Isolation of Bovine Kidney Leucine Aminopeptidase cDNA: Comparison with the Lens Enzyme and Tissue-Specific Expression of Two mRNAs†

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ABSTRACT: Aminopeptidases catalyze the hydrolysis of amino acid residues from the amino terminus of peptide substrates. Leucine aminopeptidase (LAP) from bovine lens is the best characterized aminopeptidase and the only LAP for which the amino acid sequence was determined by protein sequencing. Using this sequence information, we isolated a bovine kidney LAP cDNA and compared its deduced amino acid sequence to the published amino acid sequence for bovine lens LAP. Overall, the sequences are highly conserved. However, several differences are observed. The kidney LAP cDNA indicates a 26 amino acid extension at the amino terminus which is not found in the mature purified lens LAP. The cDNA also indicates an additional octapeptide in the C-terminal region which was not indicated in the published lens LAP amino acid sequence but which was required for best fit of crystallographic data regarding bovine lens LAP. Several other single amino acid changes were also noted. Levels of LAP transcripts were examined in bovine lens and kidney tissue as well as in cultured lens cells. Lens epithelial tissue showed only one LAP transcript (2.4 kb) whereas two transcripts (2.0 and 2.4 kb) were observed in cultured lens cells derived from epithelial tissue and in kidney tissue. Using Northern blot analysis, we correlated LAP mRNA levels with previously determined changes of LAP activity in aging lens tissue and in progressively passaged lens epithelial cells which were used to simulate aging *in vitro*. No differences were found in LAP mRNA levels in epithelial tissue from old and young lenses. LAP mRNA concentrations are regulated in a manner consistent with the transient increases in LAP activity and in intracellular proteolytic activity in the progressively passaged cells. Northern blot analysis suggests that the 2.0- and 2.4-kb LAP transcripts arise by differential splicing of a common precursor RNA and that they code for similar but distinct proteins.

Aminopeptidases constitute a large group of proteases which catalyze the hydrolysis of amino acid residues from the amino terminus of peptide substrates. They are widely distributed throughout the plant and animal kingdoms, and most have broad specificity. Aminopeptidases exist on cell surfaces, in soluble cytoplasmic forms, and several forms of these enzymes have been found in many tissues or cells [Taylor et al., 1984a; Ledeme et al., 1983; Oettgen & Taylor, 1985; Watt & Yip, 1989; and references cited within Stirling et al. (1989); Ahmad and Ward (1990); Chang and Smith (1989); and Taylor (1993a,b)].

The suggested functions of aminopeptidases are many. They are required in mammalian tissues and cells for degradation of peptides (Botbol & Scornik, 1989) and EGF-induced cell-cycle control (Takahashi et al., 1989). These enzymes also participate in metabolism of secreted regulatory molecules (hormones and neurotransmitters; Ahmad & Ward, 1990; Taylor & Dixon, 1978; Nyberg et al., 1990; Malfroy et al., 1989; Squire et al., 1991; Taylor, 1993a) and dietary constituents, in modulation of cell–cell interactions [for reviews, see Watt and Yip (1989) and references cited within], and in regeneration of plasmid stability in *Escherichia coli* (Stirling et al., 1989). In *Salmonella typhimurium*, the essentiality of some aminopeptidases is indicated since deletion of enzymes, such as methionine aminopeptidase, is lethal [Miller et al., 1989; reviewed in Taylor (1993b)].

Bovine lens leucine aminopeptidase (LAP)[1] was one of the first proteases discovered (Smith & Hill, 1960), and at present, it is the best-studied aminopeptidase with respect to composition (Carpenter & Vahl, 1973; Hanson & Frohne, 1976; Cuypers et al., 1982), structure (Taylor et al., 1979, 1992; Burley et al., 1990), and mechanism of action (Allen et al., 1983; Taylor et al., 1982b, 1992, 1993). Immunological (Taylor et al., 1984a,b), mechanistic (Hanson et al., 1967), and structural (Oettgen & Taylor, 1985) data indicate that within a species LAPs from different tissues are indistinguishable and that interspecies differences are small.

