Differences in cathepsin B mRNA levels in rat tissues suggest specialized functions

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The tissue distribution of mRNAs encoding two lysosomal proteases, cathepsin B and cathepsin D, was examined using cloned cDNAs to probe Northern and dot blots of RNAs extracted from various rat tissues. Cathepsin B mRNA showed a wide range of variation in expression in the tissues analyzed with the highest concentrations found in spleen and kidney, while the cathepsin D mRNA levels were relatively uniform in these same tissues. Significant quantities of cathepsin B mRNA were detected in total RNA from isolated islets of Langerhans but was not detectable in equivalent amounts of RNA from whole pancreas. The wide variations in tissue levels of cathepsin B mRNA suggest that tissue specific controls may regulate its expression and are compatible with the participation of this protease in specialized cellular functions other than intralysosomal protein degradation.

Thiol protease *Precursor processing* *Lysosome* *Gene expression* *Protein degradation*

1. INTRODUCTION

Cathepsin B (EC 3.4.22.1) is a thiol activated protease which, together with the related cysteine proteases, cathepsins H and L, and the aspartyl protease, cathepsin D (EC 3.4.4.23), is believed to play a major role in intracellular protein degradation in many tissues [1–3]. Cathepsin B has been purified from several mammalian sources [4–8] and the primary sequences of the rat [9], human [10] and a partial sequence of the porcine liver enzymes [11] have been determined. Isolated mature cathepsin B usually consists of a mixture of single and two chain forms having a molecular mass of approx. 27 kDa and single glycosylation site is present in the mature enzyme [9,12]. Both cathepsin B and H share important structural homologies with papain, a thiol endoproteinase from plants [9].

Although cathepsin B has been localized to lysosomes in some tissues and undoubtedly plays an important role in intracellular protein catabolism, evidence has accumulated in recent years suggesting that this protease, or its biosynthetic precursor form(s), may also have extralysosomal functions. Thus, it has been proposed that cathepsin B-like thiol proteases may be involved in the processing of some peptide hormone precursors [13–16] and, recently, a higher molecular mass form of cathepsin B has been demonstrated in secretory granules from rat islets and a rat insulinoma cell line [17]. A number of investigators have also observed enhanced secretion of both latent and active forms of cathepsin B from malignant tumor cells and have suggested that cathepsin B or similar enzymes may be involved in tumor invasiveness [18–20]. Such a multi-functional role for cathepsin B suggests that its gene(s) might be highly regulated and consequently that its expression may vary in different tissues in response to local stimuli or special demands. To investigate more fully the expression and role of cathepsin B, we recently have cloned

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and sequenced cDNAs encoding rat, mouse and human preprocathepsin B ([21], unpublished). The nucleotide sequence data indicate that procathepsin B from all 3 species contains a conserved proregion of 61 residues amino-terminal to the domain of mature cathepsin B. In this study, we have utilized cloned cDNA as a hybridization probe to measure the relative levels of cathepsin B mRNA in various rat tissues. For comparative purposes, we have measured the mRNA levels of another lysosomal enzyme, cathepsin D, in these tissues using an available cloned human cathepsin D cDNA probe [21].

2. MATERIALS AND METHODS

2.1. *Materials*

Nitrocellulose paper was BA 85 (0.45 μm) from Schleicher and Schuell. [α-^{32}P]dCTP and [α-^{32}P]CTP (spec. act., 3000 Ci/mmol) were from Amersham (Arlington Heights, IL). DEAE-membrane (NA-45) was purchased from Schleicher and Schuell. DNA was labelled with [α-^{32}P]dCTP using a nick translation kit bought from Amersham. Restriction enzymes were from New England Biolabs. A cloned cDNA for human cathepsin D was obtained from Dr J. Chirgwin (St. Louis, MO).

2.2. *RNA isolation*

Total RNA was isolated from tissues of Sprague-Dawley rats by a modified guanidine thiocyanate procedure [22]. Total RNA was also obtained from a transplantable rat insulinoma [23]. The tissues were excised and frozen at -70°C. After pulverization, aliquots were homogenized in equal volumes of SDS buffer (0.2 M Tris-HCl, pH 9.0, 0.1 M HCl, 0.025 M EDTA, 1% SDS) and a mixture of phenol/chloroform/isoamylalcohol (50:50:1) (1 g tissue/10 ml solution). The nucleic acid containing aqueous phase was extracted with the organic solvent mixture twice and precipitated with ethanol. Each RNA sample was further purified by sedimentation through a CsCl cushion essentially according to Chirgwin et al. [24] to remove residual amounts of DNA. Approx. 2000 islets of Langerhans were isolated from rat pancreas by collagenase digestion [14] and total RNA was extracted as described above.

