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LENS CALCIUM ACTIVATED PROTEINASE: DEGRADATION OF VIMENTIN

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The lens has been shown to contain a Ca^{+2} activated proteinase specific for vimentin. The proteinase is present in the soluble fraction of the cortex but not in the epithelium. It is suggested that this proteinase is expressed during terminal differentiation of the epithelial cells and may be responsible for degradation of the intermediate filaments in the fiber cells. The proteinase is inhibited by EGTA but not by several proteinase inhibitors.

Disulfide linked high molecular weight aggregates are believed to be important in the development of cataract (1). Recent work has demonstrated that these aggregates contain not only polypeptides contributed by the crystallins but that membrane and matrix components may also be present (1,2). Such work has drawn attention to the components of intracellular matrix. Recently, it was shown that the 43,000 dalton species prominent in the high molecular weight aggregates contain actin and polymerized beta and gamma crystallins (3). A polypeptide of approximately 60,000 daltons has also been observed in these aggregates (1). It is possible that this polypeptide is vimentin, a component of 57,000 daltons which is the major constituent of the IF of the lens (4,5). It is of interest that such filaments can only be observed in the epithelium and the outer region of the lens by either biochemical or morphological techniques (6,7). It is conceivable that the vimentin has been incorporated into the high molecular weight aggregates as well as possibly being degraded by proteolytic

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ABBREVIATIONS: IF, intermediate filaments; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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attack. The latter possibility is investigated in this communication.

Several proteinases have been previously reported in the lens (8-11). Leucine aminopeptidase is present in high concentration (8). Endopeptidase activity is more difficult to detect (9-11) and it has been suggested that an endopeptidase inhibitor may be present in the tissue (9). The 10,000 dalton polypeptide fraction which increases moderately with aging and dramatically in cataract (12) is believed to be produced by proteolytic cleavage of alpha and gamma crystallins (13,14). In all previous work in the lens, no proteinase activity specific for a particular protein has been observed (11).

In this report, it is shown that a Ca^{+2} activated proteinase is present in the lens which specifically hydrolyzes vimentin.

METHOD:

Calf lenses were dissected from eyes obtained from the Great American Veal Co., Newark, NJ. Anterior capsule-epithelium were dissected and examined under a Zeiss phase contrast microscope for the presence of fiber cells. The outer cortical portion of the lenses was obtained by gently shaking the decapsulated lenses in 10 mM Tris, 1 mM EGTA pH 7.5 (1 ml/lens) at 0°C for 10 minutes. The capsule-epithelium (1 capsule- epithelium/100 ul) and the outer cortical suspension were homogenized in the same buffer using a Potter-Elvehjen homogenizer. To obtain the soluble fraction, the outer cortical homogenate was centrifuged at 60,000 g for 30 minutes using a Beckman Ti 50 rotor. The pellet was resuspended in the same buffer and then centrifuged as above. The procedure was repeated 2 more times.

Vimentin was isolated from bovine lenses according to Geisler and Weber (5). The purified protein was dissolved in 6 M urea, 50 mM Tris, 0.1 mM EGTA pH 7.5 and dialyzed against 0.1 mM EGTA pH 7.5. This preparation was used as a source of exogenous vimentin.

For proteinase assay, samples (100 ul) were mixed with 50 mM calcium to obtain a final concentration of 4 mM. Preparations were incubated at 37 °C for 2 hours. The reaction was stopped by addition of an equal volume of SDS-PAGE sample buffer and boiling for 2 minutes. SDS-PAGE was performed according to Laemmli (16) or Neville (17). The degradation of vimentin was detected by the disappearance of the 57,000 dalton polypeptide band in the Coomassie Blue stained gels. An LKB soft laser densitometer was used in certain experiments to measure the degradation.

To assess the effect of protease inhibitors, the total homogenate of the outer cortex (100 ul) was preincubated in the above buffer containing 1 mM inhibitor for 30 minutes and then 4 mM calcium was added. After further incubation for 2 hours, the samples were analyzed by SDS-PAGE.