Altered leucine aminopeptidase activity has been associated with a variety of clinical conditions and pathologies, including cataracts (Taylor et al., 1982, 1983, 1984a; Taylor & Davies, 1987), cancers (Gupta et al., 1989), myeloid leukemia, and altered white blood cell counts (Scott et al., 1986). Changes

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[1] Abbreviations: LAP, leucine aminopeptidase; bp, base pair(s); kb, kilobase(s); MEM, minimal essential medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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in LAP levels have been used for diverse assays such as prenatal diagnosis of cystic fibrosis (Buffone et al., 1988) and detection of chromium toxicity (Boge et al., 1988). Upon progessive cell passage (and possible associated transformation of lens epithelial cells in culture), changes in LAP activity have also been observed (Eisenhauer et al., 1988; Courtois et al., 1978). In addition, LAP polymorphism analysis was used for genotyping mussels (Beaumont et al., 1989).

In this paper, we describe the isolation of the full-length cDNA for bovine kidney LAP and compare the cDNA-derived amino acid sequence with the published sequence for the lens enzyme. Predicted structural and mechanistic features are considered. Tissue-specific regulation of expression of LAP in kidney and lens tissues and cultured lens cells is also discussed.

MATERIALS AND METHODS

Laboratory reagents were of the highest purity available. LAP, which was used as a standard in activity assays and gels, was purified from bovine lenses as per Allen et al. (1983). Cell culture media and plasticware were obtained from Gibco.

Isolation of LAP cDNA. Total RNA was extracted from bovine kidney, enriched for poly(A+) RNA on oligo(dT)–cellulose, and used to construct a λgt10 cDNA library as described previously (Gubler & Hoffman, 1983; Chirgwin et al., 1979). The cDNA library, consisting of 1×10^6 independent recombinant phages, was screened with 5′-[γ-^{32}P]-ATP-labeled oligonucleotide probes at a concentration of 1×10^5 cpm/mL of hybridization buffers as described previously (Wallner et al., 1986). Oligonucleotides were a gift of Dr. K. Ramachandran (Biogen) and were chemically synthesized on a 380A Applied Biosystems DNA synthesizer. Three degenerate oligonucleotide probe pools were synthesized according to the published bovine lens LAP amino acid sequence (Cuypers et al., 1982) as follows: LAP1 corresponding to amino acid residues 11–20, 2048-fold degenerate, 30 bp long; LAP2 corresponding to residues 77–84, 128-fold degenerate, 24 bp long; LAP3 corresponding to residues 207–213, 48-fold degenerate, 21 bp long. Oligonucleotides were end-labeled with [γ-^{32}P]ATP, and each probe pool was hybridized individually to bovine kidney RNA in Northern blot analysis (Wallner et al., 1986) to confirm homology between lens and kidney LAP and to decrease the degeneracy of the probe. Probe pool LAP1, which hybridized to a 2.4-kb transcript, was resynthesized as two subsets (LAP5 and LAP6), each of which was 64-fold degenerate, 23 bp long, and differed by one nucleotide from each other. Oligonucleotide LAP6 hybridized to bovine kidney RNA under stringent conditions (Wallner et al., 1986) and was used to screen the bovine kidney library.

Positive recombinant phages were isolated. Mini preparations of λ DNA were digested with restriction enzymes according to manufacturer's specifications and were analyzed by Southern blot analysis (Devlin et al., 1988). Inserts of positive phages were subcloned into sequencing vector pNNO1, and DNA sequence analysis was performed as described (Wallner et al., 1987a).

Cell Culture and Northern Blot Analysis. These experiments were performed using three separate sets of cells. Each showed the same results. Bovine lens explants containing the anterior capsule and adhering epithelium were used to obtain primary cultures of lens epithelial cells (Eisenhauer et al., 1988). After a few minutes, the explant in a 60-mm dish was covered with 1.5 mL of MEM containing 10% FBS, 100 units/mL penicillin, 0.01% streptomycin, and 100 units/mL nystatin (cMEM). When the cells reached confluence, they were rinsed twice with Dulbecco's PBS and treated with 2.5 mL of trypsin/EDTA. After 5 min, the trypsin was deactivated with 3 mL of cMEM, and the resulting suspension was pooled and spun 15 min, 4 °C, 1500*g*. The cells were washed by resuspending them in cMEM. After centrifugation, the cells were again resuspended and transferred to T-150 tissue culture flasks. Cell death was monitored as described by Berger (Berger et al., 1988) by exclusion of trypan blue. Upon reaching confluence, the cells were either scraped or trypsinized, and the pellets were quick-frozen on liquid nitrogen. The pellets were resuspended in guanidinium thiocyanate buffer, and RNA was extracted as described (Chirgwin et al., 1979). RNA was analyzed essentially as described previously (Wallner et al., 1987a).

