Eye tissues contain splice variants of muscle-preferred p94 (calpain 3), such as lens-specific Lp82 and Lp85, retina-specific R88, and cornea-specific Cn94. The purpose of the present experiment was to analyze the activation and regulation of the best characterized p94 splice variant, Lp82. Recombinant rat Lp82 (rLp82) was expressed using the baculovirus system, purified with Ni-NTA affinity and DEAE-ion exchange chromatographies, and characterized by SDS-PAGE, casein zymography, and immunoblotting. After incubation with calcium, rLp82 autolyzed into two major fragments at ~60 and 22 kDa. Sequencing of the autolytic fragments showed loss of three amino acids from the N terminus and cleavage near the IS2 region. Also, Lp82 and calpain 2 were found to hydrolyze each other. Calpastatin inhibited calpain 2 activity, but not Lp82. Homology modeling suggested that the lack of inhibition of Lp82 by calpastatin was due to molecular clashes at the unique AX1 region of Lp82. Lp82 also hydrolyzed calpastatin. These results suggested that Lp82 might regulate other calpain activities and cause hydrolysis of substrates such as crystallins during lens cataract formation.

Calpins (EC 3.4.22.17) comprise a family of non-lyosomal, cysteine proteases with a neutral pH optimum and a requirement for calcium for activation (1). They are widely distributed in animal tissues, where they are involved in a variety of cellular processes involving calcium (2). Calpins consist of the ubiquitous calpains 1 (μ-calpain), 2 (m-calpain), and 10; and tissue-specific calpains such as 3 (muscle-specific p94), 8, and 9 (3–6). Recently, splice variants of calpain 3 such as lens-specific Lp82 and Lp85, retina-specific R88, and cornea-specific Cn94 were found in the eye (7–10). Tissue-specific calpains such as 3 (muscle-specific p94), 8, and 9 (Sf9) insect cells. Transfection was performed with recombinant Lp82 (rLp82) baculovirus amplified to 10^10 plaque-forming units/ml. SF9 cells were cultured for 3 days for rLp82 protein expression.

Purification of rLp82—Cultured cells were sonicated in lysis buffer containing 20 mM Tris (pH 7.5), 0.5 mM EGTA, and 2 mM dithioerythritol. The soluble protein was obtained by centrifugation at 13,000 rpm for 30 min. Soluble Lp82 was purified by Ni-NTA (Qiagen Inc, Valencia, CA) metal-affinity chromatography according to the manufacturer’s protocol under native conditions. Further purification was performed by high performance liquid chromatography using a 7.5-mm inner diameter × 7.5-cm DEAE 5PW column (TOSOH, Japan) with a linear 0.0–0.5 mM NaCl gradient in buffer A containing 20 mM Tris (pH 7.5), 1 mM EDTA, 1 mM DTT, and 2 mM dithioerythritol at a flow rate of 0.5 ml/min. Enzyme-linked immunosorbent assay was performed by incubating 50 μl of each column fraction in 0.1 M NaHCO3 buffer (pH 9.3) overnight at 4°C. The peaks were collected and concentrated by ultracentrifugation (Microcon 10, Millipore).

Activation of rLp82—Purified rLp82 was incubated with 1.4 mM calcium in 20 mM Tris buffer (pH 7.5) for 10, 20, 30, 40, 50, 60, and 90 min. Fractions containing Lp82 were visualized using goat anti-rabbit alkaline phosphatase-conjugated secondary antibody and alkaline phosphatase substrate kit (Bio-Rad). The peaks were concentrated by ultrafiltration (Microcon 10, Millipore).

