 90 min at 37°C. BANA-hydrolyzing activity was assayed according to the method of Barrett (19) at 37°C. One unit of enzyme activity is defined as that amount of proteinase inactivating 1 μmol of β-naphthylamine/min.

**Other Assays**—Protein was measured by the method of Lowry et al. (16) with bovine serum albumin as the standard. For protein concentrations lower than 200 μg/ml, the modified Lowry protein assay was utilized (25). Glucose-6-phosphate dehydrogenase activity was assayed by the method of Kuby and Noltmann (26). One unit of enzyme activity is defined as that amount which catalyzes the reduction of 1 μmol of NADP/min at pH 8.0 at 30°C.

**RESULTS**

**Proteolysis of Oxidized Ribonuclease A by the Convertase**—In the previous report (13), we demonstrated that the convertase purified from rat liver showed a potent azocaseinolytic activity that was about 6 times higher than that of cathepsin L, a thiol endopeptidase in rat liver lysosomes (24, 27). This finding prompted us to investigate the activity of the convertase on other substrates of proteinases. Of the protein and peptide substrates tested, oxidized ribonuclease A was readily cleaved by the convertase even at 0°C, and acid-cleaved ribonuclease A was also hydrolyzed. However, digestion of native bovine serum albumin was not detectable. The enzyme had little or no activity toward native ribonuclease A, cytochrome c, and oxidized insulin B chain at 0°C. As shown in Fig. 2, conversion of Form I of tyrosine aminotransferase to Forms II and III was inhibited in a dose-dependent manner by the presence of oxidized ribonuclease A, but not by native ribonuclease A in the assay mixture. Equimolar concentrations of oxidized ribonuclease A to the subunits of Form I of tyrosine aminotransferase reduced this conversion to 40\% of control. Concomitant studies of the same sample on SDS-polycrylamide gel electrophoresis showed that the production of 48,000-M, subunits of tyrosine aminotransferase from 52,000-M, subunits was also inhibited by oxidized ribonuclease A in accordance with the inhibition in the conversion of the aminotransferase forms measured by

![Fig. 1. Electrophoreograms of SDS-polycrylamide gel electrophoresis of the proteolysis of oxidized ribonuclease A by the purified convertase. Oxidized ribonuclease A (40 μg) was incubated with the purified convertase (0.4 μg) at 0°C in 100 μl of 50 mM Mops buffer, pH 7.0, containing 2 mM dithiothreitol. At the indicated times, aliquots (each) were removed and treated as described under "Experimental Procedures." A, 0 min; B, 6 min; C, 12 min; D, 30 min; E, 60 min. No cleavages were detected in the absence of the convertase.](image-url)
enzyme activity (data not shown).

Activity of the Convertase on Synthetic Substrates of Small Molecular Weight—The ability of the convertase to hydrolyze small molecular weight peptides and amides was examined by use of the following synthetic substrates: glycyl-L-phenylalanine-\(\beta\)-naphthylamide, \(\beta\)-serine-\(\beta\)-tyrosine-\(\beta\)-naphthylamide, \(\beta\)-leucine-\(\beta\)-naphthylamide, \(\beta\)-arginine-\(\beta\)-naphthylamide, \(\beta\)-leucinamide, \(\beta\)-hippuryl-\(\beta\)-arginine, \(\beta\)-hippuryl-\(\beta\)-phenylalanine, and \(\beta\)-benzoyl-\(\beta\)-tyrosine ethyl ester. No detectable cleavage of any of these compounds by the purified convertase was observed under the optimal conditions for the conversion of multiple forms of tyrosine aminotransferase (13 and see below).

