Localization of Leucine Aminopeptidase in Normal Hog Lens by Immunofluorescence and Activity Assays

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Rabbit antisera to purified bovine lens leucine aminopeptidase (EC 3.4.11.1, LAP) were prepared and shown to be specific for leucine aminopeptidase alone (Taylor et al. Cur Eye Res 2:47-56, 1982). When whole hog lens thin sections were treated first with these antisera and then with fluorescein conjugated goat anti-rabbit gamma globulin, greatest fluorescence was observed in the elongating epithelium and outer cortex, and significantly less fluorescence was observed in the lens core. That largest amounts of LAP are present in the epithelium is supported by activity measurements using leucylamide as substrate. The specific activity of LAP in the epithelium/outer cortex is 83 times that of LAP in the inner cortex. The concentration of posttranslationally (postsynthetically) modified and oxidized crystallins is greatest in the core, yet in this zone there is a paucity of aminopeptidase activity, possibly due to age-related degradation of the enzyme. It is possible that LAP is in association with membranes and present in nucleoplasm of rapidly metabolizing, elongating epithelial cells. These observations are discussed in relation to the possible role of proteolysis in cataractogenesis. Invest Ophthalmol Vis Sci 24:1172-1180, 1983

Upon aging some lens crystallin proteins undergo oxidation, aggregation, and other posttranslational modifications.1,2 The lens also contains proteases.3 Many types of cells are known to degrade oxidized and denatured proteins more rapidly than their native precursors.4-7 Thus, it may appear surprising that altered crystallins are not degraded but rather accumulate in senile and possibly other types of cataract.1 It would be useful to elucidate the role of lenticular proteases in maintaining lens transparency and in editing the lenticular protein population. This paper describes the localization in different metabolic and developmental zones of the lens of the N-terminal hydrolase leucine aminopeptidase.

Based on activity measurements using young hog and beef lenses, LAP has been described as the most prevalent lenticular protease.8,9 Preliminary evidence suggests that active LAP together with endopeptidases can catalyze the total hydrolysis of some crystallins.10 However, in aged human lens tissue the low levels of LAP activity, as contrasted with the high concentration of the enzyme10,11 (Taylor et al, Exp Eye Res, in press) imply that with increasing age this enzyme is inactivated progressively. Cataractous protein precipitation also increases progressively with increasing age of the tissue. It seemed that a combination of activity-based and nonactivity-based data regarding the localization of LAP might indicate if LAP is inactivated in the lens core, where protein precipitation seems to occur most regularly. This information would also help resolve the contradictory results regarding the localization of LAP activity of Van Kamp and Hoenders8 on one hand and of Iwig and Glasser12 and Wolff and Resnik9 on the other. Van Kamp and Hoenders showed that the LAP content of calf lenses decreases with increasing age of the tissue but these workers found no LAP in the epithelium.8 In contrast, Iwig and Glasser12 found increasing amounts of LAP activity in the epithelium, progressing from central to equatorial and elongating epithelial zones. A report by Wolff and Resnik9 seems to support this conclusion. In this paper we demonstrate by both immunofluorescence and activity assays that LAP is present, in porcine lenses, in greatest concentration in the elongating epithelium, and that it is present throughout the epithelium and outer cortex. Lesser concentrations of the intact native enzyme are present in the inner cortex and nucleus.
Surprisingly, LAP appears to also be associated with lenticular fiber cell membranes and in nuclei. These results are useful in interpreting the physiologic function of the enzyme and in suggesting modifications in the currently employed isolation procedure.