RESULTS:

To detect the degradation of the 57,000 dalton (57K) polypeptide of the lens, the outer cortical proteins were divided into soluble and insoluble fraction by centrifugation of the

homogenate in 10 mM Tris-HCl, 1 mM EGTA, pH 7.5 at 60,000g for 30 minutes. Figure 1 shows the results obtained after incubation of the soluble fraction with and without added 4 mM Ca^{+2}. In the presence of Ca^{+2}, the 57K fraction is almost completely degraded while without Ca^{+2} no change is observed. If the soluble protein fraction is heated to 100 ^{o}C for 1 minute before incubation, no degradation is observed. It has previously been shown that the 57K in the lens is vimentin. This polypeptide was, therefore, purified and added to the reaction mixture. The results (Figure 1) indicate that the endogenous 57K fraction comigrates with the exogenous

Neville (17) and similar results were obtained. When the washed insoluble protein fraction was resuspended and treated with Ca^{+2}, no degradation was detected, indicating the absence of proteinase activity.

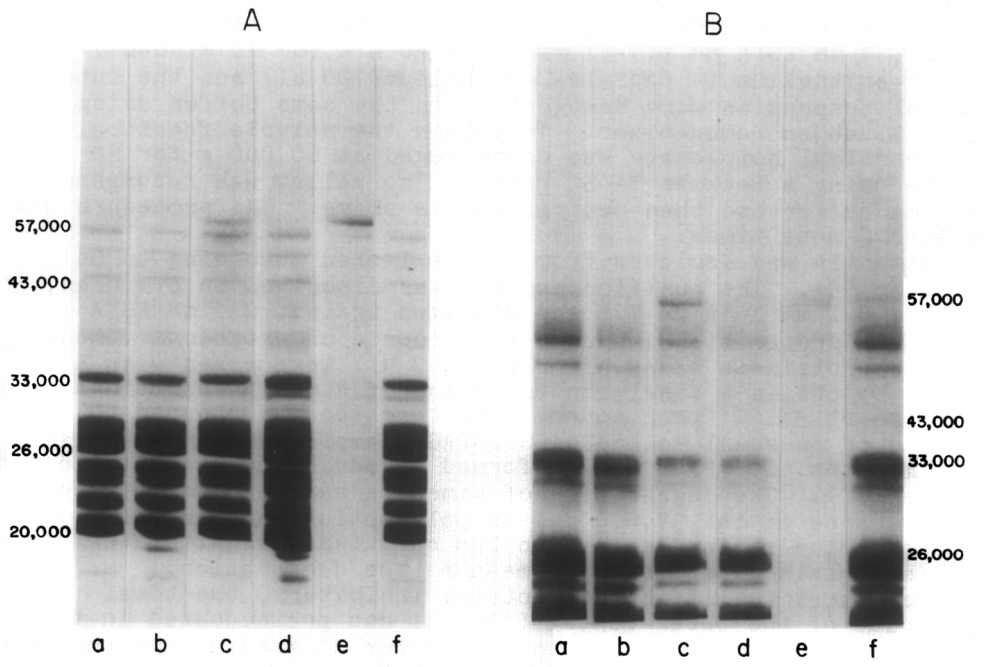

Figure 1: SDS-PAGE of the soluble fraction of the bovine lens incubated in the absence and presence of calcium, (a) and (b) respectively. (c) and (d) are similar to (a) and (b) but with purified lens vimentin added. (e) lens vimentin and (f) is same as (b) but heated to 100^{o}C before incubation with calcium. Gels in panel A and B were run according to Laemmli (16) and Neville (17) respectively.

It was of interest to determine the proteinase activity in the epithelium since in contrast to the cortex, it contains a well developed matrix. Therefore, the tissue was divided into capsule-epithelium and outer cortex. The total homogenate of these sections was used for determination of proteinase activity. The homogenates were incubated in the presence and absence of 4 mM Ca^{+2}. Figure 2 shows that the 57K component is degraded in the outer cortical homogenate but not in the capsule-epithelium. Besides the 57K polypeptide, a minor 120,000 dalton component, which may be a dimer of 57K is also degraded. To prove that the 57K in the epithelial cells is degradable by the enzyme present in the cortex, capsule-epithelium was dissected with some cortical fibers. The results, (Figure 2), indicate that all the 57K is degraded in the presence of Ca^{++} when cortical fibers are present. Therefore, either the proteinase is inactive or absent in the capsule-epithelium. The lower molecular weight proteins which are mostly crystallins as well as an actin (43,000 daltons) are not degraded under similar conditions.