Activity of Cathepsins B₁, D, H, and L during Purification of the Convertase from Rat Liver—The lysosomal localization of the convertase has been demonstrated in three laboratories including our own (10-12). Up to now, four endopeptidases, cathepsins B₁, D, H, and L, have been found in rat liver lysosomes. In order to clarify whether the convertase was identical with one of these proteinases, activities of these cathepsins were followed during purification of the convertase from rat liver and compared with that of the convertase. These results are shown in Table I. Since cathepsin H was reported by Kirschke et al. (28) to hydrolyze BANA rapidly, BANA was used as the substrate of both cathepsin B₁ and H. The final preparation of the convertase exhibited a 1490-fold purification over the mitochondrial-lysosomal extract with a 20.5% yield in the convertase activity. This preparation of the convertase was homogeneous as judged by polyacrylamide gel electrophoresis in the presence or absence of SDS, as reported previously (13). Most of BANA hydrolyase activity was separated from the convertase on CM-cellulose chromatography. On the last three steps, no activity of BANA hydrolyase was detectable. Activity of cathepsin L was greatly reduced on hydroxyapatite chromatography and was not detectable after this step. On the other hand, cathepsin D activity measured as acid hemoglobin-degrading activity was still detectable in the final fraction of DEAE-cellulose chromatography, although hydroxyapatite chromatography removed much of its activity. The yield and purification of cathepsin D were only 0.427% and 30.8-fold, respectively; these values were much lower than those of the convertase activity. Therefore, it is likely that the convertase exhibits hemoglobin-degrading activity at this pH. These results strongly suggest that the convertase is a proteinase different from cathepsins B₁, D, H, and L.

Influence of Group-specific Reagents and Proteinase Inhibitors on the Convertase Activity—We reported previously (10) that the convertase activity in extracts of crude lysosomes from rat liver was markedly inhibited by sulfhydryl-reactive reagents, such as iodoacetate and p-chloromercuribenzoate. The effects of sulfhydryl compounds and EDTA were examined by incubating the convertase with these reagents for 15 min at 0°C prior to assay of the convertase. As shown in Table II, the enzyme was activated by dithiothreitol at an optimal concentration of 2 mM. EDTA could activate the enzyme to

![Figure 2](https://example.com/figure2.png)

**FIG. 2.** Inhibition by oxidized ribonuclease A of the convertase. The activity of the purified convertase (4.17 ng) was assayed in the presence of various amounts of oxidized ribonuclease A (○) or native ribonuclease A (●), employing purified Form I of tyrosine aminotransferase (3.75 μg. 2.37 units) as substrate.

| Activity of the Convertase on Synthetic Substrates | BANA hydrolase | Cathepsin L |
|--------------------------------------------------|----------------|------------|
| Acetone, 45% precipitate                         | Acid supernatant | CM-cellulose | Hydroxylapatic | Sephadex G-75 | DEAE-cellulose |
| Total activity (units)                           | 3630            | 153        | 1.00         | 0.450       | 0.988       | 1.12        |
| Specific activity (units/mg)                     | 1.59            | 22.8       | 1.85         | 5.73        | 911         | 9.93        |
| Yield (%)                                        | 911             | 31.1       | 20.8         | 64.4        | 51.2        | 33.4        |
| Purification (fold)                              | 2350            | 35.0       | 1.85         | 5.73        | 34.7        | 27.0        |
| Cathepsin D                                      | 1870            | 134        | 7.09         | 34.7        | 27.0        | 20.5        |
| Total activity (k units)                         | 120             | 243        | 27.0         | 27.0        | 20.5        | 20.5        |
| Specific activity (units/mg)                     | 16.1            | 243        | 27.0         | 27.0        | 20.5        | 20.5        |
| Yield (%)                                        | 212             | 243        | 27.0         | 27.0        | 20.5        | 20.5        |
| Purification (fold)                              | 1070            | 583        | 31.0         | 31.0        | 30.8        | 30.8        |
| BANA hydrolase                                   | 1490            | 583        | 30.8         | 30.8        | 30.8        | 30.8        |

**Table I**

Activities of lysosomal endopeptidases during purification of the convertase from rat liver

