Yonsei Medical Journal
Vol. 45, No. 1, pp. 73-80, 2004

Accumulation and Aberrant Modifications of α-Crystallins in Anterior Polar Cataracts

Kyung-Hoon Hwang[1], Eunjoo H. Lee[2], Eek-hoon Jho[3], Jae-Ho Kim[4], Do Hyung Lee[5], Sung-Kun Chung[4], Eung Kwon Kim[6], and Choun-Ki Joo[1]

[1]Laboratory of Ophthalmology and Visual Science, Korea Eye Tissue and Gene Bank, [4]Department of Ophthalmology, College of Medicine, The Catholic University of Korea, Seoul, Korea;
[2]Graduate School of East-West Medical Science, Kyung Hee University, Seoul, Korea;
[3]Department of Life Scence, The University of Seoul, [5]Department of Ophthalmology, Inje University, Ilsan Paik Hospital, Korea;
[6]Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea.

Crystallins are the major proteins found in the lens, and the localization of specific crystallins is well known. Overexpression and accumulation of αB-crystallin has been observed in response to stress conditions or in certain diseases, such as brain tumors and neurodegenerative diseases. The purpose of this study was to examine whether α-crystallins are modified during pathological myofibroblastic changes in lens epithelial cells.

Lens epithelial cells attached to the anterior capsules of patients with nuclear or anterior polar cataracts were analyzed quantitatively for α-crystallin proteins and mRNAs using Western blot and RT-PCR analysis., respectively. The degree of modification of α-crystallins was determined by 2-dimensional gel electrophoresis followed by Western blotting.

Higher molecular weight protein bands that were immunoreactive to anti-αA- and anti-αB-crystallin antibodies around 45 kDa accumulated more in the anterior polar cataract samples than in those with the nuclear type of cataracts. Also monomeric αB-crystallins accumulated more in lens epithelial cells of patients with anterior polar cataracts. By comparison, no significant changes were found in the levels of the mRNAs encoding αA- and αB-crystallins in the different types of cataracts. Both αA- and αB-crystallin proteins seemed to undergo more extensive modification in anterior polar cataracts.

Conclusion. In addition to fibrotic changes, which accompany increased levels of extracellular matrix molecules, accumulation and abnormal modification of α-crystallins might be implicated in the pathogenic mechanism of this type of cataract.

Key Words: Alpha-crystallins, cataract, lens epithelium, modification, two-dimensional gel electrophoresis

Received January 8, 2003
Accepted December 18, 2003

This study was supported by grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ1-PG1-CH002-0003).

Reprint address: requests to Dr. Choun-Ki Joo, Department of Ophthalmology and Visual Science, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, Korea. Tel: 82-2-590-2613, Fax: 82-2-533-3801, E-mail: ckjoo@cmc.cuk.ac.kr

INTRODUCTION

The refractive properties of the transparent eye lens depend on a group of proteins called crystallins that comprise approximately 90% of the soluble proteins in this tissue. Three major groups of crystallins, namely α, β, and γ-crystallins, exist in the mammalian lens. The α-crystallins consist of the two polypeptides, αA and αB,. These polypeptides share a 55% amino acid sequence identity[1,2] and are present as heterologous complexes in the region of 800 kDa. The α-crystallins are highly expressed in the lens and to a lesser extent in other tissues, in which they have non-refractive roles. The α-crystallins share amino acid sequence similarity with the small heat shock proteins and show chaperone activity.[3] Both αA- and αB-crystallins act as molecular chaperones *in vitro*, preventing protein aggregation induced by heat and other stresses. In particular, αB-crystallin has been shown to accumulate and to be overexpressed in response to stress caused by certain diseases, such as neurodegenerative diseases and brain tumors.[4,5] Because α-crystallins are essential components for lens transparency, alterations in α-crystallins may

disrupt normal lens structure and function. It is well documented that α-crystallins undergo extensive post-translational modifications, including partial degradation,[6,7] deamidation,[8] disulfide formation,[7,8] and phosphorylation[8,9] with increasing age.[8,10,11] Extensive racemization and isomerization of aspartate and asparagine residues in both αA and αB also occur in humans.[11] Many of these modifications can cause conformational changes, which eventually lead to protein aggregation and lens opacification.[12-17]