RESULTS AND DISCUSSION

The isolation of bovine kidney LAP cDNA provided us with a means to establish the correct amino acid sequence for LAP and to proceed with structural (Burley et al., 1992; Taylor et al., 1992) and mechanistic (Taylor et al., 1992, 1993) studies of the protein. It also allowed us to begin to explore regulation of LAP expression in lens epithelial cells during different stages in culture; in lens tissue at different stages of differentiation, maturation, and development; and in kidney tissue.

Isolation and Characterization of Bovine LAP cDNA. Prior data indicated that bovine lens and kidney LAPs are indistinguishable (Taylor et al., 1984a). Thus, bovine lens LAP amino acid sequence information (Cuypers et al., 1982) was used to synthesize oligonucleotide probes which were employed in the isolation of bovine kidney LAP cDNA from a bovine λgt10 cDNA library. One positive phage, λLAP9, was isolated and determined to contain a 1211 bp cDNA insert consisting of 134 base pairs of 5′-untranslated region and sequence coding for 359 amino acids which were homologous to the published lens LAP protein sequence. However, clone λLAP9 was missing the C-terminal sequence of bovine lens LAP.

To isolate the full-length LAP cDNA, a unique oligonucleotide, LAP7 (solid overline in Figure 1), which corresponds to the most 3′ sequence of clone λLAP9, was used to rescreen the λgt10 bovine kidney cDNA library. One positive clone, λLAP5, contained a 1040 bp insert which included the coding sequence for the LAP C-terminus and 390 bp of 3′-untranslated region. Clones λLAP9 and λLAP5 had overlapping sequences, and a common *Bst*XI restriction site was used to construct a full-length LAP cDNA, pLAPE2 (Figure 1). Plasmid pLAPE2 contains a total of 2050 bp of kidney LAP cDNA sequence; 134 bp are 5′-untranslated and 377 bp 3′-untranslated sequences including a poly(A) addition signal sequence (overline in Figure 1). The coding region spans 1539 bp that translate into a 513 amino acid protein (Figure 1).

Comparison of Bovine Kidney and Bovine Lens LAP. Access to a complete amino acid sequence was essential for further structural and mechanistic studies regarding aminopeptidases. A comparison of the amino acid sequence determined by protein sequencing of bovine lens LAP (Cuypers et al., 1982) with that deduced for kidney LAP (Figure 2) indicated substantial similarity and revealed important differences. The N-terminus of lens LAP was determined to be TKGL... by N-terminal analysis of purified protein (Cuypers et al., 1982), and the same would be expected for bovine kidney LAP (Taylor et al., 1984a,b). The LAP cDNA isolated from the kidney library indicates two possible initiating methionines. One is located 27 amino acids upstream from the N-terminal