The convertase was purified from livers of 100 rats fasted overnight as described previously (13). The fractions from each step after extraction of mitochondrial-lysosomal fraction were used for the assay of activities of the convertase, BANA hydrolase, and cathepsins D and L.

| Activity | Mitochondrial lysosomal extract | Acetone, 45% precipitate | Acid supernatant | CM-cellulose | Hydroxylapatic | Sephadex G-75 | DEAE-cellulose |
|----------|--------------------------------|--------------------------|-----------------|--------------|---------------|---------------|---------------|
| Convertase | 8110                          | 2090                     | 911             | 120          | 9.93          | 2.05          | 1.12          |
| Total activity (units) | 3630                          | 153                      | 1.85            | 27.0         | 31.1          | 41.8          | 30.8          |
| Specific activity (units/mg) | 1.59                          | 22.8                     | 1.85            | 20.8         | 64.4          | 51.2          | 33.4          |
| Yield (%) | 911                           | 31.1                     | 20.8            | 64.4         | 51.2          | 33.4          | 27.0          |
| Purification (fold) | 2350                          | 134                      | 7.09            | 34.7         | 27.0          | 27.0          | 20.5          |
| Cathepsin D | 1870                          | 243                      | 27.0            | 27.0         | 20.5          | 20.5          | 20.5          |
| Total activity (k units) | 120                           | 243                      | 27.0            | 27.0         | 20.5          | 20.5          | 20.5          |
| Specific activity (units/mg) | 16.1                          | 243                      | 27.0            | 27.0         | 20.5          | 20.5          | 20.5          |
| Yield (%) | 212                           | 243                      | 27.0            | 27.0         | 20.5          | 20.5          | 20.5          |
| Purification (fold) | 1070                          | 583                      | 31.0            | 31.0         | 30.8          | 30.8          | 30.8          |

*Not detectable.*
A New Thiol Proteinase

Table II
Activation of the convertase by sulphydryl compounds and EDTA

| Experiment | Effector                  | Concentration | Relative activity of the convertase |
|------------|---------------------------|---------------|------------------------------------|
| A          | None                      | 0.1 mm        | 114                               |
|            | Dithiothreitol            | 1 mm          | 228                               |
|            |                           | 2 mm          | 250                               |
|            |                           | 5 mm          | 213                               |
|            |                           | 10 mm         | 196                               |
|            |                           | 50 mm         | 201                               |
| B          | None                      | 0.1 mm        | 114                               |
|            | EDTA                      | 1 mm          | 251                               |
|            | Dithiothreitol            | 1 mm          | 198                               |
|            | EDTA/dithiothreitol       | 1 mm          | 237                               |
|            |                           | 2 mm          | 196                               |
|            |                           | 2.5 mm        | 196                               |
| C          | None                      | 0.1 mm        | 131                               |
|            | Cysteine                  | 1 mm          | 177                               |
|            | β-Mercaptoethanol         | 0.1 mm        | 147                               |
|            |                           | 1 mm          | 190                               |
|            | β-Mercaptoethylamine      | 0.1 mm        | 146                               |
|            |                           | 1 mm          | 219                               |
|            | Glutathione               | 0.1 mm        | 154                               |
|            |                           | 1 mm          | 175                               |
|            | Dithiothreitol            | 0.2 mm        | 148                               |
|            |                           | 2 mm          | 248                               |

some extent, whereas in the presence of dithiothreitol, EDTA demonstrated no further activation. All sulphydryl-reducing compounds tested were effective in activating the convertase.

The effects of sulphydryl-reactive compounds and proteinase inhibitors on the convertase activity are summarized in Table III. p-Chloromercuribenzoate, N-ethylmaleimide, and 4,4′-dipyridyl disulfide were powerful inhibitors of the enzyme. The purified convertase was also sensitive to iodoacetate and iodoacetamide. Of active site-specific reagents for serine proteinases, PMSF showed no effect on the enzyme activity after additional incubation with excess dithiothreitol, whereas TLCK inhibited the enzyme under the same conditions. The convertase was insensitive to peptatin, primarily effective against carboxy proteinases, and to soybean trypsin inhibitor. The purified convertase was inhibited by leupeptin and antipain, although relatively high concentrations of the inhibitors were required for inhibition. These results obtained with activators and inhibitors are compatible with the hypothesis that the convertase is a thiol proteinase.