2.3. *Dot and Northern blot analysis*

Dot blot analysis was performed with a template manifold apparatus (Schleicher and Schuell, Keene, NH). Total RNA was applied at three different concentrations (10, 5 and 2 μg) for analysis of cathepsin B mRNA and at 5, 2 and 1 μg concentrations for analysis of cathepsin D mRNA. By adding yeast tRNA, the final total RNA (rat plus yeast) concentration in each dot was identical (10 and 5 μg, respectively). The RNA samples were denatured by incubation at 50°C for 15 min in 1.0 M formaldehyde and 10 × SSC (1 × SSC = 0.15 M, 0.015 M sodium citrate, pH 7.0). Samples were immediately chilled on ice and one volume of 10 × SSC was added. Both nitrocellulose sheets and 3 MM Whatman paper were previously equilibrated with 10 × SSC. Following sample application, filters were dried for 2 h at 80°C. Dot blots were hybridized to ^{32}P-labeled cDNA inserts of approx. 1000 bp corresponding to rat cathepsin B [21] and human cathepsin D [25] isolated from recombinant plasmids by digestion with *Eco*RI and electroelution after agarose gel electrophoresis. The eluted DNA fragment was bound to a DEAE-membrane, eluted by incubation at 65°C in 1.0 M NaCl, 0.001 M EDTA, 0.02 M HCl-Tris, pH 8.0, and nick-translated.

Hybridization and washing conditions have been described [21]. The relative abundance of mRNAs in the different tissue total RNA preparations was determined by scanning densitometry of filter autoradiographs.

Northern blots of total RNA were prepared according to Thomas et al. [26]. The cDNA inserts for both cathepsins were isolated and labeled as described above. Conditions for hybridization and washing for the cathepsin B clone were as described in [21]. The washing temperature was decreased to 45°C for the human cathepsin D clone in dot and Northern blots.

3. RESULTS

The relative amounts of cathepsin B and D mRNA in the various rat tissues studied were measured by dot blot hybridization analysis using a rat cDNA probe for cathepsin B and a human cDNA probe for cathepsin D. Based on highly conserved amino acid sequence homology between cathepsin D from different species, we anticipated

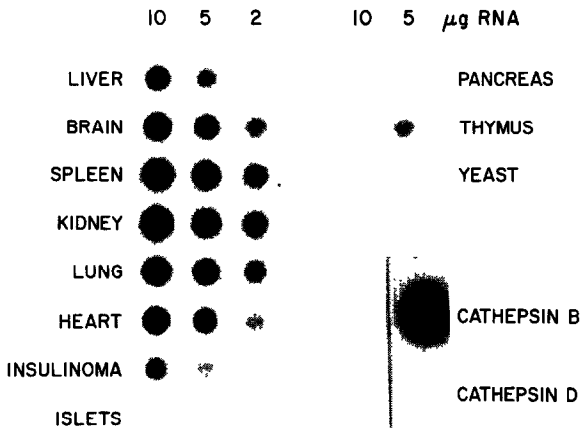

Fig.1. Distribution of cathepsin B mRNA in rat tissues. Total cellular RNA was applied at 3 different concentrations (10, 5, 2 μg) for 7 tissues (liver, brain, spleen, kidney, lung, heart and pancreas) and insulinoma or at a single concentration for islets of Langerhans and thymus RNA.

Table 1

Relative levels of cathepsin B and cathepsin D mRNAs in rat tissues

Tissues	Relative mRNA level	
	Cathepsin B	Cathepsin D
Kidney	100 (100)[a]	100
Spleen	80 (47)	134
Lung	61 (13)	96
Brain	47 (27)	85
Heart	33 (10)	96
Liver	20 (28)	83
Thymus	12	85
Insulinoma	7	65
Islets	6	103
Pancreas	ND[b]	89

[a] Relative levels of mature cathepsin B protein, measured by immunochemical methods, recalculated from data of Katunuma and Kominami [27]

[b] ND, not detectable

that the human cDNA would hybridize specifically with rat cathepsin D mRNA. The results for the distribution of cathepsin B mRNA are shown in fig.1 and summarized in table 1. The dot blots revealed that cathepsin B mRNA was most abundant in kidney. It was also abundant in spleen, lung, brain and heart, in decreasing order. The relative mRNA level found in liver (20% of that in kidney) was somewhat lower than expected in view of the frequent use of this organ as a source of enzyme. RNA obtained from islets of Langerhans and insulinoma cells gave signals in the dot blot hybridizations which were 7.0 and 8.0% relative to kidney whereas no signal was detected in similar amounts of RNA from whole pancreas. The distribution of cathepsin D mRNA in the same tissues is presented in fig.2 and summarized in table 1. Although the highest concentration of cathepsin D mRNA was found in spleen, uniformly large quantities were found in all the other tissues tested including whole pancreas. Control 'dots' of yeast RNA did not react with either cathepsin probe.