Materials and Methods

Preparation of Antisera

Leucine aminopeptidase was isolated in the Zn$^{2+}$Zn$^{2+}$ form from bovine lens tissue by a modification$^{13}$ of the procedure of Hanson.$^{14}$ After three ammonium sulfate precipitations, the enzyme appeared homogeneous in both native and SDS PAGE and gave crystals suitable for x-ray diffraction studies.$^{15}$ This enzyme was used to prepare antisera as described by Taylor et al.$^{10}$ Double immunodiffusion, immunoelectrophoresis, and microcomplement fixation were employed to demonstrate that the rabbit antibovine lens LAP antiserum produced in response to the antigen was specific for LAP alone and did not react with any other lens antigens.$^{10}$ Comparison of hog lens and bovine lens LAP was done via double immunodiffusion according to Ouchterlony.$^{16}$ In preparation for immunofluorescence, the antiserum was diluted to a final concentration of 0.76 mg/ml (1:100 dilution).

Fixation and Embedding

Hog eyes from freshly killed hogs were transported on ice and the lenses excised within 3 hrs of slaughter. Lenses were sequentially placed in >25 ml 60% EtOH/40% water, 16 hrs; 100% EtOH, 18 hrs; 50% EtOH/50% BuOH, 20 hrs; 100% BuOH 20 hrs, all at 25 C. The next series of treatments were done in a vacuum oven at 60 C. The dehydrated lenses were then placed in a 1:1 (v/v) mixture of paraffin (Tissue Prep melting point 56.5 C, Fisher Scientific)/BuOH, 15 hrs; transferred to a 4:1 paraffin/BuOH mixture, 20 hrs and finally through two changes of 100% paraffin, 24 hrs each change.

After cooling for several hours at 25 C the blocks were removed, trimmed, and the exposed tissue surface was soaked in distilled water prior to and in between sectioning. Ribbons of 4–8 $\mu$m sections were cut on an American Optical rotary microtome, and floated on drops of water at 37–39 C on clean glass slides until all the water had evaporated (about 1 hr) and the sections adhered to the slides. These sections were usable for >1 month if stored at 4 C.

Immunofluorescence

The procedure employed was adapted from Ikeda and Zwaan.$^{17}$ Tissue sections were deparaffinized and rehydrated using the following treatments in Coplin jars: xylene 15 min; 100% EtOH, 2 min; 80% EtOH, 15 sec; 40% EtOH, 15 sec; and finally deionized distilled water, 15 sec. These rehydrated sections were then rinsed for 5 min in phosphate-buffered saline (1.5 mM KH$_2$PO$_4$, 4.3 mM Na$_2$HPO$_4$, 138 mM NaCl, 2.7 mM KCl, pH adjusted to 7.2 with 1 N NaOH, PBS). The excess PBS was drained from the slide and the areas surrounding the tissue were carefully blotted dry. One drop of a 1:100 dilution of either rabbit antibovine-lens-LAP-antiserum (anti-bLAP-as) or rabbit antibovine lens homogenate antiserum in PBS was applied to each section. After incubation in a moist chamber for 30 min, 25 C the sections were rinsed in PBS 10 min. The rinse was repeated once. One drop of fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit-IgG (heavy and light chains, Cappel Labs, Cochranville, PA) which had been previously diluted 1:20 with PBS was applied to each of the LAP-antiserum coated sections. After incubation in a moist chamber 30 min, 25 C, the sections were again rinsed twice in PBS, each time for 10 min. Buffer was removed from the slide edges and mounting was accomplished with a 1:1 mixture of PBS:glycerol using 1½" coverslips surrounded by clear nail polish.

Tissue Powder Absorption of Antisera

To enhance the specificity of staining, the FITC conjugated goat gamma globulin was absorbed with hog lens tissue powder as follows.$^{17,18}$ One hundred milligrams of powder, slightly wetted with saline, was added per milliliter of antiserum. The mixture was stirred occasionally for 2 hrs, 25 C; left overnight, 4 C; and centrifuged at 9000 X g, 60 min, 3 C. This was repeated with 50 mg of tissue powder per milliliter. Localization of fluorescence was most consistent when the rabbit anti-LAP-antiserum was absorbed as follows. One milliliter of a 1:100 dilution of antiserum in PBS, pH 7.2 was incubated with 0.1 mg tissue powder 2 hrs, 37 C, with vortexing every 20 min. After centrifugation 9000 X g x 3 C, 20 min the supernatant was removed and the procedure repeated once. This antiserum was stored at −10 C in 1-ml aliquots and thawed just prior to use. This absorption process was critical for the success of experiments involving LAP-absorbed-anti-LAP-antiserum.

