An Atypical Form of αB-crystallin Is Present in High Concentration in Some Human Cataractous Lenses

IDENTIFICATION AND CHARACTERIZATION OF ABERRANT N- AND C-TERMINAL PROCESSING*

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Two unique polypeptides, 22.4 and 16.4 kDa, were prominent in some human cataracts. Both proteins were identified as modified forms of the small heat shock protein, αB-crystallin. The concentration of total αB-crystallin in most of these cataracts was significantly increased. The 22.4 kDa protein was subsequently designated as αB-g. Mass spectrometric analyses of tryptic and Asp-N digests showed αB-g is αB-crystallin minus the C-terminal lysine. αB-g constituted 10–90% of the total αB-crystallin in these cataracts and was preferentially phosphorylated over the typical form of αB-crystallin. Human αB-g and αB-crystallin were cloned and expressed in Escherichia coli. The differences in electrophoretic mobility and the large difference in native pI values suggest some structural differences exist. The chaperone-like activity of recombinant human αB-g was comparable to that of recombinant human αB-crystallin in preventing the aggregation of lactalbumin induced by dithiothreitol. The mechanism involved in generating αB-g is not known, but a premature termination of the αB-crystallin gene was ruled out by sequencing the polymerase chain reaction products of the last exon for the αB-crystallin gene from lenses containing αB-g. The 16.4 kDa protein was an N-terminally truncated fragment of αB-g. The high concentration of αB-crystallin in these cataracts is the first observation of this kind in human lenses.

The three major classes of mammalian crystallins, α-, β-, and γ-crystallins, constitute about 90% of the total protein in the eye lens and are considered to determine the refractive properties of the lens. Post-translational modification of the crystallins has been a major focus of the research in trying to elucidate causes for the loss of lens transparency or cataract development (1–4). α-Crystallins have received the most attention in this case. The two homologous subunits, αA- and αB-crystallin, make up about 30% of the proteins in young human lenses and in the outer cortex of the adult human lens. αB-crystallin is a normal constituent of most mammalian tissues but is present in the highest concentrations in lens (5, 6). In addition to the lens, αA-crystallin is found in spleen and thymus (7). α-Crystallins are present in fiber cell extracts as large heteroaggregates with apparent molecular masses reported from 300 to 1000 kDa (8, 9). In cataracts the sizes of the aggregates reportedly increase and these aggregates are thought to be responsible for the light scattering (2).

On the other hand, α-crystallins are members of the family of small heat shock proteins and are thought to provide protection against cellular stresses (10–12). Members of this family are structurally related via the α-crystallin domain, they form large aggregates, are phospho-proteins, and have chaperone-like activity (12–19). α-Crystallins prevent the aggregation of proteins induced by heat, oxidation, or chemicals. In cultured cells, expression of αB-crystallin is induced by heat shock, oxidative stress, osmotic stress, arsenite, phorbol 12-myristate 13-acetate, and hormones such as estrogen and dexamethasone (20–23). Thus, the role of αB-crystallin in many systems is considered to be that of a stress protein. In the lens, however, it is not known whether the primary function of αB-crystallin is that of a stress protein and related to its chaperone-like function or if its major role is structural. It is possible its role in the lens may change depending on the stage of development and location in the lens.

Both α-crystallins undergo post-translational modifications including truncation of both N and C termini, deamidation, racemization, phosphorylation, methionine oxidation, glycation, disulfide formation, addition of O-GlcNAc, and the addition of 72 mass units to the C-terminal lysine of αB-crystallin (4, 17–19, 24, 25). Some of these such as phosphorylation and specific cleavage may be important functionally, others are likely the result of aging and detrimental stresses. Any of these modifications are likely to alter the protein conformation which in turn could alter the aggregate size and/or the function of α-crystallin in the cell.

Two-dimensional electrophoresis of tissue proteins is the major technique used to detect post-translationally modified proteins in cell and tissue extracts. We have used this technique extensively in an attempt to identify changes in the concentration or modification of lens proteins that could be unique to developmental, aging, and cataractogenic processes.