Central lens epithelial cells do not proliferate, but survive throughout life maintaining lens homeostasis and clarity. However, the survival mechanisms adopted by these central lens epithelial cells are unknown. It is speculated that α -crystallins, like heat shock proteins, play a critical role in the various stress conditions that influence lens epithelial cells. The majority of studies showing α-crystallin modifications have concerned age-related cataracts.[18,19] Moreover, modifications occurring in lens fibers have been well examined.[20,21] Therefore, we attempted to investigate the possibility of alterations in α-crystallins that might occur in the central lens epithelial cells under non-age related conditions. Anterior polar cataracts may provide stress conditions in which the central lens epithelial cells are exposed to transforming stimuli. Our previous results showed that cells adhering to anterior capsules in patients with anterior polar cataracts are transdifferentiated into myofibroblast-like cells.[22,23] This led us to hypothesize that α-crystallins may actively participate in a reactive processes involving the central lens epithelial cells under this particular pathologic condition. As a consequence, they become altered as noted in neurodegenerative diseases. To test this hypothesis, we examined whether lens epithelial cells from patients with anterior polar cataracts possibly influence the expression levels and modifications of the α-crystallins.

MATERIALS AND METHODS

Human specimens

This study was conducted according to the tenets of the Declaration of Helsinki. Lens capsules with adherent epithelial cells were obtained during cataract surgery from patients clinically diagnosed with nuclear and anterior polar cataracts. Lens capsules of noncataractous lenses were obtained during clear lens extraction for the correction of high myopia. Cataract samples were collected from ten patients, 5 female and 5 male, ranging in age from 25 to 60. A single lens was extracted from one highly myopic male patient who was 55 years old. The capsules were immediately placed in TRIzoL reagent (GIBCO, Gaithersburg, MD, USA) for RNA preparation or were frozen for protein extraction. Lens fiber cells were obtained from patients who underwent cataract extraction. After removing anterior capsule following continuous curvilinear capsulorrhexis, lens fiber cells were obtained by phacoemulsification, aspiration, and irrigation. Lens fiber cells were collected in Falcone tubes and stored at at -20℃ for protein analysis.

Reverse transcription-PCR

Total cellular RNA was isolated from LECs attached to the anterior capsules of human cataractous lenses by using TRIzoL reagent. One μg of RNA was reverse-transcribed in a 20 μl reaction mixture by using a kit (First strand cDNA synthesis kit; Boehringer Mannheim, Indianapolis, IN, USA). Then the prepared cDNA (0.2 to 1 μl) was amplified in a 20 μl reaction mixture. Conditions for PCR were as follows: 0.4 μM primer, 0.2 mM deoxynucleoside triphosphate mixture (Perkin Elmer, Foster City, CA, USA), 50 mM KCl, 10 mM Tris- HCl (pH 8.3), 1.5 mM $MgCl_2$, and 1.0 U of *Taq* DNA polymerase (Promega, Madison, WI, USA). Reaction mixtures were incubated in a thermal controller (Model PTC-100; MJ Research, Watertown, MA, USA) for 25 to 30 cycles (denaturation at 94℃ for 30 s, annealing at 60℃ for 30 s, extension at 72℃ for 30 s). Primer sequences specific for the genes examined and predicted product sizes are shown in Table 1.

Western blot analysis

Lens epithelial cells were lysed on ice in lysis buffer (20 mM Tris-HCl [pH 7.5], 120 mM NaCl,

Table 1. Primers and Expected Sizes of PCR Products with Each Primer Pair

Gene	Size (bp)	Forward primer	Reverse primer
β-actin	350	5′-aggccaaccgcgaagatgacc-3′	5′-gaagtccagggcgacgtagcac-3′
α A-crystallin	276	5′-caggacgactttgtggaga-3′	5′-caatgctgcttaggacgag-3′
α B-crystallin	275	5′-ccttctttcctttccactcc-3′	5′-cacctcaatcacatctccca-3′
β-crystallin	337	5′-gatgcctggagtgggagtaa-3′	5′-attggatctgcgaagtctgg-3′
Filensin	606	5′-tcctccagcatccattgtg-3′	5′-agccttgtgtcttccaaacc-3′