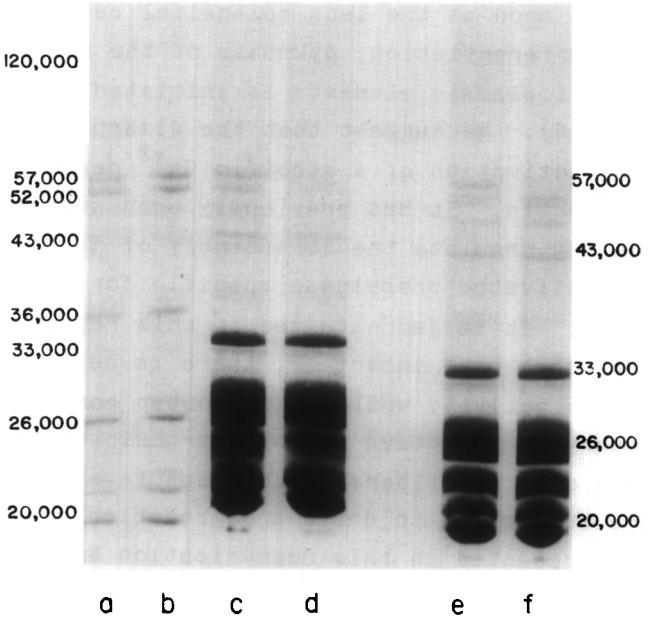

Figure 2: SDS-PAGE according to Laemmli (16) of the capsule-epithelium (a,b) and cortical (c,d) homogenates in the absence (a,c) and the presence (b,d) of calcium. Gels (e) and (f) are preparations of capsule-epithelium containing cortical fibers incubated in the absence and presence of calcium respectively.

In order to quantitate the degradation of vimentin, the gels in Figures 1 and 2 were scanned with a LKB soft laser scanner. The results indicate that in all cases where vimentin was added, the material was almost completely digested in the 2 hour incubation period. The experiments with endogeneous 57K show hydrolysis of more than 80% of the material in the presence of Ca^{+2}.

The effect of several potential proteinase inhibitors has also been investigated. The chloromethyl ketones TLCK (1-chloro-3-tosylamido-7-amino-2-heptanone) (trypsin like specificity) and TPCK (L-1-tosylamide-2-phenylethyl chloromethyl ketone) (chymotrypsin like specificity) have essentially no effect on this proteinase. The specific trypsin and chymotrypsin inhibitor, phenylmethylsufonyl fluoride also has no detectable inhibitory effect. Iodoacetamide, an alkylating agent, does not inactivate the proteinase. The proteinase is completely inhibited by 1 mM EGTA, again indicating a requirement for Ca^{+2} (Figure 2).

DISCUSSION:

IF of the lens have not been assigned a definitive biological function. It has been suggested that the IF are involved in establishing the architecture of the cell, maintaining the nucleus and cytoplasmic elements such as mitochondria in particular positions (18). As soon as the lens epithelial cells begin to undergo terminal differentiation, pyknosis of the nucleus and the disappearance of cytoplasmic elements is initiated (6). The IF also disappears (6,7). We suggest that the disappearance of the IF is related to the activation of a specific Ca^{+2} dependent proteinase for vimentin. It has previously been suggested that such proteinases may regulate the disassembly of IF (18-20) and the presence of Ca^{+2} activated proteinase specific for vimentin have been reported (21). It is demonstrated in this report that in the epithelial cells which have intact IF, there is no vimentin-proteinase activity while in the outer cortex where little intact IF is observed, an active vimentin-proteinase is found. Even the presence of a few fibers in the capsule-epithelial preparations are sufficient to cause a degradation of vimentin.

The results presented in this communication suggest that a proteinase specific for vimentin is present in the cortical soluble fraction and that the 57K polypeptide present in the epithelial and fiber cells is degradable by this enzyme. These results explain observations of vimentin degradation in the water insoluble lens preparations isolated in the presence of Ca^{+2} (22).

The failure of several common proteinase inhibitors to affect crude preparation of the lens enzyme is interesting. In Ehrlich Ascites tumor cell homogenates, the vimentin specific proteinase can be inhibited by these compounds. (However, the purified proteinase cannot be inhibited) (21). It has been suggested that such inactivation of the crude preparations is due to acylation of the essential thiol(s) in the enzyme by the inhibitor (21). The absence of essential thiol in the lens proteinase is suggested by the lack of inhibition in the presence of iodoacetamide, although, at present, the extent of alkylation of this proteinase under the conditions of these experiments is not known.

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