In this study we report the identification and characterization of two unique polypeptides observed on two-dimensional gel electrophoresis of human cataracts. One of the proteins was present in high concentration comparable to the concentration of a crystallin. Both are likely the result of stress-induced processes on the path to cataract formation.
MATERIALS AND METHODS

**Normal Lenses and Cataracts**—Normal human lenses were obtained from the National Disease Research Interchange, Philadelphia, PA. Human cataractous material was obtained from intracapsular cataract surgery done in India and from extracapsular extractions done at the National Eye Institute. Tenets of the Declaration of Helsinki for dealing with human samples were strictly followed. The lens capsule epithelia were removed, and the lenses were separated into the lens cortical and nuclear regions as described previously (26).

**Two-dimensional Gel Electrophoresis**—A proteinase inhibitor mixture containing 4-(2-aminoethyl)benzenesulfonyl fluoride, pepstatin A, α,β-epoxysuccinyl-l-leucylamido(4-guanidino)butane (E-64), bestatin, leupeptin, and aprotinin (Sigma) was added to frozen lens cortex samples. The samples were thawed and homogenized in 9 x urea, 2 or 4% Nonidet P-40, 10 mM DTT.2 Resolyte 3.5–10. Two-dimensional gel electrophoresis was done according to previously described procedures (27, 28). Non-linear, pH 3–10, 18-cm dry strips (Amersham Pharmacia Biotech) were used. Samples were loaded onto the acidic end and run under these conditions at 10,000 x g for 5 h. Cells were collected by centrifugation at 3,000 × g for 10 min at 4 °C and resuspended in 25 ml of lysis buffer, 50 mM sodium phosphate buffer, pH 7, containing 150 mM NaCl, 0.02% sodium azide, and a protease inhibitor mix (Roche Molecular Biochemicals). Cells were disrupted by sonication on ice. The bacterial lysates were then centrifuged at 10,000 × g for 30 min at 4 °C. The αB-crystallin and αBb, which were primarily soluble, were purified by gel filtration and ion exchange chromatography. The sequences of αB-crystallin and αBb were confirmed by sequencing the plasmids used for the expression and by mass spectral fingerprinting of the expressed proteins. The sequences of the recombinant proteins were identical to those of the proteins in the cataractous lenses with the exception that the recombinant proteins were not acetylated on the N termini.

**Isolation of Genomic DNA, αB-crystallin Exon 3 Amplification, and Sequencing**—Genomic DNAs were isolated from small pieces of lens tissue (5–10 mg) by a rapid desalting process using a DNA purification kit (Epigence Technologies) and following the recommendations of the manufacturer. These DNAs (200 ng) served as templates for PCR amplification of exon 3 and flanking regions of human αB-crystallin gene (nucleotide identifier (NID) g181075). Primers were 24-mers in length and localized at positions 3722 (upper) and 4172 (lower). PCR cycling conditions were 40 cycles of 94 °C for 5 s, 50 °C for 30 s, and 72 °C for 1 min, using Pfu polymerase (Promega). PCR products were sequenced by the BigDye Terminator method (PE Applied Biosystems) using the same primers.

**Chaperone-like Activity**—Chaperone-like activity was measured as the ability to protect against the DTT-induced aggregation of lactalbumin (30). The reaction was done at 23 °C in 50 mM sodium phosphate buffer, pH 6.9, containing 0.1 mM NaCl and 2 mM EDTA. Lactalbumin was at 1 mg/ml, and crystallin was at 0.2, 0.5, and 1 mg/ml. Turbidity was measured at 360 nm.