10% glycerol, 1% Triton X-100, 2 mM EDTA, 2 mM EGTA, 1 mM ditheiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20 μM leupeptin, and 1 μM aprotinin; Sigma). After centrifugation for 10 min at 10,000 × g, the supernatant was stored at -20℃. Protein concentrations were determined by using a protein assay kit (*DC* protein assay kit; Bio-Rad, Hercules, CA, USA). The lysates containing 10 μg of protein were boiled for 5 min in SDS sample buffer, fractionated by SDS-10% polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane (Hybond; Amersham, Cleveland, OH, USA) by using an electroblotting apparatus (Bio-Rad). The membranes were blocked for 1 hr in Tween-phosphate-buffered saline (PBS-Tween) containing 5% nonfat milk powder. The membranes were then incubated with primary antibody at 1 : 1,000 dilutions in PBS-Tween containing 5% nonfat milk powder at room temperature for 1 hr and then washed in PBS-Tween. The primary antibodies used in this study were rabbit anti (α-crystallin antiserum (1 : 1,000 dilution) and (β-crystallin antiserum (1 : 2,000 dilution) (Stressgen, Victoria, B.C., Canada). Blots were then incubated with a 1 : 1,000 dilution of horseradish peroxidase-conjugated anti-rabbit antibody (Amersham) at room temperature for 1 hr. After washing in three times in PBS-Tween, the blots were developed by using ECL detection reagents (Amersham) or chromogenic substrate solution (DAB substrate; Boehringer Mannheim). Pre-stained molecular weight standards were purchased from Bio-Rad and Novex.

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2-D) of lens epithelial cell lysates was performed according to the method of O'Farrell.[24] Lens epithelial cells were lysed on ice in urea-soluble sample buffer. In brief, samples were thoroughly mixed in 240 μl of Sample Buffer I (0.3% SDS, 200 mM DTT, 50 mM Tris, pH 8.0) and boiled for 5 minutes. The samples were chilled on ice for 5 minutes and 24 μl of Sample Buffer II (500 mM Tris, 50 mM $MgCl_2$, 1 mg/ml DNAse I, 0.25 mg/ml RNAse A, pH 8.0) was added to each tube. They were mixed thoroughly and incubated for up to 8 minutes on ice. Acetone was added to 80 % v/v and incubated on ice for 20 minutes. The solution was centrifuged at 13,000 xg for 10 minutes. The supernatant was discarded and the pellet was dried at room temperature for 5 minutes. The pellet was resuspended in 240 μl of Sample Buffer Mix (7.92 M urea, 0.06% SDS, 1.76 % Ampholytes, 120 mM DTT, 3.2% Triton X-100, 40 mM Tris (pH 8.0)) and stored at (70℃. Total urea-soluble proteins were separated by 2-D as previously described.[25,26] Fifty-micro-liter of the urea-soluble proteins were then applied to a gel (8 M Urea, 0.4% Triton X-100, 4 parts pH 5-8 and 1 part pH 3.5 - 10 Ampholine mixtures, Duracryl [acrylamide/Bis 30.8% T]). Isoelectric focusing was performed for the first dimension at 450 V for 16 h at 16.5℃ (Top buffer : 20 mM NaOH, Bottom buffer : 0.085% H_3PO_4), and proteins were separated by SDS-PAGE (15% gels, 16 × 18 cm, 1.5 mm, 100 - 200V) for the second dimensional analysis. The proteins were visualized by staining with silver nitrate or subsequently subjected to Western blotting.

RESULTS

To examine any changes in α-crystallins in lens

epithelial cells of anterior polar cataracts, we first attempted to compare the expression level of α-crystallin proteins in lens epithelial cells from patients with distinct types of cataracts. The expression level of αA-crystallin in lens epithelial cells from patients with clear lenses (noncataractous high myopia) was included as a control value. As shown in Fig. 1, a marked increase in higher molecular weight bands, especially around 45 kDa, ocurred in anterior polar cataracts. This may indicate non-disulfide cross-linking of native αA-crystallins.

