Exopeptidases identified as dipeptidyl peptidase III and leucine aminopeptidase, and an endopeptidase, prolyl endopeptidase, were found in the Emory Mouse cataract and the Cataract Resistant mouse lens extracts. The specific activity measured on Arg-Arg-2-NNap for DPP III and the hydrolysis of Boc-Arg-Pro-2-NNap for prolyl endopeptidase were higher in the Emory Mouse cataractous lens extract. A relatively high rate of hydrolysis of the β-naphthylamide of leucine aminopeptidase was present in both mouse categories; however, the Cataract Resistant mouse lens had approximately double the protease activity of the Emory Mouse cataract. Invest Ophthalmol Vis Sci 26:1035–1037, 1985

The late-onset cataract of the Emory Mouse may serve as an animal model for human senile cataracts. Therefore a study of biochemical changes associated with this cataract should give information that is more pertinent to the human situation than an experimental animal cataract like that produced by galactose feeding in rats or any of several types of early-onset heredity cataracts which have been found in the mouse. The biochemical studies reported so far for the Emory Mouse have revealed some effects of cataract on the lens, but a single severe metabolic lesion, such as the presence of an ATPase inhibitor in the Nakano mouse lens, remains undiscovered. The Emory Mouse cataract is characterized by marked disturbances in lens fiber morphology, membrane transport, and glutathione concentration, among other changes, especially at the late stage where protein resorption occurs. Thus, it seemed worthwhile to make a comparison of proteolytic enzymes in this cataract as compared with normal clear lenses of a cataract-resistant strain of the same Carworth Farms Webster (CFW) mouse from which the Emory Mouse was derived.

The present study surveys the activities in the Emory Mouse cataract and the Cataract Resistant mouse lens extracts of exopeptidases, such as: dipeptidyl peptidase I (EC 3.4.14.1) and II (EC 3.4.14.2), (DPP I and DPP II), which are lysosomal; dipeptidyl peptidase III (EC 3.4.14.4), (DPP III), which is a cytosolic exopeptidase; and dipeptidyl peptidase IV (EC 3.4.14.-) (DPP IV), which is membrane-bound. A neutral endopeptidase having the properties of a prolyl endopeptidase (EC 3.4.21.26), (PEP), and an aminopeptidase using β-naphthylamide of leucine (EC 3.4.11-), (LAP), were also assayed in this study.

**Materials and Methods.** The investigations utilizing animals, as described in this manuscript, conform to the ARVO Resolution on the Use of Animals in Research. The mouse lenses used were from Dr. Kuck’s colonies of the Emory Mouse and Cataract Resistant mice (a substrain having clear lenses at 2 yr of age). Morphologic and biochemical characteristics have been described elsewhere.

Lenses were dissected and homogenized in five parts of cold 0.05 M Tris, 0.05 M NaCl, pH 7.6 (20% w/w). The homogenate was centrifuged at 20,000 × g for 20 min at 4°C, the pellet was resuspended, the mixture was recentrifuged as above, and a colorless supernatant was recovered for the enzyme assays. A fluorometric assay procedure described by Swanson was employed for the measurement of hydrolysis rates on dipeptidyl-β-naphthylamides that were acquired from Bachem, Inc., Fine Chemicals (Torrance, CA). DPP I-type activity was assayed in a reaction mixture containing 1.98 ml deionized H₂O, 0.1 ml 0.2 M cacodylic acid buffer, pH 6.0, and 1.0 ml 0.8 mM Gly-Phe-2-NNap. After a 4-min preincubation at 37°C, 0.02 ml lens supernatant was added to initiate the reaction. DPP II was assayed in a reaction mixture that contained 1.98 ml deionized H₂O, 0.1 ml 0.2 M cacodylic acid buffer, pH 5.5 or 6.0, and 1.0 ml 0.8 mM Lys-Ala-2-NNap. After a 4-min preincubation at 37°C, 0.02 ml lens supernatant was added to initiate the reaction. The DPP III assay mixture included the following: 2.78 ml deionized H₂O, 1.0 ml 0.25 M glycine buffer, pH 9.0; and 0.02 ml lens supernatant. The mixture was preincubated in a water bath at 37°C for approximately 4 min. The reaction was initiated by adding 0.2 ml of 0.8 mM Arg-Arg-2-NNap. DPP IV activity was assayed in a reaction mixture containing 1.98 ml deionized H₂O; 1.0 ml 0.25 M Tris buffer, pH 8.1; and 1.0 ml 0.8 mM Gly-Pro-2-NNap. After a 4-min preincubation at 37°C, 0.02 ml lens supernatant was added to initiate the reaction. The fluorometric assay system that was used for the aminopeptidase assay consisted of 1.98 ml H₂O; 1.0 ml of 0.25 M Tris-HCl buffer, pH 7.5; and 1.0 ml of 0.8 mM Leu-2-NNap. The assay mixture was preincubated 4 min at 37°C prior to adding 0.02 ml enzyme. The PEP assay included the following: 1.28 ml H₂O; 1.0 ml 0.25 M Tris-HCl buffer, pH 8.5; 0.5 ml 8 mM EDTA; 0.2 ml 20 mM Dithiothreitol; and 1.0 ml 0.8 mM Boc-Arg-Pro-2-NNap. The assay mixture was preincubated 4 min at 37°C prior to adding 0.02 ml enzyme.