**RESULTS**

Two unique protein spots were striking on two-dimensional electrophoresis gels of the cortical fiber cell protein from some human cataractous lenses (Fig. 1A). The positions of these proteins are indicated in Fig. 1A. The spot indicated by the thick black arrow migrated at a position one charge more acidic than αB-crystallin and at a Mr of 22,400 which is approximately 600 less than αB-crystallin (Fig. 1A). The second spot, indicated by the white arrow migrated at a Mr of 16,400. Both of these proteins reacted with antibodies made against recombinant human αB-crystallin (Fig. 1B) suggesting that the proteins were related to αB-crystallin. Data that will subsequently be presented in this report show that the Mr 22,400 protein is a modified form of αB-crystallin. To simplify discussion, this protein will hereonforth be referred to as αBb. Neither αBb nor the 16.4-kDa protein has been observed on two-dimensional gels of total fiber cell protein from any normal lens examined (Fig. 2 (a–c)) or in all cataracts. The cataracts shown in Fig. 2 (d–f) that contain αBb were not clinically classified before extraction but were determined to be mixed cataracts by visual examination after intracapsular extraction. The concentration

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1 The abbreviations used are: DTT, dithiothreitol; PCR, polymerase chain reaction; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography.
of αB varied from 10 to 90% of the total αB-crystallin (αB plus αB-crystallin) in those cataracts in which it was present. In addition, the total content of αB-crystallin (αB plus αB-crystallin) was significantly increased in most of these cataracts. Representative data on three cataracts that contain high concentrations of αB-crystallin are shown in Table I. Up to 5 times the normal amount of αB-crystallin was seen. This was determined by calculating the ratio of αB-crystallin to αA-crystallin. The amount of αA-crystallin in these cataracts was present at normal concentrations relative to β-crystallins. To our knowledge, an increase in the concentration of αB-crystallin in lenses has not been previously reported.

α-crystallins are phosphorylated in vivo; however, in the lens the function of the phosphorylation is not known. As can be seen in Fig. 1A (thick black arrow), αB-crystallin was the only form of αB-crystallin that fell into a phosphorylated species was obvious; phosphorylated species of the typical form of αB-crystallin were not observed. Three phosphorylation sites have been identified for αB-crystallin, Ser 115, Ser 145, and Ser 250. Mass spectral data indicated the presence of a phosphate in the peptide corresponding to residues 2–24 in αB-crystallin.

Characterization of αB-crystallin:—The HPLC chromatograms of the tryptic digests of αB-crystallin from cataracts of two different individuals are presented in Fig. 2. There were no consistent differences between the chromatograms of αB-crystallin from the two different samples from both individuals. Edman sequencing of several tryptic peptides of αB-crystallin was performed. For each peptide sequenced there was 100% agreement with the sequence of human αB-crystallin (data not shown). These results confirm that the protein is an αB-crystallin, albeit, based on the electrophoretic migration, a modified form of αB-crystallin. Direct Edman sequencing of αB-crystallin, electrobotted onto polyvinylidene difluoride, gave no sequence, suggesting that the N terminus of the protein was blocked. In all αB-crystallin samples, a peptide eluting at 40 min corresponded to the C-terminal residues 164–174. Edman sequencing verified the identity of the peptide and the mass of the peptide agreed with its theoretical mass. The presence of C-terminal Lys 175 in αB-crystallin, however, could not be verified by this experiment because trypsin would cleave between Lys 174 and Lys 175.

Mass fingerprinting was done on trypsin and Asp-N protein digests of αB-crystallin and αB-crystallin, using MALDI-TOF mass spectrometry. The results are illustrated in Tables II and III. In Table II, the protonated molecular weights for those peptides derived by tryptic digestion of αB-crystallin and αB-crystallin, as well as the theoretical protonated molecular weights, are listed. The presence of peptides with m/z values of 1430.7 and 1431.0 in the tryptic digests of αB-crystallin from both samples indicate that the N terminus is present and acetylated. In the trypsin digests there were a few masses that could not be assigned, but there were no masses consistently present in αB-crystallin that were missing in αB-crystallin or vice versa.