Photomicroscopy

Transmitted light pictures were taken using phase contrast objectives on a Zeiss Universal Microscope. Immunofluorescent and control slides were viewed and photographed using a Zeiss #487716 exciter-bar-
Results

The specificity of rabbit antibovine lens LAP antisera used in this study was established using immunodiffusion, immunoelectrophoresis, and microcomplement fixation\(^ {10} \) (Taylor et al, Exp Eye Res, in press). The precipitin line formed between the wells containing homogeneous hog lens (hlLAP) and antiblLAP-as (Fig. 1) shows that hog lens LAP is recognized by antisera to bovine lens (blLAP). The arc of fusion formed by reaction of the antiserum with both blLAP and hlLAP indicates that the two antigens share a significant number of antigenic sites and that it is appropriate to use anti-blLAP-as to localize LAP in hog lens sections. The spur shows that blLAP has antigenic sites which are not present on the hog lens enzyme. This is expected since blLAP is the homologous antigen.

In order to demonstrate that the lens tissue remains intact during embedding, sectioning, deparaffinization, rehydration, and processing of the tissue for immunofluorescence, tissue sections were examined using phase contrast and the double label technique of Coons.\(^ {20} \) Clear views of cuboidal and elongating epithelium, nuclei, nucleoli, and cytoplasm in the phase contrast photographs (Fig. 2) show that tissue integrity has been retained. This is corroborated by the photographs of lens sections that were treated with an antiserum to a whole lens homogenate as well as the FITC conjugate (Fig. 3). This antiserum should recognize the major soluble lenticular components. Recognition of lens proteins throughout the tissue is obvious and affords a detailed view of the tissue. As in the phase contrast pictures, epithelium and outer cortex punctuated by nuclei and nucleoli, are clearly...
visible. Nucleoli appear darkest; nuclei and cell membranes are dark against the brightly fluorescent cytoplasm in these sections. These sections were photographed using an exposure time of only 15 sec as compared to 2 min exposures used for all the other immunofluorescence photographs described below. The bright level of fluorescence of these sections was expected since the antiserum was formed against the most prevalent lenticular antigens.

Many lenses were sectioned. Forty-nine sections from four axially sectioned lenses and ten sections from four equatorially sectioned lenses were treated sequentially with anti-blLAP-as and FITC-as and photographed. Ninety-two percent of the photographs of these sections showed greatest fluorescence in zones of rapid differentiation and cellular elongation (Figs. 4A, E) and lowest fluorescence levels in the core (Fig. 4C). There was some recognition of LAP in the core. However, the photographic exposure time (2 min) necessary to obtain clear views of the epithelium results in dark-appearing lens cores and creates the impression that there is no LAP recognition in the lens core. The fluorescence level of sections coated with FITC-as after being treated with either phosphate buffered saline (not shown), normal rabbit antiserum (Figs. 5A, B) or anti-blLAP-as which was preabsorbed with LAP (Figs. 5C, D) was markedly diminished by comparison with sections that were treated with anti-blLAP-as and FITC-as (Figs. 4A–E).

Outer cortex/epithelium was obvious in 100 photographs. Of these 56, 14, and 30 showed nuclei in the bow region that appeared more fluorescent than, less fluorescent than, or were of indistinguishable level of fluorescence from the surrounding cytoplasm, respectively (Figs. 4A, F). Fluorescent nuclei were also observed in epithelial zones of equatorially sectioned lenses (data not shown). Lens sections that were coated with normal rabbit antiserum and FITC-as also showed nuclei that appeared more fluorescent than the surrounding outer cortex cell cytoplasm, but the levels of fluorescence were markedly reduced when compared to the fluorescence levels of nuclei of sections coated with anti-blLAP-as. Nucleoli were less fluorescent than the surrounding nucleoplasm in sections treated with either antiserum to a bovine lens homogenate or anti-blLAP-as and FITC-as.