Hydrolysis of Calpains by Other Calpains—To inactivate calpains, purified rLp82 and recombinant calpain 2 (rcalpain 2) from rat (more than 98% purity, Calbiochem) were incubated with 5 mM iodoacetamide in 20 mM Tris buffer (pH 7.5) at 37 °C for 2 h. After quenching excess iodoacetamide with 10 mM dithioerythritol, 1.2 units of rcalpain 2 or rLp82 were added and incubated with calcium for 1 h at 37 °C. Fragments were detected by SDS-PAGE and immunoblotting.
Activation of Lp82

Sensitivity of Calpains to Inhibition by Calpastatin—0.22 units of calpain 2 or rLp82 were incubated for 1 h at 37 °C with 0.5 μM calpastatin purified from human erythrocytes (Calbiochem) and 1.5 mM calcium. Hydrolyzed calpastatin fragments were detected by SDS-PAGE. Immunoblotting and casein zymography were used to detect calpain degradation and calpain activity, respectively.

Electrophoresis and Immunoblotting—SDS-PAGE was performed on discontinuous, 8 or 12% gels (Invitrogen) using the glycine buffer system. ImmunobLOTS for Lp82 and calpain 2 were performed by electrotransferring proteins from SDS-PAGE gels to polyvinylidene difluoride membrane at 30 V (constant) for 100 min at an ice-cold temperature using Tris-glycine buffer (12 mM Tris, 96 mM glycine, 20% methanol). The anti-Lp82 antibody and anti-calpain 2 antibodies were used at 1:1000 dilution, and immunoreactivity was visualized with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium and alkaline phosphatase conjugated to anti-rabbit IgG secondary antibody (Bio-Rad).

Casein Zymography—8% (1-mm thick) gels, co-polymerized with 0.05% casein were pre-run with a buffer containing 25 mM Tris (pH 8.3), 192 mM glycine, 1 mM EGTA, and 1 mM dithiothreitol for 15 min at 4 °C. After electrophoresis, the gels were incubated with slow shaking overnight at room temperature in 20 mM Tris (pH 7.4), 10 mM dithiothreitol, and 2 mM calcium. Gels were stained with Coomassie Brilliant Blue. Bands of caseinolysis appeared white.

N-terminal Sequencing of Autolytic Fragments—rLp82 was auto-
lized by incubating in 1.4 mM calcium for 10 and 90 min at 30 °C, and 12% SDS-PAGE (1 mm thick) gels were then run and transferred onto polyvinylidene difluoride membrane at 30 V (constant) for 100 min. Membranes were stained with 0.1% Coomassie Brilliant Blue in 50% methanol for 10 min and destained with 50% methanol. Destained membranes were dried, and bands were excised for Edman N-terminal sequencing. Proteins (or peptides) were sequenced by Deb McMillen in the Biotechnology Laboratory at the University of Oregon, Eugene, OR, using an Applied Biosystems model 492 Procise N-terminal protein sequencer using N-terminal Edman degradation chemistry. Phenylthiohydantoin-derivatives were identified with an Applied Biosystems model 140 PTH Analyzer and a model 785A detector set at wavelength 269 nm.

Homology Modeling of Lp82 and Calpain 3—Protein homology mod-eling was performed on a Silicon Graphics OCTANE2 work station, using MOE (The Molecular Operating Environment, Version 2001.01, Chemical Computing Group Inc., Montreal, Canada, www.chemcomp.com). The Brookhaven Protein Data Bank (14) was used as the structural template for building homology models. The rat Lp82 and rat calpain 3 sequences were taken from the NCBI Protein Data Base (NCB accession numbers AAC04848 and AAA41790, respectively). Each sequence was aligned to the template sequence (human calpain 2, Protein Data Bank number 1KFU) using the protein alignment tools in MOE. The alignment conditions were tree-based using an initial pair-wise buildup followed by round robin and iterative refinement. The group-to-group calculation used the Gonnet substitution matrix and had a gap start penalty of 3 and a gap extend penalty of 1. The alignment was not biased by either actual or predicted secondary structure.