In Table III, the masses for the peptides derived by Asp-N endoproteinase digestion are listed. αB-crystallin samples contained peptides with m/z 3842.9 and 3842.0. These molecular weights are consistent with the C-terminal peptide, residues 140–175, that has a theoretical m/z value of 3842.4. A mass that corresponded to this peptide was absent in both αB-crystallin samples, but in both cataracts αB-crystallin contained a peptide absent in either αB-crystallin spot, with m/z values of 3715.0 and 3714.4 (Fig. 4). These masses are consistent with the theoretical m/z value of the C-terminal peptide minus Lys 175. Only one peak corresponding to residues 129–139 (DPLTITSSLS) was consistently absent in Asp-N spectra, and it was not present in either crystallin. In cataract B, there was evidence for Met 105 being present as methionine sulfoxide in both αB-crystallin and αB-crystallin. The m/z value of 1294.9 is consistent with the expected molecular weight of residues 62–72 plus a mass of 16 mass units. The theoretical m/z value of the same peptide with Met 105 oxidized is 1294.61. Between the two enzymatic digestions spectra were obtained for peptides that covered 100% of the polypeptide sequence. The regions of the polypeptide that could not be accounted for by tryptic peptides were accounted for with the peptides from Asp-N digests and the reverse was also true, making it possible to detect a difference in any residue of αB-crystallin and αB-crystallin. There were no unique modifications, amino acid substitutions, or internal deletions detected in αB-crystallin. The excellent agreement of the masses obtained on the peptide digests of αB-crystallin with the respective theoretical masses of the same peptides in αB-crystallin indicate that there is only one difference between αB-crystallin and αB-crystallin. The terminal Lys 175 is missing in αB-crystallin.

The loss of the C-terminal lysine could readily explain the migration of αB-crystallin on two-dimensional gel electrophoresis at a position 1 charge more acidic than the typical form of αB-crystallin. However, it did not seem likely that the loss of one lysine (128 mass units) could explain the migration of αB-crystallin at the lower molecular weight. The only reasonable explanations were that αB-crystallin had region(s) of structure different from αB-crystallin that remained throughout the electrophoresis even in the presence of 9 M urea, 2% Nonidet P-40 or 1% SDS and affected its electrophoretic migration, or that an adduct was lost during the subsequent analyses.

Recombinant Human αB-crystallin and αB-crystallin.—To test whether the removal of the C-terminal lysine was sufficient to induce the altered migration on electrophoresis as was observed in the second dimension of two-dimensional electrophoresis of unexpected migration, the recombinant human αB-crystallin and αB-crystallin were used to generate the two-dimensional electrophoresis pattern.
phoresis, both human αB-crystallin and human αB-crystallin minus the C-terminal lysine (αBg) were cloned and expressed in E. coli. Both were expressed as soluble proteins in this system. Neither protein was acetylated on the N-terminal methionine. The recombinant human αB-crystallin and the recombinant human αBg were cut out of a two-dimensional gel and subjected to in-gel digestion by Asp-N endoproteinase. Resultant peptides were extracted and analyzed by MALDI-TOF mass spectrometry. The portions of the spectra showing the difference between αB-crystallin and αBg are shown. The m/z value of 3842.9 corresponds to the singly charged peptide of residues 140–175, which has a theoretical average m/z of 3842.4. The m/z 3714.4 corresponds to the singly charged peptide of residues 140–174, which has a theoretical average m/z of 3714.3. The absence of a peak at m/z 3842.4 and the coincident mass at m/z 3714.4 in the spectrum of αBg indicates that Lys175 is not present in αBg.