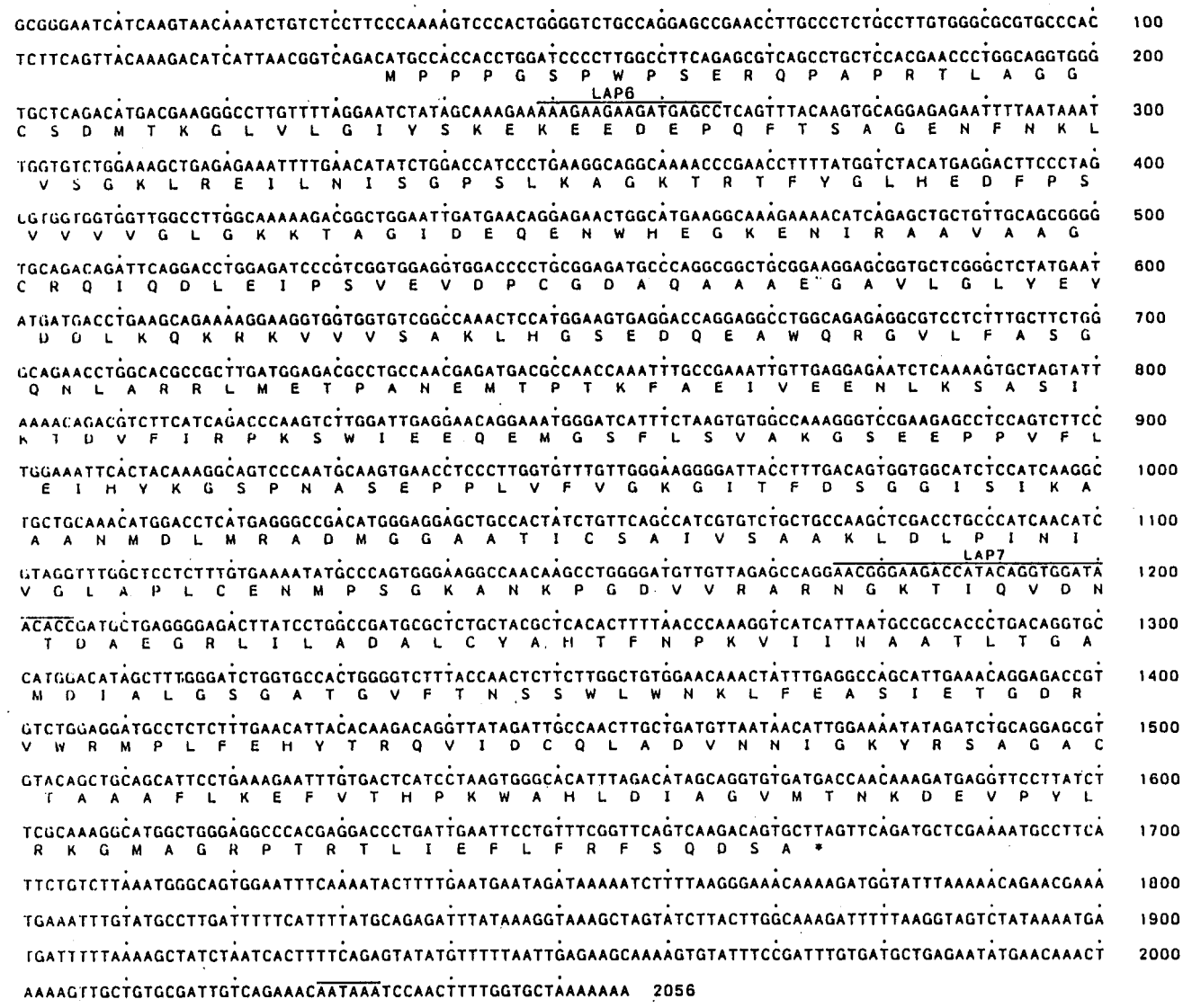

FIGURE 1: Nucleotide and amino acid sequences of pLAPE2 coding for bovine kidney LAP. The sequence of oligonucleotides LAP6 and LAP7 and the poly(A) addition sequence are overlined. The numbers correspond to the nucleotide sequence. The amino acid sequence is that of the precursor protein (see text and Figure 2).

threonine described previously (Cuypers et al., 1982) while the other immediately precedes that threonine. We assume the first ATG to be the initiation codon since there is a stop codon TAA 12 nucleotides 5′ to this ATG. In addition, the sequence preceding the first ATG is in agreement with consensus sequences found in other eukaryotic initiation regions (Kozak, 1986). A similar consensus sequence is not present in the vicinity of the second methionine. This suggests that the kidney LAP is synthesized with a 26 amino acid extension, starting with MetProPro.... Whether this is unique to kidney LAP, or whether it represents a prosequence which is processed and therefore not present in the mature purified lens LAP, remains to be determined. However, initial attempts to identify a proenzyme have been unsuccessful to date. Since all physical, immunological, and biochemical comparative studies indicate that lens and kidney LAPs are identical (Taylor et al., 1984a,b; Oettgen & Taylor, 1985) and since some other aminopeptidases do show such prosequences (Watt & Yip, 1989; Cueva et al., 1989; Guenet et al., 1992; Chang et al., 1992), it is likely that both enzymes are synthesized with a prosequence and are similarly processed.

Another important amino acid sequence difference between the deduced kidney protein sequence and the published amino acid sequence of lens LAP was found at the C-terminus. The kidney LAP cDNA sequence indicates an eight amino acid sequence at the C-terminus that is not present in the published lens LAP protein sequence (see asterisks in Figure 2). This octapeptide is flanked on both ends by trypsin recognition sequences. Cuypers et al. (1982) determined the protein sequence by isolating overlapping fragments generated either by digestion with various proteases or by CNBr cleavage. They were unable to generate overlapping fragments in this region of the C-terminus. Therefore, it is likely that this small tryptic fragment escaped detection during sequencing of bovine lens LAP and that the difference in sequences is a consequence of the techniques used.