The reaction mixture was maintained at 37°C in the water-jacketed cuvette holder of a Turner fluorometer (Model III, Sequoia-Turner; Mountain View, CA) equipped with a general purpose uv lamp (Turner
no. 110-850) and an optical bandpass filter with a central wavelength of 334.9 nm, and bandwidth of 9.9 nm (PTR Optics; Waltham, MA) and secondary Wratten filter 2A (yellow glass). The cuvette was irradiated with light at 335 nm and the rate of increase in fluorescence intensity at 410 nm was directly and continuously monitored and recorded with a strip chart recorder. The rate of hydrolysis was indicated by the rate of increase in the intensity of the fluorescence at 410 nm. A linear rate from zero time was taken to be indicative of a direct attack on the arylamide bond. The stepwise degradation of a dipeptide-β-naphthylamide would result in a delayed and nonlinear rate of fluorescence increase. A standard solution of β-naphthylamine hydrochloride in the assay buffer was used for instrument calibration. Rates of hydrolysis were established by comparing the rate of increase in fluorescence intensity produced by released β-naphthylamine. One unit of enzyme activity was defined as the amount of enzyme required to release the β-naphthylamine at the rate of 1 nmol per min under the conditions of the assay.

All the β-naphthylamide substrates were obtained from Bachem, Inc. (Torrance, CA). The purity and identity of these compounds were established by thin layer chromatography and amino acid analysis. Protein concentrations of quadruplicate samples were determined by the procedure of Bradford. Specific activities of triplicate samples were expressed as nmol of naphthylamine released/min/mg of protein in the lens solution.

The net wet weight for 12 lenses was 0.102 g with 26.69 ± 4.32 mg/ml protein concentration for the Emory Mouse cataract, and 0.138 g and a protein concentration of 78.30 ± 7.65 mg/ml for the Cataract Resistant mouse.

**Results.** A wide range of dipeptidyl-β-naphthylamide (DPP) substrates was utilized in an attempt to characterize the spectrum of peptide hydrolases present in the Emory Mouse cataract and Cataract Resistant mouse lens extracts, as shown in Figure 1. There was no hydrolysis of Gly-Phe-2-NNap at pH 6; Lys-Ala-2-NNap at pH 5.5, or pH 6; Gly-Pro-2-NNap at pH 8.1; for DPP I, II, and IV, respectively. The hydrolysis of Arg-Arg-2-NNap at pH 9.1 was characteristic of the DPP III previously described in bovine lens extracts and human cataract and human normal lens extracts.

The specific activity measured on Arg-Arg-2-NNap for DPP III was higher in the Emory Mouse cataractous lens extract than the Cataract Resistant mouse lens extract. The assays for PEP showed lower activities for each of the lens categories for the hydrolysis of Boc-Arg-Pro-2-NNap; however, higher activities were shown for the Emory Mouse cataract than the Cataract Resistant mouse lens at pH 8.0.

The very high activities for both lens categories with the leucine aminopeptidase were an interesting paradox. The aminopeptidase activities for the Cataract Resistant mouse were twice as high as for the Emory Mouse lens.

**Discussion.** The Emory Mouse cataract was developed in Dr. Kuck's laboratory and is characterized by failure to gain weight with concomitant depressed lens protein content as compared to the clear Cataract Resistant lens of the same age. This low protein content
content could partly be due to loss by proteolysis, and the evidence presented may suggest proteolysis due to increased enzyme activities in cataract lenses, with the exception of leucine aminopeptidase. This has also been shown in previous studies for human cataract lenses.\(^7\) However, rates of hydrolysis for arylamide derivatives for human cataract lenses far exceeded the rates in normal human lenses. It is entirely possible there may be a species-specific leucine aminopeptidase in the Cataract Resistant mouse lenses, Emory Mouse lenses, and human lenses. Another possible explanation for the low leucine aminopeptidase activities in the Emory Mouse cataract homogenates may be an age related inactivation or degradation of the enzyme because of structural alteration\(^10\) which may be responsible for the markedly diminished activity observed when compared to human cataract lenses.

A comparison of relative substrate specificities on Arg-Arg-2-NNap and Boc-Arg-Pro-2-NNap derivatives revealed additional apparent similarities between the human\(^7\) and mouse lens extracts.

**Key words:** cataract, proteases, mouse lens, Emory Mouse

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**References**


**Cataract Induction in Rabbits with the Nd-YAG Laser**

Glen Congelosi, Marguerite B. McDonald, and Keith S. Morgan

The study of occlusion amblyopia and its therapy has involved animal models of stimulus deprivation achieved by various means, none of which closely simulates human congenital cataract. The authors used the Nd-YAG laser as a means of inducing cataracts in rabbit eyes. Twenty rabbit eyes were treated at various frequency and power settings. High energy YAG laser pulses of 10 mJ produced cataracts that at first resembled large bubbles. Over several days, these bubbles coalesced into smaller opacities. With multiple treatments (usually 150–200 pulses), the authors could create a cataract of a specific size and position. Six months later, 100% of these laser-treated rabbit eyes showed persistent cataracts that resembled human congenital cataracts. It appears that the YAG laser can provide a reproducible and reliable method of inducing specific types of cataracts in animal eyes without damaging other ocular structures. Invest Ophthalmol Vis Sci 26:1037–1040, 1985

To assess the efficacy of various forms of surgical treatment and methods of aphakic correction in preventing or reversing amblyopia, it would be useful to have an animal model that closely simulates human congenital cataract. Various methods of inducing cataracts in animals have been tried in the past. These models have included lid closure, translucent plastic contact lenses, cycloplegics, sutured nictitating membranes, or “needling”-induced traumatic cataracts.\(^1\)\(^–\)\(^3\) However, all have demonstrated drawbacks that limit their effectiveness in mimicking human congenital cataract and the development of amblyopia.

This study was undertaken to demonstrate that the Nd-YAG laser could be used as a reliable tool in