Brightly fluorescing fiber cell membranes, as compared to cytoplasm, were obvious in 42 out of 100 photographs of outer cortical zones of axial sections and in 46 out of 49 photographs of outer cortical zones of equatorial sections (Fig. 4G). In contrast to the outer cortex, membrane fluorescence was only observed in about 10% of over 400 photographs of the inner cortex of these sections. Membranes that fluoresced more brightly than surrounding cytoplasm were not observed in epithelium. The outer cortical zone of axial sections that were treated with normal rabbit serum or phosphate buffered saline and FITC-as also showed membrane fluorescence (with respect to the surrounding cytoplasm), however, the level of fluorescence was markedly diminished when compared to the level of fluorescence in sections treated with anti-blLAP-as and FITC-as.

Since the protein concentration of lens tissue increases progressively from approximately 140 mg/ml in the outer cortex/epithelium to 230 mg/ml in the inner cortex and 300 mg/ml in the core (Table 1), the observation of greatest fluorescence in the epithelium and outer cortex implies that native LAP is not only in greatest supply in the outer cortex and epithelium but also in greatest concentration in these outer zones of the lens. This distribution of LAP was determined using nonactivity based methods but it is corroborated by activity measurements using leucylamide and leucyl-p-nitroanilide as substrates. In this study leucylamide was chosen as the primary substrate since most other commonly used substrates for LAP are also hydrolyzed by aryl amidas.10,21 Nevertheless, results from leucyl-p-nitroanilide experiments are provided below. It can be seen from Table 1 that under the conditions of this experiment the specific activity of LAP in the outer cortex/epithelium is approximately 83 times that in the inner cortex.
and 31 times that in the core. In these assays the epithelial fractions showed large optical density changes with respect to simultaneously performed control assays from which either substrate or protein was excluded. Indeed, there was no detectable optical density change due to aggregation of epithelial components. Aggregation and settling of some inner cortex and core proteins contributed significantly to the optical density changes during the assay. Attempts were made to correct for these optical density changes. Since the changes due to activity were not much larger than the changes due to aggregation and settling and because we were working at high absolute optical densities, the relative standard deviations of these assays is quite high with respect to the actual activity. Analogous leucyl-p-nitroanilide assays also shared this complication.

The epithelium/outer cortex fraction accounted for 2.4% of the lens volume, 1.3% of the protein, and contained 41% of the total LAP activity as measured in the leucylamide assay. Inner cortex contained 73% of the volume, 67% of the protein but only 28% of the LAP activity. Core accounted for 24% of the volume, 31% of the protein, and 32% of the total LAP activity. When the aryl-amide, 1-leucyl-p-nitroanilide, was employed the trend in relative specific activities of the different zones was the same as was found with the leucylamide assay, however, investigation of the absolute magnitude of the specific and total activity of each zone reveals different relative magnitudes for the zones. The specific activity of the epithelium/outer cortex was only four times that of the inner cortex or core. The majority of activity (about 1 unit/lens) was in the inner cortex and the

Figs. 4A–E. Lens axial sections adjacent to those shown in Figure 2, were treated first with a 1:100 dilution of rabbit antibovine lens LAP antiserum and then with a 1:20 dilution of FITC conjugated goat antirabbit gamma globulin. Epithelium and outer cortex, A and E; inner cortex, B and D; core, C. Figure 4F is a detail of another lens section showing enhanced fluorescence in epithelial and outer cortical nuclei as opposed to surrounding cytoplasm. Figure 4G is a detail of an equatorially sectioned lens showing apparent membrane fluorescence. See text for discussion of relative intensity of fluorescence (shown here) as opposed to actual levels of fluorescence.