**Table II**

αB-Crystallin tryptic digest products determined by MALDI-TOF mass spectrometry

| Residues | Theoretical m/z | Cataract A | Cataract B | Cataract A | Cataract B |
|----------|-----------------|------------|------------|------------|------------|
| 1–11/Ac  | 1430.7          | 1430.9     | 1430.7     | 1431.0     |            |
| 12–22    | 1374.7          | 1374.8     | 1374.8     | 1374.9     | 1374.9     |
| 23–56    | 4006.5          |            | 4006.7     |            |            |
| 57–69    | 1496.7          | 1496.9     | 1496.9     | 1497.0     |            |
| 75–82    | 921.5           | 921.5      | 921.5      | 921.7      |            |
| 83–92    | 1213.7          | 1213.8     | 1213.6     | 1213.8     | 1213.9     |
| 93–116   | 2788.1          | 2787.8     | 2787.8     | 2787.9     | 2787.0     |
| 117–120  | 588.3           | 588.3      | 588.3      | 588.3      | 588.4      |
| 121–149  | 3073.5          | 3073.2     | 3074.2     | 3073.6     | 3073.9     |
| 150–157  | 900.5           | 900.6      | 900.6      | 900.7      |            |
| 158–174  | 1822.0          | 1822.0     | 1822.5     | 1822.4     | 1822.4     |

* Theoretical monoisotopic m/z values are listed for m/z less than 2000, and theoretical average m/z values are listed for m/z greater than 2000.

**Table III**

αB-crystallin Asp-N endoproteinase digest products determined by MALDI-TOF mass spectrometry

| Residues | Theoretical m/z | Cataract A | Cataract B | Cataract A | Cataract B |
|----------|-----------------|------------|------------|------------|------------|
| 2–24     | 2875.4          | 2875.1     | 2875.5     | 2875.1     | 2875.5     |
| 25–35    | 1321.6          | 1321.8     | 1321.8     | 1321.8     | 1321.8     |
| 36–61    | 3031.5          | 3031.1     | 3031.5     | 3031.2     | 3031.0     |
| 62–72    | 1278.6          | 1278.8     | 1278.8     |            |            |
| 62–72/mso | 1294.9          |            | 1294.8     | 1294.8     |            |
| 73–79    | 850.4           | 850.5      | 850.5      | 850.5      | 850.6      |
| 80–85    | 1825.0          | 1826.3     | 1826.3     | 1826.3     | 1825.3     |
| 96–108   | 1575.8          | 1576.0     | 1576.0     | 1576.0     | 1576.0     |
| 109–126  | 2259.5          | 2259.6     | 2259.5     | 2259.7     | 2259.5     |
| 129–139  | 1121.2          |            |            |            |            |
| 140–175  | 3842.4          | 3842.0     | 3842.9     |            |            |
| 140–174  | 3714.3          | 3715.0     | 3714.4     |            |            |

* Theoretical monoisotopic m/z values are listed for m/z less than 2000, and theoretical average m/z values are listed for m/z greater than 2000.

The expected mass for peptide 62–72 upon oxidation of Met68.
two-dimensional gels of lens proteins. These results show that the removal of the C-terminal lysine is sufficient to cause the migration of aBG at a significantly lower molecular weight than aB-crystallin. The pI values determined by isoelectric focusing under non-denaturing conditions for both proteins are shown in Fig. 6. The recombinant aB-crystallin had a native pI of 6.8, and recombinant aBG had a native pI of 5.8. The theoretical pI values are 6.76 and 6.50, respectively.

Chaperone-like Activity—aCrystallins exhibit a chaperone-like activity, protecting against aggregation of proteins that is induced by heat, oxidation, and reduction by DTT (30). The effect of the removal of the C-terminal lysine on the chaperone-like activity of aB-crystallin was assessed by determining the ability of the recombinant forms of human aB-crystallin and aBG to prevent the DTT-induced aggregation of lactalbumin. As shown in Fig. 7, the removal of the C-terminal lysine had essentially no effect on the chaperone-like activity of this protein under these conditions.

16.4-kDa Protein—The 16.4-kDa protein was present at a concentration about one-tenth that of aBG and has only been observed in samples that contain aBG. Direct Edman sequencing was performed on the 16.4-kDa protein from cataracts of two different individuals. These data are shown in Fig. 8. The 16.4-kDa protein in lens 1 yielded multiple N-terminal sequences, Ser43, Ser 41, Ser 45, and Phe 47. All sequences were identical to aB-crystallin. MALDI-TOF derived data on the 16.4-kDa protein from a third individual indicated the N terminus were Ser43 and Ser41. In a fourth individual the N terminus was Pro 39 (Table IV). Two of the cleavages were between Thr and Ser, one between Leu and Ser, one between Pro and Phe, and the fourth was between Phe and Pro. The significance of these data is that multiple N termini have been identified, each different by two or multiples of two residues.