Next, we also studied αB-crystallin in an identical manner. Because clear lenses were of limited availability, and because lens epithelial cells of nuclear cataracts showed similar patterns of immunoreactivity to those of noncataractous high myopia (Fig. 1, lanes 2 and 3), we examined anterior polar and nuclear cataracts for the rest of this study. Fig. 1 (lanes 4 and 5) showed that the expression of αB-crystallin in anterior polar cataracts was enhanced. Similar results were obtained in another independent set of assays (data not shown), suggesting that these alterations could be attributed to the common nature of this particular type of cataract. Coomassie Blue staining of gel demonstrated that equal amounts of protein were applied for samples from both types of cataract (Fig. 1, lanes 6 and 7).

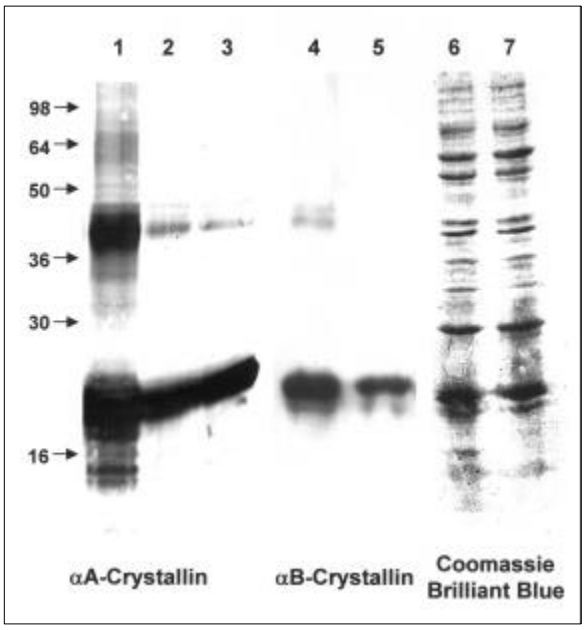

Fig. 1. Alterations of αA- and αB-crystallin proteins in lens epithelial cells of anterior polar cataracts. Lens epithelial cells attached to the anterior lens capsules from patients with anterior polar cataracts (lane 1, 4, 6), nuclear cataracts (lane 2, 5, 7), and noncataractous high myopia (lane 3) were lysed in lysis buffer. Equal amounts (10 μg) of cell lysates were examined for αA- and αB- crystallin expression by Western blot analysis. To confirm equal loading, cell lysates were separated on SDS-PAGE and stained with Coomassie Brilliant Blue. Three separate experiments showed similar results.

We then examined whether the lens epithelial specimens we obtained were contaminated with lens fiber materials or whether abnormally proliferating fiber cells existed in the specimens from anterior polar cataracts. To test this, we performed RT-PCR analysis for messages for fiber cell specific proteins such as β-crystallin and filensin. As shown in Fig. 2, the expression of β-crystallin and filensin mRNA was not detectable in lens epithelial cells whereas it was readily detectable in lens fibers that were included as a positive control. This shows that the cells adhering to the anterior lens capsules from anterior polar cataracts are mostly lens epithelial cells. It suggests that the alteration of α-A and α-B-crystallin expression is not due to contamination of lens fiber cells or abnormal proliferation of fiber cells in anterior polar cataracts.

To determine whether the observed changes in

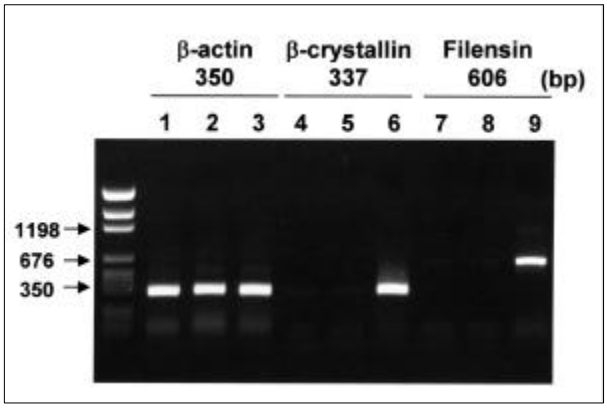

Fig. 2. Absence of mRNAs for fiber-specific proteins in lens epithelial cells from cataract patients. Total RNA was isolated from cells attached to the anterior lens capsules and from lens fibers obtained during cataract surgery. mRNA levels for β-actin, β-crystallin, and filensin were examined by RT-PCR. Two separate experiments yielded similar results. M=molecular weight standards (base pairs); lanes 1, 4, 7=anterior polar cataracts; lane 2, 5, 8=nuclear cataracts; lane=3, 6, 9 lens fibers.