Fig. 5. Lens axial sections treated first with a 1:100 dilution of normal rabbit antiserum and then with a 1:20 dilution of FITC conjugated goat antirabbit gamma globulin, A and B. Lens sections treated as in Figure 4 except that the antibovine lens LAP antiserum was incubated with LAP and centrifuged prior to application to lens sections, C and D. In A and C the epithelium/outer cortex is shown. Photographs B and D are of the inner cortex.
Table 1. LAP activity and specific activity of epithelium/outer cortex, inner cortex, and core homogenates of hog lenses using 25 mM 1-leucylamide 0.1 M Tris, pH 8.5 at 30 C. All samples were treated with MgCl₂ as per Carpenter and Vahl (10). This data is the average of six batches each of 10 lenses.

<table>
<thead>
<tr>
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<th>Specific activity</th>
<th>Total activity</th>
<th>Total protein</th>
<th>Volume occupied</th>
<th>Protein concentration</th>
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<tr>
<td></td>
<td>micro mol/min mg</td>
<td>std deviation</td>
<td>micro mol/min</td>
<td>mg/10 lenses</td>
<td>mg/10 lenses</td>
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<td>Epithelium/Outer Cortex</td>
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<td>0.10</td>
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<td>14.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Inner Cortex</td>
<td>0.006</td>
<td>0.003</td>
<td>4.86</td>
<td>702</td>
<td>3.0</td>
</tr>
<tr>
<td>Core</td>
<td>0.016</td>
<td>0.004</td>
<td>5.5</td>
<td>332</td>
<td>1.0</td>
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minority (0.08 unit/lens) was in the epithelium. The core had 0.05 units/lens. The total units in this assay is different from that in the leucylamide assay due to differing Km, kₐ and extinction changes at the wavelength used.22

Using the leucyl-p-nitroanilide assay we noted that the total activity of any pellet was about 5–10% of the activity shown in the respective supernatant and the specific activities for the resuspended pellets were about 50% of the supernatant. Similar determinations using the leucylamide assay were difficult to pursue owing to the elevated optical densities at 238 nm of concentrated protein solution.

In contrast to the extensive activation of purified LAP by Mg²⁺23 homogenates of whole hog lenses that were treated with 10 mM MgCl₂ at pH 9.5 showed only a twofold activation.

Discussion

Leucine aminopeptidase has been described as the predominant lenticular protease⁹ and most important lenticular protease.⁸,⁲⁴ It is the most studied aminopeptidase¹⁵,²²,²⁵–³⁵ yet a clear view of the role and importance of LAP in lenticular protein metabolism has been obscured by the fact that there is little protein turnover in eye lenses from most species and by the observation of little if any LAP activity in aged human lenses.³,¹⁴,³⁶,³⁷ We recently demonstrated that although the specific activity of LAP in aged human lenses is much lower than in lenses of young beef or hogs, the concentration of LAP in the lenses of these three species is similar¹⁰,¹¹ and the amino acid sequence of the respective enzymes is similar (Taylor et al, Exp Eye Res, in press). The similarity in both amino acid sequence and concentration of LAP in lenses of these mammalian species implies that information obtained from hog lenses should be applicable to understanding the role of LAP in human lens protein metabolism. Since in aged human lenses LAP is present in a state of markedly reduced activity, it is desirable to have available nonactivity-based techniques for localizing the enzyme within different metabolic zones of the intact tissue. This information should be particularly useful in determining if there are localized aberrations in proteolytic capability associated with cataractogenesis.

Hog lens LAP cross reacts with antisera to the bovine enzyme thus, given suitable whole lens sections, it is possible to localize LAP in hog lens sections via immunofluorescence. The clear views of intact cytoplasm, nuclei, nucleoli, and membrane morphology in phase contrast pictures (Fig. 2) and in the photographs of sections treated with rabbit antisera to a whole bovine lens homogenate and then anti-rabbit FITC conjugated gamma globulin (Fig. 3) show that the tissue was well preserved during embedding, sectioning, deparaffinization and preparation of the slides for immunofluorescence.