The homology modeling procedure involved building three-dimen-sional models of both sequences using human calpain 2 as a template structure. Two types of models were considered. The first assumed that Lp82 co-existed with calpain 4, thus all atoms of calpain 4 were in-cluded in the modeling and minimization. The other type of model did not consider calpain 4 as part of the final structure. In addition, models were constructed so that the outgap residues (residues that extend beyond the terminals of the template chain) were either ignored or included in the modeling procedure. Homology modeling generated ten intermediate models, from which the best scoring model was chosen and minimized to produce the final model (Amber89 forcefield, root mean square distance = 0.01).

To investigate the potential interaction of Lp82 with calpain 4, van der Waals (VDW) interaction energies were calculated and are reported on Fig. 7. The structure labeled Lp82 was constructed with the as-sumption that calpain 4 was present, with the outgap residues in-cluded. Calpain 3 models did not include the presence of calpain 4, since calpain 3 is well known not to associate with calpain 4 (16).
RESULTS

Autolysis of Lp82—To test if Lp82 is autolytic, rLp82 was incubated with calcium for 0–90 min. Unautolyzed, intact rLp82 migrated at 85 kDa instead of 82 kDa (Fig. 1, lane marked “0 min”). This is because rLp82 contains 26 additional amino acids in the N terminus from the His tag and vector protease cleavage site. Autolytic fragments at 82, 60, and 22 kDa appeared after incubation with calcium (Fig. 1A, arrows).

FIG. 3. SDS-PAGE of inactivated calpains proteolyzed by activated calpain 2 or Lp82 (A). Lane 1, inactivated rLp82 + realpain 2 without calcium; lane 2, inactivated rLp82 + realpain 2 with calcium; lane 3, inactivated realpain 2 + realpain 2 with calcium; lane 4, inactivated realpain 2 + rLp82 without calcium; lane 5, inactivated realpain 2 + rLp82 with calcium; and lane 6, inactivated rLp82 + rLp82 with calcium. B and C, immunoblots for Lp82 (B) and calpain 2 (C) with the same lane designation as above.

FIG. 4. Proteolysis of purified erythrocyte calpastatin by rLp82 or by realpain 2 as shown by SDS-PAGE (A) and by immunoblotting for Lp82 and calpain 2 (B). Casein zymography for calpain activities (C). Lane 1, realpain 2 and calpastatin with no calcium; lane 2, realpain 2 and calpastatin with calcium; lane 3, rLp82 and calpastatin with no calcium; lane 4, rLp82 and calpastatin with calcium; lane L, total soluble protein from rat lens, with the upper white band indicating Lp82 activity and the lower band indicating calpain 2 activity. For immunoblotting in B, lanes 1 and 2 were visualized with antibody against calpain 2, and lanes 3 and 4 were visualized by with antibody against Lp82.
Production of these fragments was inhibited by addition of EGTA or cysteine protease inhibitor E64 (data not shown).

The origin of the fragments from Lp82 autolysis was determined by immunoblotting using two different antibodies. One antibody recognized epitopes near the deleted IS2 region at the end of Lp82 domain III (Fig. 1B, lanes 1–3), while the second antibody recognized the N terminus (Fig. 1B, lanes 4–6). The 82-kDa autolytic fragment reacted with both antibodies, the 60-kDa fragment reacted only with the N-terminal antibody, and 22 kDa was visualized only with the IS2 antibody. These results suggested that the 82- and 60-kDa fragments contained the N-terminal region, while the 22-kDa fragment was the C-terminal region. This was confirmed by direct N-terminal sequencing of the 82- and 22-kDa fragments (Fig. 2). The 82-kDa fragment showed loss of 29 amino acids from the N terminus. This included 3 amino acids from Lp82, the His tag region, and the protease cleavage site domain from the vector (Fig. 2A). The autolytic cleavage producing the new N-terminal sequence on the 22-kDa fragment started at arginine (R) residue 524 near the deleted IS2 region (Fig. 2B). The location of autolytic sites in intact Lp82 is showed in Fig. 2C. Thus, the 82-kDa fragment was rLp82-truncated at leucine 30 in the N terminus, the 60-kDa fragment was comprised of residues from leucine (L) 30 to aspartic acid (D) 549, and the 22 kDa was from arginine (R) 550 near the IS2 region to the C-terminal alanine residue (A) 735.