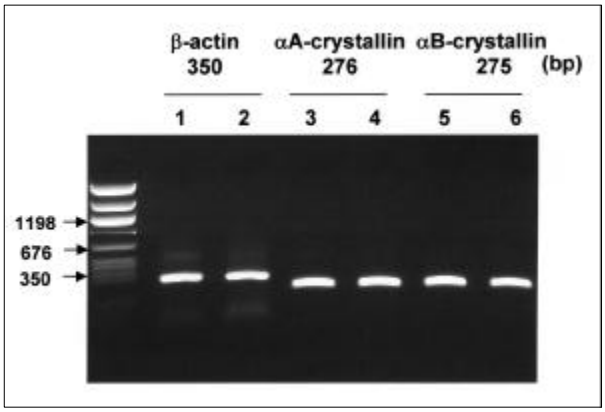

Fig. 3. The levels of αA- and αB-crystallin mRNAs in lens epithelial cells of anterior polar and nuclear cataracts. No changes in mRNA levels were observed. Total cellular RNA was isolated from lens epithelial cells of anterior polar cataracts (lane 1, 3, 5) and nuclear cataracts (lane 2, 4, 6), then subjected to RT-PCR using gene-specific primers. Three independent assays showed similar results.

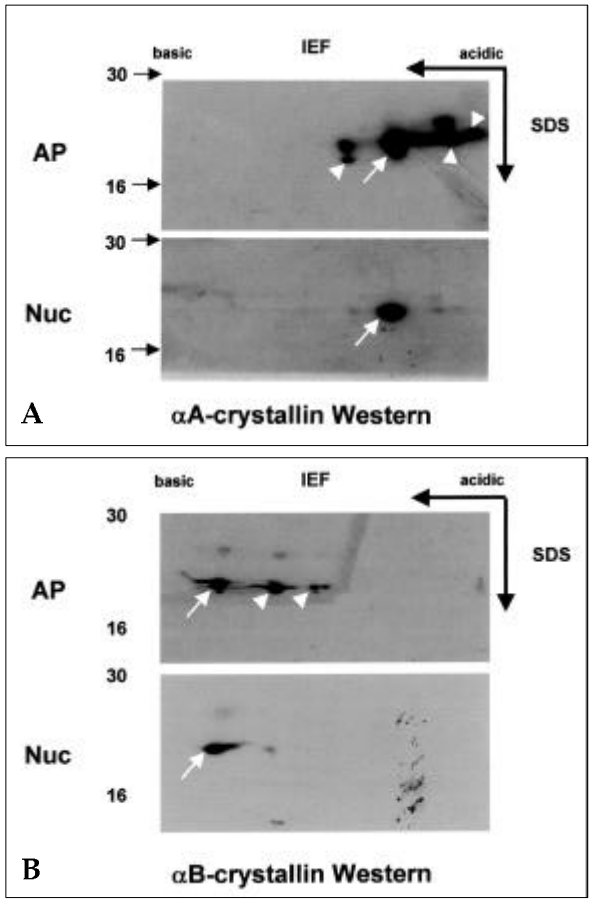

Fig. 4. Modification of αA- and αB-crystallins in lens epithelial cells from anterior polar type cataract patients. Cell lysates were prepared from lens epithelial cells attached to the anterior lens capsules from patients with anterior polar cataracts (AP) and nuclear cataracts (Nuc). Lysates were first focused in an isoelectric focusing gel and then subsequently resolved on SDS-PAGE. After separation using 2-D gel electrophoresis, each gel was tested by Western blot analysis for αA-crystallin (A) or αB-crystallin (B). Three separate experiments showed similar results. Arrow: unmodified crystallin, arrow head: modified crystallin.

protein expression reflected those in mRNA, we compared the steady state mRNA level of αA- and αB-crystallins in anterior polar and nuclear cataracts using RT-PCR analysis. Fig. 3 showed similar levels of mRNA expression in lens epithelial cells obtained from both types of cataract. This data implies that the increased level of αA- and αB-crystallins in lens epithelial cells of anterior polar cataracts is controlled at the post-transcriptional level.