Tryptic digests of this spot were similar but not identical to that of aB-crystallin. A peptide with a mass of 1140 Da that...
eluted at the same time as a peptide identified by sequencing as residues 164–174 was present. MALDI-TOF mass spectral analysis of Asp-N endoproteinase digests showed the absence of a peak at \( m/z \) 3842.4 which would correspond to residues 140–174. As shown in Table IV, experimental \( m/z \) values were matched to theoretical \( m/z \) values from Asp-N cleavage that covered most of the polypeptide. Aside from the N-terminal peptides, the only mass missing was the same one not observed in aB and aB\(_g\). A unique peak at \( m/z \) 3130.5 was observed. This mass may correspond to residues 39–66 (3129.8). These results show that the 16.4-kDa protein is derived from aB\(_g\).

**Genomic Analysis**—The mechanism by which aB\(_g\) is generated in these cataractous lenses is not known. One possibility was that aB\(_g\) is the product of a mutated gene for aB-crystallin since a single nucleotide change could convert the codon for lysine to a stop codon. To test this possibility PCR products were obtained for the last exon of the aB-crystallin gene using genomic DNA prepared from lenses containing aB\(_g\). The sequences of the PCR products indicated that there was no mutation in the gene for aB-crystallin (data not shown).

**DISCUSSION**

We have demonstrated that in some human cataracts the concentration of the small heat shock protein, aB-crystallin, is significantly increased. This could be the result of an up-regulation of its expression and/or a diminution of its degradation in the lens. Regardless, it represents a significant deviation from the normal protein composition of the lens and is the first time such an observation has been made in a human cataract. We have also provided evidence for aberrant N- and C-terminal processing of aB-crystallin. One atypical form of aB-crystallin found in high concentrations in these cataracts has been designated aB\(_g\). This species is aB-crystallin minus the C-terminal lysine. A second atypical form of aB-crystallin, found at about 10% the concentration of aB\(_g\), is a 16.4-kDa fragment of aB\(_g\). In this protein 38–46 N-terminal residues have also been removed.

The removal of the C-terminal lysine does not diminish the chaperone-like activity of aB-crystallin under the conditions used for these studies. As reported here (Fig. 7) the chaperone-like activity of the recombinant human aB\(_g\) is comparable to that of the recombinant human aB-crystallin in protecting against the DTT-induced aggregation of lactalbumin at room temperature. aA- and aB-crystallin have polar, flexible C-terminal extensions that are thought to contribute to the solubility of these crystallins and have been implicated in their chaperone-like activity (31). Substitution of Lys\(^{174}\)-Lys\(^{175}\) of aB-crystallin with Leu-Leu significantly diminished chaperone-like activity; however, removal of the last 5 residues had little effect on chaperone-like activity (32). Likewise, in aA-crystallin the introduction of tryptophan at the C terminus and removal of 17 C-terminal residues diminished chaperone activity (33–35). Maintenance of a polar, flexible C-terminal extension appears to be an important factor for maintaining chaperone-like activity (33). Thus, it is not surprising that aB\(_g\) has full activity in protecting against the DTT-induced aggregation of lactalbumin.

The only modification identified for aB\(_g\) was the loss of Lys\(^{175}\). There was nothing in the mass spectral, HPLC, and protein sequence data that suggested an additional modification. The N and C termini of the protein were otherwise intact. The N-terminal methionine was acetylated, and every peptide sequence examined matched aB-crystallin exactly. With the exception of the mass corresponding to the loss of the C-terminal lysine, there were no masses found by MALDI-TOF mass spectrometry that were consistently present in aB\(_g\) that were not present in aB-crystallin from the same cataract and vice versa. This held for both Asp-N and trypsin digests of the proteins, and, combining data from both digests, the entire sequences of both aB\(_g\) and aB-crystallin were analyzed. These data rule out splice variants and other mutations unless replacements have the same mass and would not be detected in the MALDI-TOF mass spectrometry fingerprint analysis.