Interaction between calpains—Since rodent lenses contain calpain 2 and calpastatin in addition to Lp82, we next investigated how these components of the calpain system interacted with each other. This was accomplished by inactivating rcalpain 2 and rLp82 by alkylation of the active site cysteine and then testing to see whether the proteins were proteolyzed by fresh, active rLp82 or rcalpain 2 (Fig. 3) as detected by SDS-PAGE (Fig. 3A), and immunoblotting with Lp82 (Fig. 3B) and calpain 2 antibodies (Fig. 3C). When active Lp82 was incubated with inactive calpain 2 protein in the presence of calcium, the calpain 2 protein at 80 kDa was decreased (lane 5 in Fig. 3, A–C). When calcium was omitted, Lp82 and calpain 2 were not degraded (lane 4). Of course, addition of active Lp82 to inactive Lp82 protein caused a decrease in the Lp82 band (lane 6), and this was a confirmation of autolysis described above. In the converse experiment, when active calpain 2 was added to inactive Lp82 substrate, Lp82 was totally hydrolyzed (lane 2). Calpain 2 also autolyzed itself (lane 3). Thus, Lp82 and calpain 2 hydrolyzed each other, although Lp82 seemed the more sensitive substrate for calpain 2.

Hydrolysis of Calpastatin—Calpastatin (CS) purified from human erythrocytes migrated to 65 kDa on SDS-PAGE (Fig. 4A, lane 1). Incubation of this CS with calpain 2 activated by calcium did not cause breakdown of CS (Fig. 4A, lane 2). Immunoblotting revealed that the calpain 2 did not undergo autolysis when it was incubated with CS (Fig. 4B, lane 2), and the...
CS completely inhibited calpain 2 activity (Fig. 4C, lane 2). Thus, CS was not a substrate for calpain 2, because CS inhibited calpain 2. In contrast, incubation of calcium-activated Lp82 with CS caused total hydrolysis of CS (Fig. 4A, lane 4). This was accompanied by a decrease in apparent molecular mass of the Lp82 band (Fig. 4B, lane 4) by removal of the N-terminal 29 amino acids as shown in Fig. 2, indicative of activation of Lp82. This was confirmed on zymograms (Fig. 4C, lane 4) showing a smear band of active Lp82 below the usual Lp82 band. This smear band has been noted previously during in vivo activation of Lp82 (15). These data suggested that even when CS is present, Lp82 is not inhibited and that CS is actually a substrate for hydrolysis by Lp82.

To determine the mechanism for the lack of inhibition of Lp82 by CS, a three-dimensional model of Lp82 was first constructed using the crystal structure of calpain 2 as a template (14) (Fig. 5). This was appropriate because calpain 2 and Lp82 have 51% sequence identity (7) and because the Lp82/calpain 2 match provided the best fit compared with using other templates such as papain. No significant differences between the three-dimensional structure Lp82 and calpain 2 were observed when domains IIa and b, III, and IV from each calpain were compared against each other. Similar results were observed when homology models of each domain were independently compared (data not shown). However, the helical AX1 domain I of Lp82 (30.2 Å based on model) was longer than domain I of calpain 2 (21.6 Å based on crystal structure). Since the longer extension on domain I could influence association of Lp82 to regulatory proteins, we also constructed a model of the association between the traditional calpain regulatory subunit (calpain 4) and domain I in Lp82 (Fig. 6A). We found that the longer N-terminal extension on domain I of Lp82 could interfere with the association of Lp82 with calpain 4. This was similar to the predicted lack of association between calpain 4 and calpain 3 (16), possibly because of long domain I (Fig. 6B).