Discussion

In a previous report (13) we demonstrated that the conversion of Form I of tyrosine aminotransferase to Forms II and III by the purified convertase was accompanied by the concomitant production of 48,000-Mr, subunits from 52,000-Mr, subunits of the enzyme. From these results and the observation that the purified convertase exhibited a potent azocaseinolytic activity, we concluded that the conversion of tyrosine aminotransferase forms was a proteolytic process (13). In this study, oxidized ribonuclease A was found to be readily cleaved by the purified convertase (Fig. 1). Furthermore, oxidized ribonuclease A strongly inhibited the conversion of the aminotransferase forms as well as the production of 48,000-Mr, subunits of the enzyme (Fig. 2). These results further support our previous conclusion that the conversion of the tyrosine aminotransferase forms is a proteolytic process.

High activity of the convertase toward proteases such as azocasein and oxidized ribonuclease A suggests an endopeptidase nature of the enzyme. The following observations also demonstrate endopeptidase activity of the convertase. 1) During the early stages of the digestion of oxidized ribonuclease A by the convertase, only two major polypeptide bands were observed as cleavage products on gels of SDS-polyacrylamide electrophoresis (Fig. 1). 2) Only 48,000-Mr, subunits of tyrosine aminotransferase were produced during the conversion of Form I of the enzyme (13). No other intermediate sizes of subunits of the enzyme were detected on any stages of the conversion. 3) During the cleavage of purified serine dehydrodase by the convertase at 0°C, two major polypeptides, whose molecular weights were approximately 24,000 and 10,000, were produced from 34,000-Mr, subunits of the enzymes (28) with concomitant decrease of the enzyme activity. 4) No detectable hydrolysis by the purified convertase of synthetic substrates for exopeptidases tested was observed.

Several characteristics of the purified convertase are consistent with the interpretation that the enzyme is a thiol proteinase. Typical of other thiol proteinases (30), the convertase activity was stimulated by sulphydryl-reducing compounds or EDTA (Table II). The enzyme was sensitive to

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sulfhydryl-reactive compounds such as p-chlomercuribenzoate, N-ethylmaleimide, etc. The convertase was also inactivated by TLCK, which inhibited a range of thiol proteinases irreversibly by alkylating their essential sulfhydryl groups (31, 32), although this compound is usually regarded as an active site-specific reagent for trypsin. Ayrum and Herschko (33) reported that the convertase activity in a particulate fraction from rat hepatoma HTC cells was inhibited by PMSF. In our experiment, however, PMSF showed no effect on the purified convertase activity after addition of excess diithiothreitol prior to assay of the convertase activity. PMSF has been reported to inhibit thiol proteinases, but this inhibition, unlike that of serine proteinases, was reversed by excess diithiothreitol (30). The convertase was insensitive to pepstatin, a powerful inhibitor of all carboxyl proteinases. Leupeptin and antipain, which readily inhibit thiol proteinases (34), inactivated the convertase at relatively high concentration. Of thiol endopeptidases in rat liver lysosomes, cathepsins L and B, which are quite sensitive to leupeptin and antipain (24, 27), but cathepsin H is relatively insensitive to leupeptin (28).