Finally, to better examine possible modifications of α-crystallins in lens epithelial cells of anterior polar cataracts, we performed Western blotting after two-dimensional gel electrophoresis. As shown in Fig. 4A, additional spots (black arrow heads) migrating to the acidic side of the major (α-crystallin spot (arrow) were prominent in anterior polar cataracts. Several faster migrating spots (white arrow heads) that could be truncated products were also detected at the basic side of the major αA-crystallin spot (Fig. 4A). In the blot probed with αB-crystallin, more spots were detected at the acidic side of the major αB-crystallin spot in anterior polar cataracts (Fig. 4B).

DISCUSSION

Crystallins were previously regarded as lens-specific and exclusively considered as structural proteins.[25] However, they have been found to also possess genuine chaperone activity similar to that of heat-shock proteins.[26-28] In this study, we observed abnormal changes in α-crystallins from lens epithelial cells of anterior polar type cataract (APC, Fig. 1). In addition, using 2-D gel analysis, we showed aberrant modification α-crystallins (Fig. 4). These results suggest that aberrant accumulation and modification of α-crystallins can be major causes of APC formation. In the previous

studies, Andley et, al.[29] used total lenses to analyze protein modification or expression. However, we used lens epithelial cell samples from human patients with cataracts. Thus our investigation gave us more detailed information about changes in proteins in the cataract samples. However, still more investigation is required to understand the nature of protein modification with regard to APC. The majority of reports concerning the accumulation and modifications of the α-crystallin gene and protein have included various aspects of the function of this protein in the lens.[30-33] For example, studies on the mechanisms that lead to the accumulation of α-crystallin in normal and diseased tissues, including tumors, have been reported.[4,5,34]

Modifications in the crystallins may disrupt their normal structure in the lens and cause light scattering. Early investigators of the crystallins reported post-translational charge modifications[35] and decreases in molecular weights[36] associated with protein aging. A recent study also showed that α-Crystallins in the human lens undergo extensive modifications,[37] which lead to their incorporation as major components of the insoluble colored fraction of age-related nuclear cataracts.[12] During aging, lens crystallins undergo extensive posttranslational modification. Both α-Crystallins undergo post-translational modifications: truncation of N-14 and C-termini,[14,38] deamidation,[8] racemization,[11] phosphorylation,[8,9] oxidation of methionine,[14,39] glycation,[40-42] disulfide formation,[7,8] and addition of O-GlcNAc of αB-Crystallin.[43,44]

We have used two-dimensional gel electrophoresis techniques to examine modifications in lens epithelial cell proteins that could be characteristics of the cataractogenic processes. According to our results, additional spots migrating to the acidic side of the major αA- and αB- crystallin spots were prominent in anterior polar cataract samples. This result suggests phosphorylation, deamination, oxidation, and O-linked glycosylation can be possible modifications ocurring in anterior polar cataracts. However, we still need more investigation to figure out the nature of modifications on αA- and αB- crystallins and whether these modification(s) are related to disease progression.

In summary, we showed accumulation and aberrant modifications of α-Crystallins, which may significantly influence the transparency of-lens epithelial cells. The data obtained from this study of α-crystallin in lens epithelial cells can be used as a reference to monitor changes in lens crystallins that occur with anterior polar cataracts. Further understanding of anterior polar cataract-related modifications may lead to identify therapeutic targets for anterior polar cataracts.

ACKNOWLEDGMENTS

The authors thank David Beebe at Washington University School of Medicine, St. Louis, Missouri, for his insightful comments and advice for this study.