Three internal sequence differences were also found. The kidney sequence has a Ser at position 45 instead of Pro, as indicated in the lens sequence, a Leu inserted after the Trp at position 382, and a Met replacing the Trp at position 383 (Figure 2).

There have been two determinations of the amino acid composition of purified bovine lens LAP [reviewed in Hanson and Frohne (1971)]. The amino acid composition of bovine kidney LAP deduced from the cDNA compares well with the composition of lens LAP obtained by direct amino acid analysis of both the native and the carboxamidomethylated lens LAP (Cuypers et al., 1982). If the data obtained by Cuypers and

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kidney                              MPPPGSPWPSERQPAPRTLAGGCSDM -26
kidney TKGLVLGIYSKEKEEDEPQFTSAGENFNKLVSGKLREILNISGPSLKAGK  50
       |||||||||||||||||||||||||||||||||||||||||||||||||| |||||
lens   TKGLVLGIYSKEKEEDEPQFTSAGENFNKLVSGKLREILNISGPPLKAGK  50
kidney TRTFYGLHEDFPSVVVVGLGKKTAGIDEQENWHEGKENIRAAVAAGCRQI 100
       ||||||||||||||||||||||||||||||||||||||||||||||||||||
lens   TRTFYGLHEDFPSVVVVGLGKKTAGIDEQENWHEGKENIRAAVAAGCRQI 100
kidney QDLEIPSVEVDPCGDAQAAAEGAVLGLYEYDDLKQKRKVVVSAKLHGSED 150
       ||||||||||||||||||||||||||||||||||||||||||||||||||||
lens   QDLEIPSVEVDPCGDAQAAAEGAVLGLYEYDDLKQKRKVVVSAKLHGSED 150
kidney QEAWQRGVLFASGQNLARRLMETPANEMTPTKFAEIVEENLKSASIKTDV 200
       ||||||||||||||||||||||||||||||||||||||||||||||||||||
lens   QEAWQRGVLFASGQNLARRLMETPANEMTPTKFAEIVEENLKSASIKTDV 200
kidney FIRPKSWIEEQEMGSFLSVAKGSEEPPVFLEIHYKGSPNASEPPLVFVGK 250
       ||||||||||||||||||||||||||||||||||||||||||||||||||||
lens   FIRPKSWIEEQEMGSFLSVAKGSEEPPVFLEIHYKGSPNASEPPLVFVGK 250
kidney GITFDSGGISIKAAANMDLMRADMGGAATICSAIVSAAKLDLPINIVGLA 300
       ||||||||||||||||||||||||||||||||||||||||||||||||||||
lens   GITFDSGGISIKAAANMDLMRADMGGAATICSAIVSAAKLDLPINIVGLA 300
kidney PLCENMPSGKANKPGDVVRARNGKTIQVDNTDAEGRLILADALCYAHTFN 350
       ||||||||||||||||||||||||||||||||||||||||||||||||||||
lens   PLCENMPSGKANKPGDVVRARNGKTIQVDNTDAEGRLILADALCYAHTFN 350
                                    **
kidney PKVIINAATLTGAMDIALGSGATGVFTNSSWLWNKLFEASIETGDRVWRM 400
       |||||||||||||||||||||||||||||||  ||||||||||||||||||
lens   PKVIINAATLTGAMDIALGSGATGVFTNSSW.MNKLFEASIETGDRVWRM 399
kidney PLFEHYTRQVIDCQLADVNNIGKYRSAGACTAAAFLKEFVTHPKWAHLDI 450
       ||||||||||||||||||||||||||||||||||||||||||||||||||||
lens   PLFEHYTRQVIDCQLADVNNIGKYRSAGACTAAAFLKEFVTHPKWAHLDI 449
                                 ********
kidney AGVMTNKDEVPYLRKGMAGRPTRTLIEFLFRFSQDSA 487
       |||||||||||||||||||||||  ||||||
lens   AGVMTNKDEVPYLRKGMAGRPTR.....FSQDSA 478
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FIGURE 2: Comparison of the deduced amino acid sequence of bovine kidney LAP and bovine lens LAP. Homologous residues are connected by a vertical line. Differences between the two sequences are indicated by an asterisk. The residues of the prosequence are indicated by negative numbers.