In contrast, because of a short domain I, calpain 2 was able to interact well with the calpain 4 regulatory subunit (17) (Fig. 6C). For more objective observations, VDW interaction energies were also calculated using MOE. The VDW interaction energy between Lp82 and calpain 4 was 44,079,557 kcal/mol, and VDW contacts showing potential clashes were observed between domain I of Lp82 and calpain 4 (Fig. 7B). Even when the Lp82 model (Lp82*) was constructed taking into account surroundings with calpain 4, the VDW interaction energy was still high (132,181 kcal/mol), and clashes were observed between Lp82 and calpain 4 (Fig. 7C). The VDW interaction energy was also high between calpain 3 and calpain 4 (12,897,436 kcal/mol), and the clashes were also observed (data not shown). This result was consistent with a previous report for calpain 3 (16). In contrast, the VDW interaction energy between calpain 2 and calpain 4 from the crystal data was only 36 kcal/mol, and no clash was observed (Fig. 7A). These data suggested that the
atoms in domain I of Lp82 and calpain 4 were too close together to allow association between Lp82 and calpain 4. The enormous energies (44,079,557 and 12,897,436 kcal/mol) were homology models built and minimized without considering calpain 4 as part of the environment. To obtain these energies, the model was left in the MOE window, the crystal structure for calpain 4 was opened, and there were many VDW clashes. When calpain 4 was considered part of the environment during the model building, the calpain 4 atoms were copied directly to the final model and included in the minimization. Thus, the VDW interaction for Lp82* was lower (132,181 kcal/mol), since the atoms of calpain 4 were considered present when the sequence was modeled and minimized.

**DISCUSSION**

A major finding of the present report was the site for autolysis of Lp82. Our data showed that the major autolytic site was in the IS2 region. This is in contrast to the parent gene product, calpain 3, which autolyzed in the IS1 region (18). The splice deletion of IS1 apparently eliminated this autolytic site in Lp82. This is probably the reason why Lp82 is so stable in rodent lens (12, 15) and why it retains activity for at least 18 h in vitro (19). Unexpectedly, we found that the N-terminal half retained activity as evidenced as the smear band on zymograms (15). Since Lp82 and calpain 3 are from the same gene, lack of CS inhibition could be due to similar mechanisms. CS contains four homologous inhibitory domains. Each domain contains three regions designated as A, B, and C (23, 24). Region B is essential for inhibition and binds near the catalytic site in calpains 1 and 2. Regions A and C potentiate inhibitory activity by binding to domain IV of calpains 1 and 2 and to domain VI of calpain 4 (17). Our homology model suggested that the extra amino acids in the N terminus of domain I in calpain 3 extend into the pocket used for association of calpain 4 to calpain 2 (Fig. 6). This N-terminal extension may interfere with the association of calpain 4 to calpain 3. Likewise, Lp82 also has unique N-terminal sequence termed AX1, which also extends into calpain 4 binding pocket (Fig 6). This may also interfere with the association of calpain 4 to Lp82. This was also confirmed by calculation of the VDW interaction energies (Fig. 7).
When the calpain 4 was included in the final minimization, the VDW interaction energy was calculated to be 132,181 kcal/mol. This shows that many of the VDW clashes can be alleviated in the course of the minimization; however, the interaction energy remains large. This suggests that even with energy minimization, there is not enough room to accommodate all of the atoms in the protein interface area without having some atoms violate the VDW radii of others. The lack of room is visually supported by the pictures of the interface in Fig. 6, which shows that the extension at the N terminus of both Lp82 and calpain 3 extends from the calpain 2 template into the region where calpain 4 would normally bind.