It was confirmed that the removal of the carboxyl-terminal lysine (128.09 mass units) was responsible for the faster migration of aB\(_g\) on SDS-PAGE relative to aB-crystallin. This was demonstrated using the recombinant forms of these proteins. In addition to showing that a difference of one lysine was sufficient to alter the migration on SDS-PAGE, it also supported the conclusion that the only modification of aB\(_g\) was the lack of the terminal lysine. Smulders \( et \ al. \) (33) demonstrated that a mutant of aA-crystallin with an extension of ALRKG migrated on SDS-PAGE slightly slower than mutants with ALGKG or ALDKG. Thus, addition of one more positive charge in the C-terminal extension of either aA- or aB-crystallin can slightly retard its electrophoretic mobility.

In this study, aB\(_g\) and the 16.4-kDa proteins were only observed in cataracts but not in all human cataracts. Over 70 cataracts from 60 to 90 years have been analyzed. Eight cataracts have the high concentration of aB\(_g\). Many other cataracts have aB\(_g\) at lower concentrations. So far, no correlation cannot be made between the presence of these proteins in cataracts and any cataract etiology. Neither aB\(_g\) nor the 16.4-kDa fragment have been observed in our laboratory on two-dimensional electrophoresis of normal human lens total protein, water-soluble or water-insoluble fractions, of about 50 non-cataractous lenses (newborn to 75 years). However, the presence in a normal lens of a low concentration of a-crystallin with a mass that corresponded to aB-crystallin minus the C-terminal lysine has been reported using liquid chromatography/mass spectrometry (36–38). The sensitivity of electrospray...
ionization mass spectrometry made the detection of very low levels of αB in non-cataractous lenses possible, whereas Fig. 2 clearly shows that αB is not detected by Coomassie Blue staining of normal lens proteins. The findings in other laboratories suggest that at least very low concentrations of αB may exist in normal lenses and that αB and possibly the 16.4-kDa fragment may be intermediates in the normal pathway of processing αB-crystallin (36–38). In the cataracts examined in this study, there may be increases and/or decreases in proteinases that result in the accumulation of these species. Interestingly, αA-crystallin which has Ser-Ser as the final C-terminal residues is found with only the final Ser removed (60). This form of αA-crystallin has been observed in normal human and bovine lenses. The functional significance of the modified form is not known. Furthermore, the significance of the processing of α-crystallins to cataractogenesis is not known.

Multiple proteinases are certain to be involved in the post-translational modification of αB-crystallin generating αB and the 16.4-kDa fragment. The data reported in this study rule out the possibility that αB is the result of a mutation in the αB-crystallin gene but cannot rule out a genetic component involving other genes such as proteinases or involving the regulation of proteinases. There was a high incidence of αB in cataracts from India. This supports the possibility of a genetic component. Alternatively, the high incidence in cataracts from India could indicate the presence of a particular form of stress on the lens which alters the proteinase activities.

In αB the terminal lysine is removed but not the penultimate lysine. The first cleavage would be a Lys-Lys cleavage. Carboxypeptidases have been described that are specific for basic amino acids for which the penultimate amino acid alters the rate of cleavage (39). Peptide carboxypeptidases have been described that will not cleave prolyl bonds, and endopeptidases with specificity for pairs of basic amino acids that will remove one or both of the basic residues have been described (39). Thus, the specificity for removing one but not both lysine residues is possible; however, whether any of these proteinases are in the lens is not known. If the removal of the C-terminal lysine is related to function, it is possible a specific carboxypeptidase may be induced during stress.