Northern blot analysis was also performed on the tissue RNA preparations. The size of cathepsin B mRNAs (fig.3) did not vary among the different tissues, in all instances showing the 2.3 kb size mRNA as noted earlier for rat liver [21]. No other significant bands were detected in any of the

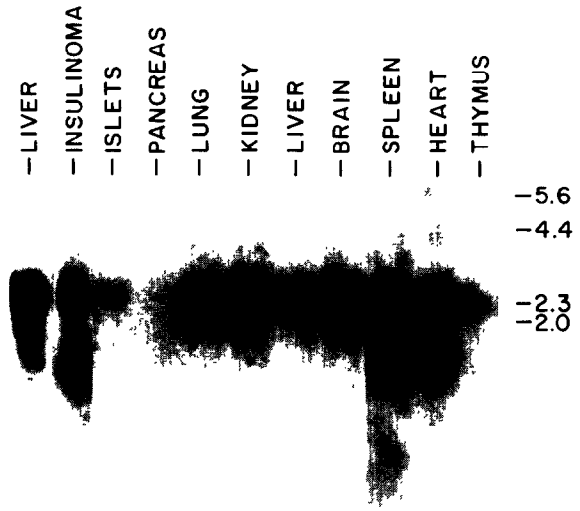

Fig.2. Determination of the size of cathepsin B mRNA in rat tissues. Total RNAs from liver (50 μg), insulinoma (50 μg), islets (15 μg), pancreas (10 μg), lung (5 μg), kidney (10 μg), liver (10 μg), brain (10 μg), spleen (7.5 μg), heart (12.5 μg) and thymus (7.5 μg) were used. The positions and sizes (in kb) of *Hin*dIII-digested λDNA are indicated.

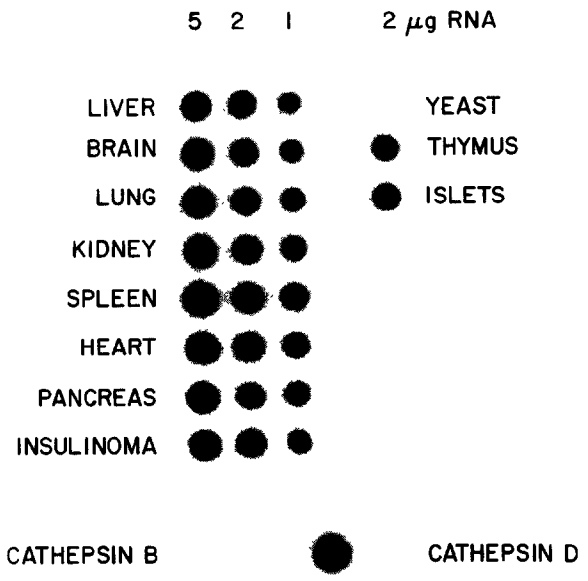

Fig.3. Distribution of cathepsin D mRNA in rat tissues. Total cellular RNA was applied at three concentrations (5, 2 and 1 μg) for tissues and insulinoma and at only one concentration for thymus and islets of Langerhans RNA.

tissues examined. These findings are consistent with other results indicating that cathepsin B is encoded by a single copy gene in several species including rats (S.J. Chan, unpublished). The size of the cathepsin D mRNA (not shown) was closely similar in size to that of cathepsin B mRNA, in agreement with the reported human cathepsin D-mRNA length of 2.2 kb [25]. The presence on the Northern blots of a distinct band for cathepsin D mRNA and the virtual absence of a band for cathepsin B mRNA in the pancreatic RNA sample confirms the results obtained above by dot hybridization, suggesting that the expression of cathepsin B in this organ is considerably higher in the islets of Langerhans than in the exocrine or ductal cells.