Carpenter for bovine lens LAP are averaged, the numbers of Ser, Pro, Trp, Thr, Gly, Ile, Leu, Phe, and Arg are identical with the predicted numbers of these residues based on the kidney cDNA sequence. This corroborates the presence of the octapeptide and the single amino acid changes in lens LAP. Further corroboration is obtained from structural studies since knowledge of all of these modifications of the sequence was required to fit—and is consistent with—the electron density maps in the crystallographic studies of lens LAP (Burley et al., 1992). Taken together, the molecular biological, biochemical, immunological, and crystallographic data strongly suggest that bovine kidney LAP and bovine lens LAP are indistinguishable (Taylor et al., 1984a). As determined from the deduced sequence, the subunit mass of kidney LAP is 52 989 Da. This is in good agreement with values obtained by sequence analysis (Cuypers et al., 1982). The subunit mass of the putative pro-kidney LAP is 55 562 Da.

Bovine LAP, E. coli xerB Aminopeptidase, and Other Aminopeptidases Constitute a New Family of Zinc Enzymes. We previously showed that with respect to amino acid sequence (Taylor et al., 1984a) and quaternary structure (Taylor et al., 1979), hog lens LAP, hog kidney LAP, and human lens LAP are also closely related to bovine lens and kidney LAP. Recently, it was demonstrated that prolylaminopeptidase activity is also due to the LAP enzyme (Turzynski & Mentlein, 1990). It was recently reported that as compared with bovine lens LAP the 503 amino acid *xerB* gene product shows overall 31% sequence identity, and in the C-terminal region, 52% sequence identity. Its metal ion activation is also similar to that observed for bovine lens LAP (Stirling et al., 1989). In lens LAP, the C-terminal region contains the active site (Taylor et al., 1992), and we hypothesize that the same pertains for the *E. coli* peptidase since all the residues which appear to be involved in zinc binding and catalysis and all residues (except Met-454) which comprise the putative substrate binding site in bovine LAP are conserved in *E. coli xerB* (Burley et al., 1990; Taylor, 1993a). These data suggest that these enzymes,

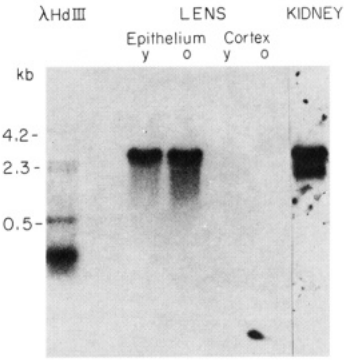

FIGURE 3: Northern blot analysis of lens and kidney RNA. Epithelium and cortex of young and old bovine lens were separated, and total RNA was extracted and enriched for poly(A) RNA. Kidney RNA was extracted from adult bovine kidney. Aliquots of 5 μg of poly(A) RNA of each tissue were electrophoresed and blotted onto a Genescreen membrane (Wallner et al., 1986). Full-length ^{32}P-labeled pLAPE2 cDNA was used as the hybridization probe.

the *xerB* gene product from *E. coli*, and a variety of other aminopeptidases from diverse species are part of a new family of zinc aminopeptidases with structural similarity, which utilize the zinc binding amino acid constellations described for bovine LAP, and which may employ a similar mechanism of catalysis (Taylor, 1993a,b). These enzymes can be distinguished from another recently identified superfamily of zinc proteases which use two His and Glu to bind zinc, Arg in substrate binding, and Glu in catalysis (Watt & Yip, 1989).