Minimized homology models could not place the outgap residues such that their atoms do not clash with atoms of the calpain 4. This could explain the high VDW interaction energies observed when the outgap residues were placed. During the homology modeling procedure, when the outgap residues were being modeled, the scoring of the homology model incorporates a term that reflects the VDW interaction energy between atoms already placed and those that are currently being placed. When the outgap residues were not included, the interaction energy in the presence of calpain 4 was 287 kcal/mol (data not shown), which is in the order magnitude of the interaction energy between calpain 2 and calpain 4 from the crystal structure (36 kcal/mol). The increase in VDW interaction energy when the model includes the outgap residues (287 kcal/mol to 132,181 kcal/mol) again suggests that the presence of the additional atoms contributes to VDW clashes that cannot be resolved with minimization. The presence of these potential clashes near the protein interface suggests that the unique AX1 region of Lp82 is responsible for blocking the calpain 4 association.

Thus, the minimization part of the homology modeling cannot relieve all of the VDW interactions, because not enough room is present to move the outgap residues around in the region occupied by calpain 4. All modeling can do is minimize the number of potential VDW clashes/contacts.

These results from homology modeling are consistent with our previous result showing that Lp82 was only poorly inhibited by CS, even in the presence of calpain 4 (15). As described above, inhibition of calpain 2 is potentiated by binding of CS to calpain 4 (17). In fact, calpain 2 used in the present experiment contained calpain 4, since the chaperone-like effect of calpain 4 was used for producing recombinant calpain 2 (25). The lack of calpain 4 binding to Lp82 may explain why CS was not an effective inhibitor of Lp82, although the crystal structure of Lp82 will be needed to fully understand the inhibition mechanism. Lack of inhibition is important physiologically because it would allow Lp82 to be active in lens in the presence of CS. Furthermore, degradation of CS by Lp82 may help explain how calpain 2 escapes inhibition by CS to become active in lens.

We also hypothesize that in young rodent cataracts, influx of calcium leads to Lp82 activation, since Lp82 has a lower calcium requirement for activation than calpain 2 (Fig. 8). Lp82 cleaves CS, which eliminates inhibition of calpain 2 by CS. Both active Lp82 and calpain 2 truncate crystallins, leading to insolubilization and precipitation of crystallins. Oxidation enhances precipitation of truncated crystallins (Fig. 8). Activated Lp82 may be the most active calpain in young rodent lenses. However, four deleted nucleotides in exon 1 produce a stop codon in the Lp82 transcripts in man. However, an Lp82-like cleavage site was observed on human α-crystalline (26). Thus, another p94 splice variant may assume the function of Lp82 in human lens.