Greatest fluorescence is seen in the exterior zones of lenses treated with antisera to a whole lens homogenate (Fig. 3). Diminished fluorescence in the interior zones of these sections can be interpreted in at least two interrelated ways. It is possible that those proteins to which the antisera were elicited are in greater supply in the exterior zones of the lens. Alternatively the antigens once present in native (or antigenically most reactive) form in cortical fiber cells may be present in degraded form and diluted among and associated with aggregates and albuminoid, which are present in greater supply in the core of the tissue. These insoluble components are removed during processing of the homogenate prior to immunization. Both of these scenarios result in the same conclusion: the crystallins to which the antisera were elicited are present in greatest supply in native conformation in the cortex as opposed to the core. This implies that age-related modification of these proteins leads to the observation of less fluorescence in the oldest lens zone or core as opposed to the cortex.³ In corroboration of our data is the observation by van Kamp and Hoenders and Broekhuyse and Kuhlmann of degeneration of cells and protein in the core of normal⁸ and cataractous³⁸ lenses, respectively. The overall level of fluorescence observed on the sections shown in Figure 3 was much greater than the level of fluorescence of sections treated with anti-bILAP-as. This
was elicited to the major lenticular components as opposed to purified LAP.

Interpretation of the immunofluorescence photographs of lens sections treated with anti-blLAP-as (Fig. 4) is straightforward since the specificity of the antiserum for native LAP has previously been established. These pictures clearly show that the highest level of LAP recognized by the antiserum is in the elongating epithelium and outer cortex. The protein concentration of lens tissue increases progressively from epithelium to core (Table 1). If a given protein is retained in its original conformation at its original concentration, with respect to other proteins in the tissue, then the core should contain the highest and the newly elongated cortex the lowest amount, respectively, of that particular protein. This is not observed. The amount of LAP recognized in these lens sections decreases progressively as the age of the tissue increases and as the protein concentration of the lens section increases. Thus, it appears that LAP in native conformation is present in highest concentration prior to fiber synthesis and as fiber is being elaborated. However, decreased fluorescence in the inner cortex and core implies that there is a subsequent age-related degradation of LAP.

In partial contradiction to our observations Van Kamp and Hoenders reported that in lens epithelium there is no LAP. These workers used leucyl-p-nitroanilide as substrate in activity assays. Thus, we sought corroboration of the immunofluorescence data from activity assays. Peptidase activity was assayed by monitoring the hydrolysis of leucylamide, leucyl-p-nitroanilide, and leucyl-beta-naphthylamide. An advantage of using leucyl-p-nitroanilide as substrate is that the extinction change upon hydrolysis (about 9900 M⁻¹ cm⁻¹) is large and it occurs at a wavelength (405 nm) far removed from the wavelength of absorbance for peptide bonds. Unfortunately, the limited solubility (less than K₅₅/2) of leucyl-p-nitroanilide makes reproducible kinetic measurements difficult to achieve with this substrate under these conditions. Activity assays using leucyl-p-nitroanilide and leucylamide both routinely reflected the success of the hog lens LAP purification scheme. However, no factor was found that would permit direct correlation of one assay with the other. This suggests that while the hydrolysis of both substrates is catalyzed by LAP, other lens enzymes such as ariydamidases or other aminoacyl-peptide hydrolases (which are reported to be distinct from LAP) may also catalyze these hydrolyses (Wagner, in press). Leucylamide is very soluble; thus, it affords assays at saturating conditions as well as maximal velocities which are two orders of magnitude greater than the maximal velocity values determined with leucyl-p-nitroanilide. Because of (1) the increased precision of the leucylamide assay, (2) the difficulty of achieving saturating conditions with leucyl-p-nitroanilide and the probability that it is hydrolyzed by other enzymes, and (3) the fact that leucine aminopeptidase is defined as the enzyme which cleaves most rapidly N-terminal leucyl-residues from peptides or leucylamide, leucylamide was used as the primary substrate, and the data corroborated with leucyl-p-nitroanilide assays. In Table 1 it is seen that the specific activity of the epithelium/outer cortex is 83 times that of the inner cortex, and 31 times that of the core. Forty-one percent of the LAP is found in the epithelium/outer cortex, a region that accounts for only 1.3% of the lens protein. Elevated concentrations of LAP in epithelium were also noted by Wolfl and Resnik using L-leucyl-beta-naphthylamide as substrate. Since the epithelial fraction used in our laboratory was prepared very similarly to that used by Van Kamp and Hoenders, the discrepancy remains to be explained. The measurements of relative specific activity in the cortex and core is subject to considerable error due to low optical density changes and high protein concentration, hence this difference cannot be considered significant.