Expression of LAP in Bovine Kidney, Lens Tissue, and Cultured Lens Epithelial Cells. The eye lens can be divided into developmentally and maturationally different zones [see Figure 1 in Taylor and Davies (1987)]. Accordingly, it provides unique opportunities to observe the fate of proteins upon maturation and aging. Epithelial cells divide and comprise the youngest tissue. Older cells are found in inner lens layers, or cortex and core. Proteins are extensively modified in the aged lens, and this has been causally related to cataract formation. In many cell systems, proteases appear to act as a protein-editing capability, selectively removing damaged, abnormal, or obsolete proteins. Since the aging lens accumulates rather than degrades damaged proteins in cataractous opacities, the fate of proteases upon aging is of interest (Taylor & Davies, 1987). LAP comprises a large proportion (>0.1%) of the lens epithelial cell protein (Taylor et al., 1983). As cell development, differentiation, and maturation proceed, crystallin synthesis predominates, and the relative level of LAP diminishes. In order to determine if the levels of LAP change because of alterations in the mRNA levels or because of protein degradation, expression of LAP was examined in the lens tissues by Northern blot analysis. Equivalent levels of the 2.4-kb LAP mRNA were found in young and old lens epithelium (Figure 3). This is consistent with the continued growth throughout life of this area of the lens. No LAP mRNA was found in the lens cortex, indicating that LAP gene transcription has ceased in this tissue. This is in contrast to our previous observation that the specific activity of LAP in cortex, particularly in young lenses, is substantial (Taylor et al., 1983) and suggests that in lens cortex LAP activity (like the structural proteins) is retained over a long period of time. This stability may be essential if LAP is part of the proteolytic repertoire, which is necessary for cells to execute efficient removal of obsolete proteins during cellular differentiation and maturation, or damaged proteins formed during a lifetime of exposure to oxygen and light.

Northern blot analysis of kidney mRNA indicated that the same 2.4-kb LAP mRNA is expressed in the kidney and

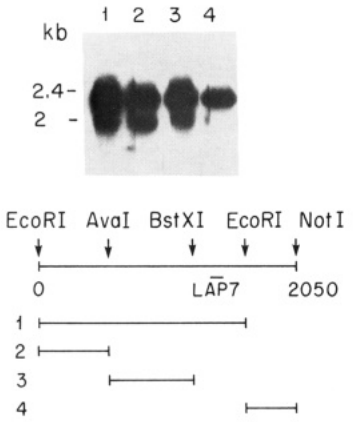

FIGURE 4: Northern blot analysis of bovine kidney RNA. Distinct restriction fragments of pLAPE2 cDNA labeled with [^{32}P]dNTP by nick-translation were used as hybridization probes. Lane 1, 1640 bp *Eco*RI fragment; lane 2, 585 bp *Eco*RI/*Ava*I fragment; lane 3, 600 bp *Ava*I/*Bst*XI fragment; lane 4, 410 bp *Eco*RI/*Not*I fragment. Oligonucleotide LAP7 corresponds to nucleotides 1176–1205 and hybridizes to the 2.4-kb transcript only (data not shown).

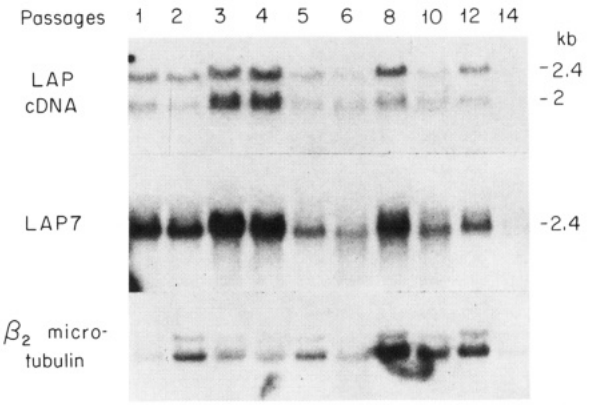

FIGURE 5: Northern blot analysis of bovine lens epithelial cell culture RNA. The numbers on the top of each lane represent the passage number (population doubling level). Each lane contains 10 μg of total RNA. To assure equal loading of mRNA to the gel, human β_2-tubulin RNA was used as an internal standard. The following ^{32}P-labeled hybridization probes were used: top panel, full-length pLAPE2 cDNA; middle panel, oligonucleotide LAP7; bottom panel, human β_2-tubulin cDNA.

expression is at similar levels in kidney and lens epithelial tissue (Figure 3). However, in contrast to lens epithelium, bovine kidney contains an additional mRNA transcript of 2.0 kb. Further Northern blot analyses, using distinct LAP cDNA (pLAPE2) restriction fragments and pLAPE2-specific oligonucleotides as hybridization probes, established that the isolated cDNA (pLAPE2) is equivalent to the larger (2.4 kb) transcript (Figure 4 and see below). Since Southern blot analysis indicated only one gene for bovine LAP (data not shown), the second transcript is most likely derived by tissue-specific differential splicing of a common precursor RNA.