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REFERENCES
1. Murachi, T., Tanaka, K., Hatanaka, M., Murakami, T. (1980) Adv. Enzyme Regul. 19, 407–424
2. Murachi, T. (1989) Biochem. Int. 18, 263–294
3. Horikawa, Y., Oda, N., Cox, N. J., Li, X., Orho-Melander, M., Hara, M., Hinokio, Y., Lindner, T. H., Mashima, H., Schwarz, P. E., del Bosque-Plata, L., Horikawa, Y., Oda, Y., Yoshiuchi, I., Collina, S., Polonsky, K. S., Wei, S., Concannon, P., Iwasaki, N., Schulze, J., Baier, L. J., Bouladoux, C., Group, L., Boerwinkle, E., Hanis, C. L., Bell, G. I. (2000) Nat. Genet. 26, 163–175
4. Sorimachi, H., Imaojoh-Ohmi, S., Enomoto, Y., Kawasaki, H., Ohno, S., Minami, Y., and Suzuki, K. (1989) J. Biol. Chem. 264, 21016–21011
5. Sorimachi, H., Ishiura, S., and Suzuki, K. (1993) J. Biol. Chem. 268, 19476–19482
6. Lee, H. J., Sorimachi, H., Jeong, S. Y., Ishiura, S., and Suzuki, K. (1998) Biol. Chem. 379, 175–183
7. Ma, H., Fukuiage, C., Azuma, M., and Shearer, T. R. (1998) Invest. Ophthalmo. Vis. Sci. 39, 454–461
8. Ma, H., Shih, M., Hata, I., Fukuiage, C., Azuma, M., and Shearer, T. R. (2000) Curr. Eye Res. 21, 710–720
9. Nakajima, T., Fukuiage, C., Higahmin, M., Nakajima, T., Hata, I., and Shearer, T. R. (1999) Curr. Eye Res. 18, 424
10. Nakajima, T., Fukuiage, C., Azuma, M., Hata, I., and Shearer, T. R. (2000) Biochim. Biophys. Acta 1519, 55–64
11. Richard, I., Broux, O., Allamand, Y., Fougerousse, F., Chiannekulchaisri, N., Bourg, N., Brenguier, L., Devaud, C., Pasturaud, P., and Roudaut, C. (1995) Cell 81, 27–40
12. Ma, H., Shih, M., Hata, I., Fukuiage, C., Azuma, M., and Shearer, T. R. (1998) Exp. Eye Res. 67, 221–229
13. Y. Ueda, C. Fukuiage, M. Shih, T. R. Shearer, and L. L. David, unpublished data.
14. H. Ma, C. Fukuiage, M. Azuma, and T. R. Shearer, unpublished data.
13. Ma, H., Shih, M., Fukiage, C., Azuma, M., Duncan, M. K., Reed, N. A., Richard, I., Beckmann, J. S., and Shearer, T. R. (2000) *Invest. Ophthalmol. Vis. Sci.* 41, 4232–4239
14. Strobl, S., Fernandez-Catalan, C., Braun, M., Huber, R., Masumoto, H., Nakagawa, K., Irie, A., Sorimachi, H., Bourenkov, G., Bartunik, H., Suzuki, K., and Bode, W. (2000) *Proc. Natl Acad. Sci.* 97, 588–592
15. Nakamura, Y., Fukiage, C., Ma, H., Shih, M., Azuma, M., and Shearer, T. R. (1999) *Exp. Eye Res.* 69, 155–162
16. Sorimachi, H., Kinbara, K., Kimura, S., Takahashi, M., Ishiura, S., Sasagawa, N., Sorimachi, N., Shimada, H., Tagawa, K., Maruyama, K., and Suzuki, K. (1995) *J. Biol. Chem.* 270, 31158–31162
17. Takano, E., Ma, H., Yang, H. Q., Maki, M., and Hatanaka, M. (1995) *FEBS Lett.* 362, 95–97
18. Kinbara, K., Ishiura, S., Tomioka, S., Sorimachi, H., Jeong, S. Y., Amano, S., Kawasaki, H., Kolmerer, B., Kimura, S., Labeit, S., and Suzuki, K. (1998) *Biochem. J.* 335, 589–596
19. Ueda, Y., McCormack, A. L., Shearer, T. R., and David, L. L. (2001) *Exp. Eye Res.* 73, 625–637
20. Shearer, T. R., Ma, H., Shih, M., Hata, I., Fukiage, C., Nakamura, Y., and Azuma, M. (1998) *Curr. Eye Res.* 17, 1037–1043
21. Nakamura, Y., Fukiage, C., Shih, M., Ma, H., David, L. L., Azuma, M., and Shearer, T. R. (2000) *Invest. Ophthalmol. Vis. Sci.* 41, 1460–1466
22. Sorimachi, H., Ishiura, S., and Suzuki, K. (1997) *Biochem. J.* 328, 7217–7232
23. Emori, Y., Kawasaki, H., Imajoh, S., Minami, Y., and Suzuki, K. (1988) *J. Biol. Chem.* 263, 2364–2370
24. Maki, M., Bagei, H., Hamaguchi, K., Ueda, M., Murachi, T., and Hatanaka, M. (1989) *J. Biol. Chem.* 264, 18866–18869
25. Graham-Siegenthaler, K., Gauthier, S., Davies, P. L., and Elce, J. S. (1994) *J. Biol. Chem.* 269, 30457–30460
26. Takemoto, L. J. (1995) *Curr. Eye Res.* 14, 837–841