REFERENCES

1. de Jong WW, Terwindt EC, Bloemendal H. The amino acid sequence of the A chain of human alpha-crystallin. FEBS Lett 1975;58:310-3.
2. Dubin RA, Ally AH, Chung S, Piatigorsky J. Human alpha B-crystallin gene and preferential promoter function in lens. Genomics 1990;7:594-601.
3. Lu SF, Pan FM, Chiou SH. Sequence analysis of frog alpha B-crystallin cDNA: sequence homology and evolutionary comparison of alpha A, alpha B, and heat shock proteins. Biochem Biophys Res Commun 1995; 216:881-91.
4. Aoyama A, Steiger RH, Frohli E, et al. Expression of alpha B-crystallin in human brain tumors. Int J Cancer 1993;55:760-4.
5. Lowe J, Errington DR, Lennox G, Pike I, Spendlove I, Landon M, et al. Ballooned neurons in several neurodegenerative diseases and stroke contain alpha B crystallin. Neuropathol Appl Neurobiol 1992;18:341-50.
6. Takemoto L. Quantitation of C-terminal modification of alpha-A crystallin during aging of the human lens [letter]. Exp Eye Res 1995;60:721-4.
7. Yang Z, Chamorro M, Smith DL, Smith JB. Identification of the major components of the high molecular weight crystallins from old human lenses. Curr Eye Res 1994;13:415-21.
8. Miesbauer LR, Zhou X, Yang Z, Sun Y, Smith DL, Smith JB. Post-translational modifications of water-soluble human lens crystallins from young adults. J Biol Chem 1994;269:12494-502.
9. Takemoto LJ. Differential phosphorylation of alpha-A crystallin in human lens of different age. Exp Eye Res 1996;62:499-504.
10. Srivastava OP, Srivastava K, Silney C. Covalent modifi-