To further analyze whether the two mRNAs code for homologous LAP proteins, specific oligonucleotide probes and restriction fragments of LAP cDNA were hybridized to kidney RNA in a Northern blot analysis (Figure 4). Restriction fragments containing the 5′ end of pLAPE2 (*Eco*RI/*Ava*I; *Ava*I/*Bst*XI; *Eco*RI) hybridize to both transcripts in kidney RNA. Restriction fragments of the 3′ sequence and oligonucleotide LAP7 homologous to nucleotides 1176–1205 (Figure 1) hybridize only to the 2.4-kb mRNA. This suggests that the 2.0-kb transcript codes for a protein highly homologous to LAP but with a distinct C-terminus. The potential splice site is located upstream of nucleotide 1170 of pLAPE2 since the oligonucleotide LAP7 does not hybridize to the 2.0-kb transcript. Extremely stringent hybridization conditions were used in this analysis. This allows us to conclude that the 5′ sequences of both messages are at least 90% homologous. Thus far, no second smaller LAP protein has been detected in the kidney or lens by standard immune precipitations or Western blot analysis (data not shown). There is, however, the possibility that both messages code for proteins of similar size which cannot be distinguished by SDS–PAGE. It is interesting to note that two aminopeptidase activities have been identified in human liver (Ledeme et al., 1983) and two different mRNAs, both originating from one gene, were also observed for kidney aminopeptidase N by Watt and Yip (1989). Whether these two RNAs are due to differential splicing and whether the 2.0-kb LAP mRNA is translated will have to await the isolation of the 2.0-kb cDNA and the characterization of the LAP gene. Since these hybridization results indicate that the amino acid sequences are different beyond residues ~300, it would appear that Asp-332 and Glu-334, implicated in catalytic function and zinc binding in bovine lens LAP, are not present in the protein coded for by the 2.0-kb transcript observed in lens epithelial cells in culture and in kidney.

In contrast to lens tissue, lens epithelial cells in culture also contain both LAP transcripts (Figure 5). This is notable since such lens epithelial cells are derived from epithelial tissue.

Progressively passaged lens epithelial cells have been used to simulate age-related changes to lens tissue (Berger et al., 1988). One of the changes noted during this process was, at early population doubling levels, a transient large increase in LAP activity (Eisenhauer et al., 1988). It was of interest to determine at what level this increase in protease activity was regulated.

As cells were passaged, the levels of both LAP transcripts increased in accordance with the transient increase in LAP activity [compare Figure 5 with Figure 1 in Eisenhauer et al. (1988)]. However, the relative amount of the two LAP transcripts differed. Early passages (passages 1 and 2) and later passages (passages 5–14) had roughly equal levels of both messages. In passages 3 and 4, the 2.0-kb message was expressed about 5-fold higher than the 2.4-kb message. This change in the relative expression of the two transcripts is further support for the hypothesis that there is differential regulation of the two RNAs. The stronger hybridization signals in the lane containing passage 8 lens cell mRNA is a result of slightly higher mRNA load. (See relative hybridization signal of β_2-tubulin.) The role and the significance of the different expression levels of these two LAP transcripts are under study.

Our data indicate that the regulation of LAP expression in lens cells is at least in part at the transcriptional level. It is interesting to note that LAP activity and expression levels are also enhanced due to treatment with interferon γ in cells from human HS153 fibroblasts, ACHN renal carcinoma, A549 lung carcinoma, and A375 melanoma [Harris et al., 1992; see Taylor (1993b) for review regarding regulation of aminopeptidase expression].

LAP is the first aminopeptidase to have its structure and mechanism of action studied in any detail. Thus, it is the best model for future studies of this class of peptidases. Isolation and characterization of the full-length cDNA for LAP made it possible to determine the corrected amino acid sequence for bovine LAP. This was essential for interpretation of crystallographic data and allowed comparison of lens LAP and other aminopeptidases. Using the cDNA as a hybridization probe, we were able to demonstrate changes in LAP mRNA

levels: in lens cells upon aging in vitro; during lens tissue development, maturation/aging; and in cells exposed to interferon γ (Harris et al., 1992). In addition, tissue-specific expression of a second LAP mRNA, which seems to be regulated independently of the larger transcript, was detected. The properties of the mutagenized enzyme and the protein coded for by the second transcript and its regulation of gene expression are under study.

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