When performing activity assays we attempted to activate LAP in the homogenates. Carpenter and Vahl indicated that Mg²⁺ incubation should yield 16-fold activation for bovine lens LAP and Van Wart and Lin shown that similar activation should be expected for the hog kidney enzyme. Since hog kidney and hog lens LAP are indistinguishable (Taylor et al, manuscript in preparation) we also expected to observe similar activation. Despite our ability to produce the expected activation with purified enzyme, activation in the homogenates was rarely greater than twofold.

It has been noted that during cataractogenesis lens membranes become leaky, and it has been hypothesized that once leaky, proteolysis may be stimulated. To date there have been few clues as to the cause of membrane damage. The appearance of brightly fluorescing membranes in many photographs of the outer cortex of lens tissue which were treated with anti-blLAP-as and FITC-as (Fig. 1G) and the observation of activity associated with this fraction is provocative but must be interpreted with caution. It is plausible that this fluorescence is due to the enhanced ability of the membranes (as opposed to the cytoplasm) to pass light which is of much greater intensity in these photographs as compared with the controls. The similarity in observed specific activity of the membrane-associated LAP indicates that it is similar to the cytoplasmic enzyme and that the LAP may not be tightly bound to the membranes.

The apparent localization of LAP in nuclei is also noteworthy. Protease presence in thymus nuclei has been known for some time, and the possibility of...
gene regulation by nuclear protein modification by proteases has been proposed. Thus, our observation of highest LAP concentrations in nucleated, anabolic lens cells is of interest.

These data suggest a modification of the currently employed purification of LAP. Instead of using whole lens homogenates it should be possible, by removing only the outer lens layers, to obtain a majority of the total activity in only a minority of the protein. Assuming a specific activity of 100 micromol/min/mg for LAP with a small fraction of Mg²⁺ per subunit it can be seen from Table 1 that there are 7.3 LAP units or 73 micrograms/lens. Since the outer zones contain only 14 mg of the total protein this implies that LAP could account for as much as 0.5% of the weight of the lens protein in this zone. This calculated % LAP in the lens epithelial protein could be even higher if the specific activity of LAP is less than 100 micromol/min/mg.

This work establishes unequivocally that in hog lens, leucine aminopeptidase is present in greatest amounts and in greatest concentration in hog lens epithelium and equatorial outer cortex and in lesser quantities or concentration in the inner cortex and core of the hog lens. It is possible that LAP is also present in a membrane associated form in most zones of the lens but most prevalent in the membranes of outer cortical cells. The LAP concentration of the nuclei is as high as that of the surrounding cytoplasm in most lens zones where nuclei are present and higher than the surrounding cytoplasm in the nuclei of rapidly dividing and elongating cells. These data together with the data shown in Table 1 show increasing protein concentration upon aging of lens tissue and decreasing LAP specific activity and total activity in the core as opposed to the epithelium and imply that like some other lens proteins LAP is progressively inactivated and/or damaged with increasing age of lens tissue.

It is reported that oxidized and denatured proteins are more rapidly hydrolyzed by proteases than are native proteins. It is also known that oxidized and crosslinked crystallins occur in substantial quantity in the cores of senile cataractous and precataractous lenses. It is possible that the absence of sufficient proteolytic capability in the inner cortex and core of the lens is related to the appearance of senile cataract in these zones.

Key words: lens, aminopeptidase, aging, proteolysis, cataract, nucleus, immunofluorescence

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