The 16.4-kDa protein is an N-terminally truncated form of αB and was only observed in those lenses in which αB was in high concentration. Multiple N termini were identified, but the major N terminus was Ser43. Since each N terminus represented the removal of two residues or multiples of two, it is reasonable to suggest that cleavage is catalyzed by a dipeptidyl peptidase. Dipeptidyl peptidases II (lysosomal) and III (cytoplasmic) are present in lens and cataracts (40, 41). However, both of these preferentially use peptides as substrates, not proteins. Dipeptidyl peptidases that utilize proteins as substrates exist but, to our knowledge, have not been described in the lens. The N termini of αB and αB-crystallin are blocked, so presumably a different proteinase made the first cleavage. Acylaminohydrolases that could cleave the acetylmethionine are present in lenses (42).

Our hypothesis is that a stress mechanism is involved in generating both the high concentrations of αB-crystallin and the subsequently modified forms, αB and the 16.4-kDa protein, and that the increased concentration of αB-crystallin is an effort by the lens to protect against loss of lens function. That a stress mechanism is involved is strongly supported by our observation that αB was present in the lens from an infant with microphthalmia, coloboma, and persistent hyperplastic primary vitreous. The latter is a condition where the vessels do not regress at the appropriate time in development and cells invade the back of the lens. Our assumption is that this is perceived by the lens as an extreme stress condition. In addition, two forms of αB-crystallin were induced in hypertonically stressed dog lens epithelial cells in culture (21). Direct evidence is not yet available, but it is highly likely, based on the migration on two-dimensional gels, that the atypical form of αB-crystallin found is αB. αB-crystallin is a member of the family of small heat shock proteins and in cultured cells, including lens cells, αB-crystallin is induced by heat shock, oxidative stress, osmotic stress, sodium arsenite, phorbol 12-myristate 13-acetate, and hormones such as estrogen and dexamethasone (20–23, 43, 44). Heat shock proteins are also phosphorylated in response to stress (45). α-Crystallins are phosphorylated in vivo and in vitro (46–48). The role of phosphorylation of αB-crystallin in lens is not understood, but αB was preferentially phosphorylated in the cataracts examined.

This is the first time an increased concentration of αB-crystallin has been described in vivo in human lenses. The increased concentration is due to the presence of αB not to an increased concentration of the typical form of αB-crystallin. Due to the nature of the lenses, it is difficult to determine whether there is truly an increased expression or a decreased degradation of αB-crystallin. If the increased concentration of αB-crystallin reflects diminished processing, the degradation of this protein must occur at a much greater rate in human lens than previously appreciated. We propose that there is an increased expression due to chronic stress conditions and altered processing of αB-crystallin generating αB in these cataracts. αB-crystallin also accumulates in brains of individuals with neurological disorders (49–51). It is unclear in these cases, as well as the cataracts, whether the presence of αB-crystallin is an attempt to rescue the tissue or if it is a contributor to the pathology. We think it is unlikely that the increased concentration of αB-crystallin and the presence of the modified forms cause the loss of lens clarity in the cataracts, but it must be considered.

The functional significance of the post-translational modification of αB-crystallin described here is not known. In the intact lens, it is unclear whether the primary function of αB-crystallin is that of a stress protein and related to its chaperone-like activity or if it is structural. The findings that αB is present in a lens with persistent hyperplastic primary vitreous and is induced by hypertonic stress in lens cells suggests a major role is stress-related. Based on a number of reports, αB-crystallin interacts with actin, intermediate filaments, membranes, and components in cell nuclei (52–57). Lys175 has been implicated as an amine donor substrate for transglutaminase reactions (58, 59). The data presented suggest αB has some difference in structure from the typical form of αB-crystallin. This is likely to alter specific protein-protein interactions. In fact, the larger than expected change in the native pI of αB supports the observations that the C-terminal lysine is involved in intramolecular interactions and is likely to affect intermolecular interactions as well. Further evidence for this is the preferential phosphorylation of αB over αB-crystallin.

Studies in progress will hopefully elucidate the mechanisms involved in the increased concentration of αB-crystallin and the function of αB in lens and in cataracts. They will also determine whether the pathway for modification of αB-crystallin is one specific to lens or is a general pathway induced by stress in other tissues.

2 D. Garland, unpublished data.
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