4. DISCUSSION

Here we have demonstrated that the expression of the cathepsin B gene, as reflected in the level of its mRNA transcripts, can vary over 10-fold in different tissues from the rat. In contrast, the mRNA levels for another lysosomal proteinase, cathepsin D, varied only 2-fold in these tissues. Although cathepsin B is related structurally to cathepsins H and L [2,9], as well as to calcium-dependent thiol proteases [28], no cross-hybridization to mRNA for these proteins was noted under the stringent conditions used. This finding, together with evidence from Southern blot analysis that rat cathepsin B is encoded by a single gene (S.J. Chan, unpublished), indicates that the observed variations in cathepsin B mRNA signal result from differences in expression of this gene, rather than arising from a complex of several related genes.

While these experiments were in progress, Katunuma and Kominami [27] published a report on the distribution of cathepsin B and H in rat tissues in which a sensitive immunochemical method was used to quantitate the amount of enzyme present. Our results generally are in agreement with theirs and both methods indicate that the kidney contains the highest level of both cathepsin B mRNA and immunoreactive enzyme. However, our estimates of the relative level of liver cathepsin B mRNA is somewhat lower than the amount of measured enzyme while brain, lung, and heart all appear to contain relatively more mRNA than measurable enzyme. A number of factors might account for such discrepancies including tissue differences in the translational efficiency of cathepsin B mRNA or the turnover rate of mature enzyme, the presence of various inhibitors or stabilizers of the enzyme protein, or differential processing and subcellular compartmentation of precursor forms of cathepsin B (vide infra). Such precursors may not be reactive with antibodies to the mature enzyme which require prior denaturation of the protein ([2,17]; D.F. Steiner, unpublished).

The wide variation in the levels of cathepsin B mRNA observed in this study and its virtual absence in exocrine pancreatic RNA, raises questions as to its function as a constitutive component of lysosomes. A possible explanation is that lysosomes themselves are not homogeneous organelles but may be regulated to contain varying mixtures of hydrolytic enzymes suitable for different sets of substrates in different tissues. Thus, the rather uniform distribution of cathepsin D mRNA in most tissues is consistent with the putative major role of this protease in general intracellular degradation of proteins. However,

cathepsin B might be more specifically involved in the degradation of susceptible proteins that only occur in certain tissues. For example, the presence of relatively large amounts of cathepsin B mRNA in spleen correlates well with the cytochemical localization of large amounts of mature enzyme in macrophages and other phagocytic cells [27] and is consistent with the possibility that cathepsin B may catalyze critical rate-limiting steps in the degradation of ingested extracellular proteins. It could thus be involved in antigen processing in macrophages, a chloroquine-sensitive process involving limited proteolysis of proteins in endocytic vesicles [29].

Our findings also support the possibility that cathepsin B or its precursor forms may have extralysosomal function(s). Much interest has been evoked by suggestions that cathepsin B-like thiol proteases may be involved in the processing of some peptide hormone precursors [13,16] and proapolipoproteins [30,31], and by its possible role in malignant tumors as a secreted protease associated with invasive behavior [18–20,32]. These putative functions imply that the sorting of procathepsin B as it passes through the Golgi stack is biphasic, allowing a portion of the precursor to enter the secretory pathways while a major fraction is transported to the lysosomes, presumably via the mannose 6-phosphate receptor pathway [33]. Differences in processing of procathepsin B in secretory vesicles as compared with the lysosomes might then result in the production of active derivatives of higher molecular mass having specialized proteolytic characteristics [16]. In normal cells or tissues low levels of secretion of lysosomal enzymes or their precursors may occur via non-regulated pathways [34], and in pathological situations such as I-cell disease [35] where the mannose 6-phosphate receptor is missing or defective, secretion via this pathway is increased. Increased entry of procathepsin B into secretory pathways in neoplasms could possibly be attributable either to defective specific sorting functions or to overproduction of the enzyme, thereby exceeding sorting capabilities [34].

In conclusion, these findings are consistent with the proposal that cathepsin B is a multi-targeted protein within the various tissues in which it is expressed. Its destinations include most certainly the lysosomes, but also the secretory pathway and perhaps, in some cells, specialized endocytic vesicles engaged in limited proteolysis of ingested external proteins. An essential element of this potential multi-functional role is that some larger precursor-derived form(s) may have specialized proteolytic activity differing from that of mature cathepsin B. Further analysis of this role for procathepsin B will be facilitated by the elucidation of the primary structure of preprocathepsin B via cDNA cloning (San Segundo, B. et al., in preparation), as well as by the expression in vitro of this precursor via recombinant DNA methods for further study of its properties and possible pathways of processing into proteolytically active forms.

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