cation at the C-terminal end of a 9 kDa gamma D- crystallin fragment in human lenses. Exp Eye Res 1994;58: 595-603.
11. Fujii N, Ishibashi Y, Satoh K, Fujino M, Harada K. Simultaneous racemization and isomerization at specific aspartic acid residues in alpha B-crystallin from the aged human lens. Biochim Biophys Acta 1994;1204: 157-63.
12. Chen YC, Reid GE, Simpson RJ, Truscott RJ. Molecular evidence for the involvement of alpha crystallin in the colouration/crosslinking of crystallins in age-related nuclear cataract. Exp Eye Res 1997;65:835-40.
13. Groenen PJ, Merck KB, de Jong WW, Bloemendal H. Structure and modifications of the junior chaperone alpha-crystallin. From lens transparency to molecular pathology. Eur J Biochem 1994;225:1-19.
14. Lund AL, Smith JB, Smith DL. Modifications of the water-insoluble human lens alpha-crystallins. Exp Eye Res 1996;63:661-72.
15. de Jong WW, Mulders JW, Voorter CE, Berbers GA, Hoekman WA, Bloemendal H. Post-translational modifications of eye lens crystallins: crosslinking, phosphorylation and deamidation. Adv Exp Med Biol 1988;231:95-108.
16. Van Kleef FS, De Jong WW, Hoenders HJ. Stepwise degradations and deamidation of the eye lens protein alpha-crystallin in ageing. Nature 1975;258:264-6.
17. Voorter CE, Roersma ES, Bloemendal H, de Jong WW. Age-dependent deamidation of chicken alpha A-crystallin. FEBS Lett 1987;221:249-52.
18. Truscott RJ, Chen YC, Shaw DC. Evidence for the participation of alpha B-crystallin in human age-related nuclear cataract. Int J Biol Macromol 1998;22:321-30.
19. Mostafapour MK, Schwartz CA. Age-related changes in the protein concentration gradient and the crystallin polypeptides of the lens. Invest Ophthalmol Vis Sci 1982;22:606-12.
20. Calvin HI, Wu JX, Viswanadhan K, Fu SC. Modifications in lens protein biosynthesis signal the initiation of cataracts induced by buthionine sulfoximine in mice. Exp Eye Res 1996;63:357-68.
21. Castellani RJ, Perry G, Harris PL, Monnier VM, Cohen ML, Smith MA. Advanced glycation modification of Rosenthal fibers in patients with Alexander disease. Neurosci Lett 1997;231:79-82.
22. Lee EH, Joo CK. Role of transforming growth factor-beta in transdifferentiation and fibrosis of lens epithelial cells. Invest Ophthalmol Vis Sci 1999;40:2025-32.
23. Lee EH, Seomun Y, Hwang KH, Kim JE, Kim IS, Kim JH, et al. Overexpression of the transforming growth factor-beta-inducible gene betaig-h3 in anterior polar cataracts. Invest Ophthalmol Vis Sci 2000;41:1840-5.
24. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. J Biol Chem 1975;250:4007-21.
25. Harding JJ, Dilley KJ. Structural proteins of the mammalian lens: a review with emphasis on changes in development, aging and cataract. Exp Eye Res 1976; 22:1-73.
26. Bhat SP, Nagineni CN. alpha B subunit of lens-specific protein alpha-crystallin is present in other ocular and non-ocular tissues. Biochem Biophys Res Commun 1989;158:319-25.
27. Dubin RA, Wawrousek EF, Piatigorsky J. Expression of the murine alpha B-crystallin gene is not restricted to the lens. Mol Cell Biol 1989;9:1083-91.
28. Iwaki T, Kume-Iwaki A, Liem RK, Goldman JE. Alpha B-crystallin is expressed in non-lenticular tissues and accumulates in Alexander's disease brain. Cell 1989;57: 71-8.
29. Andley UP, Mathur S, Griest TA, Petrash JM. Cloning, expression, and chaperone-like activity of human alpha A-crystallin. J Biol Chem 1996;271:31973-80.
30. Scalbert P, Birlouez-Aragon I. Relationship between lens protein glycation and membrane structure in human cataract. Exp Eye Res 1993;56:335-40.
31. Dickerson JE, Jr., Lou MF. A new mixed disulfide species in human cataractous and aged lenses. Biochim Biophys Acta 1993;1157:141-6.
32. Nagaraj RH, Sady C. The presence of a glucose-derived Maillard reaction product in the human lens. FEBS Lett 1996;382:234-8.
33. Garner B, Shaw DC, Lindner RA, Carver JA, Truscott RJ. Non-oxidative modification of lens crystallins by kynurenine: a novel post-translational protein modification with possible relevance to ageing and cataract. Biochim Biophys Acta 2000;1476:265-78.
34. Iwaki A, Iwaki T, Goldman JE, Ogomori K, Tateishi J, Sakaki Y. Accumulation of alpha B-crystallin in brains of patients with Alexander's disease is not due to an abnormality of the 5'-flanking and coding sequence of the genomic DNA. Neurosci Lett 1992;140:89-92.
35. Palmer WG, Papaconstantinou J. Aging of alpha-crystallins during development of the lens. Proc Natl Acad Sci USA 1969;64:404-10.
36. Loo JA, Udseth HR, Smith RD. Peptide and protein analysis by electrospray ionization-mass spectrometry and capillary electrophoresis-mass spectrometry. Anal Biochem 1989;179:404-12.
37. Tumminia SJ, Jonak GJ, Focht RJ, Cheng YS, Russell P. Cataractogenesis in transgenic mice containing the HIV-1 protease linked to the lens alpha A-crystallin promoter. J Biol Chem 1996;271:425-31.
38. Takemoto LJ. Identification of the *in vivo* truncation sites at the C-terminal region of alpha-A crystallin from aged bovine and human lens. Curr Eye Res 1995;14: 837-41.
39. Spector A. Oxidation and cataract. Ciba Found Symp 1984;106:48-64.
40. Nagaraj RH, Monnier VM. Isolation and characterization of a blue fluorophore from human eye lens crystallins: *in vitro* formation from Maillard reaction with ascorbate and ribose. Biochim Biophys Acta 1992;1116: 34-42.
41. Ortwerth BJ, Olesen PR. Characterization of the elastase inhibitor properties of alpha-crystallin and the water-insoluble fraction from bovine lens. Exp Eye Res 1992;

54:103-11.
42. Abraham EC, Cherian M, Smith JB. Site selectivity in the glycation of alpha A- and alpha B-crystallins by glucose. Biochem Biophys Res Commun 1994;201:1451-6.
43. Roquemore EP, Chevrier MR, Cotter RJ, Hart GW. Dynamic O-GlcNAcylation of the small heat shock protein alpha B-crystallin. Biochemistry 1996;35:3578-86.
44. Roquemore EP, Dell A, Morris HR, Panico M, Reason AJ, Savoy LA, et al. Vertebrate lens alpha-crystallins are modified by O-linked N- acetylglucosamine. J Biol Chem 1992;267:555-63.