Four lysosomal endopeptidases have been found in rat liver. Three of them (cathepsins B, H, and L) are thiol endopeptidases and the fourth is a carboxyl endopeptidase, cathepsin D. We reported the molecular weight of the convertase to be 33,500 to 35,000 (13), which is greater than that of cathepsins B, (Mw = 22,500) (35), H (Mw = 28,000) (28), and L (Mw = 23,000-24,000 or 22,000) (24, 27). During purification of the convertase from a mitochondri-alysosomal fraction of rat liver, the activity of cathepsins B, and H, measured as BANA-hydrolyzing activity, and of cathepsin L were completely removed from the convertase fraction after hydroxylapatite chromatography and Sephadex G-75 gel filtration respectively (Table I). Thus, the dissociation of the activity was still measurable on the final preparation of the homogeneous convertase. Since the recovery and purification of cathepsin D activity were much lower than those of the convertase activity, and the convertase was insensitive to pepstatin (Table III), it is likely that the convertase itself possesses hemoglobin-hydrolyzing activity at this pH rather than that the convertase is identical with cathepsin D. The convertase showed about the same molecular weight as BANA amidohydrolase, an enzyme distinct from cathepsin B, in rat liver, as reported by de Lumen and Tappel (36). BANA, however, was not hydrolyzed by the purified convertase, as mentioned above. Collagenolytic cathepsin, which degraded soluble and insoluble collagen at acid pH, and was separated from cathepsin B, in bovine spleen by Etherington (37, 38), has been recently and named cathepsin N (39). This enzyme was found to be a thiol proteinase having a molecular weight of 18,000 to 20,000 (39), which is smaller than that of the convertase from rat liver (13). Its group has partially purified a similar thiol proteinase from human placenta (40) and has also named this enzyme cathepsin N (41), although the human enzyme showed some properties different from those of bovine spleen cathepsin N, such as molecular weight, isoelectric point, and specific activity (39, 40). Cathepsin N partially purified from human placenta was reported to have a molecular weight of 34,600 (40), which is very close to that of the purified convertase of rat liver (13) when estimated on a Sephadex G-100 column. Our preliminary experiments have shown potent collagenolytic activity of the convertase at acid pH and 38°C. However, the degradation of collagen is suggested to be a general property of thiol proteinases (42). Most protein substrates other than collagen remained apparently undegraded by cathepsin N (43), whereas the convertase was able to digest common proteins, such as azocasein, hemoglobin, and oxidized ribonuclease A in addition to cleaving Form I of tyrosine aminotransferase. Moreover, cathepsin N from human placenta was shown to be stable at pH 7.4 (43), but the convertase was extremely unstable at this pH, unless glycerol was present at a concentration greater than 10% (13). An additional difference between cathepsin N and the convertase is the fact that crude cathepsin N preparation could be dialyzed against 10 mM sodium phosphate buffer, pH 6.5, without appreciable loss of enzyme activity (40). Under these conditions the convertase in crude preparations from rat liver was unstable and only partially soluble or aggregated. Therefore, the convertase is likely to be a hitherto unreported endopeptidase in rat liver lysosomes.

Cathepsin L was reported to account for approximately 50% of the proteinase activity of lysosomal extract from rat liver (27) and to play an important role in intracellular protein breakdown (44). The convertase showed a specific activity for hydrolyzing azocasein about 6 times higher than cathepsin L (13). From yields of both the convertase activity (6.1%) and azocaseinolytic activity (1.4%) (13), the convertase was calculated to be responsible for about one-fourth of the total azocaseinolytic activity of rat liver homogenates. Thus, this new thiol proteinase might play an important role in lysosomal protein degradation. One might raise the question why Kirschke et al. (27, 28) were not able to detect this proteinase in spite of using azocasein as substrate during purification of cathepsins B, H, and L from rat liver lysosomes. In their experiments, the convertase, if extracted from lysosomes, would probably have been inactivated during chromatography on Sephadex G-75. Their conditions made use of 10 mM potassium phosphate buffer, pH 6.9, containing 150 mM KCl as an elution buffer, in which the convertase was unstable as described previously (13). On the basis of the results described in this report, we propose the name of cathepsin T for this new thiol endopeptidase in rat liver